Educational Workshops 2017
UNFRIENDLY FUNGI; INFECTIONS, DIAGNOSIS, ANTIFUNGALS AND MANAGEMENT

READING LIST

Keynote
- Aspergillus nodules; another presentation of Chronic Pulmonary Aspergillosis
- Efficacy and Safety Outcomes in Patients with Probable or Proven vs. Possible Invasive Mould Disease from the Phase 3 SECURE Study, Evaluating Isavuconazole vs. Voriconazole for the Primary Treatment of Invasive Fungal Disease Caused by Aspergillus spp. or Other Filamentous Fungi

- Estimating the burden of invasive and serious fungal disease in the United Kingdom
- Treatment of invasive aspergillosis
- Performance of Candida Real-time Polymerase Chain Reaction, b-D-Glucan Assay, and Blood Cultures in the Diagnosis of Invasive Candidiasis
- The role of the multidisciplinary team in antifungal stewardship
- VORICONAZOLE VERSUS AMPHOTERICIN B FOR PRIMARY THERAPY OF INVASIVE ASPERGILLOSIS

Case one
- Mistaken Identity: Neosartorya pseudofischeri and its Anamorph Masquerading as Aspergillus fumigatus
- Syndromes of invasive fungal sinusitis
- Successful isavuconazole salvage therapy in a patient with invasive mucormycosis
- Tissue Penetration of Antifungal Agents
- Frequency and Evolution of Azole Resistance in Aspergillus fumigatus Associated with Treatment Failure
- The Effect of Therapeutic Drug Monitoring on Safety and Efficacy of Voriconazole in Invasive Fungal Infections: A Randomized Controlled Trial
- Pharmacodynamics of Isavuconazole in an Aspergillus fumigatus Mouse Infection Model
- Azole Resistance in Aspergillus fumigatus: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles?
- Chronic Invasive Sinus Aspergillosis in Immunocompetent Hosts: A Geographic Comparison
Case two

- Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America
- Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology
- Candida infective endocarditis
- ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients
- ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic haemohyphomycosis: diseases caused by black fungi
- Exophiala (Wangiella) dermatitidis Prosthetic Aortic Valve Endocarditis and Prosthetic Graft Infection in an Immune Competent Patient
- Antifungal susceptibility testing of Exophiala spp.: a head-to-head comparison of amphotericin B, itraconazole, posaconazole and voriconazole
- Fungal endocarditis: current challenges
- Clinical Practice Guidelines for the Management of Candidiasis: 2009 Update by the Infectious Diseases Society of America
- Isavuconazole: A New Broad-Spectrum Triazole Antifungal Agent
- A meta-analysis of medical versus surgical therapy for Candida endocarditis

Case three

- Infective endocarditis: An intensive care perspective
- Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America
- A meta-analysis of medical versus surgical therapy for Candida endocarditis
- 2015 ESC Guidelines for the management of infective endocarditis
- Candida Infective Endocarditis: an Observational Cohort Study with a Focus on Therapy

Case Four

- Penicillium marneffei Infection and Recent Advances in the Epidemiology and Molecular Biology Aspects
- Penicillium marneffei infection in HIV

Case five

- Combination Antifungal Therapy for Mold Infections: Much Ado about Nothing?
- Treatment of Endogenous Fungal Endophthalmitis: Focus on New Antifungal Agents

Icebreaker Quiz

- Guidance for the laboratory investigation, management and infection prevention and control for cases of Candida auris
Aspergillus nodules; another presentation of Chronic Pulmonary Aspergillosis

Eavan G. Muldoon1,4*, Anna Sharman2, Iain Page1,4, Paul Bishop3 and David W. Denning1,4

Abstract

Background: There are a number of different manifestations of pulmonary aspergillosis. This study aims to review the radiology, presentation, and histological features of lung nodules caused by Aspergillus spp.

Methods: Patients were identified from a cohort attending our specialist Chronic Pulmonary Aspergillosis clinic. Patients with cavitating lung lesions, with or without fibrosis and those with aspergillomas or a diagnosis of invasive aspergillosis were excluded. Demographic, laboratory, and clinical data and radiologic findings were recorded.

Results: Thirty-three patients with pulmonary nodules and diagnostic features of aspergillosis (histology and/or laboratory findings) were identified. Eighteen (54.5 %) were male, mean age 58 years (range 27–80 years). 19 (57.6 %) were former or current smokers. The median Charleston co-morbidity index was 3 (range 0–7). All complained of at least one of; dyspnoea, cough, haemoptysis, or weight loss. None reported fever. Ten patients (31 %) did not have an elevated Aspergillus IgG, and only 4 patients had elevated Aspergillus precipitins. Twelve patients (36 %) had a single nodule, six patients (18 %) had between 2 and 5 nodules, 2 (6 %) between 6 and 10 nodules and 13 (39 %) had more than 10 nodules. The mean size of the nodules was 21 mm, with a maximum size ranging between 5–50 mm. No nodules had cavitation radiographically. The upper lobes were most commonly involved. Histology was available for 18 patients and showed evidence of granulation tissue, fibrosis, and visualisation of fungal hyphae.

Conclusion: Pulmonary nodules are a less common manifestation of aspergillosis in immunocompetent patients. Distinguishing these nodules from other lung pathology may be difficult on CT findings alone.

Keywords: Aspergillus, Pulmonary nodule, Fungal infection of lung, Chronic pulmonary aspergillosis

Background

There are a number of manifestations of pulmonary aspergillosis [1]. Aspergillus spp. are ubiquitous in the environment and exposure to conidia is common. However, only a minority of people develop clinical disease, and this is often determined by host characteristics, e.g. immune compromise, genetic predisposition, underlying lung pathology, and prior pulmonary infection such as tuberculosis (TB). Classically chronic pulmonary aspergillosis (CPA) in immunocompetent patients presents as a saprophytic infection in a pre-existing cavity, often following an infection such as TB or prior lung surgery. There are a number of recognised manifestations of CPA; subacute invasive pulmonary aspergillosis (SAIA) [which may be referred to as chronic necrotising pulmonary aspergillosis (CNPA)], chronic cavitary pulmonary aspergillosis (CCPA) and chronic fibrosing pulmonary aspergillosis (CFPA) [2]. Subacute IPA occurs in the setting of some degree of immune compromise, and may present with nodules, consolidation and or cavitation on chest imaging, and a more rapidly progressive clinical course. CCPA presents with single or multiple cavities, with or without aspergilloma(s), and CFPA has this appearance with the additive features of pulmonary fibrosis, which may be progressive and destructive.

Estimates of the incidence and prevalence of CPA are difficult; however the global burden of disease is...
increasingly being recognised [3, 4]. For example, the proportion of patients with TB as an underlying risk factor for the development of CPA will vary depending on geographical location [5]. In 2011, CPA was estimated to affect 3600 patients in the UK, based on estimates of prior TB, and diagnoses of sarcoidosis [6], and 1.2 million worldwide after TB [3] and 72,000 complicating fibrocystic pulmonary sarcoidiosis [4].

There is a paucity of knowledge on CPA which presents as single or multiple nodule(s) without cavitation in immune competent hosts. The published literature is limited to case reports and small case series. Often, in these cases the diagnosis is made following removal or biopsy of the nodule(s) which is presumed to be malignant [7, 8]. In the largest case series from Korea, eleven patients with solitary pulmonary nodules were reviewed [9]. Three of the eleven patients had some evidence of cavitation on CT imaging, and all had histologically proven Aspergillus infection. In a second Korean series, seven patients were identified with biopsy proven Aspergillus disease, in the absence of immunosuppression or underlying lung disease [10]. Unfortunately in neither series was there correlation with Aspergillus IgG (precipitins), which is a cornerstone of the diagnosis of CPA [2].

The purpose of this study is to review the clinical characteristics, histological and radiological features of pulmonary nodules caused by Aspergillus spp.

Methods

Patients attending our specialist CPA clinic in the National Aspergillosis Centre (NAC) with nodular Aspergillus disease were identified. The NAC is nationally commissioned to provide specialist care for patients with chronic pulmonary aspergillosis in the UK. There are currently approximately 350 patients in follow up care of the NAC with CPA, and approximately 100 new patients referred annually. Patients were identified by one of two methods. First, patients with pulmonary nodules on chest imaging at presentation, and features consistent with a diagnosis of aspergillosis (i.e. biopsy proven disease and/or positive Aspergillus serology and/or Aspergillus spp isolated form respiratory secretions) were prospectively recorded. Second, additional case finding was performed by the retrospective review of patient correspondence and review of histopathology records. A rounded opacity, well or poorly defined, measuring up to 3 cm in diameter was defined as a nodule as per the Fleischner Society: Glossary of Terms for Thoracic Imaging [11]. Patients with aspergillomas and those with cavitating lung lesions, with or without fibrosis were excluded. Patients with a diagnosis of invasive aspergillosis were also excluded. Demographic data, details of the clinical presentation, laboratory data and radiologic findings were recorded on each patient. All radiology was reviewed by a consultant radiologist (AS) for accuracy. The ImmunoCap™ assay (Phadia, Uppsala, Sweden) was used to measure A. fumigatus IgG and the Microgen antigens and counterimmunoelectrophoresis (Microgen, Camberley, Surrey, UK) for Aspergillus precipitins. Serum mannose binding lectin (MBL) concentrations were measured by ELISA (MBL Oligomer ELISA Kit, BioPorto Diagnostics, DK), upper and lower reported detection limit of 4.00 and 0.05 mg/L respectively. For culture, sputum was digested with Sputasol® (ratio 1:1), vortexed, and 10 μL-streaked on two Sabouraud dextrose agar plates [12] and incubated at 30 °C and 37 °C for 7 days, and on bacterial media. For quantitative PCR, the MycXtra kit (Myconostica, Cambridge, UK) was used for DNA extraction using 0.5–3 mL of sample. DNA was eluted in 40 μL and 10 μL used for quantitative PCR. The MycAssay Aspergillus kit (Myconostica) was used following the manufacturer’s instructions; a crossing threshold (Ct) of >38 was negative, Ct from 36–38 a weak positive and <36 was interpreted as a strong positive [13]. The data were collected in Microsoft excel, and data analysis performed using SPSS version 20. This report is a retrospective evaluation of all patients who were managed with Aspergillus nodules, and as such is exempt from ethical review or patient consent.

Results

Thirty three patients with lung nodules and features diagnostic of CPA (histology and/or laboratory findings) were identified. Ten patients had proven disease, and the remainder deemed probable disease, based on serology and culture results (Table 1). Eighteen (54.5 %) of patients were male, the mean age was 58 years (range 27–80 years). Nineteen (57.6 %) were current or ex-smokers, in 9 (27.3 %) smoking history was not documented. The median Charleston co-morbidity index was 3 (range 0–7). On presentation all patients complained of at least one of the following symptoms: dyspnoea, cough, haemoptysis, or weight loss. Twenty nine patients (88 %) reported cough, 23 (70 %) dyspnoea, 11 (33 %) described weight loss, and 5 (15 %) haemoptysis. No patients reported a history of fever.

Radiological features

All patients had computer tomography (CT) performed. Twenty patients (60 %) had upper lobe disease alone, with either unilateral or bilateral involvement. In seven patients (6 %) all lobes were involved, the remaining patients had variable patterns of lobar involvement. In twelve patients (36 %) a single nodule was present, six (18 %) patients had between 2 and 5 nodules, 2 (6 %) had between 6 and 10 nodules and 13 (39 %) patients more than 10 nodules. The maximum nodule size
## Table 1 Characteristic of patients diagnosed with Aspergillus nodule(s)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of nodules</th>
<th>Lobes of lung involved</th>
<th>Min size (mm)</th>
<th>Max size (mm)</th>
<th>Lymphadenopathy</th>
<th>Visible on concurrent CXR</th>
<th>Symptoms</th>
<th>Aspergillus IgG</th>
<th>Sputum culture</th>
<th>Aspergillus PCR</th>
<th>Tissue Specimen</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>LLL</td>
<td>11</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td>None</td>
<td>76</td>
<td>n/a</td>
<td>lung fibrosis, fungal hyphae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Upper lobes bilaterally</td>
<td>10</td>
<td>n</td>
<td>y</td>
<td>None</td>
<td>None</td>
<td>68</td>
<td>Negative lung granuloma, necrosis, fungal hyphae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>LUL</td>
<td>16</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough, weight loss</td>
<td>14</td>
<td>Negative lung inflammation, fungal hyphae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>RUL</td>
<td>7</td>
<td>n</td>
<td>y</td>
<td>Cough, weight loss</td>
<td>40</td>
<td>Negative Lung inflammation granulomatous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>RUL</td>
<td>16</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, weight loss</td>
<td>101</td>
<td>n/a lung COP, fungal hyphae</td>
<td></td>
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<tr>
<td>6</td>
<td>1</td>
<td>RUL</td>
<td>12</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough, haemoptysis</td>
<td>N/A A. fumigatus</td>
<td>Negative Lung inflammation granulomatous</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>LUL</td>
<td>22</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>22</td>
<td>Negative Lung inflammation, fungal hyphae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>RUL</td>
<td>22</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough, haemoptysis</td>
<td>86</td>
<td>Negative Lung inflammation granulomatous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>Upper lobes bilaterally</td>
<td>27</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough, weight loss</td>
<td>54</td>
<td>A. fumigatus Negative lung fungal hyphae, necrosis</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>LLL</td>
<td>25</td>
<td>could not visualise</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>23</td>
<td>Positive Lung inflammation granulomatous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>LUL</td>
<td>35</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>32</td>
<td>Weak positive Lung inflammation granulomatous</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Probable Aspergillus Nodules

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of nodules</th>
<th>Lobes of lung involved</th>
<th>Min size (mm)</th>
<th>Max size (mm)</th>
<th>Lymphadenopathy</th>
<th>Visible on concurrent CXR</th>
<th>Symptoms</th>
<th>Aspergillus IgG</th>
<th>Sputum culture</th>
<th>Aspergillus PCR</th>
<th>Tissue Specimen</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>12</td>
<td>4</td>
<td>All lobes</td>
<td>38</td>
<td>y</td>
<td>Y</td>
<td>Dyspnoea, cough, haemoptysis</td>
<td>185</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>Upper lobes bilaterally</td>
<td>9</td>
<td>n</td>
<td>N/A</td>
<td>Cough</td>
<td>87</td>
<td>Negative</td>
<td>benign cells, polymorphs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>All lobes except RUL</td>
<td>16</td>
<td>n</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>49</td>
<td>n/a BAL</td>
<td>inflammatory infiltrate fungal hyphae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>Upper lobes bilaterally</td>
<td>12</td>
<td>y</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>68</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>all lobes except RML</td>
<td>16</td>
<td>n</td>
<td>Y</td>
<td>Dyspnoea, cough</td>
<td>65</td>
<td>A. nidulans, A. niger Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>LUL</td>
<td>14</td>
<td>n</td>
<td>Y</td>
<td>Dyspnoea, cough, weight loss</td>
<td>152</td>
<td>N/A BAL</td>
<td>benign cells, polymorphs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>RUL</td>
<td>10</td>
<td>n</td>
<td>y</td>
<td>Cough, weight loss</td>
<td>18</td>
<td>n/a</td>
<td>haemoptysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>All lobes</td>
<td>31</td>
<td>Y</td>
<td>Y</td>
<td>Dyspnoea, cough, haemoptysis, weight loss</td>
<td>52</td>
<td>A. fumigatus Weak positive lung haemosiderin deposition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>Upper lobes bilaterally</td>
<td>13</td>
<td>y</td>
<td>y</td>
<td>Dyspnoea, cough</td>
<td>190</td>
<td>A. fumigatus Weak positive lung inflammatory debris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1 Characteristic of patients diagnosed with Aspergillus nodule(s) (Continued)

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms</th>
<th>Organisms</th>
<th>Result</th>
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<tr>
<td>21</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>All lobes except RUL</td>
<td>Dyspnoea, cough 115</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>n</td>
<td>y</td>
<td>LUL</td>
<td>Dyspnoea, cough, weight loss 19</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>All lobes</td>
<td>Dyspnoea, cough 75</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>n</td>
<td>y</td>
<td>RUL</td>
<td>Cough 12</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>n</td>
<td>Y</td>
<td>All lobes</td>
<td>Cough 170</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>All lobes</td>
<td>Dyspnoea, cough, weight loss 104</td>
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<tr>
<td>27</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>All lobes</td>
<td>Dyspnoea, cough 42</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>RUL</td>
<td>Cough, haemoptysis 82</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>n</td>
<td>n</td>
<td>all except RML, RLL</td>
<td>Dyspnoea 42.5</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>n</td>
<td>y</td>
<td>LUL</td>
<td>Cough 106</td>
</tr>
<tr>
<td>31</td>
<td>1</td>
<td>n</td>
<td>y</td>
<td>RUL</td>
<td>Cough 18</td>
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<td>32</td>
<td>4</td>
<td>n</td>
<td>y</td>
<td>All lobes</td>
<td>Dyspnoea, cough, weight loss 10</td>
</tr>
<tr>
<td>33</td>
<td>2</td>
<td>n</td>
<td>N/A</td>
<td>LUL</td>
<td>Dyspnoea, cough, weight loss 108</td>
</tr>
</tbody>
</table>
ranged between 5–50 mm, mean 21 mm. Associated lymphadenopathy was present in six patients (18%). Thirty patients had a plain chest film performed concurrently with the CT imaging, in 29/30 (97%) the nodule was visible on plain film. Eight patients (24%) had undergone positron emission tomography (PET) and in all cases the fluorodeoxyglucose (FDG) uptake was low to moderate (SVUmax <5.4). Twenty three patients (70%) had a solid mass on CT imaging (Fig. 1), while the remaining patients had a mixed pattern of disease. Eleven patients had findings consistent with emphysema on CT imaging. One patient initially had a cavitating lesion which became solid on repeat imaging (Fig. 2). Only one patient, with multiple nodules, had evidence of calcification within some nodules.

**Laboratory parameters**

Aspergillus IgG antibody results were available for 32 patients. In ten patients (31%) the Aspergillus IgG was within normal limits (i.e. ≤40 mg/L), including four patients with a result between 20 mg/L and 40 mg/L. Aspergillus precipitins was positive in 4/32 (12.5%). Eight patients (24%) had lymphopenia (lymphocyte count <1.5 × 10^9/L). Twenty nine patients had MBL measured, and 11/29 (38%) were deficient (<1.0 mg/L). Twenty nine patients submitted sputum samples for analysis. Nine of the 32 patients (31%) isolated an A.
fumigatus from their sputum sample, one of whom also had A. nidulans isolated from their sputum. One patient had A. nidulans and A. niger isolated from his sputum samples. Sputum samples also yielded a number of bacterial organisms including S. aureus, H. influenzae, H. parainfluenzae, M. catarrhalis, S. marcescens, E. coli, K. pneumoniae, S. maltophilia, and P. aeruginosa. Seven patients had sputum samples which did not yield any growth of bacteria or fungi. Twenty two patients submitted sputum for Aspergillus PCR analysis, 10/22 (45 %) were positive. In four cases (4/14) Aspergillus DNA was detected by PCR but there was no growth of Aspergillus spp. by culture.

**Histology**

Histology was available on sixteen patients. Thirteen (81 %) had undergone lung biopsy, and the remainder had bronchoalveolar lavage (BAL) fluid analysed. Of those who had undergone lung biopsy, in 7/13 (54 %) fungal hyphae were visualised. Granulomatous inflammation and/or necrosis was seen in the remaining patients histology (Table 1). Of the three patients who had BAL washings available for analysis, one had fungal hyphae visualised in bronchial washings. Some had fruiting bodies (conidiophores with conidia) of Aspergillus identified (Fig. 3), suggesting that the original infection with Aspergillus occurred in an airspace or on an epithelial surface, and subsequently was filled in with inflammatory cells and Aspergillus hyphae.

**Surgery**

Nine patients underwent surgical resection of the lung nodule. One patient had recurrent disease identified on CT four years post operatively.

**Discussion**

In this, the largest published series of Aspergillus nodules to date, the characteristics of 33 patients were reviewed. These patients represent less than 10 % of the cohort of patients with CPA cared for in the National Aspergillosis Centre. However, this may be an under representation of this presentation of CPA, as cases may not be recognised, have negative Aspergillus IgG or precipitins, and/or not undergo biopsy to secure their diagnosis. However, recognition of nodules on CT scanning of the thorax is becoming more common, as screening for lung cancer is more frequently undertaken. Many nodules identified on such screening scans are removed or biopsied and do not reveal malignancy. Aspergillus nodules are one such benign entity.

In those unable to undergo biopsy or resection because of poor respiratory reserve and a risk of pneumothorax, empirical radiotherapy is sometimes given for a ‘PET positive’ suspicious lesion. We have seen at least 2 patients with chronic pulmonary aspergillosis in the area of radiotherapy, which we suspect, but cannot prove, had an Aspergillus nodule that was irradiated. We would therefore encourage clinical oncologists to consider the possibility of an Aspergillus nodule before embarking on lung irradiation. The response to radiotherapy may not be entirely problematic however, as illustrated by a small series of CPA patients explicitly treated with radiotherapy [14].

A number of other infections may also present with pulmonary nodules, which may be difficult to distinguish on radiological features alone (Table 2). The relative frequency of the differential diagnoses varies substantially by geography. In endemic areas, other fungal infections can present with persistent pulmonary nodules of masses in apparently immunocompetent persons. The appearance of such fungal infections mimics malignancy and diagnosis is often confirmed on biopsy. At one centre in Texas, USA 17 of 2,098 (0.6 %) persons presenting with pulmonary nodule were ultimately diagnosed with histoplasmosis, cryptococcosis or coccidiomycosis rather than malignancy [15]. Another case series describes 27 cases of
fungal lung infection presenting with persistent lung nodule or mass at 2 centres in Texas USA and Sao Paulo Brazil respectively [16]. All cases were referred for investigation of suspected malignancy. Diagnoses included histoplasmosis (26 %), coccidioidomycosis (22 %), aspergillosis (15 %), blastomycosis (7 %), mucormycosis (4 %) and paracoccidioidomycosis (4 %). Fourteen (52 %) of patients had a past history of treated malignancy and 15 (56 %) were symptomatic at presentation. Thirteen (48 %) of patients had cough, 7 (26 %) had chest pain and 7 (26 %) weight loss. Increased PET avidity was noted in all patients and all patients demonstrated radiological improvement or resolution with appropriate antifungal therapy.

The diagnosis of an *Aspergillus* nodule may be challenging. Almost one third of patients did not have a positive *Aspergillus* IgG, and only 12 % had detectable *Aspergillus fumigatus* precipitins. Additionally the clinical features may be non-specific, and similar to those in patients presenting with malignant disease. In this study, cough alone was the most common clinical finding. The demographics of the patients diagnosed with *Aspergillus* nodules are also similar to those diagnosed with malignant conditions of the lung. Our centre previously reported on the PET imaging in patients with CPA [17]. In that series all of the

### Table 2 Infectious differential diagnosis of pulmonary nodules

<table>
<thead>
<tr>
<th>Cause of nodule/disease</th>
<th>Underlying disease(s), geography</th>
<th>CT characteristics</th>
<th>Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspergillus nodule</strong></td>
<td>Emphysema, asthma taking corticosteroids, smoker. Not immunocompromised. Global</td>
<td>Single or multiple nodules. May affect any lobe, although upper lobes most common. Unlikely to be calcified</td>
<td>Slow to change. May cavitate over many months.</td>
</tr>
<tr>
<td><strong>Coccidioidal nodule</strong></td>
<td>None. Visit to, or inhabitant of, endemic area.</td>
<td>Usually single, upper lobes. Occasionally calcified.</td>
<td>Static over months or years.</td>
</tr>
<tr>
<td><strong>Histoplasma nodule</strong></td>
<td>None. Visit to, or inhabitant of, endemic area. May report specific exposure e.g bat cave</td>
<td>Single or multiple. Often calcified.</td>
<td>Static over months or years.</td>
</tr>
<tr>
<td><strong>Nontuberculous mycobacterial nodule</strong></td>
<td>Emphysema, corticosteroids, bronchiectasis. Global</td>
<td>Single or multiple. May be calcified.</td>
<td>Progressive</td>
</tr>
<tr>
<td><strong>Pneumocystis jirovecii</strong></td>
<td>Usually immunocompromised patients, HIV, steroids etc.</td>
<td>Single/multiple</td>
<td></td>
</tr>
<tr>
<td><strong>Nocardia spp.</strong></td>
<td>May mimic TB</td>
<td>Up to 1/3 cases occur in immunocompetent hosts. Global</td>
<td>Single or multiple</td>
</tr>
<tr>
<td><strong>Dirofilariai</strong></td>
<td>None. Mosquito borne zoonosis, travel to South East Asia</td>
<td>Single or multiple nodules or cavities</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4** Current management algorithm for *Aspergillus* nodules. **CXR** = chest radiograph; **CT** = computed tomography; **FU** = follow up
patients had positive PET imaging. Only a small number of patients in this current series had PET scans available for review, but all eight were positive with low-moderate FDG uptake.

This study is limited by being a retrospective review. Case finding was challenging, and despite a number of sources being utilised to identify cases, it is possible some cases were missed. However, this is the largest series of Aspergillus nodules published to date, and the only study to correlate radiology and histology findings with clinical features and laboratory parameters, in particular Aspergillus IgG. In patients with chronic cavitary pulmonary aspergillosis, we found the ImmunoCap Aspergillus IgG assay to be 96% sensitive and 98% specific at a cutoff of 20 mg/L and 88% sensitive and 100% specific at the current manufacturer’s cutoff of 40 mg/L, compared to a healthy younger control population [15]. So 69 to 81% of patients in this series had positive Aspergillus IgG serology, depending on the cutoff used [18]. This further highlights this previously lesser recognised manifestation of CPA.

The natural history of an Aspergillus nodule is not known. We are unable to define how long they were present before they came to medical attention, but we suspect months. We do know that some of the nodules remain stable off therapy for months or years after diagnosis. In general we treated the symptomatic patients, especially those with multiple lesions. Detection of Aspergillus in airways with culture or PCR also influenced us to treat, especially in those with difficult to control asthma or ABPA. We will report long term outcomes in a subsequent paper. We summarise our current approach to management in Fig. 4.

Conclusion

Pulmonary nodules are a less frequent manifestation of chronic pulmonary aspergillosis in immune competent patients. The natural history of these nodules is not yet defined. In this series, cough alone was a common presenting symptom. It may be difficult to distinguish Aspergillus nodules from other pathology on CT findings alone, and PET imaging would seem to be non-discriminatory. Additionally, a significant proportion of these patients do not have a detectable Aspergillus IgG, meaning biopsy is necessary to exclude malignant disease. However, chronic pulmonary aspergillosis, should be a differential diagnosis in patients presenting with single or multiple pulmonary nodules.

Acknowledgements

We would like to thank Mrs Chris Harris, for her help in data collection and case finding.

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Availability of data and material

The data set is held in the NAC, and is not publically available as it contains patient identifiable details.

Authors’ contributions

EGM data collection & analysis, wrote the paper, clinical care of patients, AS radiology review of all patients, IP case finding, data collection, PB histology review of all patients, DWD clinical care of patients, final review and approval of manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

No patient identifiable material is presented.

Ethics approval and consent to participate

This was a retrospective service evaluation of all patients managed with pulmonary nodules and as such is exempt from ethics approval. The study was checked using the NHS medical research council/health research authority online system, and deemed NOT research.

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Efficacy and Safety Outcomes in Patients with Probable or Proven vs. Possible Invasive Fungal Disease from the Phase 3 SECURE Study, Evaluating Isavuconazonium vs. Voriconazole for the Primary Treatment of Invasive Fungal Disease Caused by Aspergillus spp. or Other Filamentous Fungi

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ABSTRACT

Background: SECURE was a Phase 3 double-blind, randomized trial of isavuconazonium sulfate (ISAV) versus voriconazole (VRC) for primary treatment of invasive fungal disease (IFD) caused by Aspergillus spp., or other filamentous fungi in patients with proven/probable (PROV/PROB-IMD) or possible (PSB-IMD) invasive mould disease (IMD). Strategic objectives were to assess the primary endpoint of All-Cause Mortality (ACM) through Day 42, and key secondary endpoints of primary treatment success rates and ACM through Day 42. The study evaluated patients with documented invasive aspergillosis (IA). Secondary endpoints included radiological assessments.

Methods: Patients were randomized (1:1) to receive 4 mg/kg IV BID on Day 1, 4 mg/kg IV BID on Day 2, then 4 mg/kg IV BID or 200 mg oral BID on Day 3 onwards). Subgroup analyses of DRC-assessed patients with PROV/PROB-IMD and PSB-IMD were conducted. Analysis included all randomized patients and was conducted on an intent-to-treat basis. The primary endpoint was ACM through Day 42. Radiological assessments were conducted by an independent blinded data-review committee (DRC).

Results: 516 patients (258 per group) were in the ITT population; 272 (52.7%) had PROV/PROB-IMD (ISAV: 143; VRC: 129), and 196 (38.1%) had PSB-IMD (ISAV: 95; VRC: 101). Across treatment groups, overall and clinical success rates were higher in patients with PSB-IMD compared to PROV/PROB-IMD. Success rates and ACM were similar for ISAV- and VRC-treated patients, regardless of the DRC-assigned categories of PROV/PROB-IMD or PSB-IMD.

Safety analyses

Treatment-emergent adverse events (TEAEs) were assessed throughout the study for patients who received a dose of study drug.

CONCLUSIONS

• All-cause mortality was lower in possible IMD vs. proven or probable IMD (P<0.05), which supports the results from a previous study in which patients with possible/probable IMD had improved survival rates compared with patients treated for proven or probable IMD.

• Overall clinical success rates were significantly lower in patients with proven or probable IMD compared to those with possible IMD in both treatment groups.

• Although the superior results observed in the possible IMD group probably reflect a lower disease burden or lower proportion of IMD, it still supports the initiation of antifungal treatment prior to mycological confirmation.

The incidence of drug-related TEAEs was significantly lower in ISAV-treated patients compared with VRC-treated patients with possible/probable IMD.

References

Table 1. EORTC/MSG 2008 criteria definitions for invasive fungal disease (IFD)

Invasive fungal infections are a leading cause of mortality and infection-related morbidity, particularly in immunocompromised patients.

• Invasive aspergillosis is defined by the water-soluble prodrug of isavuconazole (ISAV), a novel, broad-spectrum, triazole antifungal agent.

– Furthermore, a recent clinical study has shown that ISAV is an effective and well tolerated treatment option for mucormycosis.

The US Food and Drug Administration recently approved oral and intravenous (IV) formulations of isavuconazonium sulfate for mucormycosis.6

Table 2. Characteristics of patients with PROV/PROB-IMD and patients with PSB-IMD

• The incidence of drug-related TEAEs was significantly lower in ISAV-treated patients compared with VRC-treated patients with proven or probable IMD.

Efficacy analyses

• The primary study endpoint was Day 42 all primary treatment with ISAV vs. VRC.

A key secondary endpoint was overall survival or primary treatment success rate at end of treatment (EOT).

– Overall results were evaluated by the DRC based on a composite endpoint including clinical, mycological and radiological response.

Safety analyses

• Treatment-emergent adverse events (TEAEs) were assessed throughout the study for patients who received a dose of study drug.

– The overall success across study groups was 35.7% (n/N=97/272) for PROV/PROB-IMD and 46.9% (n/N=92/196) for PSB-IMD (P<0.05).

– The clinical success was 61.2% (n/N=138/225) for PROV/PROB-IMD and 75.7% (n/N=67/89) for PSB-IMD (P<0.01).

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Abstract

A prospective (2005–2007) hospital-based multicentre surveillance of EORTC/MSG-proven or probable invasive aspergillosis (IA) cases whatever the underlying diseases was implemented in 12 French academic hospitals. Admissions per hospital and transplantation procedures were obtained. Cox regression models were used to determine risk factors associated with the 12-week overall mortality. With 424 case-patients included, the median incidence/hospital was 0.271/103 admissions (range 0.072–0.910) without significant alteration of incidence and seasonality over time. Among the 393 adults (62% men, 56 years (16–84 years)), 15% had proven IA, 78% haematological conditions, and 92.9% had lung involvement. Acute leukaemia (34.6%) and allogeneic stem cell transplantation (21.4%) were major host factors, together with chronic lymphoproliferative disorders (21.6%), which emerged as a new high-risk group. The other risk host factors consisted of solid organ transplantation (8.7%), solid tumours (4.3%), systemic inflammatory diseases (4.6%) and chronic respiratory diseases (2.3%). Serum galactomannan tests were more often positive (≥69%) for acute leukaemia and allogeneic stem cell transplantation than for the others (<42%; p < 10^-3). When positive (n = 245), cultures mainly yielded Aspergillus fumigatus (79.7%). First-line antifungal therapy consisted of voriconazole, caspofungin, lipid formulations of amphotericin, or any combination therapy (52%, 14%, 8% and 19.9%, respectively). Twelve-week overall mortality was 44.8% (95% CI, 39.8–50.0); it was 41% when first-line therapy included voriconazole and 60% otherwise (p < 0.001). Independent factors for 12-week mortality were older age, positivity for both culture and galactomannan and central nervous system or pleural involvement, while any strategy containing voriconazole was protective.

Keywords: Epidemiology, galactomannan, invasive aspergillosis, outcome, voriconazole

Introduction

Invasive aspergillosis (IA) remains the main cause of morbidity and mortality in patients undergoing allogeneic haematological stem cell transplantation (HSCT), but characteristics of these patients and those with myeloma [1], solid tumours [2] and solid-organ transplant (SOT) [3,4], and management of their diseases, are changing [5]. Additionally, increased numbers of cases due to underlying bronchial damage [6] and/or associated with intensive care [7,8], and fewer cases related to advanced stages of HIV infection, are now reported [9]. Therefore, the profile of patients considered at risk of IA continues to expand while the outcome seems to improve.

Trying to describe the epidemiology of IA is challenging as the diagnosis requires standardized criteria [10,11]. To overcome the limitations of setting specific [12] or single hospital
[2] studies, we implemented a dedicated network to prospectively collect all cases of IA. Our aim was to describe its incidence per hospital whatever the underlying diseases, its potential variations according to centres and transplant procedures, and to assess the contribution of diagnostic tools, the first-line antifungals used and the prognostic factors.

**Patients and Methods**

**Data collection**

A prospective surveillance programme (SAIF for ‘Surveillance des Aspergilloses Invasives en France’) was implemented in three regions (Paris-Ile de France, Grand Ouest and Rhône-Alpes) by the National Reference Centre for Mycoses and Antifungals (NRCMA, Institut Pasteur) with the participation of 12 acute care teaching hospitals and the French National Public Health Surveillance Institute (Institut de Veille Sanitaire). All new IA episodes were recorded whatever the age and the underlying disease by each local microbiologist, which limited the risk of missing cases because at least one microbiological criterion was compulsory for validation [10]. Each case was notified through a secured website using a standardized questionnaire and analysed by a local committee. To maintain adhesion to the network, the coordination committee organized semestrial meetings with participating microbiologists. Demographics, underlying conditions, diagnostic tools, dates of hospitalization, first-line antifungal therapy and outcome at day 90 were recorded. The study was approved by the Institut Pasteur Institutional Review Board.

The diagnostic investigations and therapeutic management followed local practices. Only proven and probable IA according to 2002 European Organization for Research and Treatment of Cancer and Mycoses Study Group criteria were considered [10]. The date of the first radiological or microbiological criterion was considered as the date of IA diagnosis. Dissemination was defined as more than two non-contiguous organs involved. The threshold of positivity for the galactomannan (GM) index (Platelia Aspergillus; Biorad, Marnes-la-Coquette, France) in serum was 1 for 2005 according to the manufacturer’s recommendations at that time, and 0.5 thereafter. Missing information and ambiguous answers were checked by the database manager with the corresponding microbiologist and each case was validated by three of us.

As invasive aspergillosis was managed in the referral centre where the patient was followed for his immunosuppressive condition, at least in the case of haematological malignancy or transplant procedure, we decided to use patient admissions per hospital as well as numbers of HSCT and SOT recipients in participating hospitals as denominators obtained through national health statistics [http://www.platines.sante.gouv.fr/](http://www.platines.sante.gouv.fr/).

**Statistical analysis**

Means and standard deviations (SDs) are shown when distributions were confirmed normal while median and IQR are used otherwise. We compared baseline characteristics of groups by use of the χ² test or Fisher’s exact test for categorical variables, and the t-test for continuous variables after Bonferroni adjustment at p<0.001. Other comparisons were exploratory analysis and we did not check the p-value.

Overall survival at 12 months was measured from the date of diagnosis to the last follow-up or death from any cause. For the multivariate analysis, hazard ratios and their 95% confidence intervals (95% CIs) were determined by means of the Cox regression model with shared frailty to determine factors associated with time to death. Frailties that are random effects are entered on the hazard function to model correlation within each hospital. The proportional hazard assumption was tested using weighted residuals. Clinically relevant variables with p-value <0.25 were removed following a backwards-stepwise selection procedure, leaving only variables with p-value <0.05 in the final model. Interaction terms were explored to take into account potential baseline hazards of death by hospital. Overall survival (cumulative survival probability and 95% CI) was estimated by the Kaplan–Meier method and comparisons of survival were performed by logrank tests.

The analysis took into account only cases for which the corresponding parameter was available. All variables were coded and analysed with Stata computer package version 10 (Stata Statistical Software: Stata Corporation, College Station, TX, USA).

**Results**

**Incidence**

From January 2005 to December 2007, 424 case-patients were recorded. Overall, the median incidence of IA was 0.271 per 1000 admissions (range 0.072–0.910). No significant change in the incidence was observed over time and no seasonal trend was noted. The overall incidence was 8.1% (84/1043) and 0.9% (18/2010) in allogeneic and autologous HSCT patients, respectively. Among SOT patients, the calculated IA incidence was 4.8% (7/146), 4.1% (7/172), 0.8% (9/1067) and 0.3% (11/3157) for heart, lungs, liver and kidney transplantation, respectively.
Description of the population
Out of the 424 case-patients, 31 were children (i.e. <18 years). The subsequent analyses concerned only the 393 adults (62% male patients; median age = 56 years; range, 18–84 years). Cases were classified as proven (n = 60, 15%) and probable (n = 333, 85%) IA. Overall, 305 (77.6%) patients had haematological malignancies. The main underlying risk factors/diseases were divided into eight groups (Table 1) after dispatching the patients with autologous HSCT (n = 19) and the HIV-positive patients (n = 8) according to their associated underlying disease. The major one was acute leukaemia (AL) (n = 136, 34.6%). Chronic lymphoproliferative disorders emerged as the second haematological underlying disease (n = 85, 21.6%), followed by allogeneic HSCT (n = 84, 21.4%). All patients with chronic lymphoproliferative disorders accounted for 35% (n = 106) of all haematological malignancies. Other patients had SOT (n = 34, 8.7%), solid tumours (n = 17, 4.3%), systemic inflammatory diseases with high-dose steroid therapy (n = 18, 4.6%), or chronic respiratory diseases (n = 9, 2.3%). Finally, ten (2.5%) patients had none of the above risk factors, including five with IA diagnosed at autopsy without known risk factor.

### TABLE 1. Risk factors for invasive aspergillosis and underlying diseases of the 393 adult patients of the study

<table>
<thead>
<tr>
<th>Risk factors/underlying diseases</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemia</td>
<td>136/393 (34.6)</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>90/136 (66.2)</td>
</tr>
<tr>
<td>Acute lymphoid leukemia</td>
<td>21/136 (15.4)</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>9 (6.6)</td>
</tr>
<tr>
<td>Acute transformation</td>
<td>16 (11.8)</td>
</tr>
<tr>
<td>Allogeneic HSCT</td>
<td>84/393 (21.4)</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>28 (33.3)</td>
</tr>
<tr>
<td>Acute lymphoid leukemia</td>
<td>18 (21.4)</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>Acute transformation</td>
<td>7 (8.3)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>13 (15.5)</td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>5 (6.0)</td>
</tr>
<tr>
<td>Aplasia</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (3.6)</td>
</tr>
<tr>
<td>Chronic lymphoproliferative disorders</td>
<td>85/393 (21.6)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>42 (49.4)</td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td>26 (30.6)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>13 (15.3)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (4.7)</td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td>34/393 (8.7)</td>
</tr>
<tr>
<td>Heart</td>
<td>7 (20.1)</td>
</tr>
<tr>
<td>Lung</td>
<td>7 (20.1)</td>
</tr>
<tr>
<td>Liver</td>
<td>9 (26.5)</td>
</tr>
<tr>
<td>Kidney</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>Solid tumours</td>
<td>17/393 (4.3)</td>
</tr>
<tr>
<td>Bronchio-pulmonary and others</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td>Others</td>
<td>11 (64.7)</td>
</tr>
<tr>
<td>Systemic inflammatory diseases</td>
<td>18/393 (4.6)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td>Inflammatory rheumatism</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Others</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Chronic respiratory diseases</td>
<td>9/393 (2.3)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Asthma</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Others</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>None of the above risk factors</td>
<td>10/393 (2.5)</td>
</tr>
</tbody>
</table>

Main characteristics of invasive aspergillosis
For patients with AL, IA occurred for 68% (93/136) of them during the induction phase of chemotherapy, for 27% during consolidation and for 5% during palliative care. For the 85 non-allografted patients with chronic lymphoproliferative disorders, IA occurred for 27% (23/85) during the induction phase, for 67% (57/85) during malignancy relapse/non-control, and for 6% (5/85) during palliative care. The time interval between allogeneic HSCT and the occurrence of IA was <40 days, ≥40–100< and ≥100 days for 16 (17%), 11 (13%) and 57 (68%) patients, respectively. IA occurred in the first 12 weeks following heart transplantation (6/7) and at least 100 days after surgery for the other transplant procedures (18/27).

Localization of IA was mostly pulmonary (365/393, 92.9%), either isolated (324/393, 82%) or associated with other localizations (41/393, 10%) that consisted mainly of sinus (n = 18) and central nervous system (CNS, n = 20) involvement. Isolated extrapulmonary aspergillosis was documented in 28 patients (8%) and consisted of sinusitis (n = 11) and/or CNS localizations (n = 9).

Diagnostic tools and microbiological results
**Imaging.** Chest computed tomography (CT) scan was performed in 310 (78.9%) patients, with no significant difference according to underlying diseases. Chest nodules were found in 252/310 (81.3%) patients with pulmonary IA, associated with halo sign or cavitation in 47 (15.2%) and 126 patients (40.6%), respectively. There were 58 (18%) patients with proven or probable IA, with CT signs not reported as nodule, halo sign and/or cavitation, without a statistically significant difference according to the eight groups (Table 2).

**GM serum detection.** GM serum detection was performed at least twice for 345/393 (88%) patients. Despite a change in the positivity’s threshold in 2006, no modification in the percentage of cases with two positive tests was noted over time (p 0.7). Two positive tests were recorded in 197/345 patients (57%), with variations ranging from 69% in AL and allogeneic HSCT to 40%, 26% and 0% in the chronic lymphoproliferative disorders, SOT and the chronic respiratory disease groups, respectively (p <10⁻⁴). GM detection was performed in 91 BAL fluids and was reported positive for 43 of them (47%). GM positivity in BAL fluid (index ≥ 1.5) was the only microbiological criterion in four patients (Table 2).

**Microbiological investigations.** Direct examination and culture of clinical specimens were performed in 325 (82.7%) patients. Direct examination was positive in 56% (182/325), with the
<table>
<thead>
<tr>
<th>Risk Factor/Underlying Disease</th>
<th>Acute Leukaemia (n = 136)</th>
<th>Allogeneic HSCT (n = 84)</th>
<th>Chronic Lymphoproliferative Disorders (n = 85)</th>
<th>Solid Organ Transplantation (n = 34)</th>
<th>Solid Tumours (n = 17)</th>
<th>Systemic Inflammatory Diseases (n = 18)</th>
<th>Chronic Respiratory Diseases (n = 9)</th>
<th>Others (n = 10)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) (95% CI)</td>
<td>55 (53–58)</td>
<td>44 (41–47)</td>
<td>59 (56–62)</td>
<td>54 (50–58)</td>
<td>58 (51–65)</td>
<td>62 (55–70)</td>
<td>63 (52–73)</td>
<td>55 (44–66)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Male, n</td>
<td>77 (56.6%)</td>
<td>56 (66.7%)</td>
<td>53 (62.4%)</td>
<td>25 (73.5%)</td>
<td>15 (88.2%)</td>
<td>6 (33.3%)</td>
<td>7 (77.8%)</td>
<td>5 (50.0%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Proven IA</td>
<td>20 (14.7%)</td>
<td>11 (13.1%)</td>
<td>6 (7.1%)</td>
<td>7 (20.6%)</td>
<td>6 (35.3%)</td>
<td>4 (22.2%)</td>
<td>1 (11.1%)</td>
<td>5 (50.0%)</td>
<td>0.004</td>
</tr>
<tr>
<td>CT scan and chest X-ray, No. of patients examined</td>
<td>124 (91.2%)</td>
<td>71 (84.5%)</td>
<td>66 (77.7%)</td>
<td>26 (76.5%)</td>
<td>9 (52.9%)</td>
<td>13 (72.2%)</td>
<td>9 (100%)</td>
<td>6 (60%)</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CT signs recorded in those with pulmonary IA (%)</td>
<td>104/121 (86.0%)</td>
<td>56/65 (86.2%)</td>
<td>51/65 (78.5%)</td>
<td>15/23 (65.2%)</td>
<td>5/9 (55.6%)</td>
<td>10/13 (76.9%)</td>
<td>8/9 (88.9%)</td>
<td>3/5 (60.0%)</td>
<td>0.075</td>
</tr>
<tr>
<td>Nodule</td>
<td>17/121 (14.1%)</td>
<td>9/65 (13.9%)</td>
<td>14/65 (21.5%)</td>
<td>8/23 (34.8%)</td>
<td>4/9 (44.4%)</td>
<td>3/13 (23.1%)</td>
<td>1/9 (11.1%)</td>
<td>2/5 (40.0%)</td>
<td>0.075</td>
</tr>
<tr>
<td>Other signs</td>
<td>28/121 (23.1%)</td>
<td>9/65 (13.9%)</td>
<td>17/65 (26.2%)</td>
<td>15/23 (65.2%)</td>
<td>7/9 (77.8%)</td>
<td>7/9 (77.8%)</td>
<td>9/10 (90%)</td>
<td>0/5 (&lt;10)</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum positive for galactomannan detection</td>
<td>92/121 (74.7%)</td>
<td>56/65 (86.2%)</td>
<td>58/65 (88.1%)</td>
<td>27/67 (40.3%)</td>
<td>8/31 (25.8%)</td>
<td>4/8 (50.0%)</td>
<td>4/11 (36.4%)</td>
<td>0/5</td>
<td>6/8 (75%) (&lt;10)</td>
</tr>
<tr>
<td>Direct examination, No. positive/No. tested (%)</td>
<td>46/95 (48.4%)</td>
<td>30/66 (45.5%)</td>
<td>40/76 (52.6%)</td>
<td>23/34 (67.7%)</td>
<td>13/17 (76.5%)</td>
<td>14/18 (77.8%)</td>
<td>7/9 (77.8%)</td>
<td>9/10 (90%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Positive culture, No. positive/No. tested (%)</td>
<td>50/95 (52.6%)</td>
<td>45/66 (68.2%)</td>
<td>67/76 (88.2%)</td>
<td>32/34 (94.1%)</td>
<td>15/17 (88.2%)</td>
<td>18/18 (100%)</td>
<td>9/9 (100%)</td>
<td>10/10 (100%)</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

**TABLE 2.** Characteristics, diagnostic means, treatment and outcome according to risk factors/underlying diseases in the 393 cases of invasive aspergillosis of the study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antifungal treatment, No. of patients treated</th>
<th>Voriconazole alone</th>
<th>Caspofungin alone</th>
<th>L-AmB alone</th>
<th>Voriconazole + Caspofungin</th>
<th>Voriconazole + L-AmB</th>
<th>Caspofungin + L-AmB</th>
<th>Others</th>
<th>Death within 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>134</td>
<td>74</td>
<td>16</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>51/135 (37.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84</td>
<td>44</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>47/84 (56.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>14</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>35/83 (42.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>15</td>
<td>103/15 (67.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>101/15 (67.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>49/44 (44.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>7/10 (70%)</td>
</tr>
</tbody>
</table>

*L-AmB, lipid formulation of amphotericin B.

†The comparisons are carried out among the eight groups by use of the χ² test or Fisher’s exact test for categorical variables, and the t-test for continuous variables.

bOthers included amphotericin B deoxycholate (n = 16), posaconazole (n = 7), itraconazole (n = 3) alone or in combination with two or three drugs including voriconazole (six cases), caspofungin (seven cases) or L-AmB (three cases).
lowest rate in the allogeneic HSCT group (p 0.005). Culture was positive for 76% (246/325) (from 53% (50/95) for AL to >88% for the other groups (p <10^{-3}). When positive (n = 246), cultures yielded 196 A. fumigatus (79.7%), 11 A. niger, 10 A. flavus, seven A. nidulans, five A. terreus, five other species and 12 mixtures all including A. fumigatus. No non-fumigatus species was reported in non-haematological patients (Table 2).

Treatment
First-line therapy prescribed for at least 48 h was reported for 367 (93.4%) patients. For 26 patients, there was no prescription of antifungal therapy (autopsy findings, death before final diagnosis, palliative care). Monotherapy was prescribed for 294 (80%) patients while any combination was used in 73 (19.9%), with no significant difference according to the underlying group (Table 2).

Outcome
The outcome at day 90 was available for 388/393 (99%) patients, with death recorded in 174 patients (44.8%; 95% CI, 39.8–50.0) (Table 3). Univariate analysis identified several parameters associated with death. Thus, the proportion of older patients, those with positive culture and at least two GM positive serum samples (35.8% vs. 19.3%) and those with CNS involvement (10.9% vs. 4.7%) or pleural effusion (41.7% vs. 18.0%) was higher, while the proportion of patients receiving voriconazole either alone or in combination (54.3% vs. 75.8%) was smaller when comparing the patients who died with those who survived (Table 3 and Fig. 1a,b).

![FIG. 1.](image_url) Overall survival at 3 months after diagnosis of invasive aspergillosis. (a) Patients diagnosed either with one (plain line) or with at least two mycological (dashed line) criteria. (b) Patients given any antifungal regimens containing voriconazole, either alone (plain line) or in combination (dotted line), or any antifungal regimens without voriconazole (dashed line).

In the multivariate analysis, after taking into account the within-hospital correlation, the parameters independently associated with an increased risk of death were an older age, a diagnosis based on positive culture together with two positive GM detections in serum samples, the presence of pleural

### TABLE 3. Parameters associated with deaths within 90 days after the diagnosis of IA for the 388/393 adult patients for whom the outcome was available

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. deaths (n = 214)</td>
<td>Deaths before day 90 (n = 174)</td>
</tr>
<tr>
<td>Gender male</td>
<td>135/214 (63.1%)</td>
<td>106/174 (60.9%)</td>
</tr>
<tr>
<td>Median age (IC 95%)</td>
<td>52.5 (50.5–54.5)</td>
<td>55.7 (53.5–58.0)</td>
</tr>
<tr>
<td>Underlying risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute leukaemia</td>
<td>84 (38.3%)</td>
<td>51 (29.3%)</td>
</tr>
<tr>
<td>Allogeneic graft</td>
<td>37 (17.3%)</td>
<td>47 (27.0%)</td>
</tr>
<tr>
<td>Lymphoid disorders</td>
<td>48 (22.4%)</td>
<td>35 (20.1%)</td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td>24 (11.2%)</td>
<td>10 (5.8%)</td>
</tr>
<tr>
<td>Solid tumours</td>
<td>5 (2.3%)</td>
<td>10 (5.8%)</td>
</tr>
<tr>
<td>Systemic</td>
<td>8 (3.7%)</td>
<td>10 (5.8%)</td>
</tr>
<tr>
<td>Chronic respiratory</td>
<td>5 (2.3%)</td>
<td>4 (2.3%)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (1.4%)</td>
<td>7 (4.0%)</td>
</tr>
<tr>
<td>Positive culture and positive galactomannan on ≥2 serum samples</td>
<td>29/150 (19.3%)</td>
<td>39/109 (35.8%)</td>
</tr>
<tr>
<td>Central nervous system involvement</td>
<td>10/214 (4.7%)</td>
<td>19/174 (10.9%)</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>34/189 (18.0%)</td>
<td>55/132 (41.7%)</td>
</tr>
<tr>
<td>Presence of nodule, halo sign and/or cavitation</td>
<td>155/197 (78.7%)</td>
<td>97/164 (59.2%)</td>
</tr>
<tr>
<td>Initial antifungal treatment including voriconazole</td>
<td>150/198 (75.8%)</td>
<td>89/164 (54.3%)</td>
</tr>
</tbody>
</table>
effusion or CNS involvement, while an initial antifungal treatment including voriconazole (alone or in combination) was associated with a decreased risk of death (Table 3).

Discussion

The incidence of IA was 0.271/1000 admissions, from 0.072 to 0.91 according to the hospital, without any significant temporal trend or any seasonal influence. The different recruitment of the participating hospitals and obvious differences in clinical approaches to IA diagnosis, which will be further analyzed, may explain these differences.

As expected, haematological malignancies provided the highest proportion of IA. Surprisingly, 35% of haematological-associated IA occurred in patients with chronic lymphoproliferative disorders, with 67% occurring during second-line therapies. Studies focused on this population could support our results, which suggest an intensifying treatment or the cumulative immune suppression as a possible cause of increased risk of IA in this specific group [1]. When considering the subpopulations, the incidence was 8.1% among allogeneic HSCT recipients, which is within the range of previous reports [13–15]. Of note, 70% occurred >100 days after the graft, as underlined by others [12,13] although not always reported [14]. For autologous HSCT, the 0.9% incidence is concordant with other data [12,14,16].

The incidence of IA according to the transplant procedure was in the range previously recorded [4], with a late occurrence after lungs, liver or kidney transplantation. HIV infection dramatically decreased (2%) compared with previous reports [16,17]. IA was recorded in non-haematology cancer patients, systemic inflammatory or chronic respiratory diseases and some other conditions in up to 15% of the population, higher than previously reported [16,17]. In these latter patients, we evidenced the low yield of serum GM detection. GM contribution depends on the likelihood of IA occurrence [18] and on previous antifungal therapy [19]. Another plausible explanation would be the pathophysiology of IA in deeply neutropenic patients vs. patients receiving prolonged steroid therapy [20]. Indeed, lymphoproliferative diseases correspond to patients for whom neutropenia is not the predominant risk factor [21]. The poor performance of serum GM in non-neutropenic patients and the difficulty in fulfilling the clear-cut CT-scan criteria in intensive care unit patients [11] underline the need for refining diagnostic criteria for these populations [6]. This underlines also the interest of the main mycological criterion of the present study, which remains a positive culture [22]. Also of note was the high rate (56%) of positive direct examination.

Twelve-week overall mortality was 44.8%, with differences between groups, similar to a report of 48.8% in a single-centre study [2]. For AL, the present mortality rate was 37.8%, a figure close to the 33% recently reported [23]. For allogeneic HSCT recipients, our 56% overall mortality is close to the 57.5% rate in the TRANSNET study [24] but by far higher than the 35.5% reported in the PATH Alliance registry [12]. Nevertheless, the mortality rate is lower than the 66% previously observed, suggesting a substantial improvement of IA prognosis in the allogeneic HSCT recipients [25]. In SOT patients, the mortality rate found here is concordant with the TRANSNET study (29.4% and 34.4%, respectively) [24].

Independent factors associated with death were here an older age, the combination of two positive GM tests and a positive microbiological investigation, and the involvement of pleura and/or CNS. If involvement of pleura or CNS has previously been identified as a prognostic factor [2,25,26], it is not the case for the combination of the biological diagnostic tools. This could be explained by a more advanced disease with a larger fungal burden. This emphasizes the importance of individualizing patients with unique or multiple positive tests in future clinical trials. In contrast, CT-scan signs suggestive of pulmonary IA were associated with a better prognosis, which was potentially ascribed to an earlier diagnosis [27].

Voriconazole represented the main first-line therapy prescription (51.8% of cases) while amphotericin B deoxycholate has almost disappeared. Caspofungin and antifungal combinations represented 14% and 17% of the first-line therapy prescriptions, with an increase or decrease in use over time, respectively. Of note, combination therapy was reported in 47.2% of HSCT patients [12] despite lack of benefit in this population [24] and lack of recommendation [28].

Interestingly, although not obtained during a randomized trial but taking into account variations in relation to the hospital, the use of any strategy using voriconazole was an independent factor for survival. The 90-day survival rates were 59% and 40% in voriconazole-treated and non-treated patients, respectively. These data are reminiscent of those reported in the pivotal voriconazole trial (70.8% and 57.9% in the voriconazole and amphotericin B groups, respectively) [29].

In conclusion, this prospective multicentre surveillance study allowed us to demonstrate that IA incidence markedly varied according to clinical centres. An emergence of IA in patients with heavily treated chronic lymphoproliferative disorders was recorded. Among the other major issues, the poor contribution of serum GM in all non-haematology groups, the need to better assess fungal burden by combining
fungal culture and serum antigen detection and the role of voriconazole as first-line therapy are emphasized.

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Transparency Declaration

O.L. is consultant for FAB Pharma, Gilead Sciences and Astellas, and a member of the speaker’s bureau of Pfizer, MSD, Astellas and Gilead Sciences. J.-P.G. is a member of advisory boards and/or received grant support from Astellas, Gilead, MSD, Pfizer and Schering-Plough. S.B. is consultant for Gilead Sciences, has received speaking honoraria from Pfizer and Gilead Sciences and travel grants from Astellas, Pfizer and Schering-Plough. K.S., B.L., F.M., Y. LS., B.C. and F.D.: no conflict of interests.

Appendix

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References


Estimating the burden of invasive and serious fungal disease in the United Kingdom

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KEYWORDS
Candida; Aspergillus; Cryptococcus; Morbidity

Summary
Background: The burden of fungal disease in the UK is unknown. Only limited data are systematically collected. We have estimated the annual burden of invasive and serious fungal disease.

Methods: We used several estimation approaches. We searched and assessed published estimates of incidence, prevalence or burden of specific conditions in various high risk groups. Studies with adequate internal and external validity allowed extrapolation to estimate current UK burden. For conditions without adequate published estimates, we sought expert advice.

Results: The UK population in 2011 was 63,182,000 with 18% aged under 15 and 16% over 65. The following annual burden estimates were calculated: invasive candidiasis 5142; Candida peritonitis complicating chronic ambulatory peritoneal dialysis 88; Pneumocystis pneumonia 207–587 cases, invasive aspergillosis (IA), excluding critical care patients 2901–2912, and IA in critical care patients 387–1345 patients, <100 cryptococcal meningitis cases. We estimated 178,000 (50,000–250,000) allergic bronchopulmonary aspergillosis cases in people with asthma, and 873 adults and 278 children with cystic fibrosis. Chronic pulmonary aspergillosis is estimated to affect 3600 patients, based on burden estimates post tuberculosis and in sarcoidosis.

Conclusions: Uncertainty is intrinsic to most burden estimates due to diagnostic limitations, lack of national surveillance systems, few published studies and methodological limitations. The largest uncertainty surrounds IA in critical care patients. Further research is needed to produce a more robust estimate of total burden.

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0163-4453/© 2016 The British Infection Association. Published by Elsevier Ltd. All rights reserved.
Background

Invasive fungal disease is thought to be increasing in the United Kingdom (UK) due to a variety of factors including increased survival time from previously fatal illnesses and an increase in immunosuppression from disease treatment. Understanding of the overall burden of invasive fungal disease in the UK is limited as there is no formal systematic or mandatory surveillance programme specific to fungal infections, although active surveillance networks exist for candidaemias (voluntary laboratory reporting) and specifically for candidaemias in neonates (voluntary reporting).

An analysis of laboratory reports of fungal infections was published in 2001, which highlighted the likely underestimate of the total burden due to the challenges involved in laboratory diagnosis and the voluntary nature of the laboratory reporting system. In 2008, the UK Health Protection Agency issued "Fungal Diseases in the UK: The current provision of support for diagnosis and treatment: assessment and proposed network solution". The UK community of medical mycologists has been active in developing best practice standards for the UK and beyond for the diagnosis and clinical management of fungal disease.

Next step for healthcare and research prioritisation is to quantify these burdens of invasive fungal disease with improved tools and an expanded range of serious fungal infections.

Methods

We used the UK Office for National Statistics 2011 Census data to estimate UK population size. We used this as the 2011 census is the most recent census in the UK.

We estimated the annual incidence of the following invasive fungal infections: cryptococcal disease and meningitis; Pneumocystis pneumonia; invasive aspergillosis; candidaemia; Candida peritonitis; and oesophageal candidiasis. In addition, we estimated the prevalence of chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS). Information on incidence, prevalence and total burden of these conditions in the UK is limited. Where such information was available for the UK or countries within the UK (where UK estimates were not available), we included it in the study, for example the data from the voluntary surveillance of candidaemia in England, Wales and Northern Ireland.

Where the information was not available we took a pragmatic approach. For each fungal condition, we considered which populations were most at risk of the condition, sought published estimates for incidence or prevalence measures for the fungal condition in these specific risk populations, and applied these rates to available published estimates of size of these high risk populations in the UK (or certain countries within the UK where UK estimates were not available).

Where multiple estimates of incidence or prevalence were published, we considered both internal and external validity of the studies in deciding on which estimate to use. The methods used for estimating burden of the specific fungal conditions are outlined below.

Selection criteria for published estimates of incidence: for many of the severe fungal infection, there is a paucity of published estimates of incidence, therefore we had to be pragmatic in our approach. Where more than one published estimate was available, we prioritised studies with the best applicability to the UK population (i.e. where UK studies were available we used these, if not we used studies from countries with as comparable a population as possible, where non-UK studies were selected, this is made clear in italics in the fungal infection section of the Methods) and those with the largest sample sizes (where multiple studies were considered, this is made clear in the fungal infection section of the Methods).

Pneumocystis pneumonia

First method

Prior to March 2013, no published estimates of incidence, prevalence or total burden were available for England except for people living with AIDS (PHE HIV in the UK report). The high risk populations identified and the data source used to estimate their current size included people living with AIDS and people who had received various solid organ transplants (Tx): Heart Kidney Liver and Lung or Heart and Lung.

Using the estimate of total burden amongst people living with AIDS for 2011–2013, we divided this estimate by three to obtain an average yearly estimate.

The incidence rates specific to solid organ transplant patients were found from a variety of studies.

Second method

A UK study estimating the incidence of Pneumocystis pneumonia over an 11 year period was published in March 2013. This showed that the incidence had increased significantly over the study period. We aimed to estimate the total burden for the most recent year of the study (2010) based on figures reported in the paper for each of the four data sources: Hospital Episode Statistics (HES) data – the paper reported the number of cases in 2010; Routine Laboratory Reporting – the paper reported a range for number of cases in 2008–2010, we used the central point of this range; Death Certificate Data – the paper reported the number of cases in 2010; HIV Surveillance Data – the paper did not report a number or range for total number of cases in the later years of the study, we obtained an estimate by extrapolating from figure 3 of the paper.

Cryptococcal meningitis

No published estimates of incidence, prevalence or total burden were found for the UK. We obtained an estimate based on a simple direct question to the largest mycology referral laboratories in the UK (Bristol, Leeds and Manchester) of the frequency of positive cryptococcal antigen test results. One publication was found which reported on trends in incidence and numbers of fungal meningitis, but this covered all fungal infections and was not specific to cryptococcal infection.
The high risk populations identified included newly diagnosed HIV infection. We used the PHE HIV in the UK report\textsuperscript{16} to estimate the current size of this population. The incidence rate for this high risk population was obtained from Patel et al.\textsuperscript{17}

**Invasive aspergillosis**

We took a pragmatic approach to estimating the burden of invasive aspergillosis. The high risk populations identified and the data source used to estimate their current size included: Allogeneic hematopoietic stem cell transplantation (HSCT) and autologous HSCT patients\textsuperscript{18}; solid organ transplant patients\textsuperscript{11}; people living with AIDS\textsuperscript{16}; Acute myeloid leukaemia (AML), Acute lymphoblastic leukaemia (ALL), Chronic myeloid leukaemia (CML), Chronic lymphocytic leukaemia (CLL), Non Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and Myeloma patients\textsuperscript{19}; Chronic granulomatous disease (CGD) patients\textsuperscript{20}; Chronic obstructive pulmonary disease (COPD): emergency hospital admissions\textsuperscript{21}; critical care patients\textsuperscript{22}; patients with lung cancer.\textsuperscript{19}

The incidence rates specific to the above high risk populations were found from a variety of studies: Lortholary et al.\textsuperscript{23} (for Allogeneic and Autologous HSCT patients, and for solid organ transplant patients) — these estimates were not for the UK population but the French population; Keshishtian\textsuperscript{24} (for people living with AIDS); Pagano et al.\textsuperscript{25,e} (For AML, ALL, CML, CLL, NHL, HL and myeloma patients) — these estimates were not for the UK population but the Italian population; Beauthé et al.\textsuperscript{26,f} (for CGD patients) — this estimate was not for the UK population but the French population; Guinea et al.\textsuperscript{27} (for COPD: emergency hospital admissions) — this estimate was not for the UK population but the Spanish population, another study reporting an incidence estimate was considered (Xu et al.\textsuperscript{28}) but the sample size for the study was significantly smaller than that of Guinea et al. so we did not include it; A wide range of estimates from different studies\textsuperscript{29} for critical care patients, see sensitivity analysis discussion below — these estimates were not for the UK population but the Belgian and Spanish populations; Yan X et al.\textsuperscript{30} was used for patients with lung cancer — this estimate was not for the UK population but the Chinese population.

**Critical care patients: sensitivity analysis**

The largest risk group population by far for invasive fungal infection was patients in critical care at risk of invasive aspergillosis, regardless of which type of critical care unit is considered. Any variation in incidence rate could lead to a significant change in estimated burden. We carried out a sensitivity analysis to reflect this.

Activity data is available for a broad range of critical care units in England.\textsuperscript{22} The most common type of admission to ICU amongst cases of invasive aspergillosis is medical admission, and the most common reasons for admission respiratory and cardiovascular disease,\textsuperscript{31} therefore we considered two broad groups of critical care units in the sensitivity analysis. The first was medical intensive care units (ICUs) and other ICUs where length of patient stay is likely to be similar to that of medical ICUs,\textsuperscript{8} the second was all ICUs, excluding spinal units.

There is a wide range of published estimates for incidence of invasive aspergillosis in patients in critical care: from 0.3% to 19.\textsuperscript{29} Key factors include: the type of critical care unit considered, and whether or not studies were autopsy controlled. No non-invasive diagnostic test (for example isolation of *Aspergillus* from respiratory cultures) is sensitive or specific enough to establish a definite diagnosis.\textsuperscript{32} It is difficult to distinguish colonisation with *Aspergillus* from infection with *Aspergillus*.\textsuperscript{32}

We focused on those studies that specifically examined the incidence of invasive aspergillosis in critical care units. Four such studies were found, one had a small sample size (n = 24) and did not report an incidence estimate so was not considered further.\textsuperscript{13} The other three, from which incidence rates estimates were used, are listed in Table 4 with their characteristics and the populations they apply to.

We adjusted estimates of burden to account for double counting of patients already counted in other groups. We assumed that the majority of those who developed invasive aspergillosis would require ICU admission.

**Chronic pulmonary aspergillosis**

Chronic pulmonary aspergillosis complicates a wide spectrum of underlying lung diseases of which the commonest are pulmonary tuberculosis (PTB), non-tuberculous mycobacterial lung infection, COPD, sarcoidosis, and allergic aspergillosis complicating asthma.\textsuperscript{39}

An estimate of the annual number of patients with chronic pulmonary aspergillosis after pulmonary tuberculosis (PTB) has recently been published.\textsuperscript{34} For most countries, this was based on a 22% rate of chronic pulmonary aspergillosis after PTB in those with cavities of 2.5 cm or greater and 2% in those without a residual cavity. In the absence of UK data, we assumed a rate of residual cavitation after PTB of 12% (range in other countries 21–35\textsuperscript{35–37}). To generate a five year period prevalence,
a 15% attrition rate was assumed, accounting for surgical resection and death.

A recent estimate of the rate of chronic pulmonary aspergillosis complicating sarcoidosis in the UK was also available. Numerous other antecedent underlying pulmonary conditions are found in patients with chronic pulmonary aspergillosis, and the relative proportions of these were used to estimate the total UK burden.

A separate approach was taken using referrals to the National Aspergillosis Centre from the North West England, based on population and regional variation in directly age-standardised mortality rates (DSR). Just over 100 new patients are referred annually to the National Aspergillosis Centre. It was assumed that referral was near complete in NW England to the National Aspergillosis Centre because of excellent clinical links and proximity. Using published directly age-standardised respiratory disease mortality rate for under year 75 olds (DSR) and regional populations, we derived an annual potential diagnosable burden, based on current respiratory medicine practice, which approximates to an annual incidence (Table 1 in Supplementary Materials).

Allergic bronchopulmonary aspergillosis (ABPA)

ABPA complicates asthma and cystic fibrosis (CF). The global burden of asthma has been re-estimated recently, a total of 334 million in all ages (4.8% of the global population) and 193 million adults with active asthma. The UK has one of the highest rates of asthma in the world, an estimated 16—18.2% of adults with clinical asthma, or nearly 8.2—9 million (age 15 and older). Other more recent data of asthma prescription data from the UK put the total rate at approximately 5.4 million, including children. As the prevalence in children is 88% of the adult rate, we derived an adult number of asthmatics of 4.4 million (our lowest and base case estimate).

There are no population data for ABPA or any surrogate marker such as IgE from the UK. An abstract from one hospital tracking IgE and Aspergillus IgE levels in 330 consecutive referrals to an asthma clinic found a 1.5% rate of probable ABPA with most diagnostic features and 13% with both an elevated total IgE and Aspergillus IgE. A base case estimation of ABPA rates in adults was made, using a median prevalence of 2.5% from referrals to secondary care. This 2.5% rate is derived from rates of 0.78% and 4.1% from 6 national studies in consecutive referrals to an asthma clinic found a 1.5% rate, we derived an adult number of asthmatics of 4.4 million (our lowest and base case estimate).

As SAFS is another distinctive pattern of asthma usually associated with sensitisation to multiple fungi and responsive to antifungal medication we estimated the UK burden of this entity. While recently described in children, it is rare, and so this was not estimated. Severe asthma was defined by a poor level of current clinical control including a risk of frequent severe exacerbations (or death) and/or chronic morbidity. Severe asthma includes untreated severe asthma, difficult-to-treat severe asthma, and treatment-resistant severe asthma. In a multi-country comparison of the role of fungal sensitisation in severe asthma, 21% were defined as severe. In other studies lower frequencies of severity are recorded, including a recent estimate of 3.6%. We used 5% as our base case to embrace both severe refractory and compliant difficult to control asthmatics. We have also computed a sensitivity analysis.

Fungal sensitisation becomes more common the worse the asthma, with rates ranging from approximately 25% of patients referred to a specialist to 75% for those with repeated hospital admissions. We used a rate of 60%, 61–64

Candidaemia

There is a voluntary surveillance system in England that collects laboratory reports of all microorganisms isolated (including fungi) at approximately 400 NHS and other laboratories throughout England, Wales and Northern Ireland. Blood culture has a poor sensitivity for detecting Candida species: a 2011 systematic review of the diagnostic accuracy of PCR techniques for invasive candidiasis identified 10 studies reporting the sensitivity of blood cultures. The pooled culture positivity rate in patients with proven or probable invasive candidiasis was 0.38 (95% CI: 0.29—0.46). A more recent US study using PCR and beta 1.3-α-glucan detection derived a similar figure. Therefore we made the assumption that the total number of positive blood culture samples represented 38% cases of proven or probable invasive candidiasis tested by blood culture techniques.

Candida peritonitis

We took a pragmatic approach to estimating the burden of Candida peritonitis.

The two main risk groups for this condition in the UK are: surgical ICU patients and people on chronic ambulatory peritoneal dialysis (CAPD).

Surgical ICU patients

We assumed that the majority of cases in surgical ICU patients would be counted in the estimate of total number of cases of invasive candidiasis discussed above.

CAPD patients

For the number of patients on CAPD in England every year, we used estimates from NICE.
To estimate the incidence of peritoneal candidiasis in patients on CAPD, we used an estimate reported on the Leading International Fungal Education (LIFE) website. This incidence estimate was reported as episode per patient year. In our calculation of attributable burden, we assumed that all CAPD patients in England stay on CAPD for at least a year.

Oesophageal candidiasis

The main risk group for this condition in the UK is probably people with AIDS. Oesophageal candidiasis is an AIDS-defining illness. The number of cases reported in the UK between 2011 and 2013 was reported in the PHE HIV in the UK report. We divided this figure by three to obtain a yearly estimate of burden.

Another approach to estimating the burden was also taken using published estimates of yearly incidence amongst HIV patients on anti-retroviral therapy — this estimate was not for the UK population but the USA population — and estimates of numbers of HIV patients on anti-retroviral therapy in the UK.

Mucormycosis

Occasional cases of mucormycosis occur in the UK, usually highly immunocompromised patients, occasionally in intravenous drug addicts, burn or trauma patients or people with diabetes, and rarely related to hospital transmission. Most diagnoses are made histologically or on direct microscopy specimens, culture sensitivity is low. No data are collected systematically.

To estimate the number of mucormycosis cases in the UK, we applied the French population incidence found from published studies to the UK population (no UK estimate of incidence available).

Other rarer infections

Other rarer infections are not well tracked in the UK, including imported endemic mycoses (histoplasmosis and coccidioidomycosis for example) and are rare based on the experience of the National Aspergillosis Centre. Likewise serious infections related to unusual filamentous fungi such as *Fusarium* or *Scedosporium* spp. do occur, the former in leukaemic patients, the latter in some cystic fibrosis patients and rarely as an invasive pathogen.

Results

The UK population in 2011 was 63,182,000 with 18% aged under 15 and 16% over 65.

Pneumocystis pneumonia

An average yearly total burden of 157 *Pneumocystis* pneumonia (PCP) diagnoses was found for those people living with AIDS in the UK using our first estimation approach.

The estimates of population size, population-specific incidence rate and yearly burden of disease obtained for patients who had received various transplants in the UK are outlined in Table 1.

The total estimate of burden of PCP for both people living with AIDS and solid organ transplant populations in the UK was 207. This estimate ignores other immunocompromised patients, such as haematological malignancy and severe autoimmune disease.

Second method

Our second estimation approach yielded a total UK burden of 587 cases of PCP for 2010.

Cryptococcal disease and meningitis

An estimate of up to 100 cases per year for the UK was obtained from the reference laboratories. It is unclear whether this is an underestimate or an overestimate as it is estimated that in 2011 there were a total of 51 fungal meningitis cases (all fungi, based on culture). However this 2011 estimate is based on voluntary laboratory reporting and furthermore, there is some evidence that cryptococcal infections are under-reported.

Many diagnoses of cryptococcal disease are based on cryptococcal antigen alone, and while meningitis is the commonest manifestation of disease, other organs are affected. It is likely that the vast majority of these cases were in people living with HIV and in 2013 approximately 6000 new HIV infections were diagnosed.

Invasive aspergillosis

The estimates of population size and, population-specific incidence rate and burden of disease obtained for high risk populations in the UK excluding critical care units patients are outlined in Table 2.

Therefore a total of 568–579 patients develop IA in well recognised at risk groups. Some cases in haematological patients will have been prevented with antifungal prophylaxis. Only lung Tx recipients with true IA are included, omitting those with airways infection and colonisation, all of whom are treated.

The estimates for patients with pulmonary disease are outlined in Table 3.

Therefore the estimate for the total yearly burden of IA in the UK for the all of the above groups is 2901–2912.

Sensitivity analysis

The results of the sensitivity analysis of IA in critical care are displayed in Table 5. The variation between highest and lowest burden estimates for medical type ICUs and all type ICUs (spinal units excluded) was over 10-fold. This highlights the level of uncertainty over this estimate of burden. Our view is that the rate of IA in the UK is probably at the low end of the estimates above, with ~50% of the cases occurring in COPD patients, even though IA is the most common missed infectious diagnosis at autopsy. So a total ICU caseload of between 821 and 2737 is likely, of which 50% is attributable to COPD. Adjusting downwards by 50% for probable double counting of cases of COPD emergency hospital admissions (we assumed most of these would
be admitted to ICU), and solid organ transplant recipients (n = 24) resulted in adjusted estimates of 387–1345 cases. The total estimate of burden of IA amongst the high risk populations is 2901–2912 (excluding ICU populations) and 3288–4257 (including ICU populations). This estimate ignores those with solid tumours other than lung tumours, autoimmune disease, liver failure and other conditions treated with corticosteroids.

**Chronic pulmonary aspergillosis**

Chronic pulmonary aspergillosis complicates many conditions some estimates of the annual incidence and 5 year period prevalence have been published for pulmonary tuberculosis and pulmonary sarcoidosis complicating an estimated 16,270 cases of pulmonary sarcoidosis in the UK. The anticipated annual incidence of each was 118 and 240 respectively. Together these two conditions account for about 30% of patients with CPA and so an annual diagnosable incidence is around 358 cases for these conditions and a total of 1193 cases. We compared this total, with current referral to the National Aspergillosis Centre (Table 1 of Supplementary Materials), which is actually 110 per year and should be about 204, if all cases are diagnosed and referred in NW England. Either estimate suggests major under-diagnosis.

Computing prevalence and assuming a 15% annual mortality, including 370 cases following PTB and 830 (range 415–1660). Together these 2 conditions account for about 30% of patients with CPA, consistent with a total UK burden of CPA of approximately 3600 cases. As many are asymptomatic in the early stages, this number is an overestimate of those at the more severe end of the spectrum requiring therapy.

**Allergic bronchopulmonary aspergillosis (ABPA)**

Using our base case of a rate of 2.5% for ABPA among patients with asthma, 110,667–235,070 adults would be
expected in the UK. However, the sensitivity analyses vary by over 10-fold from 34,528–385,515 affected patients. The only partial population based studies from Republic of Ireland and the USA suggests rates at the lower estimate of published estimates. Referral and discharge patterns across the UK are not uniform, so ABPA is likely to be diagnosed in some areas more often than others. However ABPA is only one fungal complication of asthma, as discussed below under SAFS.

Of the 4933 adults with CF in the UK, we estimate that 873 adults have ABPA (95% CIs: 597–1243) and 631 people over 15 years old (12.5% of 5062 patients) were documented, indicative of a diagnostic gap of 242. The annual report also described 278 children and adolescents with ABPA (7.4% of 3732 children). In addition, an estimated 1480 (95% CI: 1125–1894) have Aspergillus bronchitis. If all patients with ABPA and Aspergillus bronchitis benefit from therapy (which needs to be established), this totals 2353 patients.

Severe asthma with fungal sensitisation (SAFS)

Asthma severity and fungal sensitisation rise in parallel. Asthma severity and fungal sensitisation rise in parallel.64 There are approximately 65,000 admissions to hospital with asthma annually, approximately 40,250 in adults.78 Fungal sensitisation rates are not well studied in the UK, especially as patients may be sensitised to one or more fungi.63 In a series of 121 patients with severe asthma in the UK, sensitisation rates by either skin prick testing or IgE were Aspergillus fumigatus 45%, Candida albicans 36%, Penicillium spp. 29%, Cladosporium herbarum 24%, Alternaria alternata 22%, and Botrytis spp. 18%; 41 (34%) were not sensitised to any fungus tested.63 The minimum proportion of poorly controlled asthmatics who would be sensitised to a fungus is about 35%, rising to >75% in the worse patients.61 Using a uniform estimate of 60% fungal sensitisation of the most severe asthmatics (3.6–10%) between 95,617 and 564,169 UK adults have SAFS or severe asthma with ABPA (Table 6).

There is some duplication between ABPA and SAFS, as sensitisation to A. fumigatus is common to both and some ABPA patients have severe asthma. These patients are grouped by some authors as having ‘fungal asthma’ or ‘fungal-associated airways disease’. Part of the definition of severe asthma is continuous use of corticosteroids, which is advocated for ABPA, irrespective of the control of asthma. Therefore the overlap is uncertain, and requires detailed study. However given that 75% of SAFS patients

Table 4 Sources of estimates of the incidence of invasive aspergillosis in critical care patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Study characteristics</th>
<th>Population studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meersseman et al.73</td>
<td>127</td>
<td>Autopsy controlled</td>
<td>Patients in medical critical care units</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study aim: to determine the incidence of IA in medical ICUs</td>
<td></td>
</tr>
<tr>
<td>Garnacho-Montero et al.74</td>
<td>1756</td>
<td>Not autopsy controlled</td>
<td>Patients in any type of critical care unit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study aim: to describe the characteristics of patients with positive samples for Aspergillus species</td>
<td></td>
</tr>
<tr>
<td>Vandewoude et al.32</td>
<td>172</td>
<td>Not autopsy controlled</td>
<td>Patients in any type of critical care unit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Study aim: to describe the characteristics of patients with positive samples for Aspergillus species</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Sensitivity analysis for estimation of burden of invasive aspergillosis amongst patients in critical care in the UK.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number in Risk Group in the UK</th>
<th>Incidence Rate</th>
<th>Number of expected cases in the UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meersseman et al.73</td>
<td>166,645</td>
<td>5.8%71</td>
<td>9665</td>
</tr>
<tr>
<td>Garnacho-Montero et al.74</td>
<td>248,811</td>
<td>1.1%74</td>
<td>2737</td>
</tr>
<tr>
<td>Vandewoude et al.32</td>
<td>248,811</td>
<td>0.33%72</td>
<td>821</td>
</tr>
</tbody>
</table>
are sensitised to *A. fumigatus* and that only a minority of ABPA patients remain on long term steroids, we show a sensitivity analysis with 20%, 33% and 50% overlap in Table 4, using the mid-point estimates for ABPA (2.5%) and severe asthma (5%).

The overall estimate of adults with ‘fungal asthma’ varies by 3.4 fold, from 121,734 to 413,724, primarily dependent on the number of adults with asthma.

### Invasive candidiasis

#### Candidaemia

There were 1700 laboratory reports of candidaemia in 2013. Assuming that these represent 38% cases of proven or probable invasive candidiasis tested by blood culture techniques, the resulting estimate for the total number of cases in England, Wales and Northern Ireland in 2013 was: 4473.

Scotland had a rate of candidaemia of 4.8 cases per 100,000 population per year shortly after the millennium,79 254 bloodstream and 669 invasive *Candida* cases annually.

The total estimate of invasive candidiasis burden for the UK was therefore: 5142.

This estimate of burden of candidaemia is likely to be an underestimate as reporting from laboratories is voluntary, therefore likely to be a degree of under-reporting. Population based estimates have been reported in Northern Ireland and Scotland with rates of 6.1 and 4.8 per 100,000 population79,80 which if extrapolated to the whole population would suggest 2995–3806 cases annually, as compared to the 1700 reported for England and Wales (~ 90% of the population). Further a six sentinel hospital study in England and Wales found an incidence of 18.7 episodes of candidaemia per 100,000 finished consultant episodes (or 3.0/100,000 bed days) in 1997–199981 which translates for 2014–2015 for England only to 3497 as there were 18.7 million Finished Consultant Episodes,82 assuming no substantial change in *Candida* bloodstream rate over time.

Considering that the estimate is likely to be an underestimate, within the range of UK candidaemia burden estimates between 2995 and 5142, we selected the higher end of the range (5142) as our estimate.

These data indicate a population rate in the UK of candidaemia and invasive candidiasis of 3.1/100,000 and 10.1/100,000 respectively.

#### Candida peritonitis in CAPD patients

The estimated number of patients on CAPD in England was 1768. The estimated number of episodes per patient year attributable to Candida in this patient group was 0.5. The resulting estimate for total yearly burden in England was 88 cases.

#### Oesophageal candidiasis

An average yearly total burden of 43 diagnoses was found as AIDS indicator infections. Many additional cases occur, and one estimate was 0.5% of those on anti-retroviral treatment annually.70 If applied to the UK population of 73,300 on anti-retroviral treatment in 2013,16 this would equate to 367 episodes annually, although these data derive in part from patients without full HIV suppression, so could be an overestimate. Other patient groups also get oesophageal candidiasis, but modelling is not realistic currently.

#### Mucormycosis

The UK population in 2011 was 63,182,000,10 and the estimated population incidence of *Mucormycosis* in France was 0.09 per 100,000 population per year (averaged over 10 years). This resulted in a UK estimate of 57 cases per year.

#### Other rare infections

Based on expert opinion, there are probably fewer than 25 such patients annually in the UK.

### Totals

Table 7 summarises the estimates for total expected number of cases for each invasive fungal infection and rates per 100,000 population.

The estimated total burden of invasive fungal illness in the UK is between 241,525 and 662,987 cases per year.

### Discussion

Estimating the burden of invasive fungal infection accurately is challenging due to the lack of a dedicated mandatory systematic surveillance system, and the wide range of incidence estimates for the largest high risk populations. This is likely to be compounded by the combination of lack of clinical suspicion and limited sensitivity of traditional diagnostic tests used for invasive fungal illness, making it difficult to obtain laboratory confirmation.
for a significant number of cases. This issue is exemplified for IA as this was the commonest error in infection diagnoses missed in critical care patients examined at autopsy.75

There is a significant level of inaccuracy as our estimation methods have relied on limited published information, and there is a wide range of estimates for some of the published incidence rates. This high level of uncertainty is reflected in the results of our sensitivity analysis for the estimation of the burden of invasive aspergillosis in ICU patients, and in the difference between the estimates of PCP burden resulting from the two different calculation methods used.

The estimate of burden for PCP obtained by the first method is likely to be an underestimate as other high risk populations, notably patients with haematological malignancy and those on high dose corticosteroid regimens were not included as no overall incidence rate of PCP could be found in the literature for these groups.

The estimate of burden for PCP obtained by the second method should be considered in the light of methodological limitations outlined by the authors14: laboratories may be under-reporting as samples are not processed for Pneumocystis diagnosis unless clinically requested, and cytological techniques can also be used (cases diagnosed in this manner would not be counted in this study) and there is potential for double counting of cases captured both in the hospital admission data and the laboratory reporting data. In addition many cases are clinically diagnosed and treated, many correctly, without a respiratory sample being obtained to enable laboratory diagnosis.

The estimates of chronic respiratory disorders associated with Aspergillus and other airborne moulds are much larger than any prior estimate, even if the more conservative assumptions are made. There is certainly some double counting which we have tried to adjust for but the population prevalence range of fungal asthma of 121,734–413,724 is still wide. Epidemiological studies in primary care are required to establish a more precise estimate. Our data excludes children, in whom fungal asthma occasionally occurs.33,57

The lower end of our estimate of total invasive fungal diseases burden range is likely to be underestimated, as some condition-specific estimates are for England only. There was potential for double counting of cases, although we have attempted to account for this.

Estimates of the burden of serious fungal disease for individual countries have been published49 for Austria, Belgium, Brazil, Czech Republic, Denmark, Dominican Republic, Germany, Greece, Hungary, Ireland, Israel, Jamaica, Kenya, Mexico, Nepal, Nigeria, Qatar, Russia, Senegal, Sri Lanka, Tanzania, Trinidad and Tobago, Uganda, Ukraine, Vietnam, along with estimates of chronic and allergic aspergillosis in India.84 Burden estimates for many other countries and other prospective epidemiology studies are in press and can be used to compare the relative rates of infections to address strategies for prevention and clinical management.

We have not attempted to estimate mortality related to fungal disease in the UK, although others have done so for other countries. The overall and attributable mortality is not always clearly discernable, the estimates we have provided have much uncertainty attached to them, and adding mortality in addition is likely to add another layer of uncertainty. However, undiagnosed invasive fungal infections such as PCP and IA are always fatal without specific therapy and Candida bloodstream infections and invasive candidiasis have mortalities in excess of 90% untreated. With treatment, mortality falls especially with PCP in AIDS (~10% mortality) and ~30% with IA in non-ICU patients. An estimate of mortality would require specific therapy rates, which is unknown for most of these disorders.

Strengths and limitations of the study: We acknowledge that the estimates produced in this paper and the methods reached to achieve them are crude and vulnerable to significant error due to lack of robust surveillance information and paucity of published burden studies in the field. We have made the best attempt possible by: drawing on

### Table 7  Total estimates of burden.

<table>
<thead>
<tr>
<th>Invasive fungal infection</th>
<th>Risk group</th>
<th>Number of cases expected</th>
<th>Rates per 100,000 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumocystis pneumonia</td>
<td>All risk groups</td>
<td>207–587</td>
<td>0.33–0.93</td>
</tr>
<tr>
<td>Cryptococcal meningitis</td>
<td>Primarily AIDS patients</td>
<td>100</td>
<td>0.16</td>
</tr>
<tr>
<td>Invasive aspergillosis</td>
<td>All risk groups except critical care patients</td>
<td>2901–2912</td>
<td>4.59–4.61</td>
</tr>
<tr>
<td>Chronic pulmonary aspergillosis – all</td>
<td>Critical care patients</td>
<td>387–1345</td>
<td>0.61–2.13</td>
</tr>
<tr>
<td>Allergic bronchopulmonary aspergillosis (ABPA)</td>
<td>All risk groups</td>
<td>204–3600</td>
<td>0.32–5.70</td>
</tr>
<tr>
<td>Severe asthma with fungal sensitisation (SAFS)</td>
<td>All risk groups</td>
<td>121,734–413,724</td>
<td>192–654</td>
</tr>
<tr>
<td>Invasive candidiasis</td>
<td>All risk groups</td>
<td>5142</td>
<td>8.14</td>
</tr>
<tr>
<td>Candida peritonitis</td>
<td>CAPD patients</td>
<td>88</td>
<td>0.14</td>
</tr>
<tr>
<td>Oesophageal candidiasis</td>
<td>AIDS patients</td>
<td>43–367</td>
<td>0.07–0.58</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>All risk groups</td>
<td>57</td>
<td>0.09</td>
</tr>
<tr>
<td>Other rare infections</td>
<td>All risk groups</td>
<td>25</td>
<td>0.04</td>
</tr>
<tr>
<td>Total</td>
<td>All risk groups</td>
<td>241,525–662,987</td>
<td>382–1049</td>
</tr>
</tbody>
</table>
surveillance data where available; rigorously identifying the relevant high risk groups, the best available estimates of population size for these, and the best available population-specific incidence rates for these; being explicit about the methods used for each individual estimate; and attempted to account for under and over-estimations as well as potential double counting. We are not aware of any other comprehensive burden study for serious and invasive fungal disease in the UK and therefore would argue that although imperfect, this study is a useful contribution to the limited body of knowledge in this field.

Conclusion

There is a high degree of uncertainty around the total estimate of burden due to: diagnostic limitations, the lack of a systematic national surveillance system, the limited number of studies published on the topic and the methodological limitations of calculating the burden.

To our knowledge, this is the first attempt at a comprehensive estimation of burden of invasive fungal infection in the UK. Further studies will likely need to combine methods (pragmatic and surveillance-based), take into account any new published information on specific incidence rates, and consider using alternative data sources such as the Hospital Episodes System (HES). An accurate estimate of total burden will ultimately rely on improved diagnostic testing and laboratory reporting.

Conflict of interest

Dr Denning holds Founder shares in F2G Ltd a University of Manchester spin-out antifungal discovery company, in Novocyt which markets the Myconostica real-time molecular assays and has current grant support from the National Institute of Health Research, Medical Research Council, Global Action Fund for Fungal Infections and the Fungal Infection Trust. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Scynexis, Cidara, Biosergen and Pulmocide. In the last 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck and Pfizer.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2016.10.005.

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Burden of invasive and serious fungal disease in the UK


80. http://www.gaffi.org/media/academic-papers/.
Treatment of invasive aspergillosis

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The University of Manchester
Treatment
# Invasive aspergillosis

## Table 2. Summary of recommendations for the treatment of aspergillosis.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Therapy&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive pulmonary aspergillosis</td>
<td><strong>Primary</strong>&lt;br&gt;Voriconazole (6 mg/kg IV every 12 h for 1 day, followed by 4 mg/kg IV every 12 h; oral dosage is 200 mg every 12 h)</td>
</tr>
<tr>
<td></td>
<td><strong>Alternative&lt;sup&gt;b&lt;/sup&gt;</strong>&lt;br&gt;L-AMB (3–5 mg/kg/day IV), ABLC (5 mg/kg/day IV and 50 mg/day IV thereafter), caspofungin (70 mg day 1 IV and 100–150 mg/day; dose not established&lt;sup&gt;c&lt;/sup&gt;), posaconazole (200 mg QID initially, then 400 mg BID PO after stabilization of disease&lt;sup&gt;d&lt;/sup&gt;), itraconazole (dosage depends upon formulation)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Invasive aspergillosis

There are few randomized trials on the treatment of invasive aspergillosis. The largest randomized controlled trial demonstrates that voriconazole is superior to deoxycholate amphotericin B (D-AMB) as primary treatment for invasive aspergillosis. Voriconazole is recommended for the primary treatment of invasive aspergillosis in most patients (A-I). Although invasive

Why most and not all?
Open study of 600 mg/day for 4 d, then 400 mg/d. Treatment extended for >97 weeks, median 46

<table>
<thead>
<tr>
<th>Status</th>
<th>12 weeks</th>
<th>End of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>5%</td>
<td>26%</td>
</tr>
<tr>
<td>Partial</td>
<td>26%</td>
<td>13%</td>
</tr>
<tr>
<td>Stable</td>
<td>34%</td>
<td>4%</td>
</tr>
<tr>
<td>Failure</td>
<td>32%</td>
<td>56% (30% other causes)</td>
</tr>
<tr>
<td>Deaths</td>
<td>--</td>
<td>31%</td>
</tr>
</tbody>
</table>
Randomised study of invasive aspergillosis with voriconazole versus amphotericin B

391 pts received either
1) Voriconazole 4 mg/d BID (after loading) for 12wks (or OLAT)
or 2) AmB 1.0 mg/kg/d for 12wks (or OLAT)

mITT analysis

<table>
<thead>
<tr>
<th></th>
<th>Success (%)</th>
<th>Severe AEs (%)</th>
<th>Renal tox (%)</th>
<th>Died (all) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vori</td>
<td>53</td>
<td>13</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>[21%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB</td>
<td>32</td>
<td>24</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>[13%]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Survival after primary Rx with amphotericin B or voriconazole

Survival (percent)

Weeks

0 2 4 6 8 10 12

Number of patients at risk

144 131 125 117 111 107 102 Voriconazole

133 117 99 87 84 80 77 Amphotericin B

Overall logrank test p=0.015

Herbrecht, Denning et al, NEJM 2002;347:408
Impact of voriconazole in real life - France

5 other large case series demonstrating better outcomes with voriconazole for IA against all other therapies.

Nivoix et al, Clin Infect Dis 2008;47:1176
Random voriconazole concentrations in adults receiving 3mg/Kg BID

Very small children may metabolise voriconazole very fast and need doses of 8mg/Kg BID, then TDM

Data from Denning et al, Clin Infect Dis 2002;34:563
Intrinsic and acquired resistance among the Aspergilli

Amphotericin B resistance/insensitivity

A. terreus
A. nidulans
A. flavus

Azole resistance

Only itraconazole resistance

A. fumigatus
A. niger
Randomised study of invasive aspergillosis with Amphocil versus amphotericin B

174 pts received either
1) Amphocil 6 mg/d for >2wks after symptoms gone
or 2) AmB 1.0 - 1.5 mg/kg/d >2wks after symptoms gone
70/174 (40%) in high risk (HSCT, liver Tx, AIDS, brain)

ITT analysis

<table>
<thead>
<tr>
<th></th>
<th>Success (%)</th>
<th>Tox (%)</th>
<th>Renal tox (%)</th>
<th>Died (due to IA)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphocil</td>
<td>13</td>
<td>83</td>
<td>23</td>
<td>59 (22)</td>
</tr>
<tr>
<td>AmB</td>
<td>15</td>
<td>83</td>
<td>41</td>
<td>67 (20)</td>
</tr>
</tbody>
</table>

Bowden et al Clin Infect Dis 2002:35:359
Randomised study of invasive aspergillosis with 2 doses of AmBisome

339 pts randomised to receive either

1) L-AmB 3 mg/d for 2+wks (169 randomised; 107 in MITT)
2) L-AmB 10 mg/d for 2+wks (162 randomised; 94 in MITT)

44/201 (22%) high risk (HSCT, AIDS)

MITT analysis

<table>
<thead>
<tr>
<th></th>
<th>CR + PR</th>
<th>Stop Rx</th>
<th>Renal tox</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-AmB 3</td>
<td>50%</td>
<td>20%</td>
<td>14%</td>
<td>28%</td>
</tr>
<tr>
<td>L-AmB 10</td>
<td>46%</td>
<td>32%</td>
<td>31%</td>
<td>41%</td>
</tr>
</tbody>
</table>

Cornely et al, Clin Infect Dis 2007;44:1289
# Micafungin for invasive aspergillosis

## Table 3: Efficacy at end of therapy

<table>
<thead>
<tr>
<th></th>
<th>Primary (%)</th>
<th></th>
<th>Refractory/toxicity failure&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Total (%)&lt;br&gt;(N = 225)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micafungin in combination (n = 17)</td>
<td>Micafungin alone (n = 12)</td>
<td>Micafungin in combination (n = 174)</td>
<td>Micafungin alone (n = 22)</td>
</tr>
<tr>
<td>Complete response</td>
<td>2 (11.8)</td>
<td>0</td>
<td>13 (7.5)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Partial response</td>
<td>3 (17.6)</td>
<td>6 (50.0)</td>
<td>47 (27.0)</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>Favorable response</td>
<td>5 (29.4)</td>
<td>6 (50.0)</td>
<td>60 (34.5)</td>
<td>9 (40.9)</td>
</tr>
<tr>
<td>Stabilization</td>
<td>3 (17.6)</td>
<td>2 (16.7)</td>
<td>17 (9.8)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Progression</td>
<td>9 (52.9)</td>
<td>4 (33.3)</td>
<td>97 (55.7)</td>
<td>10 (45.5)</td>
</tr>
<tr>
<td>Not successful</td>
<td>12 (70.6)</td>
<td>6 (50)</td>
<td>114 (65.5)</td>
<td>13 (59.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Four patients who had failed previous therapy due to toxicities are included in the micafungin-alone group.
Open study of invasive aspergillosis with caspofungin as primary therapy

61 pts with chemotherapy or auto HSCT received Caspofungin 70 then 50mg IV daily

Survival by day 84 = 33/61 (54%)

### MITT population (N=61)

<table>
<thead>
<tr>
<th>Response</th>
<th>n</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>1</td>
<td>2 (0–9)</td>
</tr>
<tr>
<td>Partial</td>
<td>19</td>
<td>31 (20–44)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>9</td>
<td>15 (7–26)</td>
</tr>
<tr>
<td>Disease progression</td>
<td>31</td>
<td>51 (38–64)</td>
</tr>
<tr>
<td>Not evaluable(^a)</td>
<td>1</td>
<td>2 (0–9)</td>
</tr>
</tbody>
</table>

\(^a\)Patient refused treatment.

33% response rate

Neutropenia at enrolment (not assessable in one case)

- no: 5/9 (56)
- yes: 15/51 (29)

Survival by day 84 = 33/61 (54%)

Viscoli et al, JAC 2009;64:1274
Voriconazole versus amphotericin B
[Spectrum/activity]

Favours voriconazole
Much more active for IA (~20% better)
Active against *A. terreus*
Active against *A. nidulans*
More active *A. flavus*
Active against *S. apiospermum*

Favours Amp B
Mucorales possible
Azole resistant *A. fumigatus*
Voriconazole versus echinocandin
[Spectrum/activity]

Favours voriconazole
Much more active for IA (~20% better)
Active against *A. terreus*
Active against *A. nidulans*
More active *A. flavus*
Active against *S. apiospermum*

Favours micafungin/caspofungin
Azole resistant *A. fumigatus*
# Cytochrome P450 Interactions

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Fluc</th>
<th>Itra</th>
<th>Posa</th>
<th>Vori</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C19</td>
<td>+</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>2C9</td>
<td>++</td>
<td>+</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>3A4</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>2C9</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3A4</td>
<td></td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
The Aspergillus Website

The Aspergillus website is a worldwide comprehensive resource providing a wide range of information about the fungus Aspergillus and the diseases - such as Aspergillosis that it can cause. This site is free to use and provides an encyclopaedia of Aspergillus for doctors, scientists, patients and their relatives. Some parts of the site for example the image bank require free registration.

Aspergillosis is a group of diseases which can result from aspergillus infection and includes invasive aspergillosis, ABPA, CPA and aspergilloma. Some asthma patients with very severe asthma may also be sensitised to fungi like aspergillus (SAFS). There is a section devoted to the needs of patients, friends and family suffering from the effects of Aspergillosis.

The UK's first reference centre is supported by the Regional Mycology Lab which also provides both air sampling and mould identification services for domestic and working environments.

Aspergillosis may affect patients whose immune system may be compromised - including those with leukaemia, chemotherapy patients or those on steroids, transplant patients, cystic fibrosis, HIV or AIDS, chronic obstructive pulmonary disease (COPD), chronic granulomatous disease (CGD), severe asthma with fungal sensitivity (SAFS) and many others.

Aspergillus does not solely affect humans; birds and animals can also develop aspergillosis, and some plant diseases are also caused by the fungus. Aspergillus is found in a wide variety of environments - including damp, decaying vegetation, soil, compost, sewage, leather, and manure. It can live on the surface of a wide variety of materials, including wood, paper, and fabric.

13 years and counting
Over 2M pages read monthly in >125 countries
Supported by the Fungal Research Trust – 20 year anniversary in 2011

New section on drug interactions which you can search very quickly
Combination therapy (voriconazole + caspofungin)

Retrospective
AmB failures
Most HSCT
30/47 proven IA

Multivariate analysis
P=0.008 for combination and survival

Combination therapy may be useful for a short time early during voriconazole treatment to allow confirmation of adequate voriconazole concentrations, especially in children.
Arguments for **not** using voriconazole?

1. Amphotericin B is a broader spectrum agent – No
2. AmBisome is equivalent to voriconazole in IA – No
3. Patient was on itraconazole prophylaxis – No
4. The patient has cerebral aspergillosis – No (beware interactions, especially phenytoin)
5. The patient might have azole resistant Aspergillus – maybe
6. Major drug interactions – yes sometimes
7. Renal failure – only if IV therapy needed for any duration
8. My patient is a young child and I am worried about blood levels – yes use 9mg/Kg BD (200mg BD orally) and consider combination therapy with an echinocandin and measure levels
Choice of antifungal for invasive aspergillosis

Priority sequence

• Voriconazole (unless drug interaction)
• Micafungin/caspofungin (if not neutropenic)

OR

• AmBisome 3mg/Kg (if not ‘nephro-critical’)

3. Posaconazole (oral only, if no drug interactions)

4. Itraconazole
When not to use voriconazole as primary therapy?

Absolute contraindications
- Drug interactions (ie rifampicin, carbamazepine, phenytoin etc)
- Voriconazole used as prophylaxis (but not itraconazole or posaconazole)
- Resistance to voriconazole (esp zygomycosis, *A. lentulus* or azole resistance in *A. fumigatus*)

Relative contraindications
- Renal failure (IV only)
- Young children (need higher dose ?+ other agent)
- Severe hepatic dysfunction
- Interacting drugs (ie sirolimus)
Conclusions

• Voriconazole is the treatment of choice for invasive aspergillosis
• For those with toxicity, significant drug interactions or azole resistance, an echinocandin or lipid AmB is appropriate
• Current treatments are partially successful but more oral therapies are needed
• Isolates of *Aspergillus* should be susceptibility tested, if treatment given
After an increase in the number of reported cases of *Pneumocystis jirovecii* pneumonia in England, we investigated data from 2000–2010 to verify the increase. We analyzed national databases for microbiological and clinical diagnoses of *P. jirovecii* pneumonia and associated deaths. We found that laboratory-confirmed cases in England had increased an average of 7% per year and that death certifications and hospital admissions also increased. Hospital admissions indicated increased *P. jirovecii* pneumonia diagnoses among patients not infected with HIV, particularly among those who had received a transplant or had a hematologic malignancy. A new risk was identified: preexisting lung disease. Infection rates among HIV-positive adults decreased. The results confirm that diagnoses of potentially preventable *P. jirovecii* pneumonia among persons outside the known risk group of persons with HIV infection have increased. This finding warrants further characterization of risk groups and a review of *P. jirovecii* pneumonia prevention strategies.
A

ceddotal reports from clinicians suggest that incidence of Pneumocystis jirovecii pneumonia, previously referred to as P. carinii pneumonia or PCP, among immunosuppressed patients, especially renal transplant recipients, has increased substantially (1). To investigate this claim, we analyzed data for January 2000 through December 2010, using several national data sources: Hospital Episode Statistics, routine laboratory reporting, death certificate data, and HIV surveillance data.

P. jirovecii pneumonia gained notoriety during the AIDS pandemic (2); however, the reservoirs, modes of transmission, and pathogenesis of this organism remain poorly understood (3). Subclinical infection is considered common because studies have shown that anti-P. jirovecii antibodies develop during early childhood (4). Reactivation of latent infection after immunosuppression of the host was thought to be the main pathogenic mechanism (3); however, recent studies indicate that person-to-person spread might cause acute infection in susceptible persons (5).

Although not fully characterized, the known risk factors for P. jirovecii infection include impaired immunity because of HIV infection, hematologic malignancies, and connective tissue disorders (6). Immunosuppressive agents used to treat or prevent graft rejection have been implicated; such agents include corticosteroids, methotrexate, cyclosporine, mycophenolate mofetil, bendamustine, cyclophosphamide (7–11), and, recently, novel immunomodulating drugs, such as tumor necrosis factor–α inhibitors (12).

Prophylactically administered oral trimethoprim–sulfamethoxazole, dapsone, or atovaquone prevent the clinical manifestation of P. jirovecii infection. Also effective for decreasing P. jirovecii infection incidence among HIV-positive patients with a CD4+ count <200/μL is routine prophylactic administration of antimicrobial drugs (13,14).

Given the existence of effective chemoprophylaxis, identification of new risk groups might help prevent future increases in P. jirovecii infection incidence. Therefore, we conducted a retrospective analysis of multiple national data sources to examine trends in P. jirovecii infection.

The Health Protection Agency has approval from the National Information Governance Board for Health and Social Care for the collation of surveillance data in accordance with section 251 of the National Health Service Act 2006. No additional ethical approval was required for this study.

Materials and Methods

Hospital Episode Statistics

The Hospital Episode Statistics (HES) database contains details of all inpatient admissions to National Health Service hospitals in England. We identified all patients for whom an International Classification of Diseases, 10th Revision (ICD-10), code B59, which corresponds with P. jirovecii infection, was recorded in any of the first 10 diagnosis fields from January 2000 through December 2010. By using ICD-10 and Operating Procedure Code Supplement 4 codes, we then subdivided cases into non-mutually exclusive, condition-specific categories that are frequently cited in the literature in association with P. jirovecii (7–13,15–19). The categories covered were renal failure, hematologic malignancy, other hematologic disorders, systemic connective tissue disorders, inflammatory diseases (such as rheumatoid or psoriatic arthritis), and receipt of immunosuppressive agents or an organ transplant. Patients with chronic lung conditions, such as pulmonary fibrosis, were categorized as a single group, given the observed frequency in this study of concurrence of this condition with P. jirovecii infection. Patients who did not fit into any risk category were also included in the analysis.

We cross-checked for duplicate records and selected the record of first admission for each patient. We examined information about sex, age, and geographic distribution of patients. HIV-infected patients were excluded from analysis because the clinical records for these patients did not contain patient-identifiable information (unlike the other clinical records in the HES database), thereby making identification and exclusion of duplicate records not possible for this group.

Routine Laboratory Reporting

LabBase2 is the Health Protection Agency’s national communicable diseases database for England, Wales, and Northern Ireland; it receives semi-automated downloads of results from 99% of microbiology diagnostic laboratories (Health Protection Agency, unpub. data). Laboratory-confirmed cases of P. jirovecii infection in England during 2000–2010 were extracted from LabBase2, and duplicate laboratory samples were excluded.

Death Certificate Data

For the study period, deaths in England with an ICD-10 clinical code indicating P. jirovecii as the cause or contributory cause of death were extracted from Office for National Statistics data. Deaths from P. jirovecii infection linked to a diagnosis of HIV or AIDS were also analyzed.

HIV Surveillance Data

Data from the Health Protection Agency’s HIV and AIDS New Diagnoses and Deaths database were analyzed (20). Because HIV surveillance data are available for adults only, epidemiologic information in this study was restricted to patients ≥15 years of age. P. jirovecii infections were reported as co-infections at the time of HIV diagnosis, as subsequent AIDS diagnoses, or as the cause of death.
**Statistical Analyses**

We used the statistical software STATA/SE 11.2 (21) for all analyses. Poisson regression with an offset for resident population, which used Office for National Statistics midyear estimates, was used to calculate the annual incidence rate ratio with 95% CIs. The Pearson χ² test was used to examine changes in the proportion of cases by risk category over time (2000–2005 vs. 2006–2010).

**Results**

The absolute numbers of cases of *P. jirovecii* pneumonia in England during 2000–2010, reported by each national surveillance system, are shown in Figure 1 and Table 1. We describe data from each system separately.

**Hospital Episode Statistics**

During the study period, HES recorded 2,258 cases of *P. jirovecii* pneumonia. The number of cases increased from 157 in 2000 to 352 in 2010, an average annual increase of 9% (p<0.001).

Cases reported to HES were not restricted to a particular geographic area, and the data showed no obvious seasonal trends. Because the increase in cases began in the latter half of the decade (Figure 1), we compared data from 2000–2005 with that from 2006–2010. This comparison showed a marked change in the age distribution of patients hospitalized for *P. jirovecii* infection during 2006–2010; relatively more patients were 60–69 years of age (Figure 2). Among all age groups, there was a higher proportion of male than female patients with *P. jirovecii* infection.

During the study period, 81% of patients within the HES database who had a diagnosis of *P. jirovecii* pneumonia could be classified according to a defined risk category (Table 2). Most (40.6%) had a hematologic malignancy, and 17.5% had preexisting lung disease. Relative distribution of risk groups differed significantly between 2000–2005 and 2006–2010 for all risk categories (χ² 28.2, 7 degrees of freedom, p<0.001). The numbers of patients with *P. jirovecii* pneumonia increased significantly in all risk groups, but the difference in rates between the 2 periods was most marked among patients who had undergone transplantation, 47% of whom had undergone kidney transplantation during 2000–2010. The number of patients who were not in any of the risk groups described above dropped by 19% between the 2 periods. This test was conservative because there was some overlap between the risk categories.

**Routine Laboratory Reporting**

During the study period, LabBase2 recorded 765 laboratory-confirmed cases. Reported cases of *P. jirovecii* pneumonia remained relatively unchanged during 2000–2006 (range 41–77 cases/year, mean 55 cases/year) but increased from 76 cases in 2007 to 98–104 cases during 2008–2010 (Figure 1), particularly in older patients. The male-to-female ratio of *P. jirovecii* pneumonia patients during 2000–2010 was 2.5 to 1.0.

**Death Certificate Data**

Deaths for which *P. jirovecii* pneumonia was recorded as a cause or contributing factor rose from 57 in 2001 to 94 in 2010 (p<0.001). For several years, the numbers of *P. jirovecii* infections reported on death certificates as a contributory cause of death were greater than those captured by laboratory reports (Figure 1).

**HIV Surveillance Data**

The numbers of patients with *P. jirovecii* pneumonia and HIV infection decreased 7% per year during 2000–2010 (p<0.001) (Figure 3). Most *P. jirovecii* infection diagnoses were made at the time of HIV diagnosis. Within this group of HIV-infected patients, death from *P. jirovecii* infection remained relatively stable over this period.

**Discussion**

In this study, we found an increasing trend in rates for clinical cases recorded in HES and microbiologically confirmed and reported cases in England during 2000–2010. This finding suggests a real increase in the numbers of cases of *P. jirovecii* pneumonia diagnosed. We also found an association between *P. jirovecii* infection and a variety of chronic lung diseases not described in the literature as being associated with *P. jirovecii* infection. On the basis of these data, we propose preexisting lung disease as a new *P. jirovecii* pneumonia risk category.

The HES database yielded 2,258 cases of *P. jirovecii* pneumonia during 2000–2010, but LabBase2 found only 765. The differences in number of cases suggests substantial underreporting by laboratories, although most cases might be diagnosed on the basis of clinical or radiologic...
findings or by immunofluorescence in the cytology department without being microbiologically confirmed.

An analysis of the Health Protection Agency database of HIV-infected persons shows clear evidence of a substantial reduction in *P. jirovecii* infections during 2000–2010, consistent with an earlier diagnosis of HIV and receipt of effective antiretroviral therapy (14). *P. jirovecii* infections among HIV-infected persons declined, whereas *P. jirovecii* infections among non–HIV-infected persons increased, suggesting that other risk factors must be responsible for the increased numbers of cases.

Given the substantial illness and death associated with *P. jirovecii* infection and the resources needed to manage these cases, the increase in cases is of serious concern. Many patients need treatment in intensive care units. However, prophylactic use of antimicrobial drugs is highly effective for preventing the disease. A study in the United States suggested that almost $5 million a year could be saved in the state of Maryland alone if prophylaxis were instituted for all HIV-positive patients at risk for *P. jirovecii* infection (22).

### Potential Causes of the Observed Increase

The increased number of cases might reflect changes in ascertainment of cases and increased infections in immunosuppressed patients who have received chemotherapy. It is possible that ascertainment increased over the study period because of improved diagnostic methods; immunofluorescence staining is being replaced by more sensitive PCR methods (23). We were not able to test the hypothesis that the increased number of cases is the result of increased testing for *P. jirovecii* because the laboratory surveillance system captures positive samples only, not the total number of samples submitted. However, the change in age distribution of patients toward a much older age group suggests that increased testing is not the main reason for increased case detection.

With regard to immunosuppression, an area that has seen an increase in the use of potent immunosuppressant agents is transplant surgery. Recipients who are not well matched to donor human leukocyte antigens now receive more powerful drugs. That said, the proportion of patients receiving renal transplants with a moderate degree of human leukocyte antigen mismatch has remained stable, represented by 43.9% of patients during financial year 2009–10 (National Health Service Blood and Transplant Authority, pers. comm.). Similarly, data from the National Health Service Blood and Transplant Authority indicate that the number of renal transplantations increased by 25% during 2006–2010. Again, this increase was not proportional to that observed for *P. jirovecii* infections reported for renal transplant recipients, which was ≈388% over the same period (National Health Service Blood and Transplant Authority, pers. comm.), so the increase cannot be explained simply by an increase in the number of patients in this risk group.

The largest group of persons affected by *P. jirovecii* pneumonia is those with hematologic malignancies. This finding might reflect the 30% increase in diagnoses of these malignancies during 2000–2010 (24). However, the increase in patients in this risk group with *P. jirovecii* pneumonia was 209% over the same period.

A possible explanation for the increase in *P. jirovecii* pneumonia cases is an increase in the number of potentially vulnerable patients who did not receive appropriate prophylactic therapy. Guidelines recommend the use of antimicrobial drug prophylaxis for kidney transplant recipients and for patients with hematologic malignancies who are receiving certain chemotherapy (25–28). A Cochrane review recommends prophylaxis for patients with hematologic malignancies and for recipients of bone marrow and solid organ transplants (29). Our study identified a new group at risk for *P. jirovecii* infection: patients with
Table 2. Proportion of all Pneumocystis jirovecii–associated hospital admissions and change in population rates over time, England, UK, 2000–2010

<table>
<thead>
<tr>
<th>Risk category*</th>
<th>No. admissions (% all cases)</th>
<th>Annual rate/million population</th>
<th>Rate ratio between periods (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any transplant†</td>
<td>59 (6.3)</td>
<td>193 (14.7)</td>
<td>0.20</td>
</tr>
<tr>
<td>Other lung disease‡</td>
<td>120 (12.8)</td>
<td>276 (21.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>Hematologic disorders</td>
<td>217 (23.1)</td>
<td>354 (26.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>Hematologic malignancy</td>
<td>349 (37.1)</td>
<td>568 (43.1)</td>
<td>1.17</td>
</tr>
<tr>
<td>Connective tissue/inflammatory disease§</td>
<td>71 (7.6)</td>
<td>120 (9.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Renal failure and dialysis</td>
<td>95 (10.1)</td>
<td>208 (15.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>Immunosuppressive/chemotherapeutic drugs</td>
<td>47 (5.0)</td>
<td>90 (6.8)</td>
<td>0.73</td>
</tr>
<tr>
<td>Malignancy other than hematologic</td>
<td>92 (9.8)</td>
<td>160 (12.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Not in the above risk categories</td>
<td>255 (27.1)</td>
<td>177 (13.4)</td>
<td>0.85</td>
</tr>
<tr>
<td>Total no. cases¶</td>
<td>941</td>
<td>1,317</td>
<td>3.15</td>
</tr>
</tbody>
</table>

*Excludes HIV infection.
†Includes liver, heart, lung, kidney and bone transplants.
‡Includes tuberculosis, chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis, asthma, and interstitial lung disease.
§Includes systemic connective tissue disorder, psoriatic arthropathy, rheumatoid arthritis, and inflammatory bowel disease.
¶Because some patients belong to >1 risk category, numbers do not add up to the total number of cases.

Pneumocystis jirovecii pneumonia. To help characterize any additional groups of patients for whom prophylaxis is not currently recommended but who might be at risk. Particular focus should be given to patients with chronic lung disease, systemic inflammatory diseases, and solid tumors and to transplant recipients who do not currently fulfill the criteria for prophylaxis. When introducing new immunosuppressive agents and regimens, consideration should be given as to whether these agents might increase the patients’ risk for P. jirovecii pneumonia.

More studies involving sequencing of P. jirovecii clinical isolates identified by PCR, coupled with national surveillance, should be used to better understand transmission dynamics and thereby inform infection control policies and clarify the role of any environmental factors (1,30–32). More basic knowledge of the biology, pathogenesis, virulence factors, and the contribution of different strains will be crucial for explaining observed changes in P. jirovecii epidemiology.

To ensure adherence to current guidelines and to ensure that preventive prophylaxis is optimal for all groups at risk for this potentially life-threatening infection, auditing of prescribing practices for patients known to be at risk is warranted. Raising awareness among clinicians could also help ensure that prophylaxis is correctly used.

In conclusion, data from a variety of national sources demonstrate an increase in the number of cases of P. jirovecii in non–HIV-infected persons. P. jirovecii infections are largely preventable by use of inexpensive drugs. The current case numbers are taking a substantial toll on health care costs and human health. Further investigation leading to improved preventive strategies for this largely preventable infection is warranted.

Acknowledgments

We thank Nick Andrews and Phil Pocock for their statistical advice. We also thank the Office for National Statistics for access to death registrations and note that they bear no responsibility for our analysis or interpretation of data supplied by them.
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Dr Maini is a specialist registrar in public health and works at the Health Protection Agency, London, UK. Her research interests are focused on communicable diseases, especially respiratory infections.

References


Leptospira [lep’to-spî’ra]

From the Greek leptos (slender) and speira (coil), a genus of bacteria consisting of single, finely coiled, motile, aerobic cells. In 1886, German physician Adolf Weil described a clinical syndrome characterized by splenomegaly, jaundice, and nephritis, although the disease was likely recognized in ancient China as an occupational hazard of rice farming. The organism was first described in 1907 by Arthur Stimson, who observed spirochetes with curved ends in the kidneys of a patient thought to have died of yellow fever. He named it Spirochaeta interrograns because it looked like a question mark.

The cause of Weil’s disease was isolated independently in 1915 in Japan and Germany. In Japan, Inada et al. detected spirochetes, which they named Spirochaeta icterohaemorrhagiae, in the blood of coal miners with infectious jaundice. In Germany, 2 groups of physicians (Uhlenhuth et al. and Hubener et al.) studied soldiers afflicted with “French disease” in the trenches of northeastern France. The Germans were arguing over priority, however, and overlooked the publications by Inada’s group, which predated their own by 8 months. The genus Leptospira was suggested in 1917 by Hideyo Noguchi “on account of its fine and minute windings.”

**Sources**


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Performance of *Candida* Real-time Polymerase Chain Reaction, β-D-Glucan Assay, and Blood Cultures in the Diagnosis of Invasive Candidiasis

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**Background.** The sensitivity of blood cultures for diagnosing invasive candidiasis (IC) is poor.

**Methods.** We performed a validated *Candida* real-time polymerase chain reaction (PCR) and the Fungitell 1,3-β-D-glucan (BDG) assay on blood samples collected from prospectively identified patients with IC (n = 55) and hospitalized controls (n = 73). Patients with IC had candidemia (n = 17), deep-seated candidiasis (n = 33), or both (n = 5). Controls had mucosal candidiasis (n = 5), *Candida* colonization (n = 48), or no known *Candida* colonization (n = 20).

**Results.** PCR using plasma or sera was more sensitive than whole blood for diagnosing IC (P = .008). Plasma or sera PCR was more sensitive than BDG in diagnosing IC (80% vs 56%; P = .03), with comparable specificity (70% vs 73%; P = .31). The tests were similar in diagnosing candidemia (59% vs 68%; P = .77), but PCR was more sensitive for deep-seated candidiasis (89% vs 53%; P = .004). PCR and BDG were more sensitive than blood cultures among patients with deep-seated candidiasis (88% and 62% vs 17%; P = .0005 and .003, respectively). PCR and culture identified the same *Candida* species in 82% of patients. The sensitivity of blood cultures combined with PCR or BDG among patients with IC was 98% and 79%, respectively.

**Conclusions.** *Candida* PCR and, to a lesser extent, BDG testing significantly enhanced the ability of blood cultures to diagnose IC.

Invasive candidiasis (IC) carries significant morbidity and mortality. In part, poor outcomes stem from delayed or missed diagnoses using blood and sterile-site cultures, the current gold standard tests. Indeed, blood cultures are negative for *Candida* species in approximately 50% of autopsy-proven cases of disseminated candidiasis [1–3]; moreover, they often become positive late in the disease course. Sterile-site cultures are further limited by the need for invasive sampling. As such, there is much interest in developing rapid and more sensitive diagnostic assays. Two approaches that have gained particular attention are detection of 1,3-β-D-glucan (BDG), a major constituent of fungal cell walls, and polymerase chain reaction (PCR) amplification of *Candida* DNA. Despite their promise, neither test is a standard of clinical practice. The sensitivity of a commercial assay for BDG quantitation (Fungitell, Associates of Cape Cod) has varied from 64% to 100% [4–6], and *Candida* PCR methods are not validated.

In this study, we compared the performance of a *Candida* real-time PCR assay, which was validated at Viracor-IBT Laboratories according to relevant clinical laboratory guidelines [7], with the Fungitell BDG assay and blood cultures for diagnosing IC. We included samples from a wide range of patients with IC, including those with candidemia, catheter-related candidemia, and deep-seated candidiasis with or without positive blood cultures. To rigorously evaluate the specificity of
the assays, our control group was comprised largely of patients with mucosal candidiasis or who were colonized with Candida at nonsterile sites.

**METHODS**

We conducted a prospective study of patients at the University of Pittsburgh Medical Center between April 2009 and April 2011. Blood was collected from consenting controls and patients with IC, and serum or plasma samples were stored at −80°C. Whole blood testing was performed on fresh samples. The study was approved by our institutional review board.

**Definitions**

Invasive candidiasis included candidemia and deep-seated candidiasis [8], which were defined as the recovery of Candida species from blood or a sterile site, respectively. Controls were defined as hospitalized patients who did not have clinical or microbiological evidence of IC. Patients with mucosal candidiasis or colonized with Candida were also included as controls. Colonization was defined as the recovery of Candida species from nonsterile sites in patients without symptoms or signs of a systemic disease that were attributable to candidiasis. Blood and deep-seated cultures were considered to be concurrent if performed within 5 days of each other. Positive, indeterminate, and negative BDG results were defined as ≥80, 60 < <80, and <60 pmol/mL, respectively. Candida were speciated using standard mycological methods of carbohydrate assimilation using the API 20C kit (bioMérieux, Hazelwood, Missouri) and morphology on cornmeal agar. CHROMagar (BD Diagnostics) or other differential media to speciate Candida were not used.

**BDG Testing and Real-time PCR**

Frozen plasma and serum samples were shipped overnight, on dry ice, in batch to Viracor-IBT Laboratories for BDG and PCR testing. Whole blood samples were sent unfrozen on ice and processed within 24 hours of venipuncture. The Fungitell BDG assay was performed according to the manufacturer's instructions. For PCR, DNA from whole blood, plasma, and serum was extracted using the DNeasy Blood and Tissue kit (Qiagen, Germantown, Maryland). After adding an internal control target (engineered bacteriophage), 500 µL of specimen was manually extracted using a 35-µL elution volume. An internal control cutoff quantification cycle of 37 was required for reporting a negative result. A positive extraction control, negative extraction control, and no template control were included in every PCR run. For the design of species-specific TaqMan real-time assays, alignments of available ITS1 and/or ITS2 sequences for 4 Candida species (Candida albicans, Candida glabrata, Candida krusei, and Candida tropicalis) and 1 species complex (Candida parapsilosis complex) were created using Geneious software (Biomatters, Auckland, New Zealand.). Primers were designed to detect 2 species pairs (C. albicans/C. tropicalis and C. glabrata/C. krusei), and C. parapsilosis. The ABI 7500 Fast Instrument (Applied Biosystems, Carlsbad, California) was used with a final reaction volume of 30 µL utilizing 10 µL of template DNA. The amplification efficiencies, determined using 10-fold dilutions of plasmid standards ranging from 10 to 1 × 108 copies per reaction, were 99.3%, 96.5%, 102.5%, 97.4%, and 98.7% for C. albicans, C. glabrata, C. krusei, C. parapsilosis complex, and C. tropicalis, respectively. The analytical specificities for the Candida PCR assays tested with various targeted Candida species, nontargeted fungi, and bacteria and were 100% (7/7), 95% (37/39) and 100% (28/28), respectively (Table 1). The analytical sensitivity using targeted Candida species was 100% (7/7).

**Statistical Analysis**

Sensitivity and specificity were calculated for blood culture, BDG, and PCR. The McNemar χ2 test was used to compare sensitivity and specificity between assays [9]. Univariate analysis of contingency data was done by χ2 or Fisher exact test.

**RESULTS**

**PCR Using Different Blood Components**

In a preliminary study, we performed PCR on whole blood and plasma samples from 21 patients with IC and 27 controls. Polymerase chain reaction was more sensitive at detecting Candida in plasma than whole blood of patients with IC (57% [12 of 21] vs 14% [3 of 21]; P = .008), and specificity was comparable (81% [22 of 27] vs 96% [26 of 27]; P = .22). Next, we tested plasma and sera from 16 patients with IC and 15 controls. Plasma and serum samples did not differ in sensitivity (81% [13 of 16] vs 75% [12 of 16], respectively; P = 1.0) or specificity (67% [10 of 15] vs 73% [11 of 15], respectively; P = 1.0). For the remainder of the study, PCR was performed on plasma and/or serum samples.

**Performance of PCR and BDG**

Patients with IC had candidemia (n = 17), deep-seated candidiasis (n = 33), or both candidemia and deep-seated candidiasis (n = 5) (Table 2). Intra-abdominal infections accounted for 89% (34 of 38) of deep-seated candidiasis. Controls included 48 patients with Candida colonization (Table 3), 5 with mucosal candidiasis (esophagitis, n = 3; oropharyngeal, n = 1; vaginitis, n = 1), and 20 with no known Candida colonization.

The performance of PCR and BDG is summarized in Table 4. The sensitivity of the tests was not affected by antifungal therapy (Table 5). Using the standard BDG cutoff for positivity (≥80 pmol/mL; indeterminate result = negative), both
BDG and PCR were positive for 42% (23 of 55) of patients with IC and negative for 5% (3 of 55) (Table 6). Details of the patients with false negative results by both assays appear in Table 7. Polymerase chain reaction was positive and BDG negative for 38% (21 of 55) of patients, and BDG was positive and PCR negative for 15% (8 of 55). The sensitivity of either a positive PCR or BDG for diagnosing IC was 95% (52 of 55), and specificity was 56% (41 of 73).

### Comparison of PCR and BDG With Blood Cultures

Among the 24 patients with deep-seated candidiasis in whom blood cultures were performed concurrently, both PCR (88% [21 of 24]) and BDG (62% [15 of 24]) were more sensitive than blood cultures (17% [4 of 24]; $P = .0005$ and $P = .003$, respectively) (Figure 1). If indeterminate BDG results were considered positive, sensitivity was 67% (16 of 24; $P = .002$ vs blood culture). At either BDG cutoff, sensitivity did not differ from PCR ($P = .15$ and .23, respectively).

### Identification of Candida Species by PCR and Culture

Among the 42 patients with IC in whom blood cultures were obtained, the sensitivity of either a positive PCR or positive blood culture was 98% (41 of 42). The sensitivities of either a positive BDG or positive blood culture were 79% (33 of 42; indeterminate = negative) and 81% (34 of 42; indeterminate = positive).

### DISCUSSION

This study was designed as a head-to-head comparison of PCR and BDG in diagnosing IC. We prospectively enrolled and collected blood from hospitalized patients in 3 well-defined...
Table 2. Patient Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With IC</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 55)</td>
<td>(n = 73)</td>
<td></td>
</tr>
<tr>
<td>Underlying gastrointestinal disorder</td>
<td>36% (20)</td>
<td>14% (10)</td>
</tr>
<tr>
<td>Short-gut syndrome</td>
<td>7% (4)</td>
<td>3% (2)</td>
</tr>
<tr>
<td>Liver or biliary diseasea</td>
<td>5% (3)</td>
<td>1% (1)</td>
</tr>
<tr>
<td>Small bowel obstructiona</td>
<td>5% (3)</td>
<td>4% (3)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>11% (6)</td>
<td>4% (3)</td>
</tr>
<tr>
<td>Underlying genitourinary disorder</td>
<td>4% (2)</td>
<td>1% (1)</td>
</tr>
<tr>
<td>Trauma/motor vehicle accident</td>
<td>9% (5)</td>
<td>14% (10)</td>
</tr>
<tr>
<td>Organ transplantation</td>
<td>22% (12)</td>
<td>36% (26)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>20% (11)</td>
<td>33% (24)</td>
</tr>
<tr>
<td>Abdominal surgery within 1 month of enrollment</td>
<td>24% (13)</td>
<td>8% (6)</td>
</tr>
<tr>
<td>Extra-abdominal surgery within 1 month of enrollment</td>
<td>5% (4)</td>
<td>7% (5)</td>
</tr>
</tbody>
</table>

None of the patients had hematologic malignancy or neutropenia at the time of invasive candidiasis. Fifty-four percent (30 of 55) of patients with invasive candidiasis (IC) were receiving an antifungal agent at the time blood was collected (fluconazole, n = 20; echinocandin, n = 9; voriconazole, n = 1). The median time from the start of antifungal therapy to sample collection was 5 days (range, 1–39).

* One patient with IC had both liver disease and small bowel obstruction.

groups: candidemia, deep-seated candidiasis, and controls without IC. To maximize the rigor of the study, the latter group was overwhelmingly composed of patients who were colonized with Candida species or diagnosed with mucosal candidiasis. Our data yielded 2 particularly important findings. First, PCR was superior to BDG for diagnosing deep-seated candidiasis (sensitivity: 89% vs 53% or 66%, depending upon the BDG interpretive cutoff; P = .004 and .04, respectively). Polymerase chain reaction also was significantly more sensitive for diagnosing all cases of IC at the standard cutoff for BDG positivity (80% vs 56%; P = .03). Second, both PCR and BDG were markedly superior to blood cultures among patients with deep-seated candidiasis (88% and 62% vs 17%; P = .0005 and P = .003, respectively). Taken together, the results indicate that PCR may join BDG as a valuable adjunctive tool for diagnosing IC. Moreover, these assays are likely to identify a significant percentage of those patients with IC who are missed by blood culture, the current gold standard diagnostic test.

The limitations of blood cultures for diagnosing IC are well recognized [1–3]. Indeed, blood cultures are positive in <50% of patients with hepatosplenic candidiasis or autopsy-proven IC [1, 3]. The use of antifungals for prophylaxis or empiric therapy may further reduce the sensitivity of blood cultures [10]. Although advances in microbiology techniques have improved the recovery of Candida species, the low magnitude and short duration of candidemia suggest that the sensitivity of blood cultures will remain inadequate [11–14]. Our results are consistent with previous reports that PCR and BDG were more sensitive than blood culture for diagnosing IC [4, 15–17]. There are several potential reasons for the heightened sensitivities of these assays. In the case of PCR, the amplification of a high-copy DNA target facilitates detection of lower inocula of Candida in the blood. In fact, the lower limit of detection for the assay used in this study is 80 Candida copies/mL of serum or plasma, which corresponds to approximately 0.4 colony-forming units/mL (<1 genome). At the same time, PCR amplifies DNA from both dead and viable Candida cells as well as freely circulating DNA [13, 18]. Likewise, BDG detection is not dependent on viable organisms [5, 19]. The reasons that PCR was more sensitive than BDG in diagnosing deep-seated candidiasis are not apparent, which reflects our limited understanding of the bloodstream kinetics of Candida DNA and BDG. In animal models of IC, serum PCR remained positive after sterilization of blood, suggesting that free DNA is eliminated slowly [13, 18].

It is notable that the sensitivity of PCR for diagnosing candidemia was significantly lower than deep-seated candidiasis (P = .009). Similar results were reported previously [4]. A potential explanation for our findings is that patients were enrolled at the time of positive cultures. As such, transient or catheter-associated candidemias already may have resolved spontaneously or as a result of catheter removal. During deep-seated candidiasis, Candida DNA may be continuously

Table 3. Sites of Candida Colonization

<table>
<thead>
<tr>
<th>Sites of Colonization</th>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound (n = 14)</td>
<td>Extremity wound</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Abdominal wound</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Cheek wound</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory tract (n = 14)</td>
<td>Bronchoalveolar lavage</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Sputum</td>
<td>6a</td>
</tr>
<tr>
<td>Indwelling surgical drain (n = 10)</td>
<td>Jackson Pratt</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Biliary drain</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>2</td>
</tr>
<tr>
<td>Urine (n = 9)</td>
<td>Urine</td>
<td>6a</td>
</tr>
<tr>
<td></td>
<td>Urine distal to pelvic stone</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Urine from a patient with hematuria</td>
<td>1</td>
</tr>
<tr>
<td>Other (n = 2)</td>
<td>Catheter tip</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sinus tract</td>
<td>1</td>
</tr>
</tbody>
</table>

* One patient was colonized in both sputum and urine.

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released into the bloodstream, which could also explain the persistence of serum PCR positivity after the clearance of blood cultures in animal models of IC [13, 18]. Our results call to mind previous studies in which mannoproteinemia was detected in only 7% of patients with transient or catheter-associated candidemia compared with 76% with persistent candidemia or IC [20, 21]. On the whole, the data highlight that PCR and BDG will be most useful as diagnostic adjuncts to blood cultures rather than as replacements. Indeed, the sensitivity of blood culture combined with PCR or BDG among patients with IC was 98% and 79%–81%, respectively, which was better than any of the tests alone. Since positive blood cultures by definition are diagnostic of IC, they improve the sensitivity of nonculture-based assays without impacting

| Table 4. Performance of Polymerase Chain Reaction and 1,3-β-D-Glucan Assays |
|-----------------------------|----------------|----------------|----------------|
| Assay                       | invasive Candidiasis (n = 55) | Candidemia* (n = 22) | Deep-Seated Candidiasis* (n = 38) | Intra-abdominal Candidiasis (n = 34) |
|-----------------------------|----------------|----------------|----------------|
| PCRc                        |                 |                 |                 |
| Sensitivity                 | 80% (44/55)    | 59% (13/22)    | 89% (34/38)    | 88% (30/34) |
| Specificity                 | 70% (51/73)    |                |                |              |
| BDG (positive ≥80 pmol/mL)  |                 |                 |                 |
| Sensitivity                 | 56% (31/55)    | 68% (15/22)    | 53% (20/38)    | 56% (19/34) |
| Specificity                 | 73% (53/73)    |                |                |              |
| BDG (positive ≥60 pmol/mL)  |                 |                 |                 |
| Sensitivity                 | 69% (38/55)    | 81% (18/22)    | 66% (25/38)    | 65% (22/34) |
| Specificity                 | 63% (46/73)    |                |                |              |

P value

- PCR vs BDG (positive ≥80 pmol/mL): .03 .77 .004 .0015
- PCR vs BDG (positive ≥60 pmol/mL): .31 .23 .04 .06

Internal control detection was positive for all samples that were negative by PCR. The median time from diagnostic cultures for Candida to collection of samples for PCR and BDG was 4 days (interquartile range: 1-6 days).

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

a Candidemia and deep-seated candidiasis groups included 5 patients who had both conditions.
b Deep-seated candidiasis included patients with intra-abdominal infections and infections of other sites (bone and devitalized surrounding tissue, n = 2; lumbar spine device, n = 1; cranial abscess, n = 1).
c PCR was positive if positive result was obtained on plasma and/or sera.
d P values are for sensitivities of the respective assays, as determined by McNemar test.

<p>| Table 5. Impact of Antifungal Therapy on Polymerase Chain Reaction and 1,3-β-D-Glucan Assay Performance |
|-----------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Assay</th>
<th>invasive Candidiasis (n = 55)</th>
<th>Candidemia* (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On antifungal therapy</td>
<td>77% (23/30)</td>
<td>62% (10/16)</td>
</tr>
<tr>
<td>Not on antifungal therapy</td>
<td>84% (21/25)</td>
<td>50% (3/6)</td>
</tr>
<tr>
<td>P value</td>
<td>.74</td>
<td>.66</td>
</tr>
<tr>
<td>BDG sensitivityb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On antifungal therapy</td>
<td>56% (17/30)</td>
<td>58% (11/16)</td>
</tr>
<tr>
<td>Not on antifungal therapy</td>
<td>73% (14/25)</td>
<td>67% (4/6)</td>
</tr>
<tr>
<td>P value</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>BDG sensitivityc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On antifungal therapy</td>
<td>67% (20/30)</td>
<td>81% (13/16)</td>
</tr>
<tr>
<td>Not on antifungal therapy</td>
<td>72% (18/25)</td>
<td>83% (5/6)</td>
</tr>
<tr>
<td>P value</td>
<td>.77</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

a Candidemia and deep-seated candidiasis groups included 5 patients who had both conditions.
b Positive BDG defined as ≥80 pmol/mL (indeterminate = negative).
c Positive BDG defined as ≥60 pmol/mL (indeterminate = positive).
Along these lines, it is notable that we corroborated earlier comparisons between centers and populations in the future. The use of a validated and publically available PCR assay in this study is a major advance, which should facilitate regard, the use of a validated and publically available PCR assay in this study is a major advance, which should facilitate

Deep-seated candidiasis is a significant clinical challenge, with a reported incidence ranging from 4% to 30% of all candidiasis cases. The diagnosis of deep-seated candidiasis can be challenging due to the lack of specific symptoms and diagnostic markers. The combination of PCR and BDG was superior to whole blood [13, 18, 23, 24], which likely reflects an absence of PCR inhibitors, less cumbersome DNA extraction methods, and the more ready detection of cell-free fungal DNA within these compartments. On balance, the performance of PCR was in keeping with other reports, in which sensitivities ranged from 73% to 95% [7, 16]. In fact, the specificity of PCR and BDG in this study is likely to be lower than in most clinical practices, due to the composition of PCR primers and probes, amplification parameters in PCR studies, and the particular detection assay for BDG [6, 22]. In this regard, the use of a validated and publically available PCR assay in this study is a major advance, which should facilitate comparisons between centers and populations in the future. Along these lines, it is notable that we corroborated earlier observations that the sensitivity of PCR on plasma or sera was superior to whole blood [13, 18, 23, 24], which likely reflects an absence of PCR inhibitors, less cumbersome DNA extraction methods, and the more ready detection of cell-free fungal DNA within these compartments. On balance, the performance of PCR was in keeping with other reports, in which sensitivities ranged from 73% to 95% [7, 16]. In studies of BDG monitoring for early diagnosis of IC, sensitivity and specificity varied widely (64%–100% and 71%–98%, respectively) [4–6, 22]. The lower sensitivity of BDG in our experience may reflect the large percentage of patients with deep-seated candidiasis.

Overall, PCR and BDG demonstrated adequate specificity (70% and 63%–73%, respectively, depending on BDG cutoff). In fact, the specificity of PCR and BDG in this study is likely to be lower than in most clinical practices, due to the composition of PCR primers and probes, amplification parameters in PCR studies, and the particular detection assay for BDG [6, 22]. In this regard, the use of a validated and publically available PCR assay in this study is a major advance, which should facilitate comparisons between centers and populations in the future. Along these lines, it is notable that we corroborated earlier observations that the sensitivity of PCR on plasma or sera was superior to whole blood [13, 18, 23, 24], which likely reflects an absence of PCR inhibitors, less cumbersome DNA extraction methods, and the more ready detection of cell-free fungal DNA within these compartments. On balance, the performance of PCR was in keeping with other reports, in which sensitivities ranged from 73% to 95% [7, 16]. In studies of BDG monitoring for early diagnosis of IC, sensitivity and specificity varied widely (64%–100% and 71%–98%, respectively) [4–6, 22]. The lower sensitivity of BDG in our experience may reflect the large percentage of patients with deep-seated candidiasis.

## Table 6. Agreement Between Polymerase Chain Reaction and 1,3-β-D-Glucan Assays, Stratified by Type of Invasive Candidiasis

<table>
<thead>
<tr>
<th>Test Results</th>
<th>Total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Positive (n = 55)</th>
<th>Negative (n = 20)</th>
<th>Not Drawn (n = 9)</th>
<th>Other Deep-seated Infections (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR&lt;sup&gt;+&lt;/sup&gt;/BDG&lt;sup&gt;+&lt;/sup&gt;</td>
<td>42% (23/55)</td>
<td>40% (2/5)</td>
<td>50% (10/20)</td>
<td>44% (4/9)</td>
<td>25% (1/4)</td>
</tr>
<tr>
<td>PCR&lt;sup&gt;+&lt;/sup&gt;/BDG&lt;sup&gt;-&lt;/sup&gt;</td>
<td>33% (18/55)</td>
<td>20% (1/5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45% (9/20)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44% (4/9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75% (3/4)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCR&lt;sup&gt;-&lt;/sup&gt;/BDG&lt;sup&gt;+&lt;/sup&gt;</td>
<td>15% (8/55)</td>
<td>0% (0/5)</td>
<td>0% (0/20)</td>
<td>0% (0/9)</td>
<td>0% (0/4)</td>
</tr>
<tr>
<td>PCR&lt;sup&gt;-&lt;/sup&gt;/BDG&lt;sup&gt;-&lt;/sup&gt;</td>
<td>5% (3/55)</td>
<td>0% (0/5)</td>
<td>0% (0/20)</td>
<td>11% (1/9)</td>
<td>0% (0/4)</td>
</tr>
</tbody>
</table>

Abbreviations: BDG, 1,3-β-D-glucan; PCR, polymerase chain reaction.

<sup>a</sup> Positive BDG defined as ≥80 pmol/mL (indeterminate = negative). If positive BDG was defined as ≥60 pmol/mL (indeterminate = positive), the agreement between assays among patients with invasive candidiasis was as follows: PCR<sup>+</sup>/BDG<sup>+</sup> (53%, 29 of 55), PCR<sup>-</sup>/BDG<sup>-</sup> (4%, 2 of 55), PCR<sup>+</sup>/BDG<sup>-</sup> (27%, 15 of 55), and PCR<sup>-</sup>/BDG<sup>-</sup> (16%, 9 of 55). The sensitivity and specificity of either a positive PCR or BDG were 96% (53 of 55) and 47% (34 of 73), respectively.

<sup>b</sup> One patient had BDG in the indeterminate range (60–79 pmol/mL).

<sup>c</sup> Two patients had BDG in the indeterminate range (60–79 pmol/mL).

## Table 7. Patients With Invasive Candidiasis and False-Negative Results by Both Polymerase Chain Reaction and 1,3-β-D-Glucan Assays

<table>
<thead>
<tr>
<th>Type of Invasive Candidiasis</th>
<th>Candida Species</th>
<th>1,3-β-D-Glucan Result</th>
<th>Antifungal Therapy Prior to Sample Collection</th>
<th>Source of Invasive Candidiasis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidemia</td>
<td>C. parapsilosis</td>
<td>Indeterminate (62 pmol/mL)</td>
<td>None</td>
<td>Catheter and peripheral cultures positive</td>
<td>Catheter removed prior to sample collection</td>
</tr>
<tr>
<td>Candidemia</td>
<td>C. glabrata</td>
<td>Negative (36 pmol/mL)</td>
<td>None</td>
<td>Catheter-associated (only catheter cultures positive; multiple peripheral negative)</td>
<td>Catheter removed prior to sample collection</td>
</tr>
<tr>
<td>Deep-seated (no blood culture obtained)</td>
<td>C. albicans</td>
<td>Negative (&lt;31 pmol/mL)</td>
<td>None</td>
<td>Intra-abdominal abscess from perforated peptic ulcer</td>
<td>Immediate surgical debridement</td>
</tr>
</tbody>
</table>
of our control group. Seventy-five percent of controls were colonized with \textit{Candida} or had mucosal candidiasis, 36% were immunosuppressed, and almost half either underwent organ transplantation or had underlying gastrointestinal disease. Indeed, in several instances, our false-positive results may have represented unrecognized IC. As an example, 1 PCR-positive control was diagnosed with severe esophageal candidiasis following endoscopy for hematemesis. It is plausible that extensive mucosal disruption allowed \textit{Candida} cells or DNA, which was not detected by blood cultures due to their poor sensitivity and/or the effect of antifungal therapy, to penetrate into the bloodstream. Colonization is generally accepted as the principal factor limiting the specificity of PCR, but relatively few studies have investigated the issue for candidiasis. In a recent meta-analysis, there was a trend toward lower specificity of \textit{Candida} PCR among colonized patients \cite{16}.

False-positive BDG results have also been attributed to \textit{Candida} colonization, systemic bacterial infections, antibiotics, cellulose membranes used during hemodialysis, and cotton gauze and sponges \cite{25}. Of note, the specificity of BDG at the lower cutoff for positivity (≥60 pmol/mL) was only 50% among colonized patients, which may limit the utility of this cutoff.

A possible advantage of PCR over BDG is the ability for speciation. The PCR assay used in this study was designed to distinguish fluconazole-susceptible species (\textit{C. albicans} and \textit{C. tropicalis}) from intrinsically or potentially resistant species (\textit{C. krusei} and \textit{C. glabrata}) and \textit{C. parapsilosis}, which often demonstrates reduced echinocandin susceptibility. Overall, speciation by culture and species-specific PCR was in agreement in 82% of patients. There are several potential explanations for disagreements in the remaining cases. First, PCR speciation may be incorrect. In most instances, we do not think this was the case because there was no misidentification of \textit{Candida} isolates in preliminary experiments (Table 1). Second, the speciation in our clinical lab may be incorrect. Indeed, studies of clinical labs have indicated that \textit{Candida} species were misidentified in 8%–15% of specimens \cite{26–29}. Third, the clinical lab may have missed cases caused by multiple species. We found that 2 species were identified in 5% of blood cultures, which is consistent with rates of at least 4% reported in the literature \cite{30, 31}. At the same time, PCR identified at least 2 species in 36% of samples, suggesting that the clinical laboratory may not have isolated unique colonies for speciation. Fourth, time lags between culture and PCR sample collection may have contributed to disagreements, because it is possible that a particular infecting \textit{Candida} species was no longer in the circulation. Finally, many of the cultures were performed on deep-seated


<table>
<thead>
<tr>
<th>Culture Results</th>
<th>Candida PCR Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA/CT</td>
</tr>
<tr>
<td>Single species</td>
<td></td>
</tr>
<tr>
<td>\textit{C. albicans}</td>
<td>10</td>
</tr>
<tr>
<td>\textit{C. tropicalis}</td>
<td>1</td>
</tr>
<tr>
<td>\textit{C. glabrata}</td>
<td>4</td>
</tr>
<tr>
<td>\textit{C. parapsilosis}</td>
<td>1</td>
</tr>
<tr>
<td>Multiple species</td>
<td></td>
</tr>
<tr>
<td>\textit{C. albicans} and \textit{C. tropicalis}</td>
<td>1</td>
</tr>
<tr>
<td>\textit{C. albicans} and \textit{C. glabrata}</td>
<td>...</td>
</tr>
<tr>
<td>\textit{C. albicans} and \textit{C. krusei}</td>
<td>...</td>
</tr>
<tr>
<td>\textit{C. tropicalis} and \textit{C. glabrata}</td>
<td>1</td>
</tr>
</tbody>
</table>

Numbers in the table are the number(s) of \textit{Candida} isolates showing a particular pattern of speciation by the 2 methods. There was complete agreement in speciation between polymerase chain reaction (PCR) and culture for 45% (20/44) of patients and complete disagreement for 18% (8/44) of patients. PCR identified multiple species sets in 36% (16/44) of patients, whereas culture revealed multiple species in 9% (4/44; \textit{P} = .01, Fisher exact test).

samples, whereas PCR was performed on plasma or sera. Different species may have been present at different sites. If culture and PCR give discordant results in clinical practice, the wisest course is to treat as if the more resistant species is present. In this regard, it is notable that the most common disagreement was blood culture identifying C. albicans and PCR identifying C. glabrata or C. krusei.

It is important to acknowledge limitations of our study. First, PCR and BDG testing was performed in batches on frozen samples. As such, we cannot exclude that some negative results may have stemmed from sample instability. Second, antifungal use did not impact the performance of PCR or BDG, but the majority of patients received an azole. Thus, we could not assess the potential impact of BDG synthesis inhibition by the echinocandins on the performance of the BDG assay. Third, the number of controls with mucosal candidiasis was small; therefore, the specificity of PCR and BDG in this population needs further study. Fourth, blood for BDG and PCR was obtained after the diagnosis of IC was confirmed by culture and at only 1 time point, which precluded the evaluation of the tests for disease screening. Future studies should include serially collected blood samples from patients at high risk for IC.

In conclusion, we demonstrated that Candida PCR and, to a lesser extent, BDG testing significantly enhanced the ability of blood cultures to diagnose IC. As for all diagnostic tests, best results will be obtained if PCR and BDG are limited to situations in which there is a reasonable likelihood of IC. Employed judiciously, these assays have the potential to identify a large population of patients with deep-seated candidiasis missed by blood cultures. If our findings are validated in other studies, the results will have a major impact on the treatment of IC, our understanding of its pathogenesis, and the design of clinical trials. Follow-up studies to evaluate the impact of Candida PCR and BDG on the diagnosis, treatment, and outcome of IC are indicated.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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The role of the multidisciplinary team in antifungal stewardship

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There are a variety of challenges faced in the management of invasive fungal diseases (IFD), including high case-fatality rates, high cost of antifungal drugs and development of antifungal resistance. The diagnostic challenges and poor outcomes associated with IFD have resulted in excessive empirical use of antifungals in various hospital settings, exposing many patients without IFD to potential drug toxicities as well as causing spiralling antifungal drug costs. Further complexity arises as different patient groups show marked variation in their risk for IFD, fungal epidemiology, sensitivity and specificity of diagnostic tests and the pharmacokinetics and pharmacodynamics of antifungal drugs. To address these issues and to ensure optimal management of IFD, specialist knowledge and experience from a range of backgrounds is required, which extends beyond the remit of most antibiotic stewardship programmes. The first step in the development of any antifungal stewardship (AFS) programme is to build a multidisciplinary team encompassing the necessary expertise in the management of IFD to develop and implement the AFS programme. The specific roles of the key individuals within the AFS team and the importance of collaboration are discussed in this article.

Introduction

The primary aim of antifungal stewardship (AFS) programmes is to optimize antifungal drug use by integrating specialist experience and knowledge to tackle the issues preventing appropriate use of antifungal drugs. The formation of a multidisciplinary team with the necessary expertise is key to the development of any AFS programme. The core members of the AFS team should consist of individuals who possess sufficient knowledge of, and experience in, the clinical management of relevant patient populations, fungal epidemiology and susceptibility patterns, the laboratory diagnosis of invasive fungal disease (IFD), pharmacokinetics (PK) of antifungal drugs, dosing and drug–drug interactions. In this article the authors offer their views on the remit of the multidisciplinary team, the specific roles of key individuals within the team and the importance of collaboration.

Clinical pharmacist

Antifungal drugs are used for heterogeneous groups of patients. Consequently, knowledge of the PK behaviour of antifungal drugs is crucial in order to select the most appropriate drug at the correct dose. Those patients at risk of IFD belong to special populations, including neonates, paediatric patients, those in the ICU, pregnant women, obese patients and frail elderly patients who show substantial pathology-mediated PK variations.1 Quite often, specific information on the differences between these patient populations is lacking and extrapolation of knowledge from other subjects is required. The clinical application of knowledge of the PK behaviour of a given drug must be undertaken with caution and by someone with extensive knowledge of the field, as mistakes may lead to suboptimal or even toxic therapy. In addition to deciding on the appropriate selection of a drug, the clinician is often confronted with pathophysiological states, such as renal dysfunction, the need for extracorporeal elimination techniques, as well as drug–drug interactions that require immediate attention.

Changes in renal function have a significant impact on drugs that are cleared renally, such as fluconazole and flucytosine (5-fluorocytosine), but there are also a variety of other factors that can contribute to significant intra- and inter-patient variability in response to antifungal agents, especially drug–drug interactions. Managing potential drug interactions can pose a particular challenge to both clinicians and pharmacists, especially when several interacting agents are being administered.2,3

Theoretical data are not always sufficient to predict PK interactions, as unexpected drug interactions with antifungal drugs may occur, which adds to the complexity. It is also worth remembering that a lack of data to support an interaction does not necessarily mean the absence of the interaction. Ideally, the healthcare professional should have access to a comprehensive, up-to-date overview of drug interactions with antifungal drugs. The clinical
pharmacist should have a full understanding of the underlying mechanisms and scientific evidence for different antifungal agents, as well as extensive knowledge of drug PKs to be able to offer tailor-made advice on how to manage drug–drug interactions and select the most suitable antifungal for a given clinical condition and IFD.

It is essential that therapeutic drug monitoring (TDM) is performed for antifungal drugs since they have a narrow therapeutic range and large inter-individual variation in PK and pharmacodynamics, and can cause severe adverse effects. In the clinical setting, there is evidence to support the observation that plasma concentrations above a certain concentration may be predictive of efficacy for voriconazole, posaconazole, itraconazole and fluconazole.6,7 Though this has yet to be confirmed in prospective studies. Nevertheless, the importance of TDM of these antifungal drugs is widely accepted and TDM is recommended in guidelines for the treatment of IFD [presented at the European Conference on Infections in Leukaemia (ECIL), 2015; http://kobe.fr/ecil]. The clinical pharmacist clearly has a key role in this complex process by interpreting the results, advising on dose adaptation when required and coordinating the whole cycle of events.5

**Microbiologist**

Much of the inappropriate use of antifungal agents arises from the inability to diagnose IFD reliably. In addition to a combination of host factors, and clinical signs and symptoms to define the likelihood of the presence of IFD, several mycological tests can be used to increase the certainty of IFD being diagnosed.7,8 Understanding the accessibility, performance and interpretation of the available mycological tests is the specific expertise of the microbiologist (or medical mycologist).

Modern techniques for diagnosing fungal infection include biomarker testing to provide evidence of a fungal infection, and molecular tools such as PCR to detect the fungus itself. Biomarker tests available for the diagnosis of IFD include detection of Aspergillus galactomannan (GM) by ELISA, glycoprotein antigen detected by the immunochromatographic lateral flow device, cryptococcal antigen, Candida antigens (mannan, germ tube antigen), pan-fungal markers [1-3-β-D-glucan (BDG)] and either a fungal species-specific or pan-fungal PCR. In addition, modern developments in mass spectrometry, proteomics and breath tests are leading to the introduction of newer diagnostic techniques, although few are in mainstream use yet. A decision needs to be made between employing these tests in a screening strategy to rule out IFD (allowing a move away from empirical therapy) or as diagnostic tests to rule in disease (targeted therapy).9 Both strategies can be used within the same patient population depending on the underlying risk of IFD, prevalence and pre-test probability of disease, and use of antifungal prophylaxis.

For the diagnosis of aspergillosis, the GM test and standardized PCR methodologies show high sensitivity with a high negative predictive value, and can be used to screen at-risk patients to exclude invasive disease.10,11 However, due to limited specificity and the relative rarity of IFD, the positive predictive value of individual biomarkers is rarely sufficient to diagnose disease.11–13 Recent studies have shown the utility of combinations of biomarkers and their ability to detect infection early, before radiological evidence of disease is present, and to improve patient outcomes.14–16 All show a reduction in empirical antifungal usage and improved targeting of drugs to patients who need them. Biomarkers can also be used to monitor response to therapy and can inform decisions on when to stop treatment when the test is negative.

Microbiological culture is necessary to establish proven IFD and can provide susceptibility profiles for optimizing therapy. The microbiologist can guide appropriate sampling (e.g. large-volume blood cultures for candidaemia) and can interpret the significance of results (e.g. identifying Candida species in lower respiratory samples as contaminants). PCR can be designed to identify to a species level providing diagnostic information, but also informs on epidemiological trends. Several PCR-based methods for the detection of fungal pathogens, including mutations conferring resistance to specific antifungals (e.g. echinocandin resistance in Candida species and azole resistance in Aspergillus fumigatus), are also currently available.

The role of the microbiologist is to direct therapy more accurately by identifying patients who are unlikely to have IFD (no antifungal therapy needed), to ensure early identification of IFD before clinical symptoms develop and to diagnose those patients with IFD to enable therapy targeted to the causative fungal pathogen.

**Paediatric infectious diseases specialist**

There are differences in the underlying conditions between adults and children that predispose the latter to IFD. However, paediatric patients are also unique in terms of the epidemiology of IFD, the usefulness of non-culture-based microbiological tests and the pharmacology and dosing of antifungal agents. High-risk paediatric populations include very low birthweight infants admitted to neonatal ICUs, children with primary immunodeficiencies, infants and children with malignancies and those receiving haematopoietic stem cell transplants. IFD of very low birth weight neonates is predominantly due to invasive candidiasis, which is disseminated to the CNS in up to 23%.17 Candida albicans and Candida parapsilosis are the most common species encountered. This differs from adults and needs to be taken into account when treatment is started before cultures become positive.18 The epidemiology of invasive aspergillosis in the paediatric population is remarkable for the higher incidence of cutaneous aspergillosis19 as well as the observation that Aspergillus nidulans is the second most common Aspergillus species causing IFD in children with chronic granulomatous disease.20 The performance of several diagnostic procedures used to diagnose IFD is also different from that found for adults. Typical abnormalities on a chest CT of adults with pulmonary invasive aspergillosis are less common in children.21,22 The BDG test is not validated in children and the cut-off has yet to be determined.24

There is also a lack of Phase III clinical trials involving paediatric patients to assess the efficacy of antifungal agents. Consequently, recommendations on the treatment of IFD in neonates and children (specific antifungal agent and dosing) must often rely on evidence from efficacy trials in adults, which needs to be combined with paediatric PK and safety data, and, ideally, supportive efficacy data from published case reports and series.

Another specific issue for the paediatric population is the consideration of regulatory approval for the use of a certain
The multidisciplinary team

The first step in the development and implementation of AFS is to build a multidisciplinary team encompassing the necessary expertise in the management of IFD. The individual team members will be proficient in their clinical speciality, hold a specific interest and have expertise in complementary aspects of the management of IFD. They should also be seen as local authorities and opinion leaders (Figure 1).

It is important that the key clinical specialities are fully represented in the AFS team as unsolicited guidance is more palatable when it is provided by peers and clinical team members rather than by external individuals such as auditors or regulators. The credibility of the team depends on the knowledge and experience of its members and the roles they have within their organization. In addition, to be effective, members of the AFS team need to possess good communication and networking skills, be able to collaborate and show a willingness to share responsibilities. The exact composition of the multidisciplinary team will depend on local circumstances but as a minimum should consist of a hospital pharmacist, a microbiologist and a clinician, each of whom should be actively engaged in the management of IFD.

The role of ID specialists in AFS programmes varies between countries and healthcare systems. Nonetheless, ID specialists are often integral members of AFS teams as ID is a broad speciality of internal medicine with links to all other specialities. The spectrum of patients and specialities will determine the need to involve, on a case-by-case basis, other specialists, such as paediatric ID specialists, haematologists, ICU consultants, respiratory physicians and surgeons, in the multidisciplinary AFS team.

The multidisciplinary team should define the roles and responsibilities of each member. Establishing a programme lead and a governance structure is key to developing a functional and coherent team. Team members should be assigned tasks related to their specific expertise and networks. Their responsibilities would normally include education, developing institutional guidelines, performing audits and their physical clinical presence to provide support. Liaison with senior clinicians representing various clinical teams is vital for the AFS principles to be fully integrated into the clinical workflow.
standard clinical care. Identifying and involving opinion leaders (often called ‘AFS champions’) is strategically very important, as is official approval and the support of, and empowerment by, the hospital managers; this is a prerequisite to the success of any AFS programme.

The democratic leadership model, an inclusive strategy and consensus in decision making can prove extremely useful to AFS programmes. This approach is commonly used in clinical multidisciplinary team meetings so clinicians are used to taking advice from their peers in this setting. Obviously, this is more likely to work in settings that are familiar with democratic leadership models so different approaches may prove more useful in other settings. For example, in smaller centres with few high-risk patients and low antifungal consumption, adopting a standard antimicrobial stewardship approach may be sufficient. There may also be settings where restrictions, monitoring and penalties work best. However, true stewardship cannot thrive on fear or restrictions; it must focus on a genuine commitment to ‘doing the right thing’ for the benefit of patients. The role of the multidisciplinary team is to support the decision-making process by bringing together the available knowledge and expertise for the management of IFD. AFS is clearly needed given that the high level of antifungal drug prescribing and consumption in many centres is not commensurate with the number of patients suffering from IFD, and also because of the increasing prevalence of antifungal resistance.

Conclusions

Antifungal stewardship requires the integration of patient risk factors and interpretation of conventional tests, biomarkers, molecular diagnostics, and imaging, followed by optimal choice of antifungal therapy. The complexity of the patient population at

Figure 1. The multidisciplinary team.
risk of developing IFD necessitates a multidisciplinary team to develop and implement AFS programmes within hospitals. The members of the multidisciplinary team complement each other with respect to specific expertise in the management of IFD.

Transparency declarations

S. A. has received sponsorship for meetings, unrestricted educational grants, research grants and consultancy work from Astellas, Basilea, Gilead, MSD and Pfizer. R. Barnes has served on advisory boards, received sponsorship and travel expenses to attend meetings, and received honoraria for lectures/symposia from Merck, Sharp and Dohme, Astellas, Gilead Sciences and Pfizer. In addition, she has received educational grants, scientific fellowship awards and independent researcher grants from Gilead Sciences and Pfizer. She is a steering board member of the European Aspergillus PCR Initiative of the International Society for Human and Animal Mycology. R. Bruggemann has served as a consultant to, and has received unrestricted and research grants from, Astellas Pharma Inc., Gilead Sciences, Merck Sharpe and Dohme Corp., and Pfizer Inc. All payments were invoiced by the Radboud University Medical Center, The Netherlands. R. R.-R. has given lectures/had consultancy contracts for Gilead, Astellas, MSD and Pfizer. A. W. has received consultancy fees from Basilea and Gilead; educational grants from Pfizer and Gilead; and is supported by the Wellcome Trust Strategic Award for Medical Mycology and Fungal Immunology 097377.

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VORICONAZOLE VERSUS AMPHOTERICIN B FOR PRIMARY THERAPY OF INVASIVE ASPERGILLOSIDIS


ABSTRACT

Background Voriconazole is a broad-spectrum triazole that is active against aspergillus species. We conducted a randomized trial to compare voriconazole with amphotericin B for primary therapy of invasive aspergilliosis.

Methods In this randomized, unblinded trial, patients received either intravenous voriconazole (two doses of 6 mg per kilogram of body weight on day 1, then 4 mg per kilogram twice daily for at least seven days) followed by 200 mg orally twice daily or intravenous amphotericin B deoxycholate (1 to 1.5 mg per kilogram per day). Other licensed antifungal treatments were allowed if the initial therapy failed or if the patient had an intolerance to the first drug used. A complete or partial response was considered to be a successful outcome.

Results A total of 144 patients in the voriconazole group and 133 patients in the amphotericin B group with definite or probable aspergillosis received at least one dose of treatment. In most of the patients, the underlying condition was allogeneic hematopoietic-cell transplantation, acute leukemia, or other hemato logic diseases. At week 12, there were successful outcomes in 52.8 percent of the patients in the voriconazole group (complete responses in 20.8 percent and partial responses in 31.9 percent) and 31.6 percent of those in the amphotericin B group (complete responses in 16.5 percent and partial responses in 15.0 percent; absolute difference, 21.2 percentage points; 95 percent confidence interval, 10.4 to 32.9). The survival rate at 12 weeks was 70.8 percent in the voriconazole group and 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95 percent confidence interval, 0.40 to 0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events, but transient visual disturbances were common with voriconazole (occurring in 44.8 percent of patients).

Conclusions In patients with invasive aspergillosis, initial therapy with voriconazole led to better responses and improved survival and resulted in fewer severe side effects than the standard approach of initial therapy with amphotericin B. (N Engl J Med 2002;347: 408-15.)

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INVASIVE aspergillosis is a major infectious complication in patients with prolonged neutropenia and in transplant recipients. Its incidence ranges from 5 percent to more than 20 percent in high-risk groups.1 For decades, amphotericin B deoxycholate has been the standard therapy for invasive aspergilliosis, although responses are suboptimal (less than 40 percent) in severely immunosuppressed patients.1, 2 Amphotericin B is associated with multiple side effects, which may be ameliorated with the use of lipid formulations.1, 3

Voriconazole is a new broad-spectrum triazole that is active in vitro against various yeasts and molds, including aspergillus species.2 A noncomparative study demonstrated a response rate of 48 percent among patients with acute invasive aspergillosis.8 We undertook an open, randomized trial comparing the efficacy, safety, and tolerability of voriconazole with those of amphotericin B for the primary therapy of acute invasive aspergillosis in immunocompromised patients; both types of therapy were followed by other licensed antifungal therapy when toxic effects or insufficient response dictated.

METHODS

Conduct of the Study

Two identical protocols (protocol 150-307 in Europe, Israel, and Australia and protocol 150-602 in the United States, Canada, and the Netherlands) were compared for patients with invasive aspergillosis. Patients received either intravenous voriconazole (two doses of 6 mg per kilogram of body weight on day 1, then 4 mg per kilogram twice daily for at least seven days) followed by 200 mg orally twice daily or intravenous amphotericin B deoxycholate (1 to 1.5 mg per kilogram per day). Other licensed antifungal treatments were allowed if the initial therapy failed or if the patient had an intolerance to the first drug used. A complete or partial response was considered to be a successful outcome.

Results A total of 144 patients in the voriconazole group and 133 patients in the amphotericin B group with definite or probable aspergillosis received at least one dose of treatment. In most of the patients, the underlying condition was allogeneic hematopoietic-cell transplantation, acute leukemia, or other hematologic diseases. At week 12, there were successful outcomes in 52.8 percent of the patients in the voriconazole group (complete responses in 20.8 percent and partial responses in 31.9 percent) and 31.6 percent of those in the amphotericin B group (complete responses in 16.5 percent and partial responses in 15.0 percent; absolute difference, 21.2 percentage points; 95 percent confidence interval, 10.4 to 32.9). The survival rate at 12 weeks was 70.8 percent in the voriconazole group and 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95 percent confidence interval, 0.40 to 0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events, but transient visual disturbances were common with voriconazole (occurring in 44.8 percent of patients).

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received or were receiving interacting drugs (e.g., rifampin), were
therapy for more than 96 hours with more than 0.5 mg of
confirmed by bronchoscopy and a positive finding on histopatho-
lesions (other than lung transplantation); or tracheobronchial lesions
or culture in a patient with another immunocompromising condi-
previously allogeneic hematopoietic-cell transplantation or who had a
in the nose or paranasal sinus in a patient who had under-
positive histopathological examination or culture of aspergillus from
opacification of a sinus on CT or magnetic resonance imaging, and
culture that was positive for aspergillus; clinical evidence of sinusitis,
study-drug assignment and to adverse events and laboratory abnormalities
whose presence would suggest the use of a particular study drug,
assessed the certainty of the diagnosis at study entry and the re-
treatment on the basis of predefined criteria. The com-
respond to treatment on the basis of predefined criteria. The com-
mittee assessed the global response at week 12 and at the end of the
initial period of randomized therapy.
Digitized radiologic images were reviewed by the radiologists
on the data-review committee. Lesions were evaluated visually for
changes with the use of computerized planimetry for assistance in
estimating the percentage change. Complete responses were de-
by the resolution of all clinical signs and symptoms and more
than 90 percent of the lesions due to invasive aspergillosis that
were visible on radiology. Partial responses were defined by clinical
improvement and greater than 50 percent improvement in find-
ings on radiology. Stable responses were defined by the absence
of change from base line or an improvement of less than 50 per-
cent. Failure of therapy was defined by worsening disease. Com-
plete and partial responses were classified as successful outcomes.
Stable and indeterminate responses and failures of therapy were
regarded as unsuccessful outcomes.

Statistical Analysis
Before the two studies began, we planned to combine the results of
both in a predefined analysis. The intention-to-treat population
consisted of all patients who underwent randomization. The mod-
ified intention-to-treat population consisted of those who received at least one dose of the medication they were initially assigned to
receive and who had a base-line diagnosis of definite or probable
invasive aspergillosis as confirmed by the data-review committee.
The population included in the safety analysis consisted of all pa-
tients who received their initial study medication.

The primary objective of the studies was to demonstrate the
noninferiority of voriconazole as compared with amphotericin B
at week 12 in the modified intention-to-treat population. We esti-
mated that the rate of successful outcomes with amphotericin B
at week 12 would be 50 percent. Voriconazole would be consid-
ered not to be inferior to amphotericin B if the lower limit of the

One secondary objective was the demonstration of the super-

A sample size of 276 was required to assess the primary end

Between July 1997 and October 2000, a total of

A total of 197 patients were assigned to the voricon-

A total of 102 patients (50 in the voriconazole group and 52 in the amphotericin B group) were excluded from the modified intention-to-treat population because they did not have a confirmed diagnosis of invasive aspergillosis at base line. The most common reason for the lack of confirmation was the inability of the data-review committee to confirm the presence of a halo or air-crescent sign at base line in patients with no supporting mycologic or pathologic evidence (35 in the voriconazole group and 25 in the amphotericin B group). Other reasons included inadequate mycologic evidence (in 10 patients in the voriconazole group and 15 in the amphotericin B group), no radiologic evidence of pulmonary or sinus infection (in 1 patient in the voriconazole group and 4 in the amphotericin B group), and absence of documentation of neutropenia or immunocompromised condition before base line (in 4 patients in the voriconazole group and 8 in the amphotericin B group).

The demographic characteristics and underlying conditions of the patients in the modified intention-to-treat population are summarized in Table 1. The two groups were well matched, and there was no significant difference in these characteristics between the intention-to-treat population and the modified intention-to-treat population. Patients enrolled according to the 150-602 protocol were more likely than those enrolled according to the 150-307 protocol to have undergone allogeneic hematopoietic-cell transplantation (41 of 107 [38.3 percent] vs. 20 of 170 [15.3 percent], P<0.001), to have graft-versus-host disease (31 of 107 [29.0 percent] vs. 16 of 170 [9.4 percent], P<0.001), and to have received a definite diagnosis of invasive aspergillosis (51 of 107 [47.7 percent] vs. 57 of 170 [33.5 percent], P=0.02), and they were less likely to have neutropenia (28 of 107 [26.2 percent] vs. 95 of 170 [55.9 percent], P<0.001).

### Base-Line Characteristics of the Infection

Characteristics of the patients in terms of the site of the infection, the level of certainty of the diagnosis, and the evidence supporting the diagnosis are summarized in Table 2. The only significant difference between groups was that the voriconazole group had a higher proportion of definite cases of invasive aspergillosis (P=0.01). In the 110 infections in which the species was identified at base line, the species was

### RESULTS

**Enrollment and Base-Line Characteristics of the Patients**

Between July 1997 and October 2000, a total of 391 patients recruited by 95 centers in 19 countries underwent randomization in the studies: 252 patients were recruited in the 150-307 protocol and 139 in the 150-602 protocol.

A total of 197 patients were assigned to the voriconazole group, and 194 patients were assigned to the amphotericin B group; these patients comprised the intention-to-treat population. Twelve patients (three in the voriconazole group and nine in the amphotericin B group) did not receive any treatment and were excluded from the safety analyses. A total of 102 patients (50 in the voriconazole group and 52 in the amphotericin B group) were excluded from the modified intention-to-treat population because they did not have a confirmed diagnosis of invasive aspergillosis at base line. The most common reason for the lack of confirmation was the inability of the data-review committee to confirm the presence of a halo or air-crescent sign at base line in patients with no supporting mycologic or pathologic evidence (35 in the voriconazole group and 25 in the amphotericin B group). Other reasons included inadequate mycologic evidence (in 10 patients in the voriconazole group and 15 in the amphotericin B group), no radiologic evidence of pulmonary or sinus infection (in 1 patient in the voriconazole group and 4 in the amphotericin B group), and absence of documentation of neutropenia or immunocompromised condition before base line (in 4 patients in the voriconazole group and 8 in the amphotericin B group).

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| TABLE 1. CHARACTERISTICS OF THE PATIENTS IN THE MODIFIED INTENTION-TO-TREAT POPULATION. |
|-----------------------------------------------|-----------------|-----------------|
| CHARACTERISTIC                               | VORICONAZOLE    | AMPHOTERICIN B  |
|                                              | GROUP (N=144)   | GROUP (N=133)   |
| Age — yr                                     | 48.5            | 50.5            |
| Mean                                         | 13–79           | 12–75           |
| Sex — no. (%)                                | 98 (68.1)       | 89 (66.9)       |
| Male                                         | 46 (33.1)       | 44 (33.1)       |
| Female                                       | 70.4            | 71.0            |
| Weight — kg                                  | 39–123          | 28–118          |
| Underlying condition — no. (%)               | 37 (25.7)       | 30 (22.6)       |
| Allogeneic hematopoietic-cell transplantation | 6 (4.2)         | 6 (4.5)         |
| Autologous hematopoietic-cell transplantation |                     |                 |
| Acute leukemia                               | 58 (40.3)       | 60 (45.1)       |
| Other hematologic cancer                     | 17 (11.8)       | 18 (13.5)       |
| Solid-organ transplantation                  | 9 (6.2)         | 5 (3.8)         |
| Neutropenia — no. (%)                        | 65 (45.1)       | 60 (45.1)       |
| Yes                                          | 79 (54.9)       | 73 (54.9)       |

*Neutropenia was defined by a neutrophil count of less than 500 per cubic millimeter at base line or during the previous two weeks.
Aspergillus fumigatus (in 85 patients), A. niger (in 9 patients), A. flavus (in 7 patients), A. terreus (in 6 patients), A. glaucus (in 1 patient), A. nidulans (in 1 patient), and A. sydowii (in 1 patient).

Course of Therapy in the Modified Intention-to-Treat Population

The median duration of voriconazole treatment was 77 days (range, 2 to 84), of which intravenous therapy accounted for a median of 10 days (range, 2 to 78). The mean daily doses were 7.87 mg per kilogram (range, 4.48 to 10.87) during the intravenous phase and 416 mg (range, 200 to 750) during the oral phase. Other licensed antifungal therapy was given to 52 patients in the voriconazole group. The first other licensed antifungal therapy was amphotericin B deoxycholate in 20 patients, a lipid formulation of amphotericin B in 14, itraconazole in 17, and a combination in 1.

The median duration of amphotericin B treatment was 10 days (range, 1 to 84), and the mean daily dose was 0.97 mg per kilogram (range, 0.27 to 1.50). During the first 14 days of therapy, administration of amphotericin B was suspended for more than 1 day in 13 patients. Other licensed antifungal therapy was given to 107 patients in the amphotericin B group. The first other licensed antifungal therapy was a lipid formulation of amphotericin B in 47 patients, itracona-
cent of those in the amphotericin B group (absolute difference, 21.9 percent; 95 percent confidence interval, 12.4 to 31.2).

At the end of the initial period of randomized therapy, 53.5 percent of the patients in the modified intention-to-treat population who were receiving voriconazole had a satisfactory response, as compared with 21.8 percent of the patients treated with amphotericin B (absolute difference, 31.7 percent; 95 percent confidence interval, 21.1 to 42.6). There were similar results in the intention-to-treat population.

**Survival**

At week 12, the survival rate was 70.8 percent in the patients in the modified intention-to-treat population who were treated with voriconazole, as compared with 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95 percent confidence interval,
0.40 to 0.88) (Fig. 2). Similar results were observed in the intention-to-treat population.

Safety

Significantly fewer adverse events that were regarded by the investigators as potentially related to treatment were observed during voriconazole therapy (343 events) than during amphotericin B therapy (421 events, P=0.02), even though the median duration of therapy was much longer in the voriconazole group. Visual disturbances were more common in patients receiving voriconazole, occurring in 87 patients (44.8 percent), as compared with 8 patients in the amphotericin B group (4.3 percent, P<0.001). The most frequent descriptions of such disturbances were blurred vision, altered visual perception, altered color perception, and photophobia. All visual events were transient and resolved without intervention. Thirteen patients receiving voriconazole had hallucinations or confusion that was considered to be possibly related to the study drug, as compared with five patients in the amphotericin B group (P=0.09). There was no evidence of a relation between the episodes of hallucination or confusion and visual disturbance (P=0.20). Chills, fever, or both that were potentially related to the study drugs were recorded in six patients receiving voriconazole (3.1 percent), as compared with 46 patients receiving amphotericin B therapy (24.9 percent, P<0.001). Skin reactions (rash, pruritus, or photosensitivity) were observed in 16 patients in the voriconazole group (8.2 percent) and in 6 in the amphotericin B group (3.2 percent, P=0.05).

Fewer severe adverse events that were potentially related to the study drug occurred in the voriconazole group (26 patients [13.4 percent]) than in the amphotericin B group (45 patients [24.3 percent], P=0.008) (Table 4). The most frequent events were renal impairment (in 19 patients) in the amphotericin B group and liver-function abnormalities (in 7 patients) in the voriconazole group.

DISCUSSION

We conducted a large randomized, comparative study of the efficacy of two different drugs in the primary treatment of invasive aspergillosis. Previous studies either compared two doses of liposomal amphotericin B or used historical controls. Definitions used in this study were determined by a consensus of international investigators and proved sufficiently clear for a blinded data-review committee to use for confirmation. The largest discrepancy between the diagnoses of investigators and the determinations of the data-review committee resulted not from misinterpretation of the diagnostic criteria but from the lack of confirmation by the radiologists on the data-

Figure 2. Survival Curves for the Modified Intention-to-Treat Population According to Treatment Group.

The P value was calculated by the log-rank test.
review committee of the presence of a halo or air-crescent sign on a CT scan of the lungs in 60 cases. This open study compared two management strategies for invasive aspergillosis, one of which reflects the common clinical practice of treating patients with conventional amphotericin B and then changing drugs as dictated by the occurrence of toxic effects or a lack of response. Patients treated according to this strategy fared worse in terms of efficacy, toxic effects, and survival than those who instead began treatment with voriconazole.

The superiority of voriconazole in our study was not the result of excessive interruptions of therapy or insufficient doses in patients receiving amphotericin B. The duration of treatment is unlikely to be the only factor contributing to the better overall results with voriconazole. Acute invasive aspergillosis is a rapidly progressive infection, and its outcome is determined early in the course of therapy. In the highly immunosuppressed patients enrolled in this study, initial therapy with voriconazole proved superior to initial therapy with conventional amphotericin B. The presence of more definite cases of aspergillosis among patients in the voriconazole group did not bias the results, because the superiority of voriconazole was similar in both definite and probable cases. The difference in the rate of successful outcomes between the 150-602 and 150-307 studies can be explained by the fact that the group involved in the former study included more patients who either had a diagnosis of definite aspergillosis or had undergone allogeneic hematopoietic-cell transplantation.

The efficacy of voriconazole in invasive aspergillosis shown in this trial is consistent with the results of the recently published comparison of voriconazole with liposomal amphotericin B for empirical antifungal therapy in persistently febrile patients with neutropenia. In that study, a secondary analysis found that among the 415 patients who received voriconazole, only 8 (1.9 percent) had breakthrough mycosis (4 of the cases involving aspergillus species), compared with 21 (5.0 percent, 13 of the cases involving aspergillus species) among the 422 patients who received liposomal amphotericin B.

Voriconazole was better tolerated than amphotericin B, with fewer drug-related adverse events, severe adverse events, and discontinuations of therapy due to adverse events. Infusion-related adverse events and nephrotoxic effects are common in patients receiving amphotericin B but were not observed in patients receiving voriconazole.

Although visual adverse events were frequent in

<table>
<thead>
<tr>
<th>TYPE OF EVENT</th>
<th>VORICONAZOLE GROUP (N=194)</th>
<th>AMPHOTERICIN B GROUP (N=185)</th>
<th>P VALUE†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal impairment</td>
<td>2</td>
<td>19</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>0</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>Other metabolic event (hypoglycemia, hypoalbuminemia, worsening of adrenal insufficiency, or metabolic acidosis)</td>
<td>4</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td>Hepatic abnormalities</td>
<td>7</td>
<td>4</td>
<td>0.54</td>
</tr>
<tr>
<td>Systemic event (fever, chills, anaphylaxis, asthenia, or myalgia)</td>
<td>1</td>
<td>7</td>
<td>0.03</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0</td>
<td>4</td>
<td>0.06</td>
</tr>
<tr>
<td>Digestive tract event (nausea, vomiting, dysesthesia, abdominal pain, or pancreatitis)</td>
<td>4</td>
<td>1</td>
<td>0.37</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hematologic event (thrombocytopenia, cosinophilia, or exacerbation of paroxysmal nocturnal hemoglobinuria)</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Neurologic event (progressive encephalopathy, hallucinations, or Guillain–Barré syndrome)</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Visual events</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
patients receiving voriconazole, they were transient and generally mild or moderate, and they seldom resulted in discontinuation of treatment. Hallucination and episodes of confusion were more frequent with voriconazole than with amphotericin B, although it is unclear whether the antifungal drug was the cause of such episodes in these critically ill patients. This study shows the superiority of voriconazole over amphotericin B as initial therapy for invasive aspergillosis, in terms of response rate, survival rate, and safety.

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APPENDIX

In addition to the authors, members of the study who recruited patients were as follows: R. Allen (Sacramento, Calif.), M. Aoun (Brussels, Belgium), C. Aul (Düsseldorf, Germany), M. Bjorkholm (Stockholm, Sweden), K.L. Blanchard (Shreveport, La.), M. Boogaerts (Leuven, Belgium), E. Bouza (Madrid), E.J. Bow (Winnipeg, Man.), H.R. Brodt (Frankfurt, Germany), J. Brown (Stanford, Calif.), D. Buchheidt (Mannheim, Germany), J.Y. Cahn (Besançon, France), A. Calmaggia (La Plata, Argentina), J.M. Cisneros (Seville, Spain), C. Cordonnier ( Créteil, France), J. Daly (Worcester, Mass.), C.A. Da Cunha (Curitiba, Brazil), R. De Rock (Antwerp, Belgium), A. Del Favero (Perugia, Italy), J. Diaz Mediavilla (Madrid), M.C. Dignani (Buenos Aires, Argentina), C. Doyen (Yvoir, Belgium), J.S. Dum- mer (Nashville), B. Dupont (Paris), M. Eyges (Kaposvar, Hungary), D. Engelhard (Jerusalem, Israel), G. Fätkenheuer (Cologne, Germany), R. Feld (Toronto), D. Füre ( Lyons, France), G. Furtin (Pescara, Italy), P. Garber (Ottawa, Ont.), Z. Gaztony (Göteborg, Hungary), K. Godder (Columbia, S.C.), D. Graham (Springfield, Ill.), A. Gratzwohl (Basel, Switzerland), R. Greenberg (Lexington, Ky.), K. High (Winston-Salem, N.C.), F. Jacobs (Brussels, Belgium), V. Kremery (Bratislava, Slovakia), P. Kolmar (Washington, D.C.), W. Langer (Essen, Germany), M. Laeversc (Montreal), P. Ljungman (Huddinge, Sweden), H. Lode (Berlin, Germany), A. Louie (Stanford, Calif.), D. Maki (Madison, Wis.), J.P. Marie (Paris), D.J.E. Marriott (Sydney, Australia), D.S. McKinsey (Kansas City, Mo.), R. Merkler (Newark, Del.), S. Richardson (Toronto), L. Rickman (San Diego, Calif.), M. Ruhnke (Berlin, Germany), I. Salit (Toronto), W.M. Scheld (Charlottesville, Va.), S. Schneider (Dresden, Germany), M. Schuster (Philadelphia), R. Schwenderfecker (Wiesbaden, Germany), S.D. Shafran (Edmonton, Alta.), B. Simmons (Memphis, Tenn.), M. Slavin (Parkville, Australia), M. Sokol-Anderson (St. Louis), P. Tebas (St. Louis), C. Tsoukas (Montreal), A. Ullmann (Mainz, Germany), J. Van Burik (Minneapolis, MN), J.W. Van't Wout (Leiden, the Netherlands), E.C. Vinaya Kumar (Hyderabad, India), P. Volkov-Fernandez (Mexico City, Mexico), C. Wallrauch (Munich, Germany), H. Wandt (Nuremberg, Germany), EORTC Data Center (Brussels, Belgium): A. Marins, C. Coen, R. Sylvester; Data-Review Committee: J.E. Bennett, D.W. Denning, C. Durand, R.E. Greene, R. Herbrecht, O. Lortholary, J.W. Oestmann, T.F. Patterson, P. Ribaud, R.H. Rubin, P. Stark, J.R. Wingard. 


REFERENCES


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Mistaken Identity: Neosartorya pseudofischeri and Its Anamorph Masquerading as Aspergillus fumigatus

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Invasive fungal infections caused by Neosartorya pseudofischeri S. W. Peterson [anamorph Aspergillus thermomutatus (Paden) S. W. Peterson] are extremely rare. Phenotypically, the anamorphic state of N. pseudofischeri resembles Aspergillus fumigatus, the predominant agent of invasive aspergillosis in immunocompromised hosts. We report the recovery of three clinical isolates of N. pseudofischeri, all initially misidentified by morphological characteristics as A. fumigatus. All three isolates were correctly identified by sequencing portions of the β-tubulin and the rodlet A genes. Only one of the three isolates produced the confirmatory fruiting bodies and was thus classified as N. pseudofischeri; the other isolates did not produce asci and were therefore identified as A. thermomutatus. All three isolates had higher MICs to voriconazole in vitro compared to A. fumigatus AF293. This report emphasizes that phenotypic identification of filamentous fungi may not identify morphologically similar, but genetically distinct, members of the genus Aspergillus section Fumigati. Accurate identification of these organisms may be clinically meaningful, given their potential differences in antifungal susceptibilities.

Aspergillus fumigatus, the principal etiological agent of invasive aspergillosis, belongs to the genus Aspergillus section Fumigati and is identified in the laboratory predominantly by morphological features. Neosartorya fischeri and Neosartorya pseudofischeri also belong to section Fumigati, and their asexual (conidial) state closely overlaps that of A. fumigatus; thus, these two fungi appear morphologically very similar to A. fumigatus. Despite phenotypic similarity with A. fumigatus, N. fischeri and N. pseudofischeri have seldom been reported as etiologic agents of human aspergillosis. Phenotypically, Neosartorya fischeri (Wehmer, Malloch and Cain 1972) can be differentiated from the closely related N. pseudofischeri only by electron microscope analysis of the ascospore structure.

To date there are only seven reported cases in which N. pseudofischeri has been recovered from invasive fungal infections (Table 1). The anamorphic (asexual) form of N. pseudofischeri, Aspergillus thermomutatus, grows as whitish, fast-growing, slowly sporulating colonies (producing conidiophores with conidia [sporulating] only after prolonged incubation on laboratory medium). Recently, several slowly sporulating Aspergillus isolates have been identified as members of a new species, Aspergillus lentulus (sp. nov. S. A. Balajee and K. A. Marr [1]). As part of a screening study to identify other A. lentulus isolates among culture collections in the United States, we recovered three poorly sporulating isolates that were phenotypically identified as A. fumigatus but were not A. lentulus by sequence typing of β-tubulin (βenA) and rodlet A (rodA) gene regions. Data presented within demonstrate that these isolates are N. pseudofischeri and its anamorph, A. thermomutatus.

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MATERIALS AND METHODS

Isolates. Isolate FH274 was received from the Fungus Reference and Molecular Subtyping Unit at the Centers for Disease Control and Prevention (CDC) and was originally recovered from a patient 3 months after receipt of hemopoietic stem cell transplantation. Biopsy of a right ear wound showed branching septate hyphae by KOH stain and grew what was first identified by the local laboratory as Aspergillus versicolor. The patient was treated successfully with amphotericin B lipid complex (ABLC), followed by voriconazole (VRZ) and caspofungin (CAS); the patient died 1 month later due to progressive leukemia. This isolate was sent to the CDC, where the fungus was reidentified by morphology as A. fumigatus. At that time, no asci or ascopores were found after incubation on several media for 4 weeks. Isolates FH240 and FH242 were originally obtained from the sputum of cystic fibrosis patients in Montana and Texas, respectively. Both isolates were initially identified as A. fumigatus by referring institutions submitting the isolates to the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio (UT), for antifungal susceptibility testing. Reidentification was not performed by UT. Aspergillus fumigatus isolate AF293 was provided by David Denning and was confirmed to be A. fumigatus Fresenius by the National Collection of Pathogenic Fungi (NCPF 7367) at the Mycology Reference Laboratory, Bristol, United Kingdom, and by the Centraal Bureau voor Schimmelcultures (CBS 101553), Baarn, The Netherlands (11).

Media and antifungals. Potato dextrose agar (PDA; Becton Dickinson, Sparks, MD), CZapek-dox (CZD; Becton Dickinson, Sparks, MD) supplemented with 20% dextrose, malt extract agar (MEA; Becton Dickinson, Sparks, MD), and Sabouraud dextrose agar (SDA; Becton Dickinson, Sparks, MD) were used in the study. The antifungal agents amphotericin B (AMB; Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT), itraconazole (ITZ; Ortho Biotech, Bridgewater, NJ), and VRZ (Pfizer Pharmaceuticals, New York, NY) were dissolved in dimethyl sulfoxide, and CAS (Merck & Co. Inc., Rahway, NJ) was dissolved in distilled water. Further dilutions were made in RPMI 1640 with 1-glutamine, without bicarbonate, buffered with 0.165 morpholinepropanesulfonic acid to pH 7.0 (RPMI; Sigma Chemical Co., St. Louis, MO), as outlined in CLSI (formerly NCCLS) document M38A (9).

Molecular typing. Genomic DNA was extracted from hyphal mats of isolates AF293, FH240, FH242, and FH274 grown in SDB for 3 days as previously described (1). In brief, hyphal cells were treated with lyticase (10 U/µl; Sigma Chemical Co., St. Louis, MO) for 1 h at 37°C and then incubated in proteinase K (10 µg/ml; Sigma) and 0.5% sodium dodecyl sulfate (Sigma) for 2 h at 60°C. This suspension was then subjected to three cycles of freeze-thaw in liquid nitrogen alternating with vortexing 1 min with 0.2 g sterile glass beads (Sigma).
Genomic DNA was isolated with the DNeasy tissue kit (69504; QIAGEN, Hilden, Germany) according to the manufacturer's instructions. PCR primers were designed to amplify β-tubulin (benA-F, 5'-AATGTTGTTGCGCTTCTGG-3' and R, 5'-AGTTGTCGCGGACGGAAATAG-3') and rodA (rodA-F, 5'-GCTGCAATGGTGTTGGCAA-3' and R, 5'-AGGGCAATGCAAGGAAGACC-3') regions as previously described (1). PCR amplification was performed with 2 to 4 μl of genomic DNA as template in a total reaction volume of 50 μl consisting of PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 0.2 mM (each) dATP, dGTP, dCTP, and dTTP, 1.2 mM MgSO4, 0.2 pmol (each) primer, and 1 U of Pfu polymerase (Invitrogen-BRL Life Technologies, Carlsbad, CA) and 1× Pfu enhancer (Invitrogen). Thirty cycles of amplification were performed in a GeneAmp PCR system 9700 thermocycler (PE-Applied Biosystems) after initial denaturation of DNA at 94°C for 3 min. Thirty cycles consisted of a denaturation step at 94°C for 15 seconds, an annealing step at 55°C for 30 seconds, and an extension step at 68°C for 30 seconds, with a final extension at 68°C for 3 min following the last cycle. 

Amplicons were purified using the QiAquick PCR purification kit (catalog no. 28104) and directly sequenced on a Perkin-Elmer/ABI model 373 DNA sequencer with protocols supplied by the manufacturer. The resultant nucleotide sequences were edited using the Sequencher program, and each set of homologous gene sequences was aligned using ClustalW (17). Sequences were compared to the other available sequences in GenBank using the BLAST program of the National Center for Biotechnology Information.

Phenotypic analysis. FH274, FH240, and FH242 were grown on PDA, MEA, and CZD at two temperatures, 25°C and 37°C, for 7 to 21 days with periodic microscopic examination. When asci were produced, specimens prepared with lactophenol cotton blue were examined by differential interference contrast microscopy (magnification, 1000×) and 1000× photographs were collected.

Antifungal susceptibility testing. Susceptibilities of the isolates to AMB, ITZ, VRZ, and CAS were assayed by the CLSI M38A broth microdilution method, as previously published (1). Isolate FH240 was grown on CZD at 37°C to maximize conidial harvest, and the conidia from all three isolates tested were counted with a hemocytometer and adjusted to a concentration of 10⁶ CFU/ml. As per the CLSI recommendations (9), MICs for ITZ, VRZ, and AMB were defined as the lowest concentration of the drug that resulted in 100% growth reduction when compared to the drug-free control. For CAS, the minimal effective concentration (MEC) was defined as the minimum concentration of drug that produced morphological alterations, such as abnormal hyphal growth with highly branched tips, swollen germ tubes, and distended balloon-like hyphae, when observed under the light microscope (7). Susceptibilities were determined by duplicate measures in three different experiments. MICs for FH240 and FH242 were confirmed at the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio.

RESULTS

A large number of slowly sporulating isolates of *A. fumigatus* from various culture collections in the United States were screened for the presence of *A. lentulus*. As part of this project, we sequenced the *benA* and the *rodA* genes of the slowly sporulating isolates FH274, FH240, and FH242. Results showed that FH274 and FH240 were 100% homologous and FH242 was 99% homologous to the sequence of *N. pseudofischeri* (GenBank accession no. AF057345). Sequences of all three isolates were only 90% homologous to the *benA* sequence and 88% homologous to the *rodA* sequence of *A. fumigatus* isolate AF293 (data not shown), and the sequences were 91% homologous to the *benA* sequence and 89% homologous to the *rodA* sequence of *A. lentulus* isolate FH5 (data not shown).

All three isolates were initially identified as *A. fumigatus* by the CDC and UT by phenotypic characteristics. Since molecular data suggested that these isolates were not *A. fumigatus* but *N. pseudofischeri*, we sought to corroborate the molecular findings by phenotype analyses. All isolates were grown on PDA, CZD, and MEA at 25°C and 37°C. At the higher temperature, the isolates were fast growing on all three media and appeared as whitish velvety colonies with sparse conidiation on CZD and PDA and without conidiation on MEA. Microscopic examination revealed scant conidiophores that were smooth and hyaline, terminating in subglobose vesicles with a single series of ampulliform phialides bearing dull green globose conidia (Fig. 1a). At 25°C, FH240 and FH242 appeared as fast-growing colonies (on all three media) with absolutely no conidiation. However, FH274 appeared granular on PDA, with ascogonial initials produced within 7 days. By the 10th day, ascomata were produced and eight-spored asci were seen microscopically (Fig. 1b). The ascospores were hyaline, one-celled, and lenticular with two closely opposed equatorial crests extending beyond the spore body (Fig. 1c). Hence, isolate FH274 was identified as *N. pseudofischeri*. Both FH240 and FH242 failed to produce the confirmatory asci and ascospores in PDA, CZD, and MEA at both 25°C and 37°C and were classified as *A. thermomutatus*. All three isolates were able to grow profusely at 45°C and exhibited limited growth at 48°C. It is being increasingly appreciated that members of the *Aspergillus* species may have variable antifungal susceptibility patterns, a case in point being *A. terreus*, which has high MICs of AMB in vitro (18) and is associated with high rates of treatment failure (13, 18). Hence, we evaluated the antifungal susceptibilities of the three isolates and found that MICs were variable, although each of the isolates appeared to have relatively high MICs of the azoles, VRZ and ITZ (Table 2). One isolate (FH240) had a relatively high MIC of AMB; all CAS MECs were low. *Aspergillus fumigatus* AF293 was susceptible to all the drugs tested.

DISCUSSION

The present study brings into focus the limitations of phenotypic methods of identification of filamentous fungi, since all three *N. pseudofischeri/A. thermomutatus* isolates were initially misidentified as *A. fumigatus* by morphological typing. Limita-

### TABLE 1. *N. pseudofischeri* as a cause of invasive disease

<table>
<thead>
<tr>
<th>Yr</th>
<th>Disease</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1929</td>
<td>Sputum from patient with lung disease</td>
<td>NK*</td>
<td>NK</td>
<td>10</td>
</tr>
<tr>
<td>1971</td>
<td>Invasive aspergillosis</td>
<td>NK</td>
<td>NK</td>
<td>10</td>
</tr>
<tr>
<td>1990</td>
<td>Mycotic keratitis</td>
<td>KETOCONAZOLE</td>
<td>Evisceration of the eye</td>
<td>2</td>
</tr>
<tr>
<td>1992</td>
<td>AORTIC-related endocarditis</td>
<td>AMPHOTERICIN B</td>
<td>Progression of disease, death</td>
<td>14</td>
</tr>
<tr>
<td>1994</td>
<td>OSTEOMYELITIS</td>
<td>NK</td>
<td>NK</td>
<td>10</td>
</tr>
<tr>
<td>2002</td>
<td>Peritonitis</td>
<td>LIPOSOMAL AMPHOTERICIN, ITRACONAZOLE</td>
<td>Resolution</td>
<td>8</td>
</tr>
<tr>
<td>2004</td>
<td>Invasive aspergillosis</td>
<td>AMPHOTERICIN B</td>
<td>Resolution</td>
<td>4</td>
</tr>
</tbody>
</table>

* NK, not known.
tions of traditional fungal species characterization by phenotype are being increasingly documented, and molecular phylogenetics-based methods appear to be a more reliable and robust alternative to discriminate fungal species (3, 6, 12, 16). For example, in the human pathogenic fungus Histoplasma capsulatum, eight genetically isolated groups have been distinguished by molecular methods with biologically relevant features, such as geographic distribution and pathogenicity characteristic of each group (5). Similarly, cryptic species were revealed in several medically important fungi, including Coccioidioides immitis, Candida albicans, Cryptococcus neoformans, and A. flavus (15), and recently isolates belonging to the morphologically indistinguishable Aspergillus niger aggregate have been divided into two species, A. niger and A. tubingensis, based on molecular differences (12). We recently used multilocus sequence typing to identify a new species in the section Fumigati, A. lentulus (1). Thus, it appears that traditional phenotypic methods alone may be inadequate for fungal speciation, and an integration of molecular speciation methods with available classical techniques appears to be warranted to accurately characterize fungi, especially ascomycetes.

Consistent with the ambiguity of morphological methods of fungal characterization, two of three isolates (identified as N. pseudofischeri by molecular methods) did not produce any ascoma. Although the anamorphic states of N. pseudofischeri and A. fumigatus have overlapping phenotypic characteristics, such as conidiophore morphology and thermotolerance, it is thought that N. pseudofischeri can be clearly differentiated from A. fumigatus by the fact that N. pseudofischeri produces ascoma on MEA or CZD, whereas A. fumigatus does not (10). Some fungal isolates may not produce fruiting bodies in the laboratory, possibly due to repeated subculturing on rich medium. Reliance on such unstable phenotypic characteristics for speciation of clinical isolates may lead to misidentification of the etiological agent.

In contrast to A. fumigatus, all three isolates of N. pseudofischeri/A. thermomutatus recovered in the current study had elevated MICs of VRZ. Low susceptibility of these isolates to VRZ is an important observation and if validated in vivo may have significant implications in clinical practice. All three isolates were extremely susceptible to the echinocandin CAS, moderately susceptible to ITZ, and had variable MICs of AMB. Whether differences in susceptibility are inherent to the species or are acquired traits is not clear but, previously, a clinical isolate of N. pseudofischeri was shown to be very sensitive to AMB, ITZ, ketoconazole, and flucytosine (14).

In the present study, N. pseudofischeri was not isolated as a cause of invasive pulmonary aspergillosis but was recovered from sputum of cystic fibrosis patients and as a cause of invasive otitis. However, N. pseudofischeri clearly is able to cause infection in the

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (µg/ml)</th>
<th>Amphotericin B</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Caspofungin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. pseudofischeri</td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.015</td>
</tr>
<tr>
<td>FH274</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. thermomutatus</td>
<td></td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>FH240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. thermomutatus</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>FH242</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus AI293</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
<td></td>
</tr>
</tbody>
</table>

* For caspofungin, data are MECs rather than MICs.
right environment, as can be seen from its isolation in the past from cases of peritonitis, invasive pulmonary aspergillosis, and graft-related endocarditis (Table 1). Our investigation has also uncovered the possibility that the difficulty in distinguishing A. fumigatus from N. pseudofischeri may underestimate the frequency of disease caused by N. pseudofischeri. Detailed molecular screening and typing studies may be needed to assess the true rates of recovery of N. pseudofischeri as a cause of human disease. Correct identification of N. pseudofischeri may be important if in vitro susceptibility results predict clinical outcomes in in vivo models of infection.

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REFERENCES

Invasive fungal sinusitis should be suspected in immunocompromised or diabetic patients who present with acute sinusitis, inflammation of nasal septal mucosa, unexplained fever or cough, or the orbital apex syndrome. Histopathological studies are required to differentiate among these syndromes. Acute (fulminant) invasive fungal sinusitis has been called mucormycosis, zygomycosis and fulminant invasive sinusitis. Fever, cough, crusting of nasal mucosa, epistaxis, and headache are the most common presenting symptoms. Histopathological studies show hyphal invasion of blood vessels, vasculitis with thrombosis, and tissue infarction. Reports of granulomatous invasive fungal sinusitis come primarily from Sudan, but also from India, Pakistan, and the United States. Patients usually present with proptosis, appear to be immunocompetent and are infected almost exclusively with A. flavus. Chronic invasive fungal sinusitis can be distinguished from the two other forms of invasive fungal sinusitis by its chronic course, dense accumulation of hyphae resembling a mycetoma, and association with the orbital apex syndrome, diabetes mellitus, and corticosteroid treatment. Biopsy and orbital exploration show vascular invasion by fungal elements and only a sparse chronic inflammatory infiltrate.

**Keywords** invasive fungal sinusitis, acute (fulminant) invasive fungal sinusitis, granulomatous invasive fungal sinusitis, chronic invasive fungal sinusitis

**Introduction**

Several years ago, in the course of treating a patient with fungal sinusitis, we reviewed the published literature on the topic. To our surprise, we could find no criteria for diagnosis or consensus on the classification of fungal sinusitis. Many reports of fungal sinusitis of various types were grouped under the heading of *Aspergillus* sinusitis, regardless of invasiveness or fungal agent. Because there was no consensus on classification, we found little clinically useful information on the natural history and treatment of those diseases.

Therefore, our group began a reassessment of the syndromes of fungal sinusitis starting with their classification. Using published reports and case histories from our own clinics, clinical descriptions and diagnostic criteria for allergic fungal sinusitis, sinus mycetoma, and invasive fungal sinusitis were established. We have continued to participate with our colleagues in the treatment and follow up of patients with these syndromes [1–3]. We have learned a lot, but there is still much we do not know.

**Materials and methods**

In preparation for this paper, we reviewed the available medical literature on invasive fungal sinusitis in English using the search engines Medline, PubMed and Ovid.

**Distinguishing invasive from noninvasive fungal sinusitis**

Why some species of fungi cause fungal sinusitis and others do not is unclear, especially since fungi are the predominant airborne allergens in many parts of the United States. Nasal cultures for fungi have no role in the diagnosis of fungal sinusitis as fungi can be cultured from nasal secretions of most healthy
individuals in temperate climates, including those with allergic rhinitis.

At present, diagnosis of a specific form of fungal sinusitis can be convincingly made only when fungal elements are visualized by histopathologic examination of tissue removed from a sinus. Although visible on staining, fungi may be difficult to culture from mucoid material in the noninvasive forms of fungal sinusitis: allergic fungal sinusitis and sinus mycetoma. This may reflect the limited viability of fungal elements in these conditions. Whether or not fungi can exist in sinus mucus without causing disease is unclear. We did not identify fungal elements on silver stains of the surgical material taken from 24 consecutive patients undergoing surgery for chronic bacterial sinusitis [3]. For now, the diagnosis of fungal sinusitis requires histopathological evaluation of sinus tissue and a familiarity with the characteristics of each of the clinical syndromes.

Noninvasive fungal sinusitis

In noninvasive fungal sinusitis, fungal elements are present in mucus material within the sinus but do not penetrate sinus mucosa, submucosa, blood vessels, or bone [4]. A history of chronic sinusitis is usually present, and many patients have nasal polyps. Sinus contents often have the consistency and appearance of peanut butter or cottage cheese and may be foul-smelling. Patients are immunocompetent but often have allergic rhinitis or asthma. In patients with diabetes or in those who are otherwise immunosuppressed, the diagnosis of noninvasive fungal sinusitis is suspect without a biopsy of sinus mucosa and bone.

Invasive fungal sinusitis

Clinical setting. Except for the rare case of granulomatous invasive fungal sinusitis [5], North American patients with invasive fungal sinusitis (IFS) are almost always immunocompromised [4]. Malignancy, chemotherapy for malignancy, organ transplantation, autoimmune disease, malnutrition, or diabetes are common comorbid conditions. Fungal elements are present on histopathologic evaluation within sinus mucosa, submucosa, blood vessels, or bone. Prominent tissue necrosis is also present, although the associated inflammatory infiltrate is variable.

Fungal sinusitis should also be considered in any patient with chronic sinusitis when focal or diffuse areas of radiodensity are detected on computed tomography or with decreased T1- and T2- weighted signal intensities are present on magnetic resonance imaging of the sinuses [6]. The explanation of these radiological findings is unclear.

Most patients with IFS do not have bone erosion or extension of the disease outside of the sinuses early in their course when salvage is most likely. Recent data suggest that unilateral thickening of nasal mucosa is the most common initial finding on CT and rhinoscopy in early disease [7] (see Fig. 1). At that stage, the nasal and sinus mucosa is usually not necrotic but pale or discolored on physical examination. Patients frequently seem more ill and have more pain and fever than physical examination would suggest. Later in the disease course, infected sinuses are frequently filled with large quantities of necrotic material, mucus, and polyps, especially with chronic invasive disease.

Diagnosis. Delay in the diagnosis of invasive fungal sinusitis leads to increased mortality. Since diagnosis requires biopsies to establish tissue invasion by fungi, early endoscopic evaluation with biopsy of healthy and diseased tissue and culture of sinus contents is required when invasive fungal sinusitis is suspected [8,9]. Because concentrations of fungal elements vary from diffuse to dense, silver impregnation stains should be performed on all material submitted for histopathologic evaluation, including mucus, anytime fungal sinusitis is considered. Special stains for fungus must be obtained on all surgical specimens and should be routinely requested on material from any immunocompromised patient, including those with diabetes, hemochromatosis, neutropenic syndromes, or iatrogenic immunosuppression [10]. If endoscopic evaluation is negative and the diagnosis is still suspected, open biopsy of the sinuses should follow immediately so that adequate material may be obtained for evaluation.

Fig. 1 Coronal computed tomographic scan from a patient with invasive fungal sinusitis showing unilateral left nasal cavity soft tissue thickening of the septum, turbinates, and nasal floor, with complete opacification of the left nasal cavity. From DelGaudio et al. [7]. Used with permission. Arch Otolaryngol 2003; 129: 236–240. Copyright © (2003), American Medical Association. All Rights reserved.

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Detection of fungemia before clinical sequella develop would be a major step forward in the diagnosis and treatment of fungal sinusitis. Although an international panel has recommended that histopathology or microscopy should continue to be used to diagnose invasive fungal reactions, that committee also noted that antigen detection testing could provide supportive evidence in the appropriate clinical setting [11]. Several antigen detection systems are now available for clinical use. Some studies have suggested that antigenemia with galactomannan is detectable as early as 5–8 days before clinical signs or symptoms of invasive aspergillosis. In a meta-analysis of 27 studies using consensus criteria for the diagnosis of invasive disease, ELISA sensitivity ranged from 61–71%, specificity from 89–93%, negative predictive value from 95–98% and positive predictive value from 26–53% [12]. Performance of the assay seemed to vary among patients with malignancy, hematological and organ transplants, but specific information is not available on those patients with invasive aspergillus sinusitis. Performance is adversely affected by the administration of certain medications, including penicillins, and the presence of other organisms such as *Penicillium* spp. The utility of PCR-based testing is unclear.

**Beta-D-glucan**

The US Food and Drug Agency (FDA) has approved a serum or plasma spectrophotometric assay for beta-d-glucan (Fungitell assay), a cell wall component of all fungi except *Cryptococcus* and zygomycetes (*Rhizopus*, *Absidia*, *Cunninghamella*, *Rhizomucor*, *Syncephalastrum*, *Saksenaea*, *Apophysomyces* and *Mucor*). Sensitivity and specificity in the diagnosis of invasive fungal disease has been as high as 93 and 77% respectively [12]. Again, no specific data are available on invasive aspergillus sinusitis.

**Classification of invasive fungal sinusitis**

Criteria for diagnosis of invasive fungal sinusitis have been established and are based on confirmation of tissue invasion (Table 1) [3].

1. Mucosal thickening or air fluid levels compatible with sinusitis on radiologic imaging.
2. Histopathologic evidence of hyphal forms within sinus mucosa, submucosa, blood vessels, or bone.
3. To diagnose granulomatous invasive fungal sinusitis, histopathologic evidence of hyphal forms within sinus mucosa, submucosa, blood vessel or bone in association with granuloma containing giant cells is required. Concomitant stains for mycobacteria must be negative.

Adapted from deShazo et al. [3].

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<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Representative fungi</th>
<th>Geographic distribution</th>
<th>Host</th>
<th>Associated conditions</th>
<th>Histopathology</th>
<th>Clinical presentation</th>
<th>Treatment</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (fulminant) invasive fungal sinusitis</td>
<td>Fungi of the order of Mucorales, and Aspergillus fumigatus</td>
<td>No specific geographic location</td>
<td>Immunocompromised and rarely in immunocompetent</td>
<td>Diabetes, malignancy, immunosuppressive therapy</td>
<td>Sparse fungal elements in mucosa, submucosa, blood-vessels or bone with extensive tissue necrosis and neutrophilic inflammation</td>
<td>Chronic pan-sinusitis, nasal polypos, calcification within sinus on CT, proptosis or eye muscle entrapment in children</td>
<td>Debridement, aeration, oral and topical corticosteroids and allergen immunotherapy. No evidence that oral antifungals are helpful</td>
<td>Recurrence common</td>
</tr>
<tr>
<td>Chronic invasive fungal sinusitis</td>
<td>Aspergillus fumigatus</td>
<td>No specific geographic location</td>
<td>Immunocompromised</td>
<td>Diabetes mellitus</td>
<td>Dense accumulation of fungal elements in a mucoid matrix forming an expansile mass. Low-grade chronic inflammatory response in adjacent mucosa</td>
<td>Rhinosinusitis (often unilateral) nasal obstruction, green-brown nasal discharge, calcification in the sinus on CT.</td>
<td>Debridement, aeration. Antifungals not required</td>
<td>Excellent</td>
</tr>
<tr>
<td>Granulomatous invasive fungal sinusitis</td>
<td>Aspergillus flavus</td>
<td>Predominately in North America</td>
<td>Immunocompetent</td>
<td>None</td>
<td>Fungal elements in mucosa, submucosa, blood vessels or bone with extensive tissue necrosis and neutrophilic inflammation.</td>
<td>Fever, cough, crusting of nasal mucosa, epistaxis, headache, mental status changes.</td>
<td>Radical debridement to histopathologically normal tissue, antifungal antibiotic treatment of underlying conditions.</td>
<td>Fair when limited to sinus; poor with intracranial involvement.</td>
</tr>
</tbody>
</table>

CT, computed tomography. Adapted from deShazo et al. [4].
The prognosis of acute fulminant invasive fungal sinusitis is grave unless diagnosed early, where the best outcomes report only 50% survival. Treatment includes simultaneous surgical removal of devitalized tissue down to healthy tissue planes, and antifungal therapy, previously given at conventional doses [21]. Clinical experience has led most experts to recommend high does intravenous amphotericin B to treat invasive fungal sinusitis (amphotericin B deoxycholate 1mg/kg/day or liposomal amphotericin B 5–7.5mg/kg/day). Exceptions include *P. boydii* which is often resistant and better treated with voriconazole and *Aspergillus* species which seem more responsive to voriconazole, at least in other locations. Surgery should be performed for histopathological evaluation and to debride the devitalized tissue supporting fungal growth. When histopathological studies confirm tissue invasion, treatment with high dose antifungal therapy should be initiated immediately, without waiting for the results of fungal cultures, and for intervals determined by the clinical response. Close collaboration between medical and surgical specialists is essential in the care of these patients. Obviously, treatment of underlying immunodeficiency, or reconstitution of the iatrogenic immunodeficiency, is desirable but often difficult. The condition may recur after apparently successful treatment when immunosuppression is ongoing and requires chronic suppressive therapy.

**Chronic invasive fungal sinusitis.** Although sporadic reports of patients with a syndrome resembling chronic invasive fungal sinusitis have been published, it has only recently been recognized as a specific form of fungal sinusitis [3,4]. Chronic invasive fungal sinusitis results from a slowly progressive fungal infection that elicits limited inflammation, usually in diabetic patients. As opposed to the neutrophil-rich, highly necrotic, and angiotrophic process seen in acute invasive fungal sinusitis, there is a low-grade mixed cellular infiltrate in affected tissues [22]. Thick nasal polyposis and thick purulent mucus, like that seen grossly in allergic fungal sinusitis and mycetoma, are also common. When the infection expands out of the ethmoid sinuses medically into the orbit, orbital apex syndrome has been a common clinical presentation [23,24] (Fig. 2). This condition results from erosion of the fungal mass into the orbital apex, causing decreasing vision and abnormalities of ocular mobility. Proptosis may also occur. Chronic invasive fungal sinusitis may be advanced by the time of diagnosis, with posterior erosion out of the ethmoid sinus, resulting in cerebrovascular accidents from cavernous venous thrombosis and death. Treatment is the same as for acute invasive disease, with surgery and antifungal therapy. The role of newer oral antifungal agents in invasive fungal sinusitis is unclear but promising [25]. In infections with organisms resistant to amphotericin B, such as *Pseudallescheria boydii*, azole therapy has been reported to be lifesaving.

**Antimicrobial treatment of invasive aspergillus sinusitis.**

An increasing array of antimicrobial agents is available to treat invasive fungal sinusitis. Examples include the azoles (itraconazole), triazoles (voriconazole) and echinocandins (caspofungin) in addition to the gold-standard polyene, amphotericin B deoxycholate and its lipid-based cousins.

In the case of aspergillosis, no controlled studies are available on antimicrobial treatment of invasive fungal sinusitis. Amphotericin-B treatment requires 1–1.5 mg/kg/day, a dose almost always associated with nephrotoxicity. Therefore, lipid formulations became the standard of care for polyene therapy in invasive aspergillosis (Abelcet or AmBisome). Even then, *Aspergillus* species such as *A. terreus* may be resistant to the drug. Therefore, voriconazole has become the treatment of choice for invasive aspergillus sinusitis when the diagnosis is established as such [26]. The usual adult dose is 6mg/kg i.v. twice a day on day one followed by 4 mg/kg i.v. twice a day for 7 days with the option to decrease to 200 mg orally, twice a day thereafter. Patients with acute invasive aspergillosis who were not ventilator dependent showed a greater percentage of complete or partial response, lower mortality rate and a lower incidence of side effects...
with this regimen. Since this drug has little activity against zygomycetes, it should not be used unless the diagnosis of aspergillosis has been made.

Itraconazole is one of several second line drugs for treatments for aspergillosis since voriconazole has greater activity with fewer side effects. Caspofungin is approved for treatment of invasive aspergillosis in patients who cannot tolerate or are refractory to voriconazole. After an initial dose of 70mg i.v., 50mg/day is given i.v. as salvage therapy [27].

Conclusion

With an ever increasing number of effective therapies for cancer and autoimmune disease that also cause suppression of cell mediated immunity, fungal disease and fungal sinusitis will be a growing problem. Now that diagnostic criteria are available, multicenter trials comparing diagnostic and therapeutic approaches are greatly needed.

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References


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Successful isavuconazole salvage therapy in a patient with invasive mucormycosis

J. Ervens · M. Ghannoum · B. Graf · S. Schwartz

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Abstract A 45-year-old male with rhinocerebral mucormycosis (*Rhizopus oryzae*), refractory to liposomal amphotericin B and posaconazole, received isavuconazole salvage therapy. Initial isavuconazole plasma and tissue levels were 0.76–0.86 μg/mL and 1.09–1.38 μg/g. Plasma levels increased to 1.3–3.24 μg/mL with reduced comedication. Isavuconazole was well tolerated, and the patient has remained disease-free 24 months post-antifungal therapy.

Keywords Mucormycosis · *Rhizopus oryzae* · Isavuconazole · Drug monitoring

A 45-year-old male presented with a 2-week history of progressive left facial pain. Previous dentist examination was unremarkable. Conventional X-rays showed left-sided maxillary sinusitis, and the patient was admitted for presumed odontogenous bacterial sinusitis (day 1). Prior to admission, the patient had received prednisolone therapy (80 mg/day for 3 weeks) for a flare-up of ulcerative colitis. The medical history further revealed unilateral renal loss after trauma 20 years previously and a mild degree hypertension. The physical examination showed a slight, left-sided facial swelling. There was no visual impairment, but an enhanced, left-sided sensitivity to light and numbness of the maxillary branch of the trigeminal nerve were present. Laboratory testing showed a markedly elevated white blood cell count (20.6/nL) and creatinine level (1.5 mg/dL). Antibacterial therapy with cefuroxime and clindamycin was started.

On day 4, deterioration of the clinical condition required intensive care with mechanical ventilation. Facial swelling progressed to left-sided periorbital swelling with chemosis and absence of the pupillary reflex. The following day, the left part of the hard palate showed large areas of necrosis, and hyphae, with morphological features indicative of mucormycosis, were identified in sinonasal lavage fluid. A computed tomography scan disclosed left-sided maxillary sinusitis and orbital cellulitis with bone destruction and extended soft tissue infection of the left facial area. This prompted emergency surgical resection on day 5 which consisted of tracheostomy with left-sided maxillectomy and orbital exenteration that included the infratemporal fossa, resection of the nasal septum, left-sided turbinates and left-sided ethmoidal cells extended to the anterior skull base. The infected anterior skull base and dura were partially resected, and dural defects were covered with dura mater sealers as well as with muscle and local flaps.
Histopathological examination of resected tissue revealed the presence of extended necroses and infiltration by filamentous fungal elements that showed morphological characteristics of mucormycosis. Cultures grew a fungus with morphological features compatible with mucormycosis. The isolate was identified as *Rhizopus oryzae* species (MRL 19448) by molecular methodology using the 5.8s internal transcribed spacer 1 and referenced to accession no. HM753610, with 100% identity and a sequence length of 605 bp [1]. The minimum inhibitory concentrations (MIC) of the isolate using Etest according to the manufacturer’s instructions (bioMérieux, Marcy l’Etoile, France) were 0.5 μg/mL for amphotericin B, 3 μg/mL for posaconazole and 1 μg/mL for isavuconazole (isavuconazole test strips were provided by Basilea Pharmaceutica International Ltd., Basel, Switzerland). However, isavuconazole in vitro susceptibility against this *R. oryzae* strain varied by methodology. Although the MIC measured by Etest agar diffusion was relatively low (1 μg/mL), the MIC values obtained by both CLSI (Clinical and Laboratory Standards Institute, Wayne, PA) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) microdilution were 8 to >16 μg/mL as determined by two different reference laboratories. These in vitro data indicate possible resistance to isavuconazole, although there are no interpretive breakpoints established for this novel antifungal. The mechanism of resistance studies performed at the Center for Medical Mycology further supported the fact that this strain is resistant to isavuconazole. The fungal cellular target for azoles is a 14α-demethylase enzyme involved in sterol synthesis leading to ergosterol formation, and alteration in sterol composition is linked to antifungal resistance [2]. Our data showed that squalene, the first intermediate in the ergosterol biosynthetic pathway, was elevated in this *Rhizopus* strain (17.06 vs. 9.95% in the control susceptible strain). Similarly, zymosterol levels were greatly elevated (68.70 vs. 0.00%). In contrast, the ergosterol level in the resistant strain was significantly reduced relative to that in a isavuconazole-susceptible control strain (2.3 vs. 76.93% in the control strain). The accumulation of these ergosterol precursors and the lower levels of ergosterol observed in this strain are suggestive ofazole resistance.

Liposomal amphotericin B (5 mg/kg/day) and posaconazole (≥200 mg administered four times daily via a nasogastric tube after high-fat-containing tube feeding) were started immediately after emergency resection on day 5 and given for 92 and 52 days, respectively. Posaconazole plasma concentrations remained <0.2 μg/mL throughout the treatment (Table 1). In addition, the concentration of posaconazole in a soft tissue specimen (muscle, fat) that was obtained during surgical debridement was below the MIC of the fungal isolate despite 24 days of posaconazole therapy (0.03 μg/g; Table 1). Worsening of renal failure during therapy with liposomal amphotericin B required intermittent hemodialysis between days 23 and 28. However, filamentous fungal elements were recovered from tissue biopsies on day 61 (no cultures performed), despite 52 and 57 days of therapy with posaconazole and liposomal amphotericin B, respectively. Therefore, therapy with

<table>
<thead>
<tr>
<th>Day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Posaconazole trough level: plasma (μg/mL)</th>
<th>Posaconazole level: soft-tissue (μg/g)</th>
<th>Day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isavuconazole trough level: plasma (μg/mL)</th>
<th>Isavuconazole level: soft-tissue (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>&lt;0.16</td>
<td></td>
<td>112</td>
<td>0.86</td>
<td>1.09–1.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>&lt;0.19</td>
<td></td>
<td>113</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.09</td>
<td></td>
<td>117</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.12</td>
<td></td>
<td>141&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0.10</td>
<td>0.03</td>
<td>148</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>ND</td>
<td></td>
<td>155</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0.19</td>
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<td>187</td>
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<td>250</td>
<td>2.85</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>285</td>
<td>3.24</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>313</td>
<td>3.02</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Day 1: day of admission; day 5: start of posaconazole; day 104: start of isavuconazole (see text for dosages)

<sup>b</sup> Isavuconazole levels in 3 soft tissue biopsies (muscle, fat), simultaneously obtained 3 h after dosing, were 1.09, 1.27 and 1.38 μg/g (corresponding plasma level: 0.85 μg/mL)

<sup>c</sup> Reduction of comedication prior to day 141 (see text for details)
posaconazole and liposomal amphotericin B was discontinued on days 52 and 96, respectively, and the patient was enrolled into a clinical phase 3 trial exploring the safety and efficacy of monotherapy with the novel triazole, isavuconazole (ClinicalTrials.gov Identifier: NCT00634049).

Antifungal treatment was resumed on day 104 with isavuconazole. Initially, isavuconazole was administered intravenously (days 104–105: 200 mg t.i.d. daily; day 106–107: 200 mg daily q.d.) with a switch to oral therapy on day 108 (200 mg q.d.). During 14 days of isavuconazole therapy, the plasma trough concentration did not exceed 0.86 μg/mL, and the concentration in soft tissue samples ranged from 1.09 to 1.38 μg/g (Table 1), which although higher than the MIC of the infecting isolate was still lower than the trough concentration expected from the isavuconazole dose given (≥2 μg/mL). To enhance isavuconazole concentrations, which might have been limited by yet unknown drug–drug interactions, concomitant therapy with various drugs was either terminated between days 117 and 141 or limited to this period, while treatment with amlodipine, mesalazine (both prior to isavuconazole—ongoing after end of isavuconazole), ranitidine (terminated on day 174) and heparin (dalteparin; given prior to treatment to day 113 and on days 119–174; unfractioned heparin: days 114–118) was maintained. The drugs terminated were ciprofloxacin eardrops (days 121–127), clindamycin (days 114–118), fentanyl (days 112, 119), loperamide (days 118–119), lorazepam (days 121–132), lormetazepam (days 119–120, 124), metamizol (days 113–119), mirtazapine (prior isavuconazole—day 125), morphine (day 119), ondansetron (day 117), paracetamol (days 113–118), propofol (days 112, 119), tramadol (days 120–132) and valproate (prior isavuconazole—day 133).

There were no comedications during isavuconazole therapy which were terminated before day 117. The following drugs were administered after day 141: amoxicillin/clavulanate (days 663–669), cefuroxime (days 320–326, 357–363, 504–516), ciprofloxacin eardrops (days 293–299) and flucloxacillin (days 660–662).

Continued drug monitoring showed an increase in plasma isavuconazole trough levels, first evident on day 141, to 1.3–3.24 μg/mL. However, there was no evidence for drug accumulation (Table 1). The patient was discharged from hospital on day 174. Oral isavuconazole (200 mg q.d.) was given as monotherapy until day 609 (total duration of isavuconazole therapy: 506 days). The patient received isavuconazole as study therapy for a total duration of 84 days and for the remaining treatment period under compassionate use authorization granted by the German health authorities. Isavuconazole was well tolerated without adverse effects except for a transient grade I skin rash, which was associated with prolonged sunlight exposure. Early follow-up biopsies, obtained 16 days after the switch to isavuconazole, still showed filamentous fungal elements, but subsequent biopsies and fungal cultures (obtained for the last time more than 1 year after termination of isavuconazole therapy) gave negative results. The appearance of the facial skull cavity was without any specific evidence of an ongoing invasive fungal infection during isavuconazole therapy, with largely a similar appearance throughout. However, contrast media enhancement of the cavernous sinus was detectable by magnetic resonance imaging; this enhancement decreased during isavuconazole therapy. Two early attempts of reconstructive surgery (days 49 and 112) were unsuccessful due to ischemia of the muscle free grafts. Six months after the completion of antifungal therapy (day 796), the patient resettled in his home country, the UK, where he later received final reconstructive surgery (microvascular free fibular osteocutaneous flap as well as dental and orbital implants). At last follow-up, 2 years after the termination of isavuconazole therapy (day 1,333), the patient was well and without any signs of recurrence of the invasive fungal infection.

Invasive mucormycosis (formerly termed zygomycosis) is a devastating infection which is increasingly recognized in patients with various types of predisposing conditions (e.g. poorly controlled diabetes, iron overload, chemotherapy and hematopoietic stem cell transplantation). However, invasive mucormycosis has been reported less frequently in patients with corticosteroid-induced immunosuppression as the sole risk factor [3]. The available data on this disease have been comprehensively reviewed by Dr. Walsh and other experts, and a guideline from the third European Conference on Infections in Leukemia has been published very recently [4, 5]. The prognosis of this type of fungal infection has improved to some extent in recent years, but mortality rates in patients with pulmonary, rhinocerebral or disseminated infection still range from 48 to 100 % [6, 7]. Early therapy with amphotericin B (lipid preparations preferred) and resection of infected/necrotic areas, whenever feasible, is considered to be the treatment of choice [7–9]. Fungi of the order Mucorales are notoriously resistant to antifungal drugs other than amphotericin B, with the exception of posaconazole. Posaconazole shows clinical activity in mucormycosis, but this azole is currently available only as an oral solution and displays limited absorption and variable bioavailability [10, 11].

In our patient, liposomal amphotericin B caused nephrotoxicity, requiring hemodialysis, and the fungus persisted despite prolonged therapy with liposomal amphotericin B and posaconazole. Isavuconazole is a novel azole that shows in vitro activity against mucorales comparable to that of posaconazole [12]. A water-soluble prodrug, isavuconazonium sulfate, which is suitable for intravenous and oral administration, is under clinical development and is rapidly cleaved into the isavuconazole active moiety.
with an oral bioavailability in humans that approaches 100 % [13]. The first measured isavuconazole plasma levels in our patient were approximately 0.8 μg/mL (Table 1), with a higher tissue to plasma ratio (1.3–1.6) compared to posaconazole (0.2–0.3). The subsequent increase in isavuconazole plasma levels with reduced comedication was possibly associated with higher tissue levels, but it did not indicate drug accumulation. The long-term isavuconazole therapy for more than 16 months was well tolerated in this particular patient, with only one minor, transient side-effect. There were no remaining measurable fungal lesions after extensive resection, which precluded a radiological response assessment. However, the improved clinical condition, the transition from positive to negative biopsies after therapy switch to isavuconazole and the observed long-term survival without any further systemic antifungal therapy suggests that the treatment success was at least in part due to isavuconazole, albeit diverging susceptibility test results.

This first case report on the clinical use of isavuconazole demonstrates the beneficial clinical and pharmacokinetic properties of isavuconazole and suggests that this novelazole could be an attractive option for patients with deep-seated fungal infections, including invasive mucormycosis. However, more data from ongoing phase 3 clinical trials are needed before isavuconazole might extend the limited therapeutic armamentarium for patients with invasive mucormycosis.

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References

Tissue Penetration of Antifungal Agents

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SUMMARY

Understanding the tissue penetration of systemically administered antifungal agents is critical for a proper appreciation of their antifungal efficacy in animals and humans. Both the time course of an antifungal drug and its absolute concentrations within tissues may differ significantly from those observed in the bloodstream. In addition, tissue concentrations must also be interpreted within the context of the pathogenesis of the various invasive fungal infections, which differ significantly. There are major technical obstacles to the estimation of concentrations of antifungal agents in various tissue subcompartments, yet these agents, even those within the same class, may exhibit markedly different tissue distributions. This review explores these issues and provides a summary of tissue concentrations of 11 currently licensed systemic antifungal agents. It also explores the therapeutic implications of their distribution at various sites of infection.

INTRODUCTION

Despite recent advances in antifungal chemotherapy, invasive fungal infections (IFI) remain a significant cause of morbidity and mortality (1). Candida species, Aspergillus fumigatus, and Cryptococcus neoformans are the most common pathogens (2). However, a wide range of other fungi, often with limited susceptibility to first-line antifungal agents, may also cause infection. Mortality from IFI remains high (e.g., that from aspergillosis is ~50% [3, 4], and that from candidemia is 10 to 49% [5, 6, 7]). An understanding of the pharmacological properties of any antifungal agent is crucial for optimizing patient outcomes for all these infections (8). This may be especially true for an increasingly recognized group of patients who have not previously been considered to be at high risk of IFI, such as critically ill patients and those with chronic obstructive pulmonary disease (COPD), who may demonstrate marked pharmacokinetic (PK) variability (9, 10).

Penetration into the site of infection to achieve microbe-eliminating concentrations is a key requirement for efficacy of all antimicrobial agents (11, 12, 13, 14, 15). The importance of tissue concentrations for the various classes of antibacterial agents has been reviewed extensively, but relatively less attention has been
paid to the currently available antifungal agents (12, 16, 17, 18, 19). This review examines the tissue penetration of 11 commonly used systemic antifungal agents (amphotericin B deoxycholate [AmBd], amphotericin B lipid complex [ABLC], liposomal amphotericin B [L-AMB], fluconazole, itraconazole, posaconazole, voriconazole, 5-fluorocytosine [5FC], anidulafungin, caspofungin, and micafungin) into the clinically relevant compartments for human infection and disease. All human data, ranging from case studies through autopsies to small clinical studies in volunteers or patients, were included. We also considered key laboratory animal data, where relevant, especially if the respective information for humans is absent. Because only free drug is considered to be biologically active (20, 21, 22), tissue and fluid concentrations are placed in context with the key physicochemical properties of each agent. The major organ systems covered include the lungs, liver, kidney, spleen, and heart. Attention has also been given to drug penetration into sanctuary sites (e.g., brain and eye), with the corresponding therapeutic implications. We have also reviewed the data for key interstitial fluids, including bronchial secretions, epithelial lining fluid (ELF), pleural fluid, pericardial fluid, synovial fluid, prostatic fluid, and cerebrospinal fluid (CSF), and placed these data in a clinical context (23).

PENETRATION OF ANTIFUNGAL AGENTS INTO TISSUES: CONCEPTS, IMPORTANCE, AND CURRENT GAPS IN KNOWLEDGE

Importance of Tissue Concentrations for an Understanding of Antifungal Pharmacodynamics

The potential relevance of the tissue concentrations of any anti-infective agent must be considered in context with the pathogenesis of the invading fungal organism (24). There must be colocalization of “drug and bug” within tissue beds and tissue subcompartments. Such considerations are relevant at the level of the organ and tissue subcompartments but may be elucidated further at the cellular and even molecular levels (25, 26, 27, 28, 29).

Most agents ultimately exert their effects on microorganisms residing within tissues. However, the distribution of agents from the bloodstream to various tissue subcompartments is often characterized by considerable variability, beyond that observed in plasma alone. Consequently, target site concentrations often differ markedly from those measured in plasma, especially in sanctuary sites such as the eye or central nervous system (CNS). Furthermore, there may be discordance in the shape of the concentration-time profiles for plasma and tissues. This phenomenon is called hysteresis (Fig. 1) and may explain persistent antifungal activity when plasma concentrations are low or undetectable (e.g., as seen with L-AMB [30], caspofungin [31], and itraconazole [76]). Conversely, suboptimal target site concentrations may well explain some cases of therapeutic failure (11, 13). In addition, as most fungal infections are extracellular, interstitial fluid may be the closest measurable compartment to the site of infection. However, the important compartment for prophylaxis may be different, which in turn is related to differences in pathogenesis and the stage of infection (Fig. 2A) (32, 33).

Determinants of Distribution of Antifungal Agents into Tissues

The principal chemical and pharmacokinetic properties influencing the tissue distribution of the 11 systemic antifungal agents in this review are summarized in Table 1. The four major classes of antifungal agents, i.e., the echinocandins, polyenes, pyrimidine analogues (5FC), and triazoles, are reviewed. These compounds are all distinct in terms of their chemical structure, molecular size, lipophilicity, and metabolism, and these differences have a major impact upon their pharmacokinetic and pharmacodynamic (PD) characteristics. Furthermore, there may be significant differences within a class. For example, the lipophilicities (expressed as log D values in Table 1) of the four triazoles vary from 0.5 to >5.0, and plasma protein binding ranges from 12% to >99% (Table 1). These physicochemical properties determine the rate and extent of tissue penetration and bioavailability within a tissue, organ, or fluid (13, 34). Tissue and fluid concentrations for the three triazoles (fluconazole, voriconazole, and itraconazole), as multiples of those in blood or plasma, are shown in Fig. 3 to 5 to illustrate this.

In very general terms, small polar compounds with low plasma protein binding (e.g., fluconazole and 5FC) have volumes of dis-
A compound with an "intermediate" lipophilicity, volume of distribution, and plasma protein binding (e.g., voriconazole) is also predicted to distribute into aqueous sites but to attain relatively higher tissue concentrations than those of fluconazole or 5FC. In contrast, more lipophilic compounds (such as itraconazole and posaconazole) have much larger volumes of distribution (Table 1), tend to penetrate preferentially into tissues with high lipid content, and often exhibit tissue/plasma concentration ratios that exceed 1. Despite this, they may not necessarily penetrate well into sanctuary sites such as the brain, prostate, and eye. The polyenes (amphotericin B) and the echinocandins have variable tissue penetration but may also exhibit prolonged residence times.

A range of other factors may also have a significant impact upon tissue penetration, including (i) pharmacologic factors, e.g., route of drug administration, such as aerosol or parenteral therapy (35), or formulating drugs within lipids, e.g., amphotericin B colloidal dispersion (ABCD) and L-AMB (36), which may modify their distribution and alter their safety (37, 38) and potency (39); and

FIG 2 Different stages of invasive pulmonary aspergillosis (IPA) and the potential therapeutic importance of different tissue subcompartments. (A) In the very earliest stages of disease, the relevant subcompartments include epithelial lining fluid, alveolar epithelial cells, pulmonary endothelial cells, and pulmonary alveolar macrophages (PAMs). (B) In the early stages of established disease, a halo sign may be seen that consists of a nodule (n) surrounded by a halo (h), which is caused by active infection and inflammation around the nodule. In this case, the relevant subcompartments are within the nodule and contiguous lung. (C) In late disease, an air crescent sign may be present, which represents an organizing sequestrum. (A pulmonary sequestrum [s] is surrounded by an air crescent [ac].) The therapeutic challenge in this case is the achievement of antifungal drug concentrations within a relatively avascular area. (Reprinted from reference 262 with permission; imaging and details kindly provided by Reginald Greene.)

Distribution that approximate total body water (Table 1), achieve better penetration into aqueous sites (e.g., CSF, synovial fluid, and anterior chamber of the eye), and generally have body fluid/plasma concentration ratios that are ~1. A compound with an "intermediate" lipophilicity, volume of distribution, and plasma protein binding (e.g., voriconazole) is also predicted to distribute into aqueous sites but to attain relatively higher tissue concentrations than those of fluconazole or 5FC. In contrast, more lipophilic compounds (such as itraconazole and posaconazole) have much larger volumes of distribution (Table 1), tend to penetrate preferentially into tissues with high lipid content, and often exhibit tissue/plasma concentration ratios that exceed 1. Despite this, they may not necessarily penetrate well into sanctuary sites such as the brain, prostate, and eye. The polyenes (amphotericin B) and the echinocandins have variable tissue penetration but may also exhibit prolonged residence times.

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(ii) physiological factors, such as inflammation, which may increase tissue permeability, i.e., by disruption of normal physiological barriers such as the blood-brain barrier (29, 40); the underlying disease (41), which may result in a range of effects, including modification of plasma protein composition and hence drug binding (42, 43, 44); the recruitment of drug-containing phagocytic cells, i.e., the “dump truck phenomenon,” which may increase drug concentrations at the site of infection (12, 13, 32, 45, 46); drug export via pumps, e.g., for itraconazole and P-glycopro-

### Table 1: Principle physicochemical and pharmacokinetic properties of antifungal drugs in humans that have a potential impact on plasma concentrations and tissue penetration

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol wt</th>
<th>$\log D$ at pH 7.4</th>
<th>% Plasma protein binding</th>
<th>$t_{1/2}$ (h)</th>
<th>$AUC_{0-24}$ (mg·h/liter)</th>
<th>$V_{ss}$ (liters/kg)</th>
<th>References</th>
</tr>
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<tr>
<td><strong>Triazoles</strong></td>
<td></td>
<td></td>
<td></td>
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<td>Fluconazole</td>
<td>305</td>
<td>0.5</td>
<td>12</td>
<td>24–30</td>
<td>38</td>
<td>0.7</td>
<td>17, 18, 67</td>
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<td>Itraconazole</td>
<td>706</td>
<td>&gt;5</td>
<td>99.8</td>
<td>34</td>
<td>8.7–25</td>
<td>11</td>
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<td>700</td>
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<td>&gt;98</td>
<td>20–31</td>
<td>33–39</td>
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<td>349</td>
<td>1.8</td>
<td>58</td>
<td>6</td>
<td>13</td>
<td>4.6</td>
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<tr>
<td>AmBd (conventional amphotericin B)</td>
<td>924 (&lt;0.04)</td>
<td>−2.8</td>
<td>95–99</td>
<td>10–24</td>
<td>1–30</td>
<td>0.5–5</td>
<td>17, 144, 231–233</td>
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<tr>
<td>ABLC (Abelcet)</td>
<td>924 (1.6–11)</td>
<td>−2.8</td>
<td>95–99</td>
<td>24</td>
<td>9.5–14 ± 7</td>
<td>1.12–8.8</td>
<td>17, 144, 231, 232, 234</td>
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<tr>
<td>L-AMB (Ambisome)</td>
<td>924 (0.08)</td>
<td>−2.8</td>
<td>95–99</td>
<td>6–23</td>
<td>131 ± 126</td>
<td>0.11–0.7</td>
<td>17, 144, 233</td>
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<td><strong>Nucleoside</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorocytosine</td>
<td>120</td>
<td>−2.34</td>
<td>5</td>
<td>3–5</td>
<td>576, 1289</td>
<td>0.6–2.23</td>
<td>91, 179</td>
</tr>
<tr>
<td><strong>Echinocandins</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anidulafungin</td>
<td>1,140</td>
<td>−3.32</td>
<td>84–99</td>
<td>26</td>
<td>110.3</td>
<td>0.8</td>
<td>235, 236</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>1,093</td>
<td>−3.88</td>
<td>97</td>
<td>9–11</td>
<td>57–96</td>
<td>0.15</td>
<td>235, 236</td>
</tr>
<tr>
<td>Micafungin</td>
<td>1,291</td>
<td>−1.62</td>
<td>&gt;99</td>
<td>15–17</td>
<td>29.6 ± 4.6</td>
<td>0.24–0.39</td>
<td>182, 235</td>
</tr>
</tbody>
</table>

$^a$ From reference 18.

$^b$ Volume of distribution at steady state.

$^c$ Dose-proportional pharmacokinetics.

$^d$ Except in patients with renal impairment.

$^e$ Concentration-dependent pharmacokinetics.

$^f$ Data from oral solution and i.v. formulation in cyclodextrin.

$^g$ Values for oral and i.v. formulations, respectively.

**FIG 3** Fluconazole tissue and fluid concentrations in humans as multiples of the maximal or simultaneously measured concentration in plasma (mg/ml) after systemic administration. Tissue multiples are from tissue to plasma concentrations. Fluid multiples are from plasma concentrations. Numbers in parentheses indicate relevant references.

**FIG 4** Voriconazole tissue and fluid concentrations in humans as multiples of the maximal or simultaneously measured concentration in plasma (mg/ml) after systemic administration. Tissue multiples are from mg/g tissue values. Fluid multiples are from mg/ml concentrations. *, autopsy data; in these cases, the multiples are based on plasma $C_{max}$ values at the same dose in volunteers (188).
tein (75); variable oral bioavailability, e.g., of itraconazole (47)
and posaconazole (228); and interpatient variability in clearance,
e.g., of voriconazole (48).

Limitations of Current Understanding and Approaches

Considering tissue concentrations in isolation is of limited value.
A drug may be present at a site but at a concentration beneath the
threshold required for activity, located in the wrong subcompartment,
and not biologically available. Ideally, therefore, tissue concentrations
should be analyzed with concomitant pharmacodynamic data.
Examples of this problem include AmBd and itraconazole,
which have low concentrations in the CSF yet are effective agents for
treatment of cryptococcal meningitis (49, 50).

Tissue homogenates are frequently used to estimate tissue con-
centrations, but they are a relatively crude and potentially mis-
leading matrix when used for this purpose. Mouton and colleagues (51)
highlighted the potential pitfalls in using drug concentrations within
whole-tissue homogenates for drawing conclusions related to the activity and efficacy of a drug, especially for extracellular pathogens.
This may be a particular issue for amphotericin B (irrespective of formulation), where there is long-
standing uncertainty related to the amount of biologically available
drug in tissues. The potential reasons that tissue homogenates
may provide inaccurate information regarding the “true” concentration
at the site of infection include (i) discordance between intra- and extracellular drug concentrations versus where the
pathogen is actually located, e.g., for posaconazole (33); (ii) multifocal versus diffuse disease, resulting in altered drug penetration
at the site of infection compared with the normal contiguous tissue,
e.g., pulmonary aspergillosa (257) or cerebral cryptococ-
coma (Fig. 6); (iii) the concentration of total versus biologically active
drug, e.g., free amphotericin B versus drug that remains
complexed to lipid (29, 52, 53, 54); and (iv) incomplete extraction
of drug from tissue, e.g., for amphotericin B (29, 52, 53, 54).

Reporting tissue concentrations of anti-infective drugs in a clin-
ically useful format is also problematic. One of the most common
presentation methods is to use a ratio to plasma concentration,
which may be flawed for a number of reasons. This ratio is depen-
dent on both the denominator and the numerator, e.g., the bone
tissue/plasma concentration ratio for ABLC in rabbits is 42, while
the corresponding ratio for L-AMB is 0.66, suggesting that ABLC
penetrates bone more effectively than L-AMB. However, the actual
amphotericin concentrations achieved with the two lipid for-
mulations in bone are similar (35.4 μg/g and 39.5 μg/g for ABLC
and L-AMB, respectively) and, in both cases, superior to that
achieved with AmBd (19). Comparison of concentrations taken at
a single time point is also liable to induce errors because of hys-
teresis (Fig. 1), with a delay occurring as drug moves from the
vascular to the tissue compartment (55). For this reason, it may be
more useful to present the tissue area under the concentration-
time curve (AUC) for comparison. There are few studies that do
this for humans (56, 57, 58, 59, 60, 61, 62), and with one exception
(59), all deal with pulmonary distribution.

Most of the antifungal agents considered in this review do ex-
hibit hysteresis. This persistence of tissue concentrations may ex-
plain why, in specific situations, linking the tissue pharmacoki-
netic data with pharmacodynamic data produces a significantly
more robust PK/PD model than using plasma PK data alone (31,
63). The technique of comodeling both PK and PD data may also
produce a more insightful reflection of the impact of tissue con-
centration than the simplistic comparison of peak tissue concen-
tration with the breakpoint MIC (64).

**Antifungal Drug Concentrations in Organs, Tissues, and Body Fluids**

The papers in this review were published between January 1965
and December 2012. Inevitably, they used differing drug dosages and
formulations, with different routes of systemic administration and a
range of drug extraction and assay methods (e.g., bioassay, gas-
liquid chromatography, high-pressure liquid chromatography,
¹⁴C-autoradiography, and ¹⁸F-nuclear magnetic resonance [¹⁸F-
The data were also potentially influenced by the underlying disease of the host. Consequently, we only used data where both plasma and tissue concentrations were reported within the same study (except for some postmortem studies in which tissue concentrations alone were reported).

Most human data are from healthy adult volunteers and/or a few patients, and their applicability to young children or neonates requires further study (65). Information is most comprehensive for the older triazoles (fluconazole and itraconazole), while both human and animal data for the newer agents (posaconazole and the three echinocandins) are more limited. Human data for AmBd (discovered in the 1950s) and 5FC (discovered in the 1970s) are also surprisingly sparse.

Despite the caveats discussed in the introduction, the published data are expressed as tissue or body fluid/plasma concentration ratios. They are summarized in Fig. 7 as three differently colored ratio bands. The colors in the figure illustrate differing drug concentration ratio bands but do not imply differences in efficacy within various tissues or between drugs.

**Brain and Cerebrospinal Fluid**

The brain and CSF are sanctuary sites, as they are surrounded by lipid membranes with inward- and outward-facing transporters (66). Data from human studies suggest that fluconazole concentrations in CSF are dose dependent and vary between 50% and 100% of the concentration observed in the plasma (67, 68, 69, 70) (Fig. 3 and 7). Fluconazole is also readily detectable in human brain parenchyma. Studies with 18F-fluconazole in volunteers showed brain tissue concentrations that were similar to those in plasma, with some minor regional variation (71). However, in five surgical patients, fluconazole brain tissue/plasma concentration ratios of 0.7 to 2.4 were measured when the fluconazole plasma concentrations were at 90% of steady-state values (72). Fluconazole is a recognized therapy for cryptococcal and Candida meningoencephalitis.

In contrast, itraconazole concentrations in human CSF are very low, with CSF/plasma concentration ratios of <0.002 to 0.12 (Fig. 5 and 7) (73, 74). Itraconazole penetrates the brains of rats rapidly, and in a dose-dependent manner, up to 8 min after drug administration (25). However, tissue concentrations are less than those in the plasma (ratio of 0.2 at 60 min postdose) and subsequently decline more rapidly (half-life of 0.4 h) than those in either the plasma or liver tissue (half-life of 5 h) (25). This effect has been ascribed to its active efflux from the brain via P-glycoprotein (Fig. 6). Studies in mice by Imbert and colleagues (75) confirm the impact of P-glycoprotein on itraconazole efflux from the brain but also indicate that intracerebral infection with C. neoformans increases itraconazole exposure in the brain 2.6-fold compared with that in uninfected animals. However, in another rat study, uninfected animals given a single intravenous dose of itraconazole (10 mg/kg of body weight) had a (mean) brain tissue concentration that was 1.7 times the concentration in plasma at 1 h postdose, increasing to 21 times at 24 h postdose, as the brain concentration increased further, while the plasma concentration decreased (76). No itraconazole is detectable in the CSF of rabbits treated with oral itraconazole for cryptococcal meningitis. Nevertheless, itraconazole achieves an efficacy comparable to that of fluconazole.
fluconazole in this model, even though fluconazole is readily detectable in rabbit CSF, with a CSF/plasma concentration ratio of 0.6 to 0.8 (77). Itraconazole also exhibits efficacy in human cryptococcosis, suggesting that it does penetrate the meninges and cerebral parenchyma and achieves the concentrations required for antifungal activity (50, 78).

Voriconazole has a lipophilicity that is intermediate between those of fluconazole and itraconazole (Table 1). Voriconazole penetrates human brain tissue (79, 80) and abscess material (81), achieving peak concentrations similar to or even exceeding those seen in plasma (Fig. 4 and 7) (243). However, human CSF concentrations of voriconazole tend to be lower, with CSF/plasma concentration ratios of 0.22 to 1.0 (81, 82, 83). This is consistent with its intermediate plasma protein binding in humans of 58% (84). Voriconazole is the agent of choice for CNS aspergillosis (243). Posaconazole, which resembles itraconazole structurally but is less lipophilic (Table 1), also penetrates the CSF relatively poorly (85), with CSF/plasma concentration ratios of <0.009 (86). Its diffusion into the CSF may be increased by meningeal inflammation. Thus, CSF concentrations in two patients with bacterial meningitis and cerebral fungal infection were 44% and 230%, respectively, of those in plasma (87). In mice infected with Cryptococcus gattii or Fonsecaea monophora, a bioassay revealed that brain tissue concentrations of posaconazole were approximately 53% of those in serum at daily doses of ≤20 mg/kg but increased to 70% to 80% at a daily dose of 40 mg/kg (88, 89).

Postmortem studies of humans show that amphotericin B is detectable, but only at low concentrations, in the brain tissue of patients receiving AmBd and L-AMB (52, 53, 90). Amphotericin B concentrations in the CSF are also low after administration of intravenous AmBd (91). Similar CSF and brain data for AmBd, L-AMB, and ABLC (i.e., CSF and tissue/plasma concentration ratios of <0.3) have been recorded for rabbits (92). To overcome these potential limitations, intraventricular instillation of AmBd via an Ommaya reservoir has been used for severe cerebral infections (93, 94, 95).

The concentrations of 5FC in human CSF are similar to its corresponding serum concentrations (91, 96, 250), and a combination of 5FC with AmBd or L-AMB is a recognized first-line induction therapy for cryptococcal meningitis (97). The three echinocandins, i.e., caspofungin, micafungin, and anidulafungin, are large, amphipathic, cyclic peptides—properties that do not ordinarily favor penetration into the brain and CSF (98, 99). There are no human data for anidulafungin. However, its concentration in rabbit brains after multiple dosing is only about 10% of the maximum concentration of drug in serum ($C_{max}$) (100, 101). Delivery of $^{14}$C-anidulafungin (as total drug-derived radioactivity) into the brains of rats is delayed compared to that into the blood and other tissues, and it is not detectable in brain tissue until 24 h after a single dose (102). In contrast, CSF concentrations are similar to those in the blood within 30 min of dosing (102). The administration of caspofungin to rodents results in brain tissue concentrations and exposures that are approximately 10% of those in plasma (103, 104). In a single patient with CNS coccidioidiomycosis, CSF concentrations of caspofungin were undetectable, despite concentrations in plasma of 2.7 to 5.5 μg/ml (105). Similarly, the CSF/plasma concentration ratios of three patients receiving micafungin were low and variable, ranging from 0.002 to 0.54, while in the brain tissue of another patient, the tissue/plasma concentration ratio was only 0.17 (106, 107, 252). Micafungin penetration into rabbit brains is dose dependent, and significantly higher concentrations are measurable in the meninges than in either the cerebrum or cerebellum (108). However, the concentrations in these various subcompartments are also sufficient to achieve a significant anti-Candida effect. Animal models suggest equivalent efficacies between the echinocandins and amphotericin B formulations. The clinical value of the echinocandins for various fungal CNS infections remains to be established (18).

Eye

Endogenous fungal endophthalmitis, most commonly caused by Candida or Aspergillus spp., arises from hematogenous dissemination (109). A range of syndromes are seen, including chorioretinitis, vitritis, and pan-endophthalmitis. Successful therapy requires penetration of drug into the relevant subcompartment(s) of the eye, i.e., the choroid, retina, vitreous humor, and aqueous humor (16). For many antifungal agents, suboptimal penetration can mean that medical therapy alone is ineffective, and successful treatment may require vitrectomy and/or intracameral injection (Fig. 7).

Early human and animal data for azoles, polyenes, and 5FC have been well summarized elsewhere (16). Fluconazole (110, 111, 112), voriconazole (113, 114, 252), and 5FC (115, 116) are detectable in both the aqueous and vitreous humors of animal and/or human eyes, with and without endophthalmitis, at concentrations approximately 40% to 100% of those observed in serum. Although the use of 5FC is now uncommon, both triazoles are employed quite extensively for treating fungal opthalmic infections in humans (109, 117). The visual adverse events experienced by some patients receiving systemic voriconazole are related to plasma exposure (258) but not yet to retinal concentrations per se. These adverse events, which have been ascribed to inhibition of the B wave of ‘ON’ bipolar cells in the retina (118), do not appear to result in long-term adverse effects or toxicity (119).

Penetration of itraconazole into the eyes of rabbits after a single oral dose is minimal (120). No drug is detectable (using bioassay) in the aqueous or vitreous of uninflamed eyes, with only 0.3 μg/ml observed in the cornea, despite plasma concentrations of more than 10 times this value. With inflamed eyes, concentrations in the aqueous and vitreous are still 4- and 10-fold lower, respectively, than those in the plasma, while in the cornea they are low and unchanged relative to those in uninflamed eyes. Despite these results, itraconazole is as efficacious as ketoconazole and fluconazole against Candida albicans endophthalmitis in vivo when therapy is initiated within 24 h of infection (120). Similarly, a single patient with C. albicans endophthalmitis was treated successfully with 200 mg/day of itraconazole (capsules) and two vitrectomies (121). This was despite concentrations in the aqueous and vitreous humors that were undetectable and 0.02 μg/ml, respectively, while plasma concentrations were approximately 0.5 μg/ml.Heykants and colleagues (122) have also reported that itraconazole concentrations in human aqueous are usually only 1 to 2 ng/ml.

There are minimal data for posaconazole, but these suggest that it does penetrate into the inflamed eye. In a single patient with Fusarium solani keratitis and opthalmitis, receiving 200 mg orally (p.o.) four times daily plus topical instillation of the oral solution, the aqueous and vitreous/plasma concentration ratios were 0.6 and 0.21, respectively, and therapy was successful (244). Two patients, with rhinofacial and orbital zygomycoses, each
received 0.6 mg/kg intravenous (i.v.) AmBd (123). Penetration of AmBd into both the aqueous and vitreous of the infected eye was higher in the patient with rhinocerebral disease and extensive retinal inflammation (fluid/serum concentration ratio of 0.4) than in the second patient, who had minimal retinal inflammation (ratio of 0.06). Penetration of all formulations of amphotericin B into the eyes of rabbits is also enhanced by inflammation (124, 125, 249). Indeed, amphotericin B is not detected in noninflamed eyes, even after multiple dosing of AmBd, ABLC, or L-AMB (16, 124, 125). Consequently, intracameral injection is the favored delivery route for these agents in patients with severe keratomycosis or endophthalmitis. For AmBd, this may lead to significant local toxicity, which is somewhat ameliorated by lipid formulations (109).

All three echinocandins also show limited penetration into the aqueous and vitreous humors of laboratory animals after systemic administration, with either undetectable or low concentrations relative to those in plasma (100, 108, 109, 126, 127, 128). However, micafungin concentrations specifically in the retina and choroid of the eyes of rabbits range from 0.75 to 15.97 μg/ml and are comparable with the concentrations in plasma (129). As with amphotericin B, inflammation appears to improve the extent of echinocandin penetration (127). Potentially subtherapeutic vitreal penetration of caspofungin has been associated with treatment failure in Candida albicans endophthalmitis (130), and low concentrations of caspofungin were measured in the aqueous of one human endophthalmitis patient (113). Similarly, low micafungin concentrations in the aqueous and vitreous of a C. albicans endophthalmitis patient (0.001% of the simultaneous concentration in plasma) were associated with clinical failure (131), and the drug was also ineffective in a patient with endophthalmitis caused by Candida tropicalis, despite severe inflammation and a MIC of 0.03 μg/ml (132).

Lung
Pulmonary infection begins within the airspace (Fig. 2A). Therefore, for the agents used for prophylaxis or treatment of infection confined to the airspace, concentrations in epithelial lining fluid (ELF) and within pulmonary alveolar macrophages are of direct importance. The inhalation of aerosolized amphotericin B formulations is a potential option for prophylaxis (133, 134, 135). Antifungal drug concentrations within ELF after aerosol inhalation or systemic administration were recently reviewed (12). However, for treatment of established invasive infections, drug concentrations in the lung parenchyma may be more relevant (Fig. 2B and C). Drug concentrations may also be measurable in a number of other respiratory fluids, including bronchial secretions, sputum, pleural fluid, and pulmonary lymph (see below and Fig. 7).

Human studies suggest that 18F-fluconazole distributes rapidly into the lung tissue of volunteers, producing concentrations approximately double those in plasma (71). In 20 patients receiving a single 200-mg dose of fluconazole, the lung tissue/plasma concentration ratio range was 1.1 to 1.6 (136). Similarly, the fluconazole ELF/plasma concentration ratio in cats was 1.2 (137). Fluconazole also readily penetrates the extracellular space of the rat lung (fluid/plasma concentration ratio of 1.38), and this is unaffected by inflammation (138). Itraconazole exhibits ELF exposures that are one-third of the plasma AUC in human volunteers, while the AUC in alveolar cells is more than double that of the plasma (56). In postmortem samples from four hematology patients, the mean lung tissue/plasma concentration ratio of itraconazole was 7 (139), while Heykants and colleagues (73) reported concentrations 0.9 to 2.4 times higher than those in the plasma of four patients. However, itraconazole concentrations in bronchoalveolar lavage (BAL) fluid and airway tissue were 10-fold lower than those in plasma in a patient with allergic bronchopulmonary aspergillosis (ABPA) (140). Itraconazole has been used extensively to treat pulmonary fungal infections.

Postmortem studies show lung tissue homogenate concentrations for voriconazole that are comparable with the plasma concentrations (80, 141). In volunteers receiving an i.v. loading dose on day 1 and then 200 mg of voriconazole p.o. twice a day (b.i.d.), the ELF/plasma concentration ratio was 11 (142). However, in volunteers receiving the same i.v. loading dose on day 1, but followed by three doses of 4 mg/kg i.v. every 12 h (q12h), the ELF/plasma concentration ratio at steady state varied over 12 h from approximately 6 to 9, while for alveolar macrophages the ratio varied from approximately 3.8 to 6.5 (58). Posaconazole exhibits ELF concentrations in humans similar to those seen in the plasma, but the exposure in alveolar cells is over 30 times that in plasma in both volunteers (57) and lung transplant patients (143). It has been suggested that high intracellular posaconazole concentrations may explain its effectiveness for prophylaxis (Fig. 2A) (33). Mean lung tissue concentrations of posaconazole in rabbits have been reported to range from 0.3 μg/ml to 2.1 μg/ml after dosing at 2 to 6 mg/kg (145).

The administration of all formulations of amphotericin B results in quantifiable concentrations in the ELF in both rabbits and humans, but the plasma/ELF concentration ratios appear to differ between formulations and species. The precise state of the amphotericin in these studies is not clear (i.e., free, protein bound, or lipid associated). Furthermore, the biological relevance of the total concentrations associated with each formulation is also unclear. Human data for the various amphotericin formulations suggest that there may be some differences compared with rabbits (146, 147). Thus, intravenous ABLC produces ELF amphotericin B concentrations that are approximately 4 times those produced after administration of L-AMB in humans (147). In 18 patients undergoing thoracotomy and resection for lung cancer, a single dose of 1.5 mg/kg i.v. of L-AMB resulted in hysteresis, such that tissue/plasma concentration ratios were 0.29 and 2.5 at 10 and 25 h postdose, respectively (248). In a postmortem study, lung tissue homogenate concentrations were found to be 3 times higher with a similar dose of ABLC than with L-AMB (90). Similarly, ABLC concentrations in mouse lung homogenates exceeded those for equivalent doses of L-AMB (39). Pulmonary inflammation may increase amphotericin concentrations following administration of L-AMB (148). The amphotericin B formulations remain frontline agents for the therapy of pulmonary fungal infections.

There are no published data for echinocandin concentrations within human lung tissue. However, the concentrations of caspofungin in alveolar macrophages were >5 times the corresponding concentrations in plasma in a single patient (149). Both anidulafungin and micafungin are also accumulated in the alveolar macrophages of volunteers, attaining concentrations approximately 14 and 4 times higher than those in plasma, respectively (58, 62). In 18 lung transplant patients receiving a single 150-mg i.v. micafungin dose, ELF/plasma and alveolar cell/plasma concentration ratios varied with time postdose. Mean ratios ranged from 0.1 to 1.53 at 3 h and from 1.1 to 6.2 at 24 h postdose (62). The vast majority of anidulafungin and micafungin found in the ELF is
present within macrophages rather than in the fluid itself (58, 61, 62). Caspofungin, micafungin, and anidulafungin exhibit lung tissue exposures in rodents that exceed those in plasma by 1.1-fold, 2.8-fold, and 10-fold, respectively (102, 103, 150).

**Pulmonary Lymph Fluid**

There are no human data for antifungal drug concentrations in pulmonary lymph, but Hoeprich and colleagues (151) examined the concentrations of 5FC and AmbD in sheep cannulated via the afferent duct of the right caudal mediastinal lymph node. All drugs tested (also including ketoconazole, the triazole Bay n733, and AmbD methyl ester [AME]) appeared promptly in the lymph after a single intravenous dose, with their concentrations subsequently decaying exponentially. In general, the concentrations of all five drugs in lymph slightly exceeded those in plasma measured shortly after the end of the 30-min infusion period (maximum ratio for lymph to plasma of 1.0 to 1.9), except for AME, where lymphatic concentrations were lower. Koizumi and colleagues (152) also examined AmbD concentrations in sheep lung and lymph after an i.v. infusion. The concentrations in the lymph were similar to (or slightly exceeded) those in the plasma, depending on the duration of the infusion. Given the range of lipophilicities and plasma protein binding of the above antifungal agents, these proper-

**Plurale Fluid**

Data on antifungal drug pleural fluid concentrations are limited (Fig. 7). Voriconazole penetrates into the pleural fluid, producing trough concentrations in humans that are similar to paired plasma concentrations (153, 154). For AmbD, pleural fluid concentrations are approximately 50% of those in plasma (91, 247). However, pleural fluid amphotericin concentrations following the administration of L-AMB or ABCD are approximately 5% to 25% of their plasma exposures (60, 155). Penetration of the echinocandins into pleural fluid appears to be low. Thus, for anidulafungin in one patient with Candida empyema and for three micafungin patients, pleural fluid concentrations were less than 1% and 10%, respectively, of those measured in the plasma (107, 251).

**Bronchial Secretions**

Watkins and colleagues (140) demonstrated, for one patient, that itraconazole accumulates to approximately twice the plasma concentration in bronchial biopsy tissue and is also detectable (at only ng/ml concentrations) in BAL fluid and bronchial washings. However, no allowance was made for the significant dilution factor involved with their sampling methods. They concluded that itraconazole is present in “relatively high” concentrations in pulmonary fluids and tissues. In contrast, amphotericin B was detected, but only briefly postdose and at low concentrations, in the tracheal secretions of humans (91) and the tracheas of dogs following administration of AmbD (91), although penetration may be dose dependent (156). For 5FC, concentrations in dog bronchial secretions are approximately 75% of corresponding plasma concentrations (156).

**Saliva, Sputum, Buccal Mucosa, and Esophagus**

The attainment of effective antifungal drug concentrations within the saliva, sputum, and bronchial fluid is critical for therapy of oropharyngeal, esophageal, and bronchial infections. Fluconazole (67, 157, 158) and itraconazole (73, 159) have both been detected in the saliva and sputum of patients (Fig. 7). Consistent with their physicochemical properties (Table 1), the concentration ratios for fluconazole in saliva and sputum compared with serum are ~1, while for itraconazole they are generally much lower (73) and very variable (159). Itraconazole can also be detected in esophageal tissue, at 3 times the concentration in plasma (160), and in bronchial exudates (73). However, clinical data suggest that fluconazole is superior to itraconazole for treating oropharyngeal and esophageal candidiasis (161, 162). Voriconazole is present in the saliva of volunteers, and concentrations increase over time, using a standard dose. Thus, salivary exposure on day 1 is approximately 25% of that in plasma and increases to 88% of that in plasma with multiple dosing (163). Fluconazole and voriconazole show comparable efficacies in immunocompromised patients with esophageal candidiasis (164). While there are no published data for posaconazole concentrations in saliva, sputum, or mucosal and esophageal tissues, this drug is, as effective as fluconazole in treating HIV patients with oropharyngeal candidiasis (165).

Buccal mucosal concentrations of amphotericin B increase in a dose-dependent manner in humans after L-AMB administration and attain concentrations approximately 7 to 43 times those in plasma (166). A wide range of amphotericin B concentrations were also detectable in esophageal autopsy samples from seven patients after AmBd administration (54).

The concentrations of 5FC in human saliva are slightly lower than those in the plasma, but the 5FC concentrations measured in the bronchial secretions of dogs are comparable to serum concentrations (91).

There are no human or laboratory animal data giving the concentrations of caspofungin or micafungin at these sites. Anidulafungin is present in both the saliva and esophagus in rabbits with oropharyngeal and esophageal candidiasis, but only at concentrations between 1% and 33% of those in plasma (167). However, all three echinocandins show efficacy at the end of therapy equivalent to that of fluconazole after intravenous administration to patients with AIDS and oropharyngeal or esophageal candidiasis (168, 169, 170). There are no data to indicate whether any efficacy differences between fluconazole and the echinocandins seen on longer-term follow-up of these patients are related to residual tissue concentrations.

**Heart**

Fluconazole and voriconazole concentrations in human heart tissue are comparable to those in plasma, based on 18F-NMR studies in healthy volunteers and autopsy data, respectively (71, 80). The pericardial fluid/plasma concentration ratios of fluconazole in 20 patients ranged from 0.9 to 1.0 (136). Data from a single patient with disseminated aspergillosis also suggest that voriconazole diffuses into the pericardial fluid, at a concentration comparable to the plasma concentration (153). Autopsy data also indicate that myocardial voriconazole concentrations are similar to those in other body organs, including the lung and kidney (80). In contrast, itraconazole exposure in the hearts of mice after a single 10-mg/kg i.v. dose is only 8% of that in plasma (171). However, in rats, at 1 h postdose, the concentration is 6 times the level in plasma, and both the absolute concentration and the plasma ratio increase further after 24 h (76). There are no published human heart tissue concentration data for itraconazole. Nevertheless, itraconazole can cause congestive heart failure (172) via negative
inotropic effects, although the precise mechanism is unknown (173).

Postmortem studies of patients following administration of AmBd or L-AMB show a wide range of concentrations (<0.1 to 9.1 \( \mu g/g \)) of amphotericin in heart tissue and myocardium (52, 90). In the hearts of dogs, the AmBd concentration after 14 days of dosing with 0.6 \( \mu g/kg/day \) is approximately 7 times the corresponding plasma value (37), while in rats given a single AmBd dose of 1.0 \( \mu g/kg \), it is approximately 3 times higher (225).

As with fluconazole, the concentration of \(^{18}\)F-5FC in rat heart tissue is similar to that in blood (174).

Caspofungin is detectable in the rodent heart after a single dose, at a concentration approximately 20% of the peak plasma concentration, which then declines at a lower rate than in the plasma (103, 104). In contrast, anidulafungin exposure in the heart tissue obtained at postmortem (52, 54). There is a relationship between the concentration of amphotericin in human livers is approximately 13 times those in plasma 1 h after a single intravenous dose of 1.0 \( \mu g/kg \), it is approximately 3 times higher (225).

Liver

Given its major role in metabolism and clearance, many xenobiotics are likely to achieve higher concentrations in the liver than in the plasma. Twenty minutes after intravenous administration, the concentration of \(^{18}\)F-fluconazole in human livers is approximately 3 times the paired plasma concentration, while in rabbits it is twice that in the plasma (71). Itraconazole also accumulates in the liver (Fig. 7) (122), and it reached a concentration in one patient that was over three times that in plasma (73). However, in the livers of rats, itraconazole achieves concentrations that are approximately 13 times those in plasma 1 h after a single intravenous dose, and this increases further over 24 h (76). The plasma concentration declines 9-fold over this period, resulting in a tissue/plasma concentration ratio exceeding 150 at 24 h postdose (76).

In contrast, the nucleoside \(^{18}\)F-5FC, which is even more polar than fluconazole, attains concentrations in rat livers that are similar to those in plasma (174).

Hepatic concentrations of amphotericin are detectable from tissue obtained at postmortem (52, 54). There is a relationship between the plasma exposure of L-AMB and liver tissue concentrations of amphotericin B in human autopsy samples. After L-AMB dosing, the mean amphotericin B concentration that was achieved was 102 \( \mu g/liver\), but with substantial interpatient variability (90). Amphotericin B has a long residence time in hepatic tissue of mice. Concentrations (measured using bioassay) are detectable 14 days after dosing with L-AMB (38). However, Andes and colleagues (39) have shown that ABLC exhibits lower concentrations in mouse liver homogenates than equivalent doses of AmBd or L-AMB (at least following intraperitoneal i.p. administration).

The exposures of anidulafungin and caspofungin in the livers of rodents are raised approximately 10- and 16-fold, respectively, compared with plasma concentrations (102, 103). This is largely related to delayed clearance from the liver. However, micafungin appears to behave differently, with a lower peak concentration in the livers of rats and an AUC that is similar to that of the plasma (150). For caspofungin, specific hepatic transporters that mediate uptake into rat liver have been identified (26).

Kidney

Approximately 80% of a fluconazole dose is eliminated as unchanged drug in the urine. Consequently, urinary concentrations are approximately 10 times those in human plasma (Fig. 7) (67). Fluconazole also readily penetrates kidney tissue, with peak tissue concentrations of \(^{18}\)F-fluconazole that are approximately 4 times the peak in human plasma (71). Similar to fluconazole, voriconazole is largely excreted via the urine (78%) and feces in humans, but mostly as metabolites, with less than 2% excreted as unchanged drug (84). Postmortem studies of eight patients showed that voriconazole was detectable in kidney tissue, at a mean concentration of 6.47 \( \mu g/g \), but with significant interindividual variability (80). In contrast to fluconazole, itraconazole concentrations in urine are very low due to its negligible renal excretion (122).

The kidneys are a primary site of toxicity for all polyenes. Postmortem studies show that amphotericin B (from AmBd or L-AMB) is readily detectable in kidney tissue (52–54, 90). The renal concentration of amphotericin B in rat kidneys after AmBd administration is 10 times that in the serum, while the corresponding renal concentration after L-AMB administration is one-third that of AmBd and only 4 times the serum concentration (177). This is consistent with the reduction in amphotericin B-associated renal toxicity after its administration as L-AMB (or other lipid amphotericin formulations) rather than AmBd (178). The clearance of amphotericin B from the kidneys of rodents is prolonged, and the drug is detectable for at least 48 h after a single administration of AmBd (177) and at least 14 days after a prolonged course of L-AMB (38). In mouse kidney homogenates, concentrations of amphotericin B following administration of L-AMB or ABLC at a dose of 80 mg/kg i.p. are comparable to those observed with 20 mg/kg i.p. of AmBd (39).

Like fluconazole, 5FC is principally eliminated in the urine as unchanged drug (97%), and plasma clearance is closely related to creatinine clearance (91, 179). The concentration of \(^{18}\)F-5FC in rat kidneys is 3 times that in blood 2 h after dosing, with very high concentrations (60 times the plasma concentration) in the urine (174).

All three echinocandins readily penetrate into the kidney tissue of laboratory animals. After a single dose, \(^{13}\)C-anidulafungin exposure in rat kidney tissue is approximately 10 times that in plasma (102). In addition, anidulafungin exhibits an extended residence time in the kidney, with a terminal half-life that is twice that in plasma (102). Anidulafungin also accumulates in rabbit kidneys after multiple dosing (100). After a single dose administered to mice, caspofungin exhibits a longer mean residence time in the kidneys (31) and has a tissue/plasma concentration ratio over 24 h of approximately 7 (103). In contrast, micafungin concentrations in rat kidneys exceed those in plasma 5 min after dosing, by 1.6-fold, but then decline in parallel with plasma concentrations (150). All three echinocandins exhibit low concentrations (<2% of the dose) (104, 181, 182) of unchanged drug in human urine. There are reported cases of the efficacy of the echinocandins in patients with candiduria (183, 184), but this may reflect the attainment of high concentrations in renal parenchyma.

Spleen

Fluconazole penetrates into the spleen in both humans and rabbits, although to different extents (71, 185, 213). Higher concen-
trations of 18F-fluconazole are seen in human spleens than in any other organ, with a tissue/blood concentration ratio of approximately 6 (Fig. 7). However, in rabbit spleens, concentrations are similar to those in the blood (71, 213) but slightly less than those in the plasma (185). Human data are limited for itraconazole, but splenic concentrations in two patients were 2- to 3-fold higher than the plasma concentrations (73, 186). However, a study in rats showed a progressive accumulation of drug in the spleen over the dosing interval, to approximately 10 times the plasma concentration (76). In contrast, mice receiving itraconazole at 20 mg/kg i.v. had splenic concentrations at 5 hours postdose that were 3 times those in the plasma, but they were similar to the plasma concentrations by 24 h (187). There are no laboratory animal data for voriconazole, but it is detectable in human splenic tissue at postmortem (80, 141). The splenic concentration (mean, 5.6 μg/g) is similar to the plasma steady-state concentrations in volunteers receiving 200 mg b.i.d. p.o. (188).

Human postmortem studies indicate that AmBd and L-AMB are detectable in the spleen at concentrations exceeding those of all other organs except the liver (52–54). Furthermore, tissue concentrations are dose dependent (53, 90). In dogs receiving 0.6 mg/kg/day of AmBd for 14 days, splenic concentrations are >160 times those in the plasma (37). After multiple dosing to mice, the splenic concentrations of amphotericin B derived from the three formulations are in the rank order ABLC > L-AMB > AmBd (35, 189).

There is little published information for 5FC, but concentrations of 18F-5FC in rat spleens are similar to those in blood (174). There are no human data for the echinocandins, but all three agents are detectable in the spleens of laboratory animals (127). In the rat spleen, anidulafungin exposure is 10 times greater than that in plasma after a single dose of 5 mg/kg, and peak splenic concentrations exceed those measured in rabbits following multiple dosing at 10 mg/kg (100, 102). In contrast, the tissue/plasma concentration ratio of caspofungin is only ~1 after a single dose in mice (103) or rats (104). Micafungin concentrations in rabbit spleens are also similar to those in plasma, even after multiple dosing (127).

Pancreas

Pancreatic antifungal drug concentrations are rarely reported for laboratory animals or humans. The most comprehensive data are for fluconazole, where 15 patients undergoing pancreatic surgery received a single fluconazole i.v. infusion of 400 mg (190). Pancreatic tissue concentrations increased for up to 2 h postdose, and the mean tissue/plasma concentration ratio at the time that tissue was sampled was approximately 1.0 (Fig. 7). Fluconazole penetration into pancreatic pseudocysts is slow, and concentrations attained in two cysts were lower than those in the plasma, at 0.4 and 0.8 times the plasma concentrations (190). Fluconazole concentrations in rat pancreatic tissue are similar to those in humans, with concentrations approximately 88% to 91% of those in plasma (190).

Penetration of AmBd into human pancreatic tissues has been demonstrated only in autopsy samples. Tissue concentrations are highly variable, ranging from <0.1 to 18.6 μg/g (52).

There are no data for caspofungin or anidulafungin, but a micafungin pancreatic pseudocyst fluid concentration of 0.38 μg/ml was recorded for a single patient 24 h after a prior dose (106).

Peritonem

Intra-abdominal fungal infections are difficult to treat, particularly in patients requiring peritoneal dialysis (191). Fluconazole, 5FC, and amphotericin B are typically used as primary therapy, although limited experience in patients suggests that voriconazole, posaconazole, caspofungin, and micafungin could also be used for treating fungal peritoneal infection.

The polar agents fluconazole and 5FC achieve peritoneal concentrations after i.v. administration to uninfected laboratory animals of approximately 100% and 50% of those in serum, respectively (91, 192). Furthermore, in adults or children undergoing peritoneal dialysis, dialysate concentrations of fluconazole (following systemic administration) are similar to or exceed those in the plasma (193, 194). Limited clinical data suggest that the peritoneal concentrations of 5FC in humans are approximately 65% to 100% of those in serum (195, 196).

Five patients receiving voriconazole for peritonitis complicating peritoneal dialysis had concentrations in the peritoneal dialysate that were approximately 50% of those in the plasma after a single oral voriconazole dose (197).

The peritoneal concentrations of amphotericin B following AmBd administration are variable and less than 50% of serum concentrations (91, 195, 198) and, on occasion, are undetectable (196). Weiler and colleagues (199) have demonstrated that similar amphotericin B ascitic fluid concentrations are attained following administration of either L-AMB or ABLC for 7 to 13 days at 3 to 5 mg/kg/day.

A single patient receiving micafungin had a concentration in ascitic fluid of 1.02 μg/ml, giving an ascites/plasma concentration ratio of 0.15 (107).

Genital System

Fungal infections of the genital system, particularly vaginal candidiasis, are some of the most commonly experienced fungal infections of humans. Fluconazole is used extensively for treating urgenital infections caused by Candida spp. Consequently, there is a relative abundance of clinical data related to the concentrations of fluconazole within gynecological tissues and secretions (67, 200, 201), testicular (71) and prostatic (71, 202) tissues, and prostatic fluids (203) (Fig. 7). In the vagina and its secretions and in other gynecological tissues, the fluconazole tissue or secretion/plasma concentration ratio is at least 1. The tissue/plasma concentration ratio of fluconazole in the testicles of volunteers receiving 18F-fluconazole (71) is also ~1. However, in the prostate, which is a sanctuary site, the ratios range from 0.3 in prostatic hyperplasia patients (202) to 2.0 in volunteers (71). In the prostatic fluid of patients with AIDS and cryptococcal meningitis, the fluconazole fluid/plasma concentration ratio range is 0.6 to 0.9 (203). The human data for itraconazole indicate that its concentrations in vaginal and other gynecological tissues and in cervical mucus are between 1.6 and 20 times those in plasma but that the vaginal fluid/plasma concentration ratio is <0.5 (73, 122). There are no published human or animal data for the other antifungal agents following systemic administration.

Bone

The concentration of 18F-fluconazole in bone is approximately 33% of the plasma concentration in humans and 100% in rabbits (Fig. 7) (71). After two i.v. doses, fluconazole is also detectable in the nucleus pulposus of the rabbit spine, but with a very wide
concentration range (0 to 63.5 µg/g) that is apparently unrelated to concentrations in plasma (204). In three patients, fluconazole synovial fluid concentrations were 0.88 to 1.0 times those in plasma (136, 205, 206). Similarly, the mean synovial fluid/plasma concentration ratio of fluconazole after 10 days of dosing to horses was 0.5 (207). Itraconazole may accumulate in bone, and the bone/plasma concentration ratio in a single patient was 4.7 (73). Voriconazole is detectable in human medullary and cortical bone, with especially high concentrations (approximately 5 times the plasma concentration) in the former (208). The concentration of voriconazole in synovial fluid from a single patient was approximately one-third the plasma concentration (208), while in horses, the mean voriconazole synovial fluid/plasma concentration ratio was 0.6 (209).

Amphotericin concentrations are high in the bone marrow of dogs and rabbits following administration of any of the currently available formulations (37, 210). Certainly the administration of amphotericin B in lipid formulations is considered an example of drug targeting, with particular respect to the kidneys and bone marrow (36). The lowest bone marrow concentrations are observed following administration of AmBd, but concentrations are still approximately 5 times those in plasma (37). In human synovial fluid, the measured AmBd fluid/plasma concentration ratio is approximately 0.4 (91), while in a single neonate with C. albicans osteoarthritis, the synovial fluid/plasma amphotericin concentration ratio of a random sample following 35 days of AmBd and 10 days of L-AMB was 1.4 (211).

There are limited data available on bone and synovial fluid concentrations of 5FC in humans and animals. Polak (91) reported bone and synovial fluid concentrations of 30% and 41% of those in plasma, respectively. However, in a premature infant with Candida arthritis, the synovial fluid concentration was approximately 83% of that in the plasma (212). In rats given 18F-5FC, bone and blood concentrations are comparable (174).

Anidulafungin concentrations in the bone of neonatal rats after a single dose are less than those in plasma, with a bone/plasma concentration ratio of 0.21 (175). No data are available for caspofungin or micafungin, although these drugs have been used to treat a few patients with bone/joint infections, in combination with AmBd or a triazole.

Muscle

The concentrations of 18F-fluconazole in human skeletal muscle are similar to those in the myocardium, both of which have a concentration ratio to blood of 1.8 (71). However, in rats and rabbits, the ratio is somewhat lower (0.58 to 0.74) (138, 185, 213). In contrast, itraconazole accumulates in skeletal muscle relative to plasma, attaining a muscle/tissue concentration ratio of 2.4 in a single patient (73) but one of over 7 in rats (76) (Fig. 7).

In human autopsy samples, skeletal muscle concentrations of amphotericin following the administration of AmBd ranged from 0 to 1.2 µg/g and were lower than those in any other tissue (54). In the rat, concentrations of amphotericin B in muscle (0.21 to 0.27 µg/g) were also lower than those in other tissues but were still approximately 10-fold higher than plasma concentrations following multiple dosages of AmBd (214). Simultaneously collected heart muscle tissue concentrations were approximately 20-fold higher than those in plasma. In autopsy samples from patients receiving L-AMB, the mean myocardial amphotericin concentration was 3.18 µg/g (90).

There are no human data for 5FC, but in rats receiving 18F-5FC, the skeletal muscle/blood concentration ratio is 1.1 (174). Human data are also lacking for the echinocandins. However, skeletal muscle concentrations of anidulafungin in rats are comparable to those in plasma (102), whereas for caspofungin, skeletal muscle concentrations in mice are less than 50% of those in plasma (103, 104).

Skin and Nails

The prolonged exposure of antifungal agents within the skin, nail, and nail bed is an important factor determining the outcome of treatment of dermatomycosis (215). Fluconazole concentrations within the dermis are similar to those in plasma (216, 217), but concentrations in the stratum corneum are up to 40 times those in plasma (217, 218) (Fig. 7). The clearance of fluconazole from the stratum corneum is significantly slower than that from the plasma and other skin layers, with concentrations that decline 2 to 3 times more slowly than the plasma concentrations (215, 217, 218). Interestingly, once-weekly oral dosing of 150 mg for 2 weeks results in higher fluconazole concentrations in the stratum corneum relative to those in the epidermis/dermis, sweat, and serum than those obtained by daily dosing at 50 mg for 12 days (217). In fingernails, fluconazole concentrations are dose proportional and, at steady state, are approximately twice those in the plasma. Fluconazole is also detectable in nails up to 4 months after cessation of therapy (219). Slow clearance from both skin and nails is also seen for itraconazole. It binds tightly in the stratum corneum and does not readily distribute back to the plasma compartment (215, 220). The drug also accumulates in sebum. Consequently, those areas of skin with active sebaceous glands contain higher concentrations of itraconazole (e.g., the back, with twice the plasma concentration) than those that do not (e.g., the palm, with less than the plasma concentration) (122). Concentrations of itraconazole in blister fluid increase more slowly than those in the plasma, attaining a maximal concentration approximately 0.7 times that in the plasma (221). Itraconazole also has a very long residence time in nails after the cessation of therapy (122). Maximal concentrations of itraconazole in fingernails and toenails are 0.95 µg/g and 1.5 µg/g, respectively, 4 and 6 months after cessation of pulse therapy (222). The concentration of posaconazole within the human dermis is comparable to that in plasma (59). However, in toenails, its concentration is both dose and time dependent, attaining a maximum approximately 3 times greater than that in plasma after 24 weeks of therapy (223). There are no human data for voriconazole, but in guinea pigs, voriconazole skin concentrations are approximately twice those in blood, while in skin microdialytes, the voriconazole concentrations are only 50% of those in blood (224). Patients receiving voriconazole therapy have been shown to suffer from significant phototoxicity on exposure to sunlight, although a relationship to the voriconazole concentration or retinol levels in skin remains to be established (254). In a few patients, long-term voriconazole exposure may result in skin cancer (255, 256).

AmBd skin concentrations in rats receiving a single intravenous dose of 1.0 mg/kg are approximately 30% to 50% of those in plasma and decrease with time in parallel with the plasma concentrations (225). Laboratory animal studies show that clearance of anidulafungin and caspofungin from rat skin is delayed compared to that from plasma, but these drugs never attain the peak concentrations mea-

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sured in plasma (102, 104). After a single i.v. dose of anidulafungin, peak skin concentrations are approximately 80% of those in plasma, while for caspofungin, skin concentrations in rats peak at some 2 h postdose, but with a skin/plasma concentration ratio of only 0.3. However, caspofungin clearance from the skin is such that by 288 h postdose, residual skin concentrations, while only 15% of their peak, are 4 times those remaining in the plasma (104).

UNDERSTANDING TISSUE CONCENTRATIONS FOR OPTIMAL USE OF EXISTING AGENTS AND DEVELOPMENT OF NEWER ANTIFUNGAL AGENTS

Current State of the Art

This review provides a summary of tissue concentration data for key antifungal drugs in humans and some animals. While there is a sizeable body of literature on this topic, many of the data are of variable quality, and the implications for the clinical care of patients with invasive fungal infections are frequently unclear. In addition, the human data are almost exclusively from adults, meaning that the implications for young children and neonates remain uncertain (65). The interpretation of many studies is further compounded by a multitude of different methodological approaches. Nevertheless, the following general conclusions seem reasonable.

First, small polar agents with low protein binding (e.g., fluconazole and 5FC) distribute more evenly and into a wider range of tissues than the larger, more lipophilic (itraconazole) or amphipathic (e.g., amphotericin B and echinocandins) agents.

Second, the more lipophilic or amphipathic agents may have longer residence times within tissues and may also accumulate to concentrations that exceed those in the plasma.

Third, agents with relatively low molecular weights, such as fluconazole, 5FC, and voriconazole, penetrate more readily into tissue beds.

Fourth, the formulation may have a significant impact on serum and tissue pharmacokinetics, although the pharmacodynamic implications of these differences frequently remain unclear.

Fifth, the measurable concentration of a drug within a tissue may not necessarily be an indication of its biological activity in that compartment.

Sixth, within a single drug class and with apparently closely related structures, there may be marked differences in tissue distribution (e.g., the triazoles).

Finally, a degree of caution is always advisable in extrapolating data from laboratory animals to humans.

Beyond State of the Art

A detailed understanding of tissue concentrations is an important component of drug development (13). In this regard, the following are worthy of consideration.

First, comodeling both PK and PD data (if possible) provides key insights into the importance of tissue concentrations (64).

Second, single point estimates of tissue concentrations are of relatively limited value. Estimating concentration-time profiles (and thereby calculating the AUC in tissues) is possible using population pharmacokinetic modeling techniques. Relatively few studies have done this for humans to date (56–62), and all but one (59) deal with pulmonary distribution.

Third, designing antifungal regimens that optimize exposure at the site of infection rather than plasma exposure requires further consideration and study but may be pivotal in the design of optimum regimens for new antifungal agents (259).

Fourth, as has long been understood (51), tissue homogenates are not the ideal matrix for estimating tissue concentrations. Non-invasive methods such as magnetic resonance spectroscopy with spectroscopic imaging (e.g., 18F-5FC [260]) or positron emission tomography (e.g., 18F-fluconazole [71, 213]) can be used in laboratory animals or humans. Direct molecular analysis of whole-body animal tissue or isolated organs by matrix-assisted laser desorption ionization (MALDI) mass spectroscopy also represents a promising approach, without the requirement for radiolabeled drug (261).

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Azoles are the mainstay of oral therapy for aspergillosis. Azole resistance in Aspergillus has been reported infrequently. The first resistant isolate in Manchester, UK, was detected in 1999. In a clinical collection of 519 A. fumigatus isolates, the frequency of itraconazole resistance was 5%, a significant increase since 2004 (p<0.001). Of the 34 itraconazole-resistant isolates we studied, 65% (22) were cross-resistant to voriconazole and 74% (25) were cross-resistant to posaconazole. Thirteen of 14 evaluable patients in our study had prior azole exposure; 8 infections failed therapy (progressed), and 5 failed to improve (remained stable). Eighteen amino acid alterations were found in the target enzyme, Cyp51A, of which were novel. A population genetic analysis of microsatellites showed the existence of resistant mutants that evolved from originally susceptible strains, different cyp51A mutations in the same strain, and microalterations in microsatellite repeat number. Azole resistance in A. fumigatus is an emerging problem and may develop during azole therapy.

Invasive aspergillosis in immunosuppressed patients is difficult to diagnose, is problematic to treat, and results in a high mortality rate (1). Chronic and allergic pulmonary and sinus aspergillosis are increasingly recognized in numerous clinical settings. Treatment with itraconazole, voriconazole, and, recently, posaconazole is the backbone of therapy for these conditions because azoles are the only licensed class of oral drugs for treatment of aspergillosis (2,3). Amphotericin B and caspofungin are licensed intravenous agents for invasive aspergillosis but have limited utility for chronic and allergic aspergillosis.

Itraconazole resistance in Aspergillus spp. was first reported in 1997 in 3 clinical isolates obtained from California in the late 1980s (4); since then, only a few clinical cases have been published (5–9). The emergence of itraconazole resistance alone is of concern, but widespread azole cross-resistance would be devastating.

The primary mechanism of resistance described for A. fumigatus clinical isolates is mutation in the target protein. The cyp51A gene encodes the target of azoles, lanosterol 14α-demethylase, and this enzyme catalyzes a step in the biosynthetic pathway of ergosterol (an essential cell membrane component of filamentous fungi). Mutations in the open reading frame of the cyp51A gene can result in structural alterations to the enzyme, which in turn may inhibit binding of drugs. Mutational hotspots confirmed to cause resistance have been characterized in the gene at codons 54 (6,10–13), 220 (6,14,15), and 98 (16–18). Other mutations in the cyp51A gene have been reported, and additional resistance mechanisms have been postulated (11,19,20). The environmental or antifungal pressures driving azole resistance are unclear because few clinical azole-resistant Aspergillus strains have been studied in any detail; many reports simply describe individual patient cases. In this study, we...
investigated the frequency of A. fumigatus itraconazole resistance in a referral laboratory collection, defined the azole cross-resistance pattern, identified mutations in the cyp51A gene, and investigated any epidemiologic links between resistant isolates.

Materials and Methods

Isolates
Isolates deposited in the Regional Mycology Laboratory Manchester (RMLM) culture collection (between 1992 and 2007) were identified as A. fumigatus by macro- and micromorphologic characteristics. All isolates were screened for growth at 50°C, thus confirming A. fumigatus and excluding A. lentulus. Aspergilli were subcultured onto Sabouraud glucose agar (Oxoid, Basingstoke, UK) for 48 h at 37°C. Thirty-four azole-resistant and 5 susceptible isolates from 17 patients were studied from the RMLM collection (prefixed F); 36 isolates were respiratory specimens, 1 was cerebral, and 2 were from unknown sites. In addition, 18 azole-resistant isolates from a single aspergilloma case-patient (prefixed A, patient 3) collected at autopsy were also investigated.

Patients
Pertinent details from patients were extracted from the clinical records. All but 6 were under the care of 1 investigator (D.W.D.). Information was collected on underlying disease(s), type of aspergillosis, antifungal treatment, azole plasma levels, and characteristics of therapeutic failure.

Susceptibility Testing
Susceptibilities were determined by a modified European Committee on Antimicrobial Susceptibility Testing (EUCAST) method (21). The modification was a lower final inoculum concentration (0.5 × 10^4 as opposed to 1–2.5 × 10^4 CFU/mL). Isolates were tested at a final drug concentration range of 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 mg/L against itraconazole (Research Diagnostics Inc, Concord, MA, USA), voriconazole (Pfizer Ltd, Sandwich, UK), posaconazole (Schering-Plough, Kenilworth, NJ, USA), and amphotericin B (Sigma, Poole, UK). RPMI-1640 (Sigma) was supplemented to 2% glucose (Sigma). Inocula were prepared in phosphate-buffered saline with 0.05% Tween 80 (Sigma); Inocula were loaded into flat-bottomed 96-well microtiter plates (Costar Corning, Lowell, MA, USA) and incubated at 37°C for 48 h. A no-growth end point was determined by eye. MIC testing was performed on RMLM isolates in triplicate, and a consensus mean was derived (median or mode). Susceptibilities of the aspergilloma isolates were determined once, except for 6 that were tested 3 times. Values of >8 mg/L were classed as 16.

Clinical or epidemiologic breakpoints/cutoffs have not been declared by the Clinical and Laboratory Standards Institute (CLSI) or EUCAST for azoles and Aspergillus spp. However, proposed epidemiologic cutoff values have been mooted for the latter (22), and we have recently proposed clinical breakpoints (23). Cutoffs used in this study were itraconazole and voriconazole >2 mg/L and posaconazole >0.5 mg/L (we have not defined an intermediate zone of susceptibility).

Sequencing
DNA was extracted by using commercially available kits (FastDNA Kit, Qiagen, Cambridge, UK; Ultraclean Soil DNA Isolation Kit, MO BIO Laboratories Inc., Cambridge; and DNeasy plant tissue kit, Qiagen, Crawley, UK). The entire coding region of the cyp51A gene was amplified as previously described (7), except 3 mmol/L MgCl_2 was used and both strands were sequenced using 8 primers (7). Twelve of the aspergilloma (A) isolates were sequenced with only 1 primer, covering the region of interest in this case. Sequences were aligned against the sequence from an azole-susceptible strain (GenBank accession no. AF338659), and mismatches were identified by using AlignX (VectorNTI, Invitrogen, Paisley, UK). Mutations were confirmed by repeating the PCR and sequencing both strands by using the closest 2 primers. Isolates with an alteration in the cyp51A gene at codon 98 were also investigated for promoter modifications by sequencing this region (17). GenBank accession numbers for the cyp51A sequences determined in this study are EU807919–EU807922 and FJ548859–FJ548890.

Microsatellite Typing
Six microsatellite loci (3A, 3B, 3C, 4A, 4B, 4C) were amplified as previously described (24). Initially some amplicons were sequenced, whereas later ones were sized by using capillary electrophoresis on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Warrington, UK). Electrophoresis data were analyzed by using Peak Scanner Software version 1.0 (Applied Biosystems); amplicon sizes were adjusted by using a correction factor derived from sequenced alleles to determine the actual sizes of alleles (25). Concatenated multilocus microsatellite genotypes were created for each isolate and used to generate allele-sharing genetic distance matrices, D_A. Here, D_A = 1 – (the total number of shared alleles at all loci / n), where n is the total number of loci compared (26). Subsequently, phylogenetic comparisons using 5 of the loci (not 3B) were performed with the software PAUP* 4.0 (www.palau.csit.fsu.edu) by using the neighbor-joining algorithm with the minimum-evolution option active. The strength of support for relationships was assessed by using 1,000 bootstrap resamples of the dataset.

Azole Resistance in A. fumigatus
Results

Susceptibility

The susceptibility of 519 A. fumigatus RMLM culture collection isolates was determined. All isolates were tested for susceptibility against itraconazole and amphotericin B; 456 and 118 isolates were also tested against voriconazole and posaconazole, respectively. Subsequently, all itraconazole-resistant isolates were tested against voriconazole and posaconazole. Geometric means, ranges, MIC50 (median MIC), and MIC90 (90% of the isolates tested had a MIC at or below this level) values are shown in Table 1. Amphotericin B susceptibility was retained in the 34 itraconazole-resistant isolates tested. Of these, 65% (22) were cross-resistant to voriconazole and 74% (25) were cross-resistant to posaconazole. We did not identify any isolates that were resistant to voriconazole or posaconazole while remaining susceptible to itraconazole.

Five percent of 400 isolates were resistant to itraconazole (when duplicate isolates from the same patient with similar susceptibility profiles were removed from the analysis). The overall frequency of itraconazole resistance in this collection (with repeat specimens included) was 7% (n = 519). The first case ofazole resistance in this collection was seen in 1999. The frequency of resistance since 2004 (8%) has increased significantly (Fisher exact test, p < 0.001), compared with the period prior to 2004 (Figure 1).

Azole Exposure in Patients with Azole-Resistant Isolates and Response to Therapy

Of the 17 patients identified for respective review, limited data were available for 3 patients. Of the remaining 14 patients with antifungal data (Table 2), azole exposure of 1–30 months before the identification of the first resistant isolate was evident for all except patient 7. Thirteen patients received itraconazole as initial therapy, and 12 of these patients had at least 1 therapeutic concentration of itraconazole (>5.0 mg/L) documented at steady state during their treatment course (online expanded version of Table 2, available from www.cdc.gov/EID/content/15/7/1068-T2.htm). The infection in patient 1 (treated with voriconazole only for 18 months) failed therapy, and the 1 isolate identified had MICs of >8 mg/L for both itraconazole and voriconazole.

Of the 14 patients with available data, 2 had invasive disease; 9 had chronic diseases with ≥1 aspergillomas; 2 had allergic bronchopulmonary aspergillosis; and 1 had Asper- gillus bronchitis. At least 5 of the patients died of progressive infection, despite alternative therapies for some.

Mutations in the cyp51A Gene

A summary of Cyp51A amino acid substitutions and azole cross-resistance patterns identified in 34 resistant isolates from our clinical culture collection is shown in Table 3 and listed by line in the online Appendix Table (available from www.cdc.gov/EID/content/15/7/1068-appT.htm). The sequences of all 5 azole-susceptible isolates examined were identical to that of a previously published cyp51A gene sequence from an azole-susceptible isolate (AF338659). No cyp51A mutations were found in 3 itraconazole-resistant isolates (from 3 patients). In addition to the L98H substitution, 2 isolates from 2 patients had a 34-bp sequence that was duplicated in the promoter region (16,17) of the cyp51A gene. One isolate had 2 amino acid substitutions, H147Y and G448S. Three isolates from 2 patients had the same 6 mutations, 3 nonsynonymous ones (F46Y, M172V, E427K), along with 3 synonymous (silent) alterations at codons 89, 358, and 454 (data not shown), and an isolate from a third patient had additional mutations (N248T, D255E) as well as these 6. Four novel mutations were found (H147Y, P216L, Y431C, and G434C). The isolate bearing the P216L mutation was resistant to itraconazole and posaconazole, where-

Table 1. MICs for 519 Aspergillus fumigatus isolates from RMLM culture collection, 1992–2007*

<table>
<thead>
<tr>
<th>Isolate group (no. isolates)</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM (range)</td>
<td>MIC50/</td>
<td>GM (range)</td>
<td>MIC50/</td>
</tr>
<tr>
<td>RMLM collection (519)</td>
<td>0.46/&lt;0.015–&gt;8</td>
<td>0.25/0.92</td>
<td>0.92/1</td>
<td>0.22/0.03–&gt;8</td>
</tr>
<tr>
<td>Azole resistant (34)</td>
<td>16.0/&gt;8</td>
<td>3.69/&gt;8</td>
<td>1.70/&gt;8</td>
<td>0.22/0.06–0.5</td>
</tr>
<tr>
<td>Percentage resistant</td>
<td>100% 65%</td>
<td></td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>Aspergilloma (18)</td>
<td>16.0/&gt;8</td>
<td>2.16/&gt;4</td>
<td>1.92/&gt;8</td>
<td>0.125/0.125</td>
</tr>
</tbody>
</table>

*pRMLM, Regional Mycology Laboratory Manchester; GM, geometric mean. Values >8 mg/L were classed as 16 mg/L for GM analysis. See also the online Appendix Table, available from www.cdc.gov/EID/content/15/7/1068-appT.htm.
†n = 28.
‡n = 6.
4 patients had more than 1 susceptible/resistant pair, whereas an overlapping group of isolates from 5 patients consisted of a 4, 5, 6, 8, 9, and 13 were compared by microsatellite typing.

At autopsy. All isolates were resistant to itraconazole (>8 mg/L), and 1 of 2 different mutations at codon 220 was detected in the clinical isolates; statistically supported clustering is not evident. Therefore, none of the azole-resistant isolates have been transmitted from patient to patient, indicating that they have all evolved independently from different original strains. The only statistically supported clades contain isolates that only differ from each other by 1 of the 5 markers.

Discussion

Itraconazole resistance and azole cross-resistance in Aspergillus spp. have been reported infrequently, which suggests that they are infrequent events to date. A contributing factor to this low prevalence has been variability in testing between laboratories. Since the initial report of resistance in isolates collected before the licensure of itraconazole, substantial improvements in susceptibility testing methods that allow confidence in reported azole MICs have been implemented. Recommended methods are now promulgated by the CLSI method M38-A2 (27) and EUCAST (21), and work is ongoing to establish internationally agreed interpretative cutoffs (22) and clinical breakpoints (23).

By using such methods, some researchers have documented and published the frequency of itraconazole resistance in clinical Aspergillus fumigatus isolates (8,28–32); frequency ranged between 2% and 6%. However, most of these studies included fewer isolates (<200) than our study (519) and covered the pre-2004 era. The frequency of itraconazole resistance in our collection before 2004 was 1%; since 2004, however, it has been remarkably high at 8%. The high frequency probably reflects, at least in part, the special referral base for patients with chronic and allergic aspergillosis at our center, although there has been no material change in catchment area in the past decade. Referral numbers are rising, however, and susceptibility testing of isolates of patients receiving therapy has been more frequent since 2003.

Another remarkable aspect of this study is the diversity of cyp51A mutations. Both previously published and novel alterations were identified in our resistant isolates (Table 3). In contrast, a recent series of 32 itraconazole-resistant isolates from the Netherlands was published; 94% had the same 2 alterations: an L98H-aa substitution in Cyp51A, in combination with a duplication in the promoter region (32). This combination of mutations was found in 2 of our isolates from 2 patients.

Several authors have identified hot-spot regions associated with resistance in clinical isolates at codons 54 (6,10–13,22), 98 (16–18,22,32,33), and 220 (6,14,15,22,32) in the cyp51A gene. We previously reported an alteration at codon 138 (G138C) in multiple isolates from 1 patient (7). A single clinical isolate with a mutation at codon 448 (G to S) has also been previously reported (34). In addition, G138R and G448S mutants have been generated in the laboratory. Isolates with these alterations are panazole-resistant, as the isolates with Y431C and G434C showed pan-azole resistance phenotypes.

Patient 3 had 2 respiratory samples taken while she was alive, in addition to 18 aspergilloma isolates sampled at autopsy. All isolates were resistant to itraconazole (>8 mg/L), and 1 of 2 different mutations at codon 220 was detected in the cyp51A gene. Isolates with a methionine-to-lysine substitution were highly cross-resistant to voriconazole (4 mg/L) and posaconazole (>8 mg/L), whereas those with an alteration to threonine had variable voriconazole (0.5–4 mg/L) and posaconazole (0.125–1 mg/L) MICs.

Aspergillus fumigatus isolates

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Multiple isolates from 5 of 7 patients had identical or nearly identical genotypes. The isolates from 2 of these 5 patients (3 and 6) differed by 1 and 2 trinucleotide repeat units, respectively, at the most polymorphic locus (3A). Three matched sets (isolates pre- and postdevelopment of resistance) were identified, where resistance almost certainly evolved from an originally susceptible strain.

Figure 2 shows an unrooted tree of the phylogenetic relationships, derived from 5 of the 6 microsatellite markers, for the isolates from these 7 patients plus 18 A. fumigatus isolate controls. Only bootstrap values >90 are shown. Strains from these 7 patients are distributed among other

Figure 1. Azole resistance in clinical Aspergillus fumigatus isolates received in the Regional Mycology Laboratory Manchester, UK, 1997–2007. Overall azole resistance for each year is shown above each column as a percentage. Data do not include sequential isolates from the same patient.

Microsatellite Typing

The relatedness of isolates obtained from patients 3, 4, 5, 6, 8, 9, and 13 were compared by microsatellite typing (Figure 2). The isolates from 5 patients consisted of a susceptible/resistant pair, whereas an overlapping group of 4 patients had more than 1 cyp51A mutation. All isolates were from the lower respiratory tract, except the resistant isolate from patient 5, which was from a cerebral lesion.

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laboratory and were azole resistant (35). Mutations in codons 46, 172, 248, 255, and 427 have been found in azole-susceptible strains by us (A. Albarrag, unpub. data) and others (22) and so are not associated with resistance. The resistant isolates with these mutations must therefore have another resistance mechanism. Four novel cyp51A

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Location</th>
<th>No. isolates</th>
<th>Aspergillus disease</th>
<th>Other diseases, y</th>
<th>Treatment, duration</th>
<th>Serum azole levels, mg/L†</th>
<th>Outcome/survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cambridge, UK</td>
<td>1</td>
<td>CCPA with aspergilloma</td>
<td>Breast cancer, 1990; M. malmoense pulmonary tuberculosis, 1999 and 2005</td>
<td>Vori 200–400 mg, 18 mo</td>
<td>ND</td>
<td>Clinical and radiologic failure/alive</td>
</tr>
<tr>
<td>2</td>
<td>Copenhagen, Denmark</td>
<td>1</td>
<td>ABPA</td>
<td>CF, concomitant bacterial colonization with Staphylococcus aureus and Achromobacter</td>
<td>Itra 200 mg, 14 mo (plus previous courses)</td>
<td>ND</td>
<td>Unknown/alive</td>
</tr>
<tr>
<td>3</td>
<td>Manchester, UK</td>
<td>2‡</td>
<td>CCPA with aspergilloma CFPA</td>
<td>Pulmonary TB with residual bilateral UL scarring and LUL cavity, 1986; smoke inhalation, 1989</td>
<td>Itra 400 mg, 90 mo</td>
<td>15.0–26.0§</td>
<td>Clinical failure/died</td>
</tr>
<tr>
<td>4</td>
<td>Manchester, UK</td>
<td>3</td>
<td>CCPA with aspergilloma</td>
<td>COPD, squamous cell carcinoma with LUL segmentectomy, 1992</td>
<td>Itra 400 mg, &gt;2 mo</td>
<td>2.9–11.3</td>
<td>No improvement/died</td>
</tr>
<tr>
<td>5</td>
<td>Montreal, Quebec, Canada</td>
<td>2</td>
<td>Cerebral aspergillosis, 1998 Nov</td>
<td>AML-M2, 1997; RUL lobectomy, 1997; AlloHSCT, 1988; GVHD</td>
<td>Itra 400 mg, 4 mo</td>
<td>ND</td>
<td>Regression of cerebral abscess, IPA with respiratory failure/died</td>
</tr>
<tr>
<td>6</td>
<td>Manchester, UK</td>
<td>2</td>
<td>CCPA with aspergilloma</td>
<td>COPD, M. szulgai pulmonary infection, 2003; celiac disease</td>
<td>Itra 200–400 mg, 1 mo</td>
<td>&lt;0.8 (200 mg), 5.3–7.7 (400 mg)</td>
<td>Clinical failure/died</td>
</tr>
<tr>
<td>7</td>
<td>Manchester, UK</td>
<td>1</td>
<td>Acute invasive pulmonary infection</td>
<td>COPD, possible bronchiectasis</td>
<td>Itra 600–400 mg, 1 mo; vori 400 mg; 12 d</td>
<td>17.0–21.0 (itra)</td>
<td>No improvement, switched to vori, developed toxicity/died without IPA</td>
</tr>
<tr>
<td>8</td>
<td>Northampton, UK</td>
<td>2</td>
<td>ABPA</td>
<td>Bronchiectasis, asthma, AVR, antitrypsin deficiency syndrome; M. xenopi pulmonary infection, 2007</td>
<td>Itra 200–400 mg, 9 mo</td>
<td>0.0–5.2</td>
<td>Initial improvement, then failure/alive</td>
</tr>
<tr>
<td>9</td>
<td>Liverpool, UK</td>
<td>12</td>
<td>CCPA with bilateral aspergilloma, CFPA</td>
<td>Pulmonary sarcoidosis, 1988</td>
<td>Itra 200–400 mg, 30 mo</td>
<td>0.9–10.3</td>
<td>Clinical failure/died</td>
</tr>
<tr>
<td>10</td>
<td>Manchester, UK</td>
<td>2</td>
<td>Aspergillus bronchitis</td>
<td>Bronchiectasis, onychomycosis, 2007; α-1-antitrypsin deficiency</td>
<td>Itra 400 mg pulse, 3 mo</td>
<td>ND</td>
<td>Itra resistance identified, treated with posa/alive</td>
</tr>
<tr>
<td>11</td>
<td>Manchester, UK</td>
<td>2</td>
<td>CCPA with aspergilloma</td>
<td>RUL pneumonia, 2002</td>
<td>Itra 400 mg, 1.5 mo</td>
<td>20.0–25.6</td>
<td>No improvement/alive</td>
</tr>
<tr>
<td>12</td>
<td>Manchester, UK (Malawi origin)</td>
<td>1</td>
<td>CCPA with 2 aspergilloma</td>
<td>Pulmonary TB, 1995; HIV positive, HAART</td>
<td>Itra 400 mg, 18 mo</td>
<td>2.5–8.4</td>
<td>Improvement then progression/alive</td>
</tr>
<tr>
<td>13</td>
<td>Preston, UK</td>
<td>4</td>
<td>CCPA with aspergilloma</td>
<td>COPD, bronchiectasis, M. avium pulmonary infection, 2002 and 2006</td>
<td>Itra 600 mg, 10 mo</td>
<td>2.6–4.5</td>
<td>Progression/alive</td>
</tr>
<tr>
<td>14</td>
<td>Birkenhead, UK</td>
<td>1</td>
<td>CCPA with LUL aspergilloma</td>
<td>Sarcoidosis, COPD, celiac disease; aspergilloma removed as part of left lung transplant, 2007*</td>
<td>Itra 400 mg, 11 mo</td>
<td>13.8–17.8</td>
<td>Unchanged, switched to vori/unknown</td>
</tr>
</tbody>
</table>

*CCPA, chronic cavitary pulmonary aspergillosis; M, Mycobacterium; vori, voriconazole; ND, not determined; ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis; itra, itraconazole; CFPA, chronic fibrosing pulmonary aspergillosis; TB, tuberculosis; UL, upper lobe; LUL, left upper lobe; COPD, chronic obstructive pulmonary disease; AML, acute myeloid leukemia; RUL, right upper lobe; AlloHSCT, allogeneic hematopoietic stem cell transplant; GVHD, graft versus host disease; IPA, invasive pulmonary aspergillosis; AVR, aortic valve replacement; posa, posaconazole; HAART, highly active antiretroviral therapy. An expanded version of this table, showing complete data on all 17 patients, is available online at (www.cdc.gov/EID/content/15/7/1068-T2.htm).
†Determined by bioassay (target range 5–15 mg/L).
‡Plus aspergilloma isolates studied, taken at autopsy.
§Received a generic formulation of itra, resulting in lower concentrations (i.e., 4.6 mg/L) and then probably was noncompliant at end of treatment period.
¶Successfully completed with vori treatment.
mutations, 3 of which were unassociated with any other mutations (in codons 147, 216, 431, and 434), were identified in this series, although their association with resistance remains to be confirmed experimentally. The H147Y substitution is probably unimportant for resistance because it was found with G448S in 1 isolate and the cross-resistance profile of this isolate was identical to an isolate that had only G448S. We did not find any examples of previously reported mutations at codons 297 and 495 (17,32) or 22, 394, 491, and 440 (14) in our collection. Three of our resistant isolates had no mutations in their cyp51A gene, indicating the presence of other resistance mechanisms.

The position and type of amino acid substitution within the Cyp51A protein determines the pattern of azole cross-resistance (Table 3), which is consistent with predicted structural properties of the demethylase enzyme and its interaction with chemically different azole drugs (36). Resistance to itraconazole is usually associated with a reduction in posaconazole susceptibility, predictably because the 2 drugs are structurally similar; they have variably elevated posaconazole MICs compared with wild type isolates (22,30). Many of the isolates reported here reflect this MIC shift. Isolates with alterations at codons 98 (including the duplication in the promoter region), 138, 431, and 434 demonstrated cross-resistance to voriconazole and posaconazole. All isolates with substitutions at codons 54 and 216 remained susceptible to voriconazole. Some isolates in this study showed cross-resistance between itraconazole and voriconazole and not posaconazole, unlike the results in previous reports (22,32). However, this cross-resistance could be because of differing breakpoints; therefore, determination of an internationally agreed cutoff for posaconazole will be necessary to guide clinicians. No difference in amphotericin B MICs was seen in our azole-resistant isolates compared with susceptible ones, although the clinical utility of MIC testing of amphotericin B in Aspergillus spp. is suboptimal.

More than 1 azole-resistant A. fumigatus isolate was obtained from 6 of the 17 patients described. Microsatellite typing demonstrated that the isolates from each patient had evolved from a single original strain, because they were either identical at 6 markers or differed only in the most polymorphic marker. The resistant isolates from 4 patients had different cyp51A mutations. Given that a single colony is picked from the primary isolation plate and referred for susceptibility testing, additional mutants may have been found had multiple colonies been tested. Within this dataset, the chance of 2 isolates being identical by chance alone within a recombining population is infinitesimal given the high allelic variability that we observed (mean number of alleles per locus = 14; p value recovering the same multilocus genotype twice ≈14%). The existence of susceptible and resistant isolates that are genetically identical from 3 patients, and the phylogeny performed on the multiple-resistant isolates from an additional 4 patients, almost certainly demonstrates that the isolates from each patient had evolved from a single original strain, because the recombination of azole resistance occurred in these patients independently and repeatedly from unrelated strains. The presence of genetically identical isolates with different cyp51A codon mutations in 3 patients (and 1 almost identical) suggests that they must have evolved independently from the same original strain, because the resistance mutations are not being accumulated sequentially.
as has been shown to happen in *Candida albicans* (37). The isolates from 2 patients had differing numbers of repeats of microsatellite marker 3A, which is further proof that strains are evolving in the lung. In contrast, Snelders et al. (32) suggested that many of their patients were infected with a primary resistant strain from the environment.

The referral base for these isolates includes a specialized clinical service for the management of aspergillosis. Many of our resistant isolates came from this group, in particular from 9 patients with chronic cavitory pulmonary aspergillosis with ≥1 aspergillomas, which may explain the high frequency of resistance in our center. Because surgery is not an option for most patients with chronic cavitory pulmonary aspergillosis, these patients usually require long-term (if not lifelong) antifungal therapy, under which conditions as we have shown, strains of *A. fumigatus* may evolve resistance. Another contributory explanation could be our systematic application of susceptibility testing of *Aspergillus* spp. isolates in all cases in which treatment is to be given.

In 6 of 10 patients, steady state itraconazole plasma level data were at or above minimum therapeutic levels (i.e., <5 mg/L), as determined by bioassay (38,39). Low plasma levels of itraconazole were attributable to limited bioavailability in some patients, low doses (i.e., 200 mg daily, the standard UK registered dose), drug interactions in patients with concomitant atypical mycobacterial infection, and use of generic itraconazole (40). Low plasma levels of itraconazole, in combination with the high proportion of patients in this study with prior azole exposure (13 out of 14), indicates that resistance primarily emerged during or after azole therapy.

Our observations are of concern on several fronts. We found a sudden rise in the frequency of azole resistance in *A. fumigatus* since 2004, and many isolates showed cross-resistance between all the currently licensed azole options. Clinical data indicate that resistance has occurred during and after azole therapy in all but 1 of these cases. The infections caused by azole-resistant isolates fail therapy or at best do not respond. The molecular epidemiology shows that resistance evolved in infecting strains within the lung, rather than by superinfection with a resistant stain from the environment. Because azoles are the only useful class of oral drugs for aspergillosis (and many other serious filamentous fungal infections), clinical management of these chronically infected cases is therefore problematic. Vigilance is called for to identify azole-resistant aspergilli, and novel classes of oral antifungal would be welcome for those infected with azole-resistant strains.

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References


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The Effect of Therapeutic Drug Monitoring on Safety and Efficacy of Voriconazole in Invasive Fungal Infections: A Randomized Controlled Trial

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1Department of Internal Medicine, and 2Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine, Republic of Korea

Background. Blood levels of voriconazole, a first line therapy for invasive aspergillosis, may correlate with adverse events and treatment response. However, no randomized controlled studies have been conducted to evaluate the clinical utility of routine therapeutic drug monitoring (TDM) of voriconazole. This study aimed to determine whether routine TDM of voriconazole reduces drug adverse events or improves treatment response in invasive fungal infections.

Methods. This was a randomized, assessor-blinded, controlled, single center trial. One hundred ten adult patients were randomly assigned to TDM or non-TDM groups. In the TDM group, voriconazole dosage was adjusted (target range, 1.0–5.5 mg/L) according to the serum trough level measured on the fourth day after initiation of voriconazole. The non-TDM group received a fixed, standard dosage. Voriconazole-related adverse events were monitored, and treatment response was assessed three months after the initiation of therapy.

Results. Baseline characteristics including the CYP2C19 genotype were comparable between the two groups. While the incidence of adverse events was not different between the TDM group and the non-TDM group (both 42%; P = .97), the proportion of voriconazole discontinuation due to adverse events was significantly lower in the TDM group than in the non-TDM group (4% vs 17%; P = .02). A complete or partial response was observed in 81% (30 of 37) of patients in the TDM group compared to 57% (20 of 34) in the non-TDM group (P = .04).

Conclusions. Routine TDM of voriconazole may reduce drug discontinuation due to adverse events and improve the treatment response in invasive fungal infections.

Clinical Trial Registration. NCT00890708.
the same patient [9]. This intra-personal variability along with nonlinear saturable pharmacokinetics of voriconazole in adults may raise the concern that the adjustment of the voriconazole dosage based on TDM at a single time point may result in a suboptimal voriconazole blood level at a later time. We conducted this randomized, controlled trial in order to determine whether routine TDM of voriconazole reduces drug-related adverse events and improves the treatment outcome in invasive fungal infections.

METHODS

Patients
This was a prospective, randomized, assessor-blinded, controlled trial conducted from November 2008 through December 2011 at Seoul National University Hospital, a 1600-bed, tertiary-care teaching hospital in South Korea. Patients aged ≥15 years and within 4 days of beginning intravenous or oral voriconazole for invasive fungal infections or for empirical treatment were enrolled. Exclusion criteria were death, discharge, or transfer before the day of blood sampling for TDM, enrollment in another clinical trial, or declined consent. Invasive fungal infection was defined and classified according to the definitions of the Invasive Fungal Infection Group of the European Organization for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectious Diseases [10]. Neutropenia was defined by a neutrophil count of <500 per cubic millimeter.

Study Design
Patients were randomly assigned to the TDM group or non-TDM group using computerized 1:1 random selection. Patients in both groups initially received the standard dosage of voriconazole, irrespective of the administration route: 2 loading doses of 6 mg/kg every 12 hours, followed by a maintenance dose of 4 mg/kg twice per day.

In the TDM group, blood sampling was done on the fourth day after the initiation of voriconazole. The voriconazole dosage was adjusted 24–48 hours after blood sampling based on the results of TDM. The timing of the TDM blood collection was calculated based on data from the literature so that it coincided with the target trough concentration range (1.0–5.5 mg/L), and was determined to be the fourth day after the initiation of therapy [5, 11]. The dosage was increased by 100% if the trough level was <1.0 mg/L; it was lowered by 50% if the trough level was >5.5 mg/L and there was no drug-related adverse event. If the trough level was >10.0 mg/L or if an adverse event was suspected in a patient with a level >5.5 mg/L, 1 dose was skipped and subsequent doses were reduced by 50%. If the voriconazole dosage or administration route was altered or if the interacting drug was introduced or halted, follow-up TDM was repeated on the fourth day. This adjustment was repeated until the trough level was within the target range.

For the future determination of voriconazole levels in the non-TDM group, we collected and stored blood samples in that group on the same day that blood was collected from the TDM group. The standard dosage of voriconazole was maintained in the non-TDM group. In both groups, CYP2C19 typing and extra blood sampling to check intraindividual variation were performed, but these results were not used to adjust drug dosage.

Patients were followed up to 3 months after starting voriconazole. Discontinuation of voriconazole due to adverse events or treatment failure was decided independently by attending physicians who were blinded to treatment groups and any related information such as blood levels or genotype. The Institutional Review Board of Seoul National University Hospital approved the study protocol. All patients or their legal representatives provided written informed consent before study entry.

Measurement of Voriconazole Level and CYP2C19 Genotyping
The measurement of the voriconazole trough level and CYP2C19 genotyping were performed in the study center by the methods described in our previous publication [12]. In brief, quantitative analysis of voriconazole was performed using high-performance liquid chromatography (1200 series, Agilent Technologies) coupled with tandem mass spectrometry (API3200, Applied Biosystems/MDS Scie). For CYP2C19 genotyping, CYP2C19*2, *3, and *17 allele detection was conducted using TaqMan allelic discrimination assays on an ABI Prism 7500 Sequence Detection System (Applied Biosystems). The CYP2C19 genotype was classified as homozygous extensive metabolizer (*1/*1), heterozygous extensive metabolizer (*1/*2, *1/*3), heterozygous ultra-rapid metabolizer (*1/*17), or poor metabolizer (*2/*2, *2/*3, *3/*3).

Adverse Events and Treatment Response
Adverse events were monitored with a questionnaire for up to 3 months and assessed by investigators blinded to the group allocation and voriconazole level. Adverse events and their relationship with voriconazole were defined according to the criteria of the National Cancer Institute [13]. Grade 3–4 adverse events were considered severe, and a voriconazole-related adverse event was defined as one with a possible or stronger relationship.

Only patients with invasive fungal infection were included in the analysis of the treatment response that occurred 3 months after the initiation of voriconazole; patients treated empirically were excluded [10]. Treatment response was categorized as complete, partial, stable, undetermined, or treatment failure [14]. Complete response indicates resolution of
all clinical signs and symptoms attributable to fungal infection and complete or very nearly complete radiographic resolution. Partial response indicates major improvement or resolution of clinical signs and symptoms attributable to fungal infection and at least a 50% improvement in radiologic findings. A stable response indicates some improvement but <50% radiologic improvement. Undetermined response includes follow-up loss, death for reasons other than fungal infection, and voriconazole discontinuation due to adverse events. Treatment failure includes voriconazole discontinuation due to the progression of fungal infection and death caused by invasive fungal infection. For the analysis of the treatment response, patients with an undetermined response were excluded, and treatment success was defined as complete or partial response.

**Statistical Analysis**

The primary objective of the study was to determine whether routine TDM of voriconazole reduced the incidence of voriconazole-related adverse events in all patients included in the study. The sample size necessary to detect a 3-fold decrease in the incidence of adverse events in the TDM group was calculated. We assumed that the incidence of voriconazole-related adverse events in the control group would be 35% based on previous literature and our experience [3, 15], which was equal to 53 patients in each group being required in order to detect a difference of this magnitude (power, 0.8; type I error, 5%). Considering dropouts, 55 patients were planned for recruitment in each group.

Secondary objectives were to compare the incidence of voriconazole discontinuation due to adverse events in all analyzed patients and to determine the treatment success rate in patients with invasive fungal infection. Subgroup analyses including only CYP2C19 homozygous or heterozygous extensive metabolizers and only patients with an initial voriconazole level >8.0 mg/L were performed.

The χ² test or Fisher’s exact test was used to compare categorical variables including adverse events, drug discontinuation, and treatment success or failure. The Student t test was used to compare voriconazole levels, and time-to-event data was compared by the log-rank test using the Kaplan–Meier method. Statistical analyses and randomization were performed using SPSS (Statistical Package for the Social Sciences) software (ver. 19.0; SPSS Inc.). All tests were 2-tailed. A P value <.05 was considered statistically significant.

**RESULTS**

**Baseline Patient Characteristics**

Of the 153 patients screened for this study, 43 patients were excluded (Figure 1), which left 110 remaining patients that were randomized into the TDM group (n = 55) or non-TDM group (n = 55). Two patients in the non-TDM group withdrew their consent during the study, and thus, 108 patients were ultimately included in the analyses. The mean age was 56 ± 15 years; 31 (29%) patients were female, and all were Korean (Table 1).

**Characteristics of Voriconazole Therapy**

There were no distinct differences in overall therapy characteristics between the TDM group and the non-TDM group (Table 2). The initial trough level of voriconazole was within the target range in 52 (51%) patients (Figure 2A). Of 53 patients in the TDM group with an appropriate voriconazole trough level, 26 (49%) patients had initial levels within the target range. Of 27 patients with an inappropriate trough level, 6 (22%) patients were not given a dose adjustment because of drug discontinuation or death before the dose adjustment, and the other 21 (78%) patients received a dose adjustment; the dosage was decreased in 16 (62%) patients, whereas the dosage was increased in 5 (19%). Finally, the voriconazole trough level reached the target range in 41 (77%) patients. When the target range was validated with our own data, a therapeutic range of 2–5.5 mg/L seemed more appropriate (see Supplementary Material).

Phenobarbital was coadministered with voriconazole in 1 patient in the TDM group. Other drugs, including omeprazole, which is known to have substantial interactions with voriconazole, were not administered. To evaluate the intra-individual variation, follow-up blood sampling was performed in 31 patients with voriconazole levels within the therapeutic range. The median intraindividual difference was 1.05 mg/L (range, 0.04–3.45 mg/L).

**Adverse Events**

There was no significant difference in the incidence of adverse events between the TDM and non-TDM groups (Table 3). Visual disturbance or encephalopathy could be evaluated in only 92 (85%) patients who were communicable.

Voriconazole was discontinued due to adverse events in 2 (4%) patients in the TDM group, which was significantly less than the 9 (17%) in the non-TDM group (P = .02). The most common adverse event causing drug discontinuation was hepatic enzyme elevation (n = 5) followed by arrhythmia (n = 1), rash (n = 1), agitation (n = 1), confusion (n = 1), hyponatremia (n = 1), and visual hallucination (n = 1). The median time from treatment initiation with voriconazole to the development of the adverse event was 5 days (range, 0–16) in the TDM group and 3 days (range, 0–16) in the non-TDM group (P = .86; Figure 3).

**Treatment Response**

Of 108 patients, overall mortality was 11 (20%) in the TDM group and 18 (34%) in the non-TDM group at 6 weeks after the
initiation of voriconazole therapy and 13 (24%) in the TDM group and 21 (40%) in the non-TDM group at 12 weeks after treatment initiation ($P = .14$). Of 94 patients with invasive fungal infections, 23 (24%) were classified as having undetermined response, 11 (12%) patients discontinued voriconazole due to adverse events, 10 (11%) died due to causes other than fungal infection, and 2 (2%) were lost to follow-up.

Treatment success in the TDM group was significantly greater than in the non-TDM group (Table 4). When only probable or proven fungal infections were included, treatment success was observed in 86% (25 of 29) of patients in the TDM group and in 63% (20 of 32) in the non-TDM group ($P = .04$), and treatment failure was more prevalent in the non-TDM group than in the TDM group (31% vs 10%, respectively; $P = .04$).

Among patients included in treatment response analyses, the final voriconazole level was available in 35 patients in the TDM group and 32 patients in the non-TDM group (Figure 2B). The mean final trough level of voriconazole was $3.2 \pm 2.1$ mg/L in the TDM group and $4.3 \pm 3.1$ mg/L in the non-TDM group ($P = .10$). Treatment failure was observed in 3 (60%) of 5 patients who had a final voriconazole trough level that was <1 mg/L and in 8 (57%) of 14 patients who had a level >5.5 mg/L.

**Subgroup Analyses**

In the subgroup analyses including only CYP2C19 homozygous or heterozygous extensive metabolizers, voriconazole-related adverse events occurred in 47% (20 of 43) of patients in the TDM group and 38% (17 of 45) in the non-TDM group ($P = .41$), and voriconazole was discontinued due to adverse events in 5% (2 of 43) of patients in the TDM group and 18% (8 of 45) in the non-TDM group ($P = .09$). Treatment success occurred in 82% (22 of 27) of the patients in the TDM group and 62% (18 of 29) in the non-TDM group ($P = .11$).

Of 16 patients with an initial voriconazole level >8.0 mg/L, 6 (40%) were heterozygous extensive metabolizers and 4 (27%) were poor metabolizers. In this subgroup, adverse events included severe hepatic enzyme elevation ($n = 1$), hallucination ($n = 2$), and confusion ($n = 2$), and voriconazole treatment was halted in only 1 patient. In the 5 patients who received a dose adjustment, the voriconazole level reached the
target range in 3 patients after a 50% dose reduction and in 2 patients after a 75% dose reduction. Of 11 patients (excluding 5 who underwent a voriconazole level adjustment), 6 (55%) died; the median time from a toxic level to death was 7.5 days (range, 6–13 days).

**DISCUSSION**

In this randomized, controlled trial, routine TDM of voriconazole did not decrease the overall incidence of voriconazole-related adverse events. However, it did significantly reduce the incidence of voriconazole discontinuation due to adverse events and improve the success rate in the treatment of invasive fungal infections. To the best of our knowledge, this is the first randomized, controlled study to evaluate the utility of routine TDM of voriconazole in the clinical setting. Although previous studies either did not use TDM for intervention or did not have a control group [5–8, 16, 17], our randomized study evaluated the outcomes of clinical practice based on voriconazole TDM in comparison with conventional voriconazole therapy. The baseline patient characteristics illustrate that randomization was successfully performed. Another advantage of our study design is that group assignment and voriconazole levels were blinded to the assessors of adverse events and attending physicians who were responsible for decisions on the duration of voriconazole therapy.

The proportion of CYP2C19 poor metabolizers has been measured and has been shown to be higher in Asians (15%–20%) than in Caucasians (2%–3%) [18]. The proportion of poor metabolizers in the present study was 13%, and the inclusion of only Korean patients may be one reason why the voriconazole concentrations in our study were higher than in previous reports [5, 9]. Another reason is that we used a weight-based dosage rather than a fixed dosage even in the oral form [8, 9, 19, 20]. Despite higher mean levels of

<table>
<thead>
<tr>
<th>Table 1. Baseline Patient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM (n = 55)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
</tr>
<tr>
<td><strong>Sex, female</strong></td>
</tr>
<tr>
<td><strong>Ethnicity, Korean</strong></td>
</tr>
<tr>
<td><strong>Underlying condition</strong></td>
</tr>
<tr>
<td>Hematologic disease</td>
</tr>
<tr>
<td>Steroid use</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Neutropenia</td>
</tr>
<tr>
<td><strong>CYP 2C19 genotype</strong></td>
</tr>
<tr>
<td>Homozygous extensive metabolizer</td>
</tr>
<tr>
<td>Heterozygous extensive metabolizer</td>
</tr>
<tr>
<td>Poor metabolizer</td>
</tr>
<tr>
<td>Heterozygous ultra-rapid metabolizer</td>
</tr>
<tr>
<td><strong>Invasive fungal infection</strong></td>
</tr>
<tr>
<td>Proven</td>
</tr>
<tr>
<td>Probable</td>
</tr>
<tr>
<td>Possible</td>
</tr>
<tr>
<td>Empirical use</td>
</tr>
<tr>
<td><strong>Site of infection</strong></td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td><strong>Fungal organisms</strong></td>
</tr>
<tr>
<td><em>Aspergillus</em></td>
</tr>
<tr>
<td><em>Candida</em></td>
</tr>
<tr>
<td><em>Phialophora</em></td>
</tr>
</tbody>
</table>

Data are the mean ± SD or No. (%). Abbreviation: TDM, therapeutic drug monitoring.

* Others include lung disease (n = 6), solid tumor (n = 2), esophageal perforation (n = 2), drug-induced cytopenia (n = 1), kidney transplantation (n = 1), and none identified (n = 3).

*b* The CYP 2C19 genotype was available in 52 patients in the TDM group and 50 patients in the non-TDM group.

*c* Data from invasive fungal infections (n = 94).

*d* Others include brain (n = 2), sinus (n = 2), liver (n = 2), kidney (n = 1), leg (n = 1), and blood (n = 1).

*e* Data from proven or probable fungal infections (n = 81).

<table>
<thead>
<tr>
<th>Table 2. Voriconazole Use Between Therapeutic Drug Monitoring (TDM) and Non-TDM Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM (n = 55)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Reason for voriconazole use</strong></td>
</tr>
<tr>
<td>First-line use</td>
</tr>
<tr>
<td>Failure of other antifungal agent</td>
</tr>
<tr>
<td>For oral administration</td>
</tr>
<tr>
<td>Intolerance to other antifungal agent</td>
</tr>
<tr>
<td><strong>Administration route</strong></td>
</tr>
<tr>
<td>Intravenous followed by PO</td>
</tr>
<tr>
<td>Intravenous only</td>
</tr>
<tr>
<td>PO only</td>
</tr>
<tr>
<td>Loading dose, mg/kg/day</td>
</tr>
<tr>
<td>Maintenance dose, mg/kg/day</td>
</tr>
<tr>
<td>Initial voriconazole trough level, mg/L</td>
</tr>
<tr>
<td>&gt;5.5 mg/L</td>
</tr>
<tr>
<td>&lt;1.0 mg/L</td>
</tr>
<tr>
<td>Duration of voriconazole use, days</td>
</tr>
</tbody>
</table>

Data are the mean ± standard deviation or No. (%). Abbreviations: PO, per oral; TDM, therapeutic drug monitoring.
voriconazole in this study, adverse events were similar to those in previous investigations [2, 5, 21]. Elevation of hepatic enzymes was the most common adverse event, followed by hallucination and visual disturbance [3, 14].

Unlike previous studies, there was no difference in the incidence of adverse events between the TDM and non-TDM groups, which could be partly explained by a relatively early onset of adverse events. Considering that our study population included a relatively large number of poor metabolizers in whom the half-life of voriconazole may be prolonged (up to 32 hours), the timing of the blood sampling for TDM was determined as the fourth day after starting voriconazole therapy [12, 22, 23]. After dosage adjustment based on the TDM result, it would take an additional several days (up to 5–7 days) to reach the new steady-state concentration. However, because approximately 90% of voriconazole-related adverse events developed within 10 days after starting therapy, voriconazole TDM would not have reduced the incidence of adverse events in the study. However, incidence of adverse events might be lower if blood sampling for TDM can be done earlier in a population in which poor metabolizers are rare.

This study demonstrated that voriconazole TDM significantly reduced drug discontinuation due to adverse events. In the non-TDM group, the attending physician who did not know whether the voriconazole level was toxic or therapeutic could not wait in initiating voriconazole treatment cessation when confronting an intolerable drug-related adverse event. On the contrary, in the TDM group, the attending physician could wait because adverse events were expected to be alleviated after dosage adjustment. However, the time delay between adverse events and drug discontinuation suggests that the attending physicians tried to continue voriconazole as long as they could (Figure 3).

Our dose adjustment strategy generally worked well, although more fine adjustments may be needed for 0.5–1.0 mg/L because a 100% dose increase overshot the target level in 2 of 3 patients with an initial level in this range. Our data demonstrated considerable intraindividual variation of serial voriconazole levels, as previously reported [9]. Furthermore, validation of the therapeutic range with our own data suggests that a lower cutoff of 1.0 mg/L can be low for treatment success [16, 19]. Considering these findings together, voriconazole TDM may have to be repeated if the level is in the range of 1–2 mg/L.

In this study, higher overall mortality in the non-TDM group can be explained by more frequent treatment failure in this group, to which more the common discontinuation of voriconazole might contribute. Treatment failure was common with both toxic and subtherapeutic levels of voriconazole, and these findings were noted in previous studies [8, 16]. Poor patient conditions aggravated by the toxic effect of voriconazole might contribute to treatment failure. On the

Figure 2. Initial or final voriconazole trough level. A, The solid circle or square denotes a severe adverse event, the gray circle or square denotes a non-severe adverse event, and x indicates drug discontinuation due to an adverse event. A dotted line represents the mean value. B, A solid circle or square denotes a complete response, an empty circle or square denotes a partial or stable response, and x indicates treatment failure. A dotted line represents the mean value. Abbreviation: TDM, therapeutic drug monitoring.
contrary, decreased clearance of voriconazole due to poor medical condition might contribute to a high level of voriconazole in patients with treatment failure. In the subgroup analyses including only patients with high voriconazole levels, a relatively short duration from toxic level to death and the absence of extraordinary adverse events such as arrhythmia may favor the latter explanation.

This study has a few limitations. First, only Korean adult patients were included at a single center, so caution may be needed when extrapolating these results to other ethnic groups or pediatric populations with pharmacokinetics different from those of adult patients [24]. However, our subgroup analyses that excluded CYP2C19 poor metabolizers showed results that were similar to those from the overall analyses. In addition, considering the higher proportion of patients with subtherapeutic voriconazole levels in African American and Caucasian populations [5, 9], voriconazole TDM may be more useful to reduce treatment failure in non-Asian populations. Second, the sample size may have been too small to detect a difference in the incidence of voriconazole-related adverse events between the TDM and non-TDM groups.

In conclusion, routine TDM of voriconazole may not reduce the incidence of drug-related adverse events because of the early occurrence of adverse events compared with the time needed for optimizing voriconazole levels based on TDM. However, voriconazole TDM did reduce drug discontinuations

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### Table 3. Voriconazole-Related Adverse Events in Therapeutic Drug Monitoring (TDM) vs Non-TDM Groups

<table>
<thead>
<tr>
<th>Possible or stronger relationship</th>
<th>TDM (n = 55)</th>
<th>Non-TDM (n = 53)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All events</td>
<td>23 (42)</td>
<td>22 (42)</td>
<td>.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elevation of hepatic enzymes</td>
<td>15 (27)</td>
<td>14 (26)</td>
<td>.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Encephalopathy&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 (15)</td>
<td>7 (13)</td>
<td>.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Others&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 (9)</td>
<td>8 (15)</td>
<td>.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Severe events</td>
<td>7 (13)</td>
<td>5 (9)</td>
<td>.586&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elevation of hepatic enzymes</td>
<td>4 (7)</td>
<td>3 (6)</td>
<td>&gt;.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>2 (4)</td>
<td>0</td>
<td>.49&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Others</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>&gt;.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Probable or likely relationship

| All events                       | 12 (22)     | 9 (17)          | .53<sup>a</sup> |
| Elevation of hepatic enzymes     | 3 (6)       | 4 (8)           | .71<sup>d</sup> |
| Encephalopathy                   | 6 (11)      | 5 (9)           | .80<sup>a</sup> |
| Others                           | 3 (6)       | 2 (4)           | >.99<sup>d</sup> |
| Severe events                    | 2 (4)       | 2 (4)           | >.99<sup>d</sup> |
| Elevation of hepatic enzymes     | 0           | 1 (2)           | .49<sup>d</sup> |
| Encephalopathy                   | 1 (2)       | 0               | >.99<sup>d</sup> |
| Others                           | 1 (2)       | 1 (2)           | >.99<sup>d</sup> |

### Drug discontinuation due to adverse events

<table>
<thead>
<tr>
<th>TDM (n = 55)</th>
<th>Non-TDM (n = 53)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (4)</td>
<td>9 (17)</td>
<td>.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are No. (%).

Abbreviation: TDM, therapeutic drug monitoring.

<sup>a</sup> From the $\chi^2$ test.

<sup>b</sup> Encephalopathy includes visual hallucination (n = 11) and confusion (n = 4).

<sup>c</sup> Others include visual disturbance (n = 5), rash (n = 3), electrolyte abnormality (n = 3), bad dreams (n = 1), and arrhythmia (n = 1).

<sup>d</sup> From Fisher’s exact test.
due to adverse events and improved the treatment response in invasive fungal infections.

### Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the author(s). Questions or messages regarding errors should be addressed to the author.

### Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

### References


### Table 4. Treatment Response in Therapeutic Drug Monitoring (TDM) vs Non-TDM Groups

<table>
<thead>
<tr>
<th></th>
<th>TDM (n = 37)</th>
<th>Non-TDM (n = 34)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment success</td>
<td>30 (81)</td>
<td>20 (59)</td>
<td>.04</td>
</tr>
<tr>
<td>Complete response</td>
<td>21 (57)</td>
<td>13 (38)</td>
<td>.12</td>
</tr>
<tr>
<td>Partial response</td>
<td>9 (24)</td>
<td>7 (21)</td>
<td>.71</td>
</tr>
<tr>
<td>Stable response</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td>.60</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>6 (16)</td>
<td>12 (35)</td>
<td>.07</td>
</tr>
</tbody>
</table>

Abbreviation: TDM, therapeutic drug monitoring.

TDM of Voriconazole • CID 2012:55 (15 October) • 1087
Pharmacodynamics of Isavuconazole in an Aspergillus fumigatus Mouse Infection Model

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Johan W. Moutona,b

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Azole resistance is an emerging problem in Aspergillus fumigatus which translates into treatment failure. Alternative treatments with new azoles may improve therapeutic outcome in invasive aspergillosis (IA) even for strains with decreased susceptibility to current azoles. The in vivo efficacy of 0.25, 1, 4, 16, 64, 128, 256, and 512 mg/kg of body weight/day prodrug isavuconazonium sulfate (BAL8557) (isavuconazole [ISA]-equivalent doses of 0.12, 0.48, 1.92, 7.68, 30.7, 122.9, and 245.8 mg/kg/day, respectively) administered by oral gavage was assessed in an immunocompetent murine model of IA against four clinical A. fumigatus isolates: a wild-type isolate (ISA MIC EUCAST, 0.5 mg/liter) and three azole-resistant isolates harboring substitutions in the cyp51A gene: G54W (ISA MIC EUCAST, 0.5 mg/liter), M220I (ISA MIC EUCAST, 4 mg/liter), and TR34/L98H (ISA MIC EUCAST, 8 mg/liter). The maximum effect (100% survival) was reached at a prodrug isavuconazonium sulfate dose of 64 mg/kg for the wild-type isolate, 128 mg/kg for the G54W mutant, and 256 mg/kg two times per day (q12) for the M220I mutant. A maximum response was not achieved with the TR34/L98H isolates with the highest dose of prodrug isavuconazonium sulfate (256 mg/kg q12). For a survival rate of 50%, the effective AUC0–24/MIC EUCAST ratio for ISA total drug was 24.73 (95% confidence interval, 22.50 to 27.18). The efficacy of isavuconazole depended on both the drug exposure and the isavuconazole MIC of the isolates. The quantitative relationship between exposure and effect (AUC0–24/MIC) can be used to optimize the treatment of human infections by A. fumigatus, including strains with decreased susceptibility.

Invasive aspergillosis (IA) caused by Aspergillus fumigatus is an important opportunistic fungal infection in immunocompromised patients with an overall mortality ranging between 30 and 88% (1–4). Azole antifungals, such as voriconazole (VRC) and posaconazole (POS), are recommended drugs to manage Aspergillus diseases (5, 6). Voriconazole is currently recommended as a first-choice treatment for IA, and posaconazole is indicated for prophylaxis and salvage therapy (5, 6). However, the management of IA has become more complicated due to the emergence of azole resistance in A. fumigatus (7–16). Surveillance studies indicate that azole resistance is increasing in multiple European countries and in the Middle East, Asia, and Africa (17–21). Therefore, alternative treatment regimens need to be investigated to improve the outcome of patients with azole-resistant IA.

There are basically two alternative options with respect to the management of azole-resistant IA: treatment with a new antifungal formulation or combination therapy. Recent data suggest that combination therapy using a triazole and an echi-nocandin may be a beneficial treatment strategy for triazole-resistant isolates (22–24). However, for voriconazole, both in vitro interactions and in vivo studies indicated that the level of synergistic effect is lost at high voriconazole MICs (MIC of ≥8 mg/liter) (22, 23). As a consequence, this is a major drawback in the treatment of patients with azole-resistant IA. Therefore, it is important to explore the efficacy of new antifungal drugs against azole-resistant IA.

Isavuconazole (ISA) is an investigational broad-spectrum triazole currently being investigated in phase III clinical studies for the treatment of severe invasive fungal infections, including the SECURE (invasive aspergillosis and other filamentous fungi), VITAL (rare fungi), and ACTIVE (candidemia/invasive candidiasis) programs (http://clinicaltrials.gov). Isavuconazole is administered as a water-soluble prodrug, isavuconazonium sulfate, that is available in both intravenous (i.v.) and oral formulations. The prodrug is rapidly converted to the active moiety, isavuconazole, and nonactive metabolite upon administration. It is now granted Food and Drug Administration (FDA) fast-track status in the United States and received “orphan drug” designation in the United States and European Union for the treatment of invasive aspergillosis and mucormycosis (25). FDA also recently designated isavuconazole as a Qualified Infectious Disease Product (QIDP) for the oral and intravenous treatment of invasive aspergillosis, invasive mucormycosis, and invasive candidiasis (news release, 16 July 2014, Basilea, Basel, Switzerland). In Europe, an application also has recently been submitted to the European Medicines Agency (EMA), seeking approval for isavuconazole to be used for the treatment of invasive mold infections (news re-
lease, 17 July 2014, Basle, Basel, Switzerland), on the basis of the SECURE registration study (26).

There are only limited preclinical data on the in vivo efficacy of isavuconazole in azole-resistant IA (27). Therefore, the objective of the present study was to investigate the pharmacodynamics (PD) and dose-response and exposure-response relationships of isavuconazole against wild-type and clinical azole-resistant A. fumigatus isolates harboring different substitutions in the Cyp51A gene in an immunocompetent murine model of disseminated aspergillosis. Survival and reduction in kidney fungal burden determined by real-time quantitative PCR were used as primary and secondary endpoints (respectively) to determine the dose-effect and the exposure-effect relationships of isavuconazole for susceptible as well as azole-resistant isolates in comparison with the other azoles.

(Parts of these results were presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 10 to 13 September 2013, and at the 24th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Barcelona, Spain, 10 to 13 May 2014.)

MATERIALS AND METHODS

Fungal isolates. Four clinical A. fumigatus isolates obtained from patients with proven IA (classified according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group [EORTC/MSG] consensus definitions) (28) were used in the experiments: a wild-type isolate without mutations in the cyp51A gene (AZN8196) and three azole-resistant isolates harboring substitutions in the cyp51A gene: G54W (V 59-73) and M220I (V 28-77) isolates that have become resistant during patient azole therapy and a TR44/L98H (V 52-35) isolate that has become resistant through environmental azole exposure.

Strain identification and the cyp51A gene substitutions were confirmed by sequence-based analysis as described previously (9). In addition, microsatellite genotyping of the isolates was performed to confirm that they are genetically distinct (29). The isolates were stored in 10% glycerol broth at −80°C and were cultured on Sabouraud dextrose agar (SDA) supplemented with 0.02% chloramphenicol for 5 to 7 days at 35 to 37°C. All isolates were cultured again on SDA for 5 to 7 days at 35 to 37°C before preparation of the inoculum.

Preparation of inoculum for antifungal susceptibility testing. The suspensions of conidia were harvested in normal saline containing 0.025% Tween 20. The appropriate dilutions in normal saline were made to obtain a final inoculum concentration of 2 × 10^6 to 5 × 10^5 CFU/ml (30).

In vitro antifungal susceptibility testing. In vitro antifungal susceptibility testing (MICS and minimum effective concentrations [MECs]) was performed by using the EUCAST (European Committee for Antimicrobial Susceptibility Testing) (30) and CLSI (Clinical and Laboratory Standards Institute) (31) broth microdilution guidelines. The final concentrations of the antifungal agents ranged from 0.016 to 16 mg/liter for amphotericin B (Amb), itraconazole (ITC), voriconazole (VRC), posaconazole (POS), isavuconazole (ISA), and anidulafungin (AFG). Aliquots of 100 μl of each drug at a concentration two times the targeted final concentration were dispensed in microtiter plates (Costar, Corning, NY). Trays were maintained for a period of less than 1 month at −10°C until the day of testing. After the microtitration trays were defrosted, 100 μl of the inoculum was added to each well, corresponding to a final concentration of 2 × 10^3 to 5 × 10^5 CFU/ml for each isolate. The microtiter plates were incubated at 35 to 37°C for 48 h.

Growth inhibition was quantified by using a visual mirror. Candida parapsilosis (ATCC 22019) and Candida krusei (ATCC 6258) were used for quality control (QC) in all experiments. All incubations were performed in three independent replicates, and the breakpoints reported by Verweij et al., have been used for classifying azole-susceptible and azole-resistant isolates (32).

The MIC was defined as the lowest concentration that completely inhibited growth in comparison to the drug-free well (control) as assessed by visual inspection. The MEC was defined as the lowest concentration in which abnormal, short, and branched hyphal clusters were observed in contrast to the long, unbranched hyphal elements that were seen in the growth control well (30).

Antifungal agents. Isavuconazole (BAL4815) and the prodrug isavuconazonium sulfate (BAL8557) were provided by Astellas Pharma B.V. For in vitro studies, isavuconazole was dissolved in dimethyl sulfoxide (DMSO) prior to susceptibility testing. The concentration of DMSO in the culture medium tubes was adjusted at 1%, and the concentration of antifungal agents was 2× final concentration.

For in vivo studies, the prodrug was dissolved in sterile water prior to oral administration in each experiment. Amounts of prodrug dissolved were corrected for its 89% purity. The conversion factor for determining the equivalent isavuconazole active dose from the prodrug dose was 0.48 (provided by Astellas Pharma B.V.) on a milligram-per-kilogram-of-body-weight basis. Thus, for every 1 mg/kg of prodrug administered orally, the equivalent in vivo isavuconazole dose was considered 0.48 mg/kg.

In the pharmacokinetic study, only isavuconazole (active drug BAL4815) concentrations were quantified. The purity of isavuconazole powder (BAL4815) for in vitro susceptibility testing was 99%.

Infection model. The efficacy of isavuconazole monotherapy was determined in an immunocompetent mouse model of disseminated aspergillosis following intravenous inoculation. Animals were infected via injection of 0.1 ml of the conidial suspension into the lateral tail of the mouse, corresponding to the 90% lethal dose (LD90) for each isolate (23, 33, 34). A total of 756 outbred CD-1 (Charles River, the Netherlands) female mice, 4 to 5 weeks old, weighing 20 to 25 g, were randomized into groups of 14 mice (11 for survival analysis; 3 for quantitative real-time PCR [qPCR]) to control or prodrug doses.

Before performing the experiment, the isolates were cultured once on SDA for 5 days at 35 to 37°C and subcultured twice on 15-cm Takashio slants for 5 days at 35 to 37°C. The conidia were harvested in 20 ml of sterile phosphate-buffered saline (PBS) plus 0.1% Tween 80 (Boon B.V. Meppel, the Netherlands). The conidial suspension was filtered through sterile gauze folded four times to remove any hyphae, and the number of conidia was counted in a hemocytometer. After the inoculum was adjusted to the required concentration, the conidial suspension was stored overnight at 4°C. The 90% lethal dose (LD90) was determined for each isolate, separately. The LD90 was 2.4 × 10^7 (wild-type control), 1 × 10^7 (G54W strain), 5 × 10^6 (M220I strain), and 2.5 × 10^7 (TR44/L98H strain) conidia. Confirmatory postinfection viability counts of the injected inocula were determined to ensure that the correct inoculum had been injected.

Treatment was started 24 h after infection and continued for 14 days. The prodrug solution was administered in doses of 0.25, 1, 4, 16, 64, 128, and 256 mg/kg by oral gavage once a day in a volume of 0.12 ml or divided into two or three daily doses where applicable. The highest dose (256 mg/kg) was used two times per day, corresponding to 512-mg/kg prodrug isavuconazonium sulfate (BAL8557)/day in groups of animals where 100% efficacy was not achieved with a once-daily dose. The above-mentioned dosages were equivalent to 0.12, 0.48, 1.92, 7.68, 30.7, 61.4, and 122.9 mg/kg, respectively, of the active moiety isavuconazole. The control group received single doses of saline. In addition, dose fractionation studies were performed to determine which pharmacokinetic/pharmacodynamic (PK/PD) index correlated with efficacy. Mice were infected with the A. fumigatus isolate through the lateral tail vein, and after 24 h, treatment was initiated according to total daily dosing every 8 h (q8) or 12 h (q12) for 14 days. The animals were housed under standard conditions, with drink and feed supplied ad libitum. The animal studies were conducted in accordance with the recommendations of the European Community (Directive 2010/63/EU revising Directive 86/609/EEC on the pro-
fection of animals used for scientific purposes adopted on 22 September 2010), and all animal procedures were approved by the Animal Welfare Committee of Radboud University (RU-DEC 2012-050).

In all survival studies, the monitoring was performed by experienced individuals blinded to the animal treatment. The infected mice were examined at least three times daily. Clinical inspections focused on dehydration, torticollis, staggering, severe weight loss (a decrease of 15% within 48 h or 20% within 24 h), or body temperature drop to below 33°C. Mice demonstrating these clinical signs were humanely terminated according to strict protocols. On day 15 postinfection, all surviving mice were humanely euthanized under isoflurane anesthesia, and blood and internal organs were collected.

Survival and reduction in fungal burden were the primary end-point in groups of 11 and 3 mice, respectively. The survival in days postinfection was recorded for each mouse in each group and considered primary outcome-effect measure to assess the therapeutic efficacy of isavuconazole (35). On day 3 postchallenge, a quantitative real-time PCR (qPCR) was performed in groups of 3 mice. In these groups, the mice were sacrificed on day 3 postinfection (before they began to die from infection) and the fungal load in the kidney was determined. In the systemic aspergillosis model with i.v. infection, the kidneys are the main target organs, which may that indicate intraluminal localization of the fungus in the renal tubules initially protects them from inflammatory cells. The reduction in kidney fungal burden was then correlated with the survival of the remaining 11 mice from each corresponding group at day 15 postinfection.

Determination of fungal burden in kidney. Left and right kidneys from each animal were homogenized using a TissueLyser (Qiagen; TissueLyser Type MM 301) and UV irradiated beads with magnetic metal cores (3 mm) and 30 Hz in 2 min. Tissue samples were transferred to MagNA Lyser Green bead tubes (Roche Applied Science). Five hundred microliters of Tris-EDTA (TE) buffer was added, and homogenization was performed for 20 s at 6,500 rpm by using the MagNA Lyser instrument. Supernatant was used for DNA isolation by using the automated MagNA Pure (MP96) system and the MagNA Pure LC total nucleic acid isolation kit according to the manufacturer's protocol (Roche Applied Science). Phocine herpesvirus (PhHV) was added to all samples as an internal extraction and inhibition control. The concentration of total isolated DNA was measured by using the NanoDrop ND-1000 spectrophotometer (Thermo Scientific). Aspergillus loads were determined by qPCR using the LC480 instrument and the probe master kit (Roche Applied Science), as previously described (36). Briefly, thermocycling conditions were 95°C for 5 s, 50 cycles of 95°C for 15 s and 60°C for 45 s, and finally 40°C for 30 s, one time. The multiplex 28S rRNA gene of Aspergillus sp., was detected by using primers F 5′-TCTCCTGCTTTAGCCTGGTT T-3′ and R 5′-TGGCTCTTGTTACCCAGCG-3′ and probe 6-carboxy-fluorescein (FAM)-AGTGACAGCCCTACGGAGGAA-BHQ. Additionally, the PhHV isolation control was detected by using primers F 5′-TCTCCTGCTTTAGCCTGGTT-T-3′ and R 5′-TGGCTCTTGCTTCCACCG ACCTG-3′ and probe FAM-AGTGACAGCCCTACGGAGGAA-BHQ (Blackberry quencher). For the 28S rRNA detection, an 8-fold dilution series of the cloned PCR product was used to calculate the number of copies per milliliter. The ratio of copies per milliliter and total DNA isolated (nanograms per milliliter) was calculated to determine the Aspergillus load of each organ sample.

Pharmacokinetic analysis of isavuconazole in mice. A total of 210 outbred CD-1 (Charles River, the Netherlands) female mice, 4 to 5 weeks old, weighing 20 to 22 g, were used for the PK experiments. On day 0, mice were infected with the wild-type A. fumigatus isolate through the lateral tail vein, and after 24 h, treatment with the prodrug isavuconazonium sulfate was initiated at dosages of 0.25, 1, 4, 16, 64, and 128, and 256 mg/kg of body weight by oral gavage (ISA-equivalent doses of 0.12, 0.48, 1, 4, 16, 64, and 256 mg/kg, respectively). At day 2 of treatment (day 3 after infection), blood and bronchoalveolar lavage (BAL) fluid samples were drawn from 3 mice for each individual predefined sampling time point (10 time points in total): immediately before administration of drugs (0.0 h) and subsequently at 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 h postdose. The blood samples were drawn through the orbital vein or heart puncture into lithium-heparin-containing tubes and were cooled and centrifuged for approximately 10 min at 1,000 × g within 30 min of collection. Plasma was aspirated, transferred in two 2-ml plastic tubes, and stored at −80°C. BAL fluid samples were obtained using a technique described previously (37). After being sacrificed under isoflurane anesthesia followed by cervical dislocation, the mice were secured on a plastic platform. The trachea was exposed by a 1-cm incision on the ventral neck skin for insertion of the cannula and sutured in place. Lungs were instilled 4 times with 0.5 ml of sterile 0.9% saline, with the fluid being immediately aspirated. The aspirates recovered from the instillations were pooled per mouse, placed on ice after each aliquot, and subsequently stored at −80°C.

Analytical assay of isavuconazole. Isavuconazole (BAL4815) concentration in plasma and BAL fluid was measured by a validated ultraperformance liquid chromatography (UPLC) method with fluorescence detection. Details of the analytical assay are described elsewhere (38). Briefly, samples were pretreated using a protein precipitation procedure (acetone-nitrite-methanol, 50/50, and formic acid, 0.1%). A seven-point calibration curve with three quality control (QC) samples was used. All measurements were done in duplicate. The dynamic range of the isavuconazole assay was 0.0495 to 29.69 mg/liter, and the accuracy range, which was dependent on the concentration, was 96.1% to 101.5%. Interday coefficient of variation was 0.46 to 1.8%, and extraction recovery was 84.6%. The assay was validated for the mouse plasma matrix according to the FDA and EMA directive for bioanalytical methods validation with the exception of triadazin precision as well as freeze-thaw cycles. These experiments were not considered necessary based on the experience with human plasma.

Pharmacokinetic analysis. Geometric mean concentrations of isavuconazole were calculated for each time point (n = 3 mice). Pharmacokinetic parameters (area under the concentration-time curve from 0 to 24 h [AUC0–24], maximum concentration of drug in serum [Cmax], the trough concentration 24 h after the start of treatment [C24 h]), half-life (t1/2), volume of distribution [V], clearance [CL], and terminal elimination rate constant [k] were calculated using noncompartmental analysis (Phoenix version 6.3). The area under the concentration-time curve from 0 h to infinity (AUC0–∞) was calculated using the linear up–log down trapezoidal rule. In addition, Cmax and t1/2 were directly observed from the data. Half-life was calculated by ln 2/k, in which k was determined by linear regression of the terminal points of the log-linear plasma concentration-time curve. V was calculated using the formula V = dose/AUC × k, and CL was calculated as dose/AUC0–24.

(i) Calculation of isavuconazole concentration in ELF. Concentrations of isavuconazole in BAL fluid from three mice per time point were determined as described for plasma. Urea in plasma and BAL fluid aspirate was determined utilizing a modified enzymatic assay (Quantichrom urea assay kit, DIUR-500; BioAssay Systems) (39, 40). The concentration of isavuconazole in epithelial lining fluid (ELF) was then determined by using the ratio of urea concentration in BAL fluid to that in plasma. The drug concentration in ELF was then estimated, as described previously (37, 39, 44): drug concentrationELF = drug concentrationBAL fluid × ureaBAL fluid/ureaELF.
visual inspection. Dose/MIC and AUC0–24/MIC ratios were calculated by dividing the dose (milligrams per kilogram) or AUC by the MIC. Dose/MIC and AUC0–24/MIC ratio data were log10 transformed to approximate a normal distribution prior to statistical analysis. The 50% effective PK/PD indexes (EI50, EI80, and EI90) best correlating with efficacy were determined. In addition, the 50% effective doses (ED50) of isavuconazole best correlating with efficacy were determined. For comparison between strains, an F test was performed to define whether ED50 differed among the four groups. Statistical significance was defined as a P value of <0.05 (two-tailed).

RESULTS

In vitro susceptibility. The characteristics and in vitro susceptibilities of the four A. fumigatus isolates are shown in Table 1. All isolates grew well after 48 h of incubation at 35 to 37°C. Variable isavuconazole activity was found in azole-resistant isolates, in which isavuconazole showed cross-resistance to voriconazole, but not with itraconazole and posaconazole. In comparison to a MICEUCAST of 0.5 mg/liter for the wild-type isolate, isavuconazole showed similar activity against the isolate harboring the G54W resistance mechanism (MICEUCAST, 0.5 mg/liter) but reduced in vitro activity against M220I and TR34/L98H isolates, with MICEUCAST of 4 and 8 mg/liter, respectively. There was no difference in the amphotericin B (AmB) and anidulafungin (AFG) activity between the isolates.

Pharmacokinetics of isavuconazole. A total of 210 samples from 210 mice (3 mice per time point, 10 time points, 7 different dosages) were analyzed. All 210 mice were alive at the time of sample collection. The observed plasma concentrations-versus-time profiles of isavuconazole are shown in Fig. 1. The corresponding pharmacokinetic parameters are tabulated in Table 2 for plasma and in Table 3 for epithelial lining fluid (ELF). The dose-normalized isavuconazole AUC in plasma ranged from 0.54 to 0.84 mg · h/liter/(mg/kg) for single doses (dose expressed as the prodrug, concentration expressed as isavuconazole) ranging from 16 mg/kg to 256 mg/kg, was slightly lower for the 4-mg/kg dose, and could not be reliably determined for lower doses. The concentrations of isavuconazole in ELF correlated well with those obtained in plasma but were lower, including the maximum total drug concentrations (Cmax) of isavuconazole (Table 3). A significant relationship between mean isavuconazole concentrations in plasma and ELF was noted by linear regression analysis (r2 = 0.86, P < 0.0001) (Fig. 2). The penetration of isavuconazole in ELF based on total drug was between 35.8 and 72.5% with a mean of

![FIG 1](https://example.com/fig1.png) Plasm concentrations of isavuconazole (BAL4815) following oral administration of the prodrug isavuconazonium sulfate (BAL8557) at 0.25-, 1-, 4-, 16-, 64-, 128-, and 256-mg/kg dosages to immunocompetent infected mice. ISA-equivalent doses are 0.12, 0.48, 1.92, 7.68, 30.7, 61.4, and 122.9 mg/kg, respectively. Each symbol corresponds to the geometric mean and standard deviation of the mean plasma levels for three mice.
54.1% (Table 3). For the ELF data from the 128-mg/kg dose, $k_{el}$ (terminal elimination rate constant) could not be determined with the consequence that the predicted AUC$_{0→∞}$ could not be calculated reliably. Hence, the AUC$_{0→12}$ in plasma and ELF were determined. The variable penetration of isavuconazole into ELF might be explained due to the difference in lysis of the cells available in interstitial spaces over the course of infection that limits passage through alveolar epithelial cells in various levels; in addition, measurements at the same time in plasma and a third compartment are liable to significant variation.

**Efficacy of isavuconazole.** (i) Survival and quantitative PCR as outcome parameter to monitor therapeutic efficacy of ISA. Figure 3 shows the survival curves of isavuconazole-treated mice by prodrug dose. The survival curves for all control groups receiving saline by oral gavage showed a mortality of 100%. The results show that for each prodrug dose, survival decreased as the MIC increased. Similarly, when the prodrug dose was increased, an improved response was observed. The maximum effect (100% survival) was reached at a prodose of 64 mg/kg for the TR34/L98H isolate. The 50% effective dose (ED$_{50}$) based on the susceptible profile for isavuconazole (MIC, 0.5 mg/liter), indicating that higher doses of isavuconazole were required to achieve similar efficacy.

The Hill-type model with a variable slope fitted the relation-ship between the dose and 14-day survival well, with $R^2$ values of 1 for the wild type, 0.99 (G54W isolate), 0.95 (M220I isolate), and 0.91 (TR34/L98H isolate). The 50% effective dose (ED$_{50}$) based on survival was 24.15 mg/kg prodrug isavuconazonium sulfate (BAL8557) (95% confidence interval [CI], 23.96 to 24.33 mg/kg) for the wild type, 28.93 (95% CI, 24.23 to 34.54 mg/kg) for the TR34/L98H isolate. The 50% effective dose (ED$_{50}$) based on survival was 24.15 mg/kg prodrug isavuconazonium sulfate (BAL8557) (95% confidence interval [CI], 23.96 to 24.33 mg/kg) for the wild type, 28.93 (95% CI, 24.23 to 34.54 mg/kg) for the G54W isolate, 109 (95% CI, 50.69 to 234.6 mg/kg) for the M220I isolate (MIC, 0.5 mg/liter), indicating that higher doses of isavuconazole were required to achieve similar efficacy.

(ii) Dose-response analysis. The dose-response curves for the dosing regimens and control groups of isavuconazole administered to the immunocompetent mice are shown in Fig. 5. Isavuconazole treatment improved the survival of the mice in a dose-dependent manner. A dose-response relationship was observed that depended on the isavuconazole dose and the azole resistance mechanisms. The dose-response curve for mice infected with the isolates with higher MICs to isavuconazole (≥ 4 mg/liter) was shifted to the right compared to those infected with isolates with the susceptible profile for isavuconazole (MIC, 0.5 mg/liter), indicating that higher doses of isavuconazole were required to achieve similar efficacy.

![Graph showing the relationship between isavuconazole (BAL4815) concentrations in plasma and ELF.](image)

**Tables**

**Table 2** Pharmacokinetic parameters of isavuconazole (BAL4815) following oral administration of various dosages administered as prodrug isavuconazonium sulfate (BAL8557) ranging from 0.25 to 256 mg/kg (ISA-equivalent doses ranging from 0.12 to 122.9 mg/kg) a

<table>
<thead>
<tr>
<th>Dose group (mg/kg)</th>
<th>C$_{max}$ (mg/liter)</th>
<th>C$_{last}$ (mg/liter)</th>
<th>Half-life (h)</th>
<th>AUC$_{INF_pred}$ (h · mg/liter)</th>
<th>AUC$_{INF_D_pred}$ (h · mg/liter/kg)</th>
<th>ClssF (liters/h · kg)</th>
<th>VzF (liters/kg)</th>
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</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>0.11</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
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<td>0.08</td>
<td>1.12</td>
<td>0.97</td>
<td>0.24</td>
<td>4.09</td>
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</tr>
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<td>0.60</td>
<td>1.68</td>
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<tr>
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<td>1.87</td>
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</tr>
<tr>
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<td>1.31</td>
<td>11.46</td>
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<tr>
<td>256</td>
<td>25.62</td>
<td>0.15</td>
<td>3.06</td>
<td>146.68</td>
<td>0.57</td>
<td>1.76</td>
<td>7.75</td>
</tr>
</tbody>
</table>

*Abbreviations: C$_{max}$, last observed quantifiable concentration; AUC$_{INF\_pred}$, Predicted area under the plasma concentration-time curve from time zero to infinity; AUC$_{INF\_D\_pred}$, dose-normalized AUC$_{INF\_pred}$; ClssF, total systemic clearance; VzF, volume of distribution.*

**Table 3** Penetration ratio of isavuconazole (BAL4815) in ELF compared to plasma based on total drug a

<table>
<thead>
<tr>
<th>ISA dose (mg/kg)</th>
<th>AUC$_{INF_pred}$ (h · mg/liter)</th>
<th>AUC$_{INF_pred}$ ELF/plasma ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>ELF</td>
</tr>
<tr>
<td>64</td>
<td>34.70</td>
<td>25.14</td>
</tr>
<tr>
<td>128</td>
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<td></td>
</tr>
<tr>
<td>256</td>
<td>146.68</td>
<td>52.57</td>
</tr>
</tbody>
</table>

*Because of variability in data and lower limit of quantification, the AUC$_{INF\_pred}$ (predicted area under the plasma concentration-time curve from time zero to infinity) could not be reliably determined for the 128-mg/kg dose.*
isolate, and 483.8 (95% CI, 103.2 to 2,266 mg/kg) for the TR 34/L98H isolate. Notably, the dose-response curves were significantly different ($P < 0.05$) between the wild-type *A. fumigatus* isolate with an isavuconazole MIC EUCAST of 0.5 mg/liter and the azole-resistant isolates harboring M220I and TR 34/L98H substitutions in the *cyp51A* gene with isavuconazole MIC EUCASTs of 4 and 8 mg/liter, respectively (Table 4).

As a comparison, the ED$_{50}$ based on survival was 11.6 mg/kg isavuconazole active moiety (95% confidence interval [CI], 11.5 to 11.7 mg/kg) for the wild type, 13.9 (95% CI, 11.6 to 16.6 mg/kg) for the G54W isolate, 52.3 (95% CI, 24.3 to 112.6 mg/kg) for the M220I isolate, and 232.3 (95% CI, 49.52 to 1,089 mg/kg) for the TR34/L98H isolate.

(iii) Exposure-response analysis. The AUC for each dose (Table 2) was used to calculate the AUC$_{0-24}$/MIC ratio for each isolate, as shown in Fig. 6. The exposure-response relationship has a sigmoidal shape. Increased isavuconazole exposure was required to obtain maximum efficacy in mice infected with the M220I (MIC, 4 mg/liter) and TR$_{34}$/L98H (MIC, 8 mg/liter) strains compared to those infected with the wild-type and G54W strains (MIC, 0.5 mg/liter).

The Hill equation with a variable slope fitted the relationship between the 24-h AUC/MIC ratio and 14-day survival well ($R^2 = 0.96$), as statistically significant pharmacodynamic indices (PDIs) for isavuconazole single-agent regimens ($P < 0.05$). The 50% effective pharmacodynamic index (total AUC$_{0-24}$/MIC$_{EUCAST}$) for isavuconazole was 24.73 (95% confidence interval, 22.50 to 27.18), to be the PD index most closely predictive of efficacy. Using MICs determined with the CLSI method, the effective AUC$_{0-24}$/MIC$_{CLSI}$ ratio for ISA total drug was 50.48 (95% confidence interval, 44.90 to 56.74).

The relationship between the *in vivo* efficacy and other PDIs, such as the cumulative percentage of a 24-h period that the drug concentration exceeded the MIC under steady-state PK conditions and the peak-level $C_{\text{max}}$/MIC, was also determined (data not shown). However, AUC$_{0-24}$/MIC appeared to be the most important pharmacodynamic index correlating with efficacy.

(iv) Dose fractionation studies. Figure 7 shows the results of the dose fractionation study. There is no difference between exposure-response relationships of the groups treated with various dosing intervals.

**DISCUSSION**

In the present study, the efficacy of isavuconazole was demonstrated against *A. fumigatus* wild-type and *cyp51A* azole-resistant isolates in an immunocompetent murine model of disseminated aspergillosis. Efficacy was dependent both on the drug exposure time and on the isavuconazole MIC of the resistance phenotype of the isolates. The loss of efficacy was completely or partly compensated by increasing the doses of the prodrug isavuconazonium sulfate for the azole-resistant strains. However, for mice infected with the TR$_{34}$/L98H isolate, which had a high MIC (8 µg/ml),
Isavuconazole was not efficacious. Importantly, a maximal effect of 100% survival was achieved in all strains tested except for the strain containing the TR34/L98H mutation. This is of significant importance, since the prevalence of azole resistance in *A. fumigatus* is increasing, and cross-resistance is a growing concern (12, 21, 32, 45, 46).

A possible limitation of the experimental design used to explore the PK/PD relationships of isavuconazole in our study is that the effects were observed in nonneutropenic animals and the route of infection was dissemination rather than inhalation (the normal route of infection). In addition, a significant difference between tail and intrapulmonary infection is the difference in inoculum size. A lower inoculum size is needed in immunosuppressed models. The effects observed could therefore be an under- or overestimation of the exposure required. However, in our previous studies with azoles, we showed that using survival as a gold standard endpoint in our model provides useful exposure-response relationships. Studies with azoles in neutropenic (27, 47, 48) and nonneutropenic (23, 49, 50) models have shown that the exposure–response relationships are of the same order of magnitude; in fact, a slightly lower drug exposure target may be required in the neutropenic model. This could possibly be because of the lower inoculum used in this model.

Isavuconazole is an investigational broad-spectrum triazole developed for the treatment of severe invasive and life-threatening fungal diseases (25, 51–54). This compound demonstrated *in vitro* activity and *in vivo* efficacy against a broad range of yeasts and molds, including *Aspergillus* spp., *Fusarium* spp., *Candida* spp., the *Mucorales*, *Cryptococcus* spp., and black yeasts and their filamentous relatives (25, 55–63).

In the current study, our model indicated that the primary driver of efficacy appears to be AUC/MIC. For a survival rate of 50%, the effective AUC0–24/MICCLSI ratio for isavuconazole total drug was 50.48 (95% confidence interval, 44.90 to 56.74). Similarly, the exposure–response relationships of isavuconazole have been defined in a recent experimental immunosuppressed murine model of invasive pulmonary aspergillosis (IPA), for which a very strong relationship was observed between the PD index AUC/MIC ratio and treatment outcome (27). In that study, 10 *A. fumigatus* isolates were used, including four wild-type isolates and six cyp51 mutants. The MICCLSI range was 0.125 to 8 mg/liter. Following infection, groups of mice were treated orally with the prodrug BAL8557 at 40 to 640 mg/kg/12 h.

**FIG 4** Efficacy of isavuconazole against 4 *A. fumigatus* isolates expressed as *A. fumigatus* DNA load (copies/nanogram of DNA) in kidneys at 72 h postchallenge. For all groups, *n* was 3. Doses were administered as the prodrug isavuconazonium sulfate (BAL8557). The highest dose (256 mg/kg) was used two times per day, corresponding to 512 mg/kg prodrug isavuconazonium sulfate (BAL8557)/day, in groups of animals for which 100% efficacy was not achieved with a once daily dose of 256 mg/kg.
for 7 days. A dose-response relationship was observed for each isolate, with higher doses of isavuconazole achieving a larger microbiologic effect. The static-dose range was 65 to 617 mg/kg/12 h, for which the median total- and free-drug 24-h AUC/MIC ratio PD targets for net stasis were 503 and 5, respectively. The 1-log₁₀ killing-dose range was 147 to 455 mg/kg/12 h, and the corresponding median free-drug AUC/MIC ratio was 11.1 (27). Since the AUC of isavuconazole given 200 mg once daily (q.d.) was reported as approximately 90 mg · h/liter in healthy subjects approaching steady state (64), we therefore conclude that the A. fumigatus strains with MICs of 0.5 mg/liter would be covered, and attainment is most likely reached for strains with MICs of up to 2 or potentially 4 mg/liter.

The efficacy of isavuconazole has also been investigated in an immunosuppressed murine model of disseminated Aspergillus flavus infection (65). Isavuconazole demonstrated impressive antifungal activity against A. flavus infection, leading to prolonged survival, equivalent to similar doses of itraconazole and voriconazole and superior to either drug administered at 10 mg/kg/dose. The excellent efficacy of isavuconazole occurred despite much lower exposure as demonstrated by 4-fold-lower AUCs. Isavuconazole was at least as effective as itraconazole or voriconazole at reducing organ burden and was able to clear all burden in 33 to 83% of mice treated with >15 mg/kg/dose (65).

Three previous studies have examined drug exposure and the efficacy of isavuconazole in a murine model of invasive candidiasis (48, 66–68) and showed a very strong relationship between the PD index AUC/MIC ratio and treatment outcome. One study investigated the efficacy of isavuconazole using a neutropenic mouse model of disseminated C. krusei and Candida tropicalis infections. Isavuconazole was as effective as voriconazole and much more effective than fluconazole at reducing brain burden. All doses of isavuconazole (6, 15, 30, 60, 90, 120, or 150 mg/kg equivalent active compound) reduced brain burden (P < 0.05) in the C. krusei model and kidney burden in the C. tropicalis model (48).

Another study investigated the PK/PD properties of isavuconazole in a neutropenic murine model of invasive candidiasis (IC) against clinical isolates of Candida species, including Candida albicans, Candida glabrata, and C. tropicalis, with both a 24-h and a 96-h treatment duration (48). This study has shown that the pharmacodynamic index most closely correlated with efficacy is the ratio of the 24-h area under the concentration-time curve (AUC) to the MIC, and a target 24-h free-drug AUC/MIC ratio near 25 was associated with 50% of maximal microbiologic efficacy (48).

### TABLE 4 Comparison of efficacies of isavuconazole among four A. fumigatus isolates based on ED₅₀

<table>
<thead>
<tr>
<th>A. fumigatus strain</th>
<th>ISA MIC (mg/liter)</th>
<th>ISA ED₅₀ (mg/kg)</th>
<th>Comparison with wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value</td>
</tr>
<tr>
<td>Wild type</td>
<td>0.5</td>
<td>24.15</td>
<td>0.16</td>
</tr>
<tr>
<td>G54W strain</td>
<td>0.5</td>
<td>28.93</td>
<td></td>
</tr>
<tr>
<td>M220I strain</td>
<td>4</td>
<td>109</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TR₄₅/L98H strain</td>
<td>8</td>
<td>438.8</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

ᵃ dfₚ, degrees of freedom numerator; dfₑ, degrees of freedom denominator.
Similarly, using a nonneutropenic murine model of disseminated *Candida albicans* infection, it has been shown that the pharmacodynamic driver most likely to predict the outcome of itraconazole treatment is the AUC/MIC ratio (68).

In addition, in a neutropenic mouse model of intratracheal infection, Luo et al. investigated the efficacy of isavuconazole against a brain isolate of *Rhizopus delemar* (isavuconazole MIC100 and minimum fungicidal concentration [MFC] values of 0.25 μg/ml) (69). Isavuconazole was effective against isolates with MIC and MFC values ranging between 0.125 and 1.00 μg/ml. A high dose of isavuconazole (215 mg/kg of isavuconazonium sulfate three times daily [t.i.d.]) prolonged the survival time and lowered the tissue fungal burden of cyclophosphamide-cortisone acetate-treated mice. In addition, isavuconazole was as effective as a high-dose liposomal amphotericin B (15 mg/kg, given once daily through tail vein injection) treatment (69).

In the present study, we also found that the dosing frequency did not have an impact on survival. Given the half-life of the drug in mice, which is much shorter than that in humans, a once-daily dose should therefore suffice and be adequate to treat infections in humans. Of note, in clinical studies to date, positive efficacy and safety data have been reported in patients with invasive aspergil-
The relationship between exposure and effect (AUC0–24/MIC) can be used to optimize the treatment of human infections by A. fumigatus, including strains with decreased susceptibility.

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Azole Resistance in *Aspergillus fumigatus*: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles?

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Azole resistance in *Aspergillus fumigatus* has emerged as a global health problem. Although the number of cases of azole-resistant aspergillosis is still limited, resistance mechanisms continue to emerge, thereby threatening the role of the azole class in the management of diseases caused by *Aspergillus*. The majority of cases of azole-resistant disease are due to resistant *A. fumigatus* originating from the environment. Patient management is difficult due to the absence of patient risk factors, delayed diagnosis, and limited treatment options, resulting in poor treatment outcome. International and collaborative efforts are required to understand how resistance develops in the environment to allow effective measures to be implemented aimed at retaining the use of azoles both for food production and human medicine.

Keywords. emergence of azole resistance; azole fungicides; aspergilloma; invasive aspergillosis; chronic pulmonary aspergillosis.

*Aspergillus fumigatus* is a saprophytic mold that causes allergic, chronic, and acute invasive diseases in humans and animals [1]. The fungus is ubiquitous due to an abundant asexual reproduction cycle, producing many billions of spores, and its ability to survive in very different environments. The fungus is thermotolerant, able to resist temperatures as high as 60°C, and is important for the degradation of organic matter. Although *A. fumigatus* is not a primary pathogen for living animals or plants, it has evolved as an important cause of opportunistic fungal diseases in humans. Several decades ago, invasive aspergillosis was a much-feared complication of immunosuppressive treatments as the disease was associated with high morbidity and mortality [2–4]. The survival rates of immunocompromised patients with invasive aspergillosis have improved dramatically due to many factors, one of which is the availability of azole antifungal drugs. This class comprises a number of agents with activity against aspergilli, including itraconazole (available for clinical use since 1997), voriconazole (since 2002), posaconazole (since 2006), and, most recently, isavuconazole [5]. Each of these agents has proved beneficial for the treatment of acute invasive and chronic pulmonary aspergillosis, the prevention of invasive aspergillosis, and for difficult-to-treat disease, such as central nervous system *Aspergillus* disease [6, 7]. Recent studies also show that there is a role for azole therapy in patients with severe asthma with fungal sensitization as its use improved their quality of life and pulmonary function [8]. Moreover, azole drugs are the only anti-*Aspergillus* agents that are orally available, and therefore play an important role in long-term or ambulatory therapy such as for chronic pulmonary aspergillosis [9].

However, the clinical advances that have been made possible through the use of azole drugs might be threatened by the emergence of azole resistance in *A. fumigatus* [10–12]. We aim to describe the epidemiology and spread of azole resistance in *A. fumigatus*, the clinical implications, and directions of research that will help to understand and possibly contain this problem.

RESISTANCE DEVELOPMENT IN *A. FUMIGATUS*

Generally, 2 routes of resistance development are distinguished: through long-term azole patient therapy and via the application of azole compounds in the environment [13, 14]. Although the clinical characteristics of these routes are very different (Table 1), the fundamental prerequisites for azole resistance development are the same: Any setting that brings together active- and azole compounds has a risk of mutations developing that confer resistance to azole compounds [14, 17]. Such conditions could be present in a patient with an aspergilloma receiving azole therapy. Within the pulmonary cavity, asexual reproduction of *A. fumigatus* occurs and spores are produced abundantly, many of which may harbor azole resistance mutations. Genetic analysis of *A. fumigatus* from dissected aspergillomas and clinical cultures from patients with aspergilloma indeed confirm that *A. fumigatus* undergoes multiple genetic changes during infection, including those
conferring azole resistance [14]. This may be reflected in diagnostic specimens as multiple azole resistance mechanisms may be present in culture, concomitant with azole-susceptible colonies [17]. Although *A. fumigatus* is a eukaryotic microorganism, the complex cellular composition does not preclude rapid resistance development in response to antimicrobial exposure, as seen with bacterial pathogens. However, horizontal gene transfer, which is common in the spread of bacterial resistance, is not commonly seen in fungi. Acquired resistance has been exclusively described in patients with a cavity or aspergilloma [14, 17]. Resistance mechanisms that are recovered in culture are characterized by point mutations in the Cyp51A gene, which is the target of the antifungal azoles (Table 1). However, although the Cyp51A gene is considered a hot-spot for resistance mutations, many isolates with an azole-resistant phenotype are found to have no mutations in this gene, which suggests that other resistance mechanisms are present, some of which have been identified but many of which remain unknown [18].

Resistance mutations are also believed to develop in the environment when the fungus is exposed to azole compounds that exhibit anti-*Aspergillus* activity [18]. Although *A. fumigatus* is not a phytopathogen, manyazole fungicides were found to have activity against *A. fumigatus* isolates [19, 20]. Some of the azole fungicides are of the triazole class and have a similar molecular structure to the medical triazoles [20]. It was hypothesized that *A. fumigatus* develops resistance due to use of azole fungicides to combat phytopathogens for crop protection. Because of the molecule similarity of fungicides with medical triazoles, the latter also lose activity. In addition to abundant asexual reproduction, parasexual and sexual reproduction probably also occurs in the environment, thereby increasing the fungus’s ability to undergo genetic recombination and thus overcome cellular stress caused by fungicide exposure. Azole fungicides have a broad range of applications, including plant and crop protection, prevention of postharvest spoilage, and preservation of materials. Azole fungicides are used globally, thus creating an environment where azole-resistant *A. fumigatus* can thrive. In contrast to the United States, where environmental azole resistance in *A. fumigatus* appears to be uncommon [21], health authorities in Europe have been called to action. The European Centre for Disease Prevention and Control brought together experts from agricultural, veterinary, and medical fields to discuss the problem of emerging azole resistance in *Aspergillus* [22].

Clinically, environmental resistance is characterized by a complete lack of patient risk factors. Only residency in or visiting of a geographic area with known environmental resistance can be considered a risk.

### EPIDEMIOLOGY

In vitro susceptibility testing of *A. fumigatus* isolates is not routinely performed in most clinical microbiology laboratories, thus underestimating the true prevalence of resistance. Studies that had investigated the frequency of azole resistance in *Aspergillus* culture collections report finding the first resistant isolates up to 17 years earlier [23]. TR34/L98H was first found in the Netherlands in 1998 [13], and recently a TR34/L98H isolate was reported from Italy, also originating from 1998 [23]. The TR46/Y121F/T289A resistance mechanism was also first reported in the Netherlands in 2009 [24], but a recent study reported the recovery of TR46/Y121F/T289A from a patient in the United States already in 2008 (Table 2) [25]. Surveillance studies and case series suggest the global presence of azole resistance in *A. fumigatus* [15, 16, 25–39], including reports from Europe, the Middle East, Asia, Africa, Australia, and most recently, North and South America (Figure 1) [25, 35].

It remains unclear when and where these resistance mechanisms first emerged, although genotyping of epidemiologically and geographically unrelated strains shows a lower genetic diversity among isolates harboring TR34/L98H and TR46/Y121F/T289A compared with wild-type isolates, which suggests that each mutation might have originated from a common ancestor [24, 40, 41]. Our current understanding is that resistance traits can migrate rapidly. Isolates harboring TR46/Y121F/T289A from the Netherlands were found to be genetically highly related to resistant isolates from India [30]. Whole-genome sequencing and population analysis indicated that azole-resistant alleles are segregating into diverse genetic backgrounds.

### Table 1. Characteristics of Patient-Acquired Resistance and Environmental Resistance in *Aspergillus fumigatus*

<table>
<thead>
<tr>
<th>Patient-Acquired Resistance</th>
<th>Environmental Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic pulmonary aspergillosis with cavitary lesion or aspergilloma</td>
<td>All <em>Aspergillus</em> diseases, including allergic bronchopulmonary aspergillosis, acute invasive aspergillosis, chronic colonization in cystic fibrosis</td>
</tr>
<tr>
<td>Previous or ongoing azole therapy in all patients</td>
<td>Two-thirds of patients have no history of azole therapy</td>
</tr>
<tr>
<td>Clinical failures to azole therapy</td>
<td>Clinical failures to azole therapy</td>
</tr>
<tr>
<td>Multiple resistance mutations may be present in a single clinical sample</td>
<td>Only 1 azole resistance mechanism present in most patients</td>
</tr>
<tr>
<td>Both azole-susceptible and azole-resistant phenotypes simultaneously present in culture</td>
<td>Both azole-susceptible and azole-resistant phenotypes simultaneously present in culture</td>
</tr>
<tr>
<td>Multiazole and panazole resistance phenotypes</td>
<td>Multiazole and panazole resistance phenotypes</td>
</tr>
<tr>
<td>Point mutations in the Cyp51A gene, including substitutions at G54, P219, M220, G138, Y431, and G448 non-Cyp51A-mediated resistance mechanisms: HapE unknown resistance mechanisms</td>
<td>Mutations in the Cyp51A gene in combination with a transcriptional enhancer (tandem repeat) in the promoter region of the gene: TR46/L98H, TR34, and TR46/Y121F/T289A</td>
</tr>
<tr>
<td>High genetic diversity between azole-resistant isolates from unrelated patients</td>
<td>Low genetic diversity between azole-resistant isolates from unrelated patients</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> colonies may show an abnormal colony morphology, lack of sporulation or reduced growth rate</td>
<td>No apparent fitness cost</td>
</tr>
</tbody>
</table>

*Recently the presence of 2 point mutations was reported in the environment: G54 and M220 [15, 16].*
which will result in increasing genetic diversity over time [42]. As far as we know, azole resistance, due to mutations in the Cyp51A gene, is not associated with a fitness cost [43]. The consequence is that resistant isolates would be predicted to compete with wild-type isolates in the field and persist in the environment.

Surveillance studies have shown that between 64% and 71% of patients with Aspergillus disease due to environmental azole-resistant A. fumigatus had no history of prior azole therapy [24, 44]. Furthermore, azole resistance may occur in any Aspergillus disease, including acute invasive aspergillosis, chronic pulmonary aspergillosis, or allergic manifestations such as allergic

Table 2. Country and Year of First Recovery of TR34/L98H and TR46/Y121F/T289A Resistance Mechanisms in Aspergillus fumigatus and Year of Publication*

<table>
<thead>
<tr>
<th>TR34/L98H</th>
<th>TR46/Y121F/T289A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>First Case</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>2009–2011</td>
</tr>
<tr>
<td>France</td>
<td>2010</td>
</tr>
<tr>
<td>United States</td>
<td>2010</td>
</tr>
<tr>
<td>Germany</td>
<td>2012</td>
</tr>
<tr>
<td>Taiwan</td>
<td>2011</td>
</tr>
<tr>
<td>Kuwait</td>
<td>2013</td>
</tr>
<tr>
<td>Colombia</td>
<td>2015</td>
</tr>
</tbody>
</table>

Abbreviations: C, clinical; E, environmental.

* Due to space restriction, we were not able to include all individual publications. We have cited reviews, which included reports from individual countries over the years.

Figure 1. Shaded areas show countries that have reported the TR34/L98H and TR46/Y121F/T289A resistance mechanism in clinical or environmental Aspergillus fumigatus isolates.
bronchopulmonary aspergillosis. It is possible that azole prophylaxis or azole monotherapy provides a selective advantage for azole-resistant *A. fumigatus* and might increase the risk for azole-resistant breakthrough infections [45]. Unfortunately, the environmental resistance route was found to be the dominant route for resistance cases. In the Netherlands, between 82% and 89% of azole-resistant cases were due to TR34/L98H and TR46/Y121F/T289A (P. Verweij, personal communication), whereas this was the case in 64% of cases in Belgium [27] and 87% of cases in Turkey [26].

**DETECTION OF AZOLE-RESISTANT DISEASE**

Aazole-resistant *Aspergillus* disease is difficult to diagnose, as *Aspergillus* cultures are negative in the majority of patients. Biomarkers based on *Aspergillus* cell wall components, such as galactomannan or 1,3-β-D-glucan, are unable to detect azole resistance. At best, circulating biomarkers may indicate treatment failure if they continue to increase during azole therapy. Several investigators have used in-house molecular tests to detect azole resistance mutations directly in patient samples, using both tissue and respiratory secretions [46,47]. Recently, a commercial polymerase chain reaction (PCR)–based assay became available (AsperGenius, PathoNostics, Maastricht, the Netherlands) that enables the detection of several *Aspergillus* species and includes markers for the detection of the TR34/L98H and TR46/Y121F/T289A resistance mechanisms. Preliminary clinical validation studies indicate that this approach is feasible when bronchoalveolar lavage fluid is tested, although only very few *Aspergillus* culture-negative and resistance PCR-positive cases have been described [48]. The sensitivity of the resistance PCR might be a limiting factor, as only a single copy of the *Cyp51A* gene is present in each *Aspergillus* cell, in contrast with the multigene targets that are commonly used for detection of *Aspergillus* species. This is especially a concern when only serum is tested, although only very few *Aspergillus* culture-negative and resistance PCR-positive cases have been described [48].

Azole resistance can be tested when *A. fumigatus* is recovered through culture. However, even in culture-positive patients, resistance may be missed due to concomitant presence of azole-susceptible and azole-resistant colonies [38]. Furthermore, positive cultures may have to be sent to reference laboratories, due to limited availability or experience with fungal resistance testing on site, thus causing delay of effective therapy.

**MANAGEMENT OF AZOLE-RESISTANT ASPERGILLUS DISEASES**

All studies to date show that azole resistance is associated with treatment failure [24,36,44,45]. Mortality rates in case series of patients with culture-positive azole-resistant invasive aspergillosis varied between 50% and 100% [24,44,45]. Preclinical experimental models also indicate that an elevated azole minimum inhibitory concentration (MIC) significantly reduces the efficacy of azole monotherapy [49], but controlled trials that compare azole-resistant with azole-susceptible cases in relation to treatment success have not been performed. Nevertheless, it seems important to identify patients with azole-resistant *Aspergillus* disease as early as possible to initiate effective therapy. Furthermore, azole resistance mechanisms generally reduce the activity of all azoles. In vitro susceptibility testing of TR34/L98H isolates showed that 99.6% of isolates were resistant to itraconazole, 92.4% to voriconazole, and 97.8% to posaconazole. For TR46/Y121F/T289A 100% of isolates were resistant to voriconazole, whereas 82.7% were resistant to itraconazole and 94.9% to posaconazole [50]. The recently introduced new azole isavuconazole also had high MICs in strains with reduced susceptibilities to other triazoles, mirroring changes in voriconazole susceptibility [5]. These results indicate that the clinical role of azoles in azole-resistant aspergillosis will, at best, be very limited.

In the absence of management guidelines, an expert panel recently discussed the approach they would use in patients with documented azole-resistant *Aspergillus* disease, or in regions where azole resistance has been reported [51]. As clinical evidence is generally lacking, the panel members relied on anecdotal experience, preclinical studies, and expert opinion with respect to treatment decisions. Most experts recommended moving away from azole monotherapy in patients in whom azole resistance was documented, switching to liposomal amphotericin B or voriconazole in combination with an echinocandin. In areas with confirmed environmental resistance, the threshold at which first-line therapy with azole monotherapy should be avoided was the subject of much debate, but most experts would consider moving away from azole monotherapy when resistance rates exceeded 10%. In that situation, azole-echinocandin combination therapy or liposomal amphotericin B was deemed an appropriate alternative choice [51]. It is therefore important to determine if azole resistance is present in a hospital by regular resistance testing of (stored) clinical *A. fumigatus* isolates. Most surveillance studies indicate that the frequency of azole resistance is still below the 10% threshold [52]. These studies relied on screening of unselected clinical *A. fumigatus* isolates, through, for instance, the use of agar plates supplemented with different azoles [52]. Although this approach is useful to determine the frequency of resistance, the role of environmental mutations, and trends over time, 2 recent Dutch studies indicated that the frequency of resistance may vary considerably between departments or risk groups within the same hospital. In one study, a resistance rate of 26% was found in *A. fumigatus* culture-positive patients in the intensive care unit, which was higher than in all other departments in the hospital (14%; *P* = .06) [53]. The authors suggested that patients with (undiagnosed) azole-resistant invasive aspergillosis...
might fail azole therapy while in the department. Subsequent clinical deterioration of the patient requires intensive care support where cultures become positive due to progressive disease. Another study reported the highest azole resistance rates in hematology patients, when primary Aspergillus fumigatus cultures were analyzed for resistance [54]. Therefore, general resistance surveillance might not reflect resistance rates in specific high-risk patient groups and detailed audits will be required to determine which primary treatment strategy would be appropriate.

A “POSTAZOLE” ERA?

Compared with antibacterial resistance, the looming problem of azole resistance in A. fumigatus may seem relatively insignificant as the number of patients affected is low and the question can be raised if drug resistance in an opportunistic pathogen is altogether a threat to public health. After all, Aspergillus diseases affect only specific patient groups with chronic lung disease or those with immunosuppression. Although the number of azole-resistant cases is still low, there is every reason to assume that new azole resistance mechanisms will continue to emerge in the environment and rapidly migrate across the world, as has been the case with TR34/L98H and TR46/Y121F/T289A [12, 13, 24]. Increasing azole resistance rates will challenge our current primary treatment recommendation (ie, voriconazole monotherapy), necessitating alternative treatment strategies such as azole-echinocandin combination therapy or liposomal amphotericin B in hospitals or wards where the 10% resistance threshold is exceeded [51]. In addition, the number of cases of breakthrough aspergillosis in patients on azole prophylaxis will increase and certain manifestations of invasive aspergillosis, such as central nervous system aspergillosis, will be virtually untreatable as the use of voriconazole will be precluded. The advances made with the clinical use of the azole class will be at least partly lost and, unless new drug targets are discovered, the overall mortality of Aspergillus diseases will increase (Table 3).

RETYAINING THE AZOLE CLASS

In medicine we are confronted with the consequences of azole resistance selection in the environment, and given the prominent role of azole compounds both for management of fungal disease in humans and animals and for food production, the optimum strategy to overcome azole resistance would be to aim to retain the use of azoles for both applications. Measures that prohibit the use of specific azoles in the environment may severely compromise global food production and may not be effective. Although 5 azole fungicides were identified that might play a role in the emergence of resistance mutations [11, 20, 22], many azole fungicides show activity against A. fumigatus and thus may contribute to providing an environment with a selective advantage for azole-resistant strains, thus facilitating its persistence and spread.

An integrated approach focusing on clinical management, public health surveillance programs, and resistance selection in the environment is necessary to improve the survival of patients with azole-resistant aspergillosis, to track the emergence and spread of resistance mechanisms, and to understand how azole resistance develops in A. fumigatus in the environment.

Investigations in the environment should incorporate all applications of azoles including those in agriculture, biocides, and medicine. Recently, 2 Cyp51A point mutations, G54 and M220, were recovered from the environment in Germany [15], Romania, India, and Tanzania [16]. These mutations were previously considered to be associated with the patient route of resistance development, but the recovery from the environment suggests that these mutations either develop in the environment or

### Table 3. Reported Mortality Rates in Patients With Invasive Aspergillosis in Different Time Periods

<table>
<thead>
<tr>
<th>Era</th>
<th>IA</th>
<th>CNS IA</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azole era</td>
<td>27.5% [57] 9-wk mortality: 39 of 142 patients receiving voriconazole monotherapy.</td>
<td>45.6% [7] 35.4% [59] Retrospective analysis of 81 patients with CNS IA treated with voriconazole.</td>
<td>Review of 141 cases of CNS IA in immunocompromised patients, of whom 140 died.</td>
</tr>
<tr>
<td>Azole resistant</td>
<td>100% [44] Culture-positive patients with proven and probable IPA treated with voriconazole (5/5)</td>
<td>86% [24, 44, 60] 7 cases of azole-resistant CNS IA have been reported, of which 6 were fatal.</td>
<td>88% [45] 8 HSCT patients with culture-positive, azole-resistant IA, of whom 7 died.</td>
</tr>
<tr>
<td></td>
<td>100% [54] ICU patients with culture-positive azole-resistant IA died (10/10), compared with 21 of 28 (75%) with azole-susceptible IA.</td>
<td>88% [24, 44, 60] 7 cases of azole-resistant CNS IA have been reported, of which 6 were fatal.</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** c-AmB, conventional amphotericin B; CNS, central nervous system; HSCT, hematopoietic stem cell transplant; IA, invasive aspergillosis; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis.
that through use of azoles in hospitals and veterinary practices these mutations migrate to the environment. By understanding how azole resistance develops and persists in the environment, effective measures can be designed and implemented to prevent resistance development. It was suggested that the application of azole fungicides is crucial for the risk of resistance selection in Aspergillus fumigatus rather than the volume of use [61]. If this is the case, changes in current practices may reduce the risk of resistance selection without losing the azole class as a whole. An integrated approach would require an international and multidisciplinary collaboration including healthcare professionals, epidemiologists, researchers from veterinary medicine, mycologists, and experts in fungal genetics. Furthermore, governments and other policy makers should recognize that action is urgently warranted if we want to retain the clinical use of azoles and evade a “postazole” era. However, if we are successful in preventing azole resistance selection in the environment, only time will tell if the clinical burden of azole-resistant Aspergillus disease will also diminish.

Note
Potential conflicts of interest. P. E. V. has received research grants from Astellas, Basilea, F2G, Gilead Sciences, Merck, and Pfizer; has been a consultant to Basilea, F2G, Gilead Sciences, Merck, and Pfizer; and has received speaker’s fees from Basilea, Gilead Sciences, and Merck. J. F. M. has received grants from Astellas, Basilea, and Merck; has been a consultant to Astellas, Basilea, and Merck; and has received speaker’s fees from Merck, United Medical, and Gilead Sciences. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Chronic Invasive Sinus Aspergillosis in Immunocompetent Hosts: A Geographic Comparison

Brandon J. Webb · Holenarasipur R. Vikram

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Abstract

Purpose To investigate potential differences in clinical presentation, histopathology, and outcomes of chronic invasive sinus aspergillosis (CISA) based on geographic region and species of Aspergillus isolated.

Materials and Methods A retrospective analysis of published cases of CISA with a comparison of North American and worldwide cases comprised a systematic search of the English language literature. Thirty-four articles were identified detailing 15 North American and 76 global cases of CISA with cranio-cerebral extension in clinically immunocompetent patients.

Results North American patients with CISA were older, had a more rapidly progressive course, and appeared to have higher rates of treatment failure and mortality. Anatomic distribution and presenting symptoms were similar between the two groups. North American cases were mostly due to A. fumigatus, while A. flavus was the predominant pathogen worldwide. While granulomatous inflammation was a rare observation in North American cases, it was seen in the majority of cases worldwide. CISA due to A. fumigatus was encountered in older adults, was associated with a chronic inflammatory response, an accelerated clinical course, and a trend toward treatment failure and higher mortality. Patients with A. flavus were younger, demonstrated granulomatous inflammation, and pursued an indolent, clinically responsive course.

Conclusion Observed differences in clinical presentation, histopathology, and outcome might involve a complex interplay between the human host, Aspergillus species, and local climactic conditions.

Keywords Aspergillus · Chronic invasive · Fungal · Sinusitis · Immunocompetent

Introduction

Invasive sinus Aspergillus infection has been reported in the last decade with increased frequency, most commonly in the setting of hematologic malignancy, neutropenia, HIV infection, and other states of immunosuppression [1, 2]. Less commonly, chronic invasive sinus aspergillosis (CISA) has been reported in apparently immunocompetent patients. While most reports describe an entity unique to the Middle East or the Indian subcontinent, cases are increasingly encountered elsewhere, including in North America. Potentially important differences in microbiology and clinical course have been noted in these geographically
distinct cases. Herein, we present a case CISA in an immunocompetent patient from the United States and provide a comparison of reported cases from North America and the rest of the world.

**Case Report**

A 69-year-old healthy Caucasian male farmer with a history of mild seasonal allergic rhinitis presented with unusually severe nasal obstruction, rhinorrhea and left sinus pressure. He subsequently developed severe left retro-orbital pain. Computed tomography (CT) of the orbits revealed no evidence of abscess or orbital inflammation and limited views of the paranasal sinuses did not suggest any abnormality. Over the next 21 days, he completed a course of amoxicillin and a corticosteroid taper, during which he developed left sixth cranial nerve palsy. Additional CT imaging of the sinuses demonstrated a left sphenoid sinus soft tissue density with erosion of the posterior wall. Paranasal and mastoid air cells were otherwise clear. White blood cell count was \(6.3 \times 10^9/L\), and the erythrocyte sedimentation rate was 33 mm/h. The patient underwent transsphenoidal resection of the clival mass, and preliminary pathology suggested plasmacytoma. On further review of resected tissue specimens, multiple fungal elements with narrow, septated hyphae and acute angle branching indicative of *Aspergillus* sp. were noted. No Charcot-Leyden crystals or eosinophils were identified. Enzyme immunoassay for *Aspergillus* galactomannan was positive and the organism was identified as *A. fumigatus* by polymerase chain reaction.

He was started on intravenous liposomal amphotericin B 350 mg daily and voriconazole 300 mg orally twice a day, and was transitioned to voriconazole monotherapy after 4 weeks. There was no evidence of immunosuppression (as defined below). A dihydrorhodamine assay to assess neutrophil oxidative burst was also negative. Diplopia persisted, and 6 weeks after beginning voriconazole monotherapy, the patient developed acute mental status changes. Magnetic resonance imaging revealed a large infarct in the left superior cerebellar artery distribution and a left midbrain intracerebellar hemorrhage. Despite a repeat attempt to debulk the sphenoid sinus mass, the patient developed a large pontine hemorrhage and died 140 days after the onset of his initial symptoms.

**Methods**

A case of CISA was defined as histologically or microbiologically confirmed sinus infection with *Aspergillus* sp. along with evidence of hyphal invasion of the sinus wall, orbit, or presence of intracranial extension. Patients were excluded if they met any of the following criteria for immunodeficiency: history of solid organ or hematologic malignancy, organ transplantation, HIV infection, cancer chemotherapy, neutropenia, oral or nasal corticosteroid therapy prior to symptom onset, diabetes mellitus, and chronic renal or liver disease. Additional exclusion criteria were: previous sinus or orbital surgery, or cases describing features suggestive of allergic fungal rhinosinusitis (AFRS) (nasal polyposis, eosinophilic infiltration or Charcot-Leyden crystals). Articles published before 1980, which approximately corresponds with both the introduction of modern azole antifungals active against *Aspergillus* sp., and the initial descriptions of AFRS, were not included.

A comprehensive search of the English language medical literature was performed between 1980 and October 2009 using Medline and PubMed (U.S. National Library of Medicine) databases. Search terms “*Aspergillus*”, “aspergilloma”, “sinus”, “fungal sinusitis”, “rhinosinusitis”, “sino-orbital”, “immunocompetent”, and “invasive” were utilized in various combinations. References of articles were also searched for additional cases. Sixty-six articles describing 187 patients were manually reviewed. Fifty patients were excluded on the basis of underlying immunosuppression. Individual patient data was unavailable for 28 patients; 10 patients failed to meet criteria for anatomic involvement or invasive disease, and eight patients were suggestive of AFRS. Our final cohort consisted of 91 cases of CISA identified from 34 articles.

Cases were sorted by geographic region of origin (See Table 1). Outcomes were categorized based treatment response (cure or remission), and mortality. We also analyzed treatment response to first round of therapy (medical, surgical, or both). Statistical analysis of categorical variables was performed with the Chi-square test with Yate’s correction for continuity.

### Results

Fifteen cases originated from North America [3–13] (See Table 2). Seventy-six cases were included from other worldwide locations, of which the Indian subcontinent, the Middle East and Africa accounting for the majority [1, 9, 14–33]. No significant difference in gender distribution between the North American and global cohort was noted. Anatomic distribution of infection and presenting symptoms was also similar between the North American and worldwide cases.

Important variation between cohorts was found with respect to age, species, histopathology, and time of onset. North American patients were older and experienced a much shorter mean time from symptom onset to diagnosis than those in the global cohort. Where species was identified, *Aspergillus fumigatus* was responsible for 11/13 (84.6%) North American cases, whereas *A. flavus* was identified in 40/51 (78.4%) of global cases. Chronic inflammation or hyphal invasion of adjacent tissues was predominant in the North American group, while granulomatous inflammation was reported in the majority of global cases. Although choice of anti-fungal therapy showed similar variation between the two groups, significantly fewer cases from North America reported combined anti-fungal therapy and surgical intervention. North American patients failed initial therapy or suffered relapse at a substantially higher rate than patients in the global cohort. Mortality in the North American group was 42.9%, compared to 26.5% of global cases.

When clinical and laboratory data was compared between the two predominant *Aspergillus* species, (Table 3), statistically significant differences were noted with respect to age and inflammatory response. Cases of *A. fumigatus* infection presented with non-granulomatous inflammation in older patients. In this group, symptom duration was shorter and increased rates of treatment failure and mortality was observed, although statistical significance was not achieved.

A brief analysis of management strategies among all cases was also performed (Table 4). Treatment failure and mortality rates were not associated with degree of surgical intervention. Likewise, mortality was similar whether surgery alone, anti-fungal therapy alone or a combined approach was undertaken. However, with regard to antifungal regimen, patients receiving azoles with activity against *Aspergillus* (ketaconazole, itraconazole, voriconazole) alone or in combination with amphotericin B survived more often, compared to patients receiving amphotericin B alone.

### Discussion

*Aspergillus* species are the most common cause of fungal rhinosinusitis worldwide [34]. Although some debate exists regarding classification of fungal sinus infection [35], this disease can be broadly categorized as non-invasive (inclusive of mycetoma and allergic fungal rhinosinusitis), [35, 36] or invasive. Invasive fungal sinusitis comprises of three subcategories: acute invasive, chronic invasive and granulomatous [34, 37]. The acute or fulminant invasive form was first described by McGill et al. in 1980 [37]. It is marked by vascular hyphal invasion, hemorrhage, and infarction, time course less than 4 weeks, and a predilection for the immunocompromised host [35, 38].
Less distinction, however, exists between the chronic invasive and granulomatous forms, calling into question the clinical relevance of the aforementioned classification [8, 39]. The granulomatous form has been extensively described among immunocompetent patients in arid tropical regions in whom non-caseating granulomas are common and *A. flavus* is the predominant pathogen [1, 14, 17, 18, 21, 40, 41]. In contrast, the chronic invasive form was originally reported in association with diabetes mellitus or corticosteroid use, with a sparse inflammatory response [38]. It is now clear, however, that non-granulomatous *Aspergillus* invasion of the sinus wall can occur in the absence of clinically significant immunodeficiency [3, 9].

Our data confirms laboratory distinction between chronic invasive and granulomatous fungal sinusitis

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical and laboratory data in patients with CISA by geographic location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>North America (%)</td>
</tr>
<tr>
<td>Number (N)</td>
<td>15</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9/15 (60)</td>
</tr>
<tr>
<td>Male</td>
<td>6/15 (40)</td>
</tr>
<tr>
<td>Age–years (range)</td>
<td>57.3 (21–77)</td>
</tr>
<tr>
<td>Species</td>
<td></td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>2/13 (15.4)</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>11/13 (84.6)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
</tr>
<tr>
<td>Granulomatous</td>
<td>1/15 (6.7)</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>7/15 (46.7)</td>
</tr>
<tr>
<td>Tissue/vascular invasion</td>
<td>7/15 (46.7)</td>
</tr>
<tr>
<td>Anatomic extension</td>
<td></td>
</tr>
<tr>
<td>Intracranial (intradural)</td>
<td>5/15 (33.3)</td>
</tr>
<tr>
<td>Sinus wall, orbit or cranial base (extradural)</td>
<td>10/15 (66.7)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>7/15 (46.6)</td>
</tr>
<tr>
<td>Proptosis</td>
<td>6/15 (40.0)</td>
</tr>
<tr>
<td>Nasal obstruction</td>
<td>4/15 (26.7)</td>
</tr>
<tr>
<td>Diplopia</td>
<td>6/15 (40.0)</td>
</tr>
<tr>
<td>Periorbital pain</td>
<td>6/15 (40.0)</td>
</tr>
<tr>
<td>Symptom onset to diagnosis</td>
<td></td>
</tr>
<tr>
<td>Mean (months)</td>
<td>3.71</td>
</tr>
<tr>
<td>Surgical treatment</td>
<td></td>
</tr>
<tr>
<td>Surgery alone</td>
<td>2/15 (13.3)</td>
</tr>
<tr>
<td>Antifungal alone</td>
<td>7/15 (46.7)</td>
</tr>
<tr>
<td>Surgery + antifungal</td>
<td>6/15 (40.0)</td>
</tr>
<tr>
<td>Antifungal therapy</td>
<td></td>
</tr>
<tr>
<td>AmB* alone</td>
<td>6/15 (40.0)</td>
</tr>
<tr>
<td>Azole alone</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>Combined AmB + Azole</td>
<td>7/15 (46.7)</td>
</tr>
<tr>
<td>1st line treatment failure</td>
<td>13/15 (86.7)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>Cure/remission</td>
<td>8/14 (57.1)</td>
</tr>
<tr>
<td>Mortality</td>
<td>6/14 (42.9)</td>
</tr>
</tbody>
</table>

* AmB amphotericin B
and sheds light on notable differences based on geographic location and involved species of *Aspergillus*. In North America, *A. fumigatus* is associated with a rapid invasion of adjacent tissues in older patients. Inflammatory response is non-granulomatous and patients tend to have a poor prognosis. *A. flavus*, however, was identified in the vast majority of worldwide cases; patients tend to be younger, with a protracted clinical course, and predominant granulomatous histology. Response to treatment and overall mortality were also better in the worldwide group, although the number of cases were insufficient to draw statistical conclusions.

Several factors underlie the dichotomous geographic distribution of *Aspergillus* species. Although both species are considered ubiquitous saprophytic organisms [42, 43], *A. fumigatus* appears to be particularly tolerant to variations in temperature [44] and has been detected in greater concentration in cooler air samples of Europe and North America [42, 44–46]. Similarly, *A. flavus* is the most commonly isolated species from environmental samples in areas where granulomatous fungal sinusitis predominates [42, 47, 48]. This is likely attributable to the tropical climate which also promotes a microaerophilic sinus environment conducive to the growth of *A. flavus* [1].

In addition to geographic variations, significant differences based on *Aspergillus* species involved was also noted pertaining to host response, clinical course and perhaps prognosis. *A. flavus* was strongly

### Table 3 Clinical and laboratory data in patients with CISA by species

<table>
<thead>
<tr>
<th></th>
<th><em>Aspergillus fumigatus (%)</em></th>
<th><em>Aspergillus flavus (%)</em></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (N)</td>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Age–years [range]</td>
<td>57.6 [21–77]</td>
<td>38.1 [17–65]</td>
<td>0.0002</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue/vascular Invasion</td>
<td>11/19 (57.9)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Granulomatous</td>
<td>6/19 (31.6)</td>
<td>23/26 (88.5)</td>
<td></td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>4/19 (21.1)</td>
<td>3/26 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Anatomic extension</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Intradural</td>
<td>10/19 (52.6)</td>
<td>11/27 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Extradural</td>
<td>9/19 (47.4)</td>
<td>16/27 (59.3)</td>
<td></td>
</tr>
<tr>
<td>Onset to diagnosis</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Mean (months)</td>
<td>6.6</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td>First line treatment failure</td>
<td>13/20 (65.0)</td>
<td>9/26 (34.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Cure/remission</td>
<td>11/19 (57.9)</td>
<td>21/25 (84.0)</td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td>8/19 (42.1)</td>
<td>4/25 (16.0)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4 Management strategies among all cases

<table>
<thead>
<tr>
<th>Treatment Strategy</th>
<th>Number of cases</th>
<th>Response (%)</th>
<th>Mortality (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of surgical intervention</td>
<td></td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Resection</td>
<td>54</td>
<td>38 (70.4)</td>
<td>16 (29.6)</td>
<td></td>
</tr>
<tr>
<td>Debridement/partial resection</td>
<td>18</td>
<td>13 (72.2)</td>
<td>5 (27.8)</td>
<td></td>
</tr>
<tr>
<td>Biopsy only</td>
<td>14</td>
<td>11 (78.6)</td>
<td>3/14 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Antifungal therapy</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>AmB* alone</td>
<td>28</td>
<td>15 (53.6)</td>
<td>13 (46.4)</td>
<td></td>
</tr>
<tr>
<td>Azole</td>
<td>20</td>
<td>20 (100)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Combined AmB + Azole</td>
<td>29</td>
<td>21 (72.4)</td>
<td>8 (27.6)</td>
<td></td>
</tr>
<tr>
<td>Medical vs. surgical therapy</td>
<td></td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>5</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Anti-fungal alone</td>
<td>16</td>
<td>13 (81.3)</td>
<td>3 (18.7)</td>
<td></td>
</tr>
<tr>
<td>Surgery + anti-fungal</td>
<td>64</td>
<td>46 (71.2)</td>
<td>18 (28.8)</td>
<td></td>
</tr>
</tbody>
</table>

* AmB amphotericin B
associated with a granulomatous response, whereas infection with *A. fumigatus* elicited chronic inflammation or simple hyphal tissue invasion. Mechanisms of immunologic response to *Aspergillus* infection in the apparently immunocompetent host remain unclear [43] and may involve poorly characterized subcellular deficiencies [1, 49, 50]. The tendency for *A. fumigatus* chronic fungal sinusitis to occur in older patients, with rapid progression and poor outcomes suggests unique host–pathogen interactions, which are not yet completely understood [51, 52].

Our study has a number of limitations. Given the retrospective nature of data collection, available information was limited to published reports. The number of cases of CISA meeting inclusion criteria was fewer than those of the granulomatous variety. We could not adjust for genetic, nutritional, or socioeconomic heterogeneity between patient populations. Investigations to exclude an underlying immunodeficiency were not consistent for most of the reported cases. Publication bias could have led to selection of cases based on disease severity and poor outcomes. Lastly, we acknowledge the potential for some overlap with allergic fungal rhinosinusitis, given rare reports of local invasion with this entity.

Significant differences in treatment strategies were noted, especially pertaining to surgical or antifungal monotherapy versus a combined approach. Although our pooled data does not suggest a strong correlation between outcome and treatment modality, possibility exists that differences in treatment strategies may have affected mortality. Clearly, larger studies of patients with CISA are needed. Although antifungals with potent activity against *Aspergillus* sp. (lipid formulations of amphotericin B, voriconazole, echinocandins, and posaconazole) were administered to less than 10% of cases, mortality was lower among patients receiving these agents compared those who received amphotericin B. These data corroborate other studies that confirm the superiority of newer azoles over amphotericin B for invasive aspergillosis. [20, 23, 31, 33, 51, 53].

**Conclusion**

Chronic invasive fungal sinusitis is being recognized with increased frequency in immunocompetent patients. *Aspergillus* sp. are the most common etiologic agents. Clear differences exist between the two subcategories of this disease (chronic invasive and chronic granulomatous), which may be partially attributable to predominance of *A. fumigatus* among North American cases, and *A. flavus* in Africa, the Middle East and the Indian subcontinent. This microbiological distinction may also underlie differences in host response, clinical course, and prognosis. A prospective study of patients with clearly defined and histopathologically proven CISA in immunocompetent hosts can provide us with a better understanding of optimal management strategies and outcomes for this potentially serious illness.

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**Conflict of Interest** None.

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Report of the 2nd Meeting of the Global AMR Surveillance System (GLASS) Collaborative Platform

15-16 December 2016
Kempinski Hotel
Geneva, Switzerland

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Abbreviations

AFR        antifungal resistance
AFST       antifungal susceptibility testing
AGISAR     Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR        antimicrobial resistance
AST        antibacterial susceptibility testing
BSI        bloodstream infection
CAESAR     Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CC         Collaborating Centre
CDC        US Centers for Disease Control and Prevention
CIA        critically important antimicrobials
CLSI       Clinical and Laboratory Standards Institute
CPG        clinical practice guideline
CRE        carbapenem-resistant Enterobacteriaceae
DRC        Democratic Republic of the Congo
EARS-Net   European Antimicrobial Resistance Surveillance Network
ECDC       European Centre for Disease prevention and Control
EGASP      Enhanced Gonococcal Antimicrobial Surveillance Programme
EQA        external quality assessment
ESBL       extended-spectrum beta-lactamase
EUCAST     European Committee on Antimicrobial Susceptibility Testing
FAO        Food and Agriculture Organization of the United Nations
FIND       Foundation for Innovative New Diagnostics
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>GASP</td>
<td>Gonococcal Antimicrobial Surveillance Programme</td>
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<tr>
<td>GLASS</td>
<td>Global Antimicrobial Resistance Surveillance System</td>
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<tr>
<td>GFN</td>
<td>WHO Global Foodborne Disease Network</td>
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<tr>
<td>ICU</td>
<td>intensive care unit</td>
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<tr>
<td>LSHTM</td>
<td>London School of Hygiene and Tropical Medicine</td>
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<td>MIC</td>
<td>minimum inhibitory concentration</td>
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<td>MM</td>
<td>molecular methods</td>
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<td>MRSA</td>
<td>methicillin-resistant <em>Staphylococcus aureus</em></td>
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<td>NAAT</td>
<td>nucleic acid amplification test</td>
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<td>NAP</td>
<td>national action plan</td>
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<td>NGO</td>
<td>nongovernment organization</td>
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<td>NRL</td>
<td>national reference laboratory</td>
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<td>OIE</td>
<td>World Organisation for Animal Health</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>POC</td>
<td>point-of-care</td>
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<td>PPS</td>
<td>point prevalence study</td>
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<tr>
<td>ReLAVRA</td>
<td>Latin American Antimicrobial Resistance Surveillance Network</td>
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<td>STI</td>
<td>sexually transmitted infection</td>
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<td>TB</td>
<td>tuberculosis</td>
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<td>TOR</td>
<td>terms of reference</td>
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<td>TP</td>
<td>target product</td>
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<td>TPP</td>
<td>target product profile</td>
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<td>WGS</td>
<td>whole genome sequencing</td>
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<td>WHA</td>
<td>World Health Assembly</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Summary Report

Introduction

On 15-16 December 2016 WHO hosted a meeting with WHO Collaborating Centres, partner technical institutions and international AMR surveillance networks on the implementation of the WHO global antimicrobial resistance surveillance system (GLASS). The purpose of the meeting was to support development, dissemination and implementation of global AMR surveillance. Expected outputs from the meeting were:

- A common understanding of the status of GLASS development and support to global AMR surveillance efforts
- Defined next steps to address some AMR surveillance challenges:
  i. AMR in invasive fungi
  ii. Detection of emerging colistin resistance in *Enterobacteriaceae* and application of molecular methods for AMR surveillance
  iii. Rapid alert component for emerging/new types of AMR within GLASS

1. Global surveillance of AMR and antimicrobial use: an update from WHO and partners

During Session One, participants were updated on the following:

1.1. Overview of GLASS and update on early implementation.

GLASS is on track with its early implementation road map. By December 2016, 38 countries have expressed interest in enrolling in GLASS and 29 have completed procedures for enrolment. Next steps include:

- Development of a rapid detection and alert framework
- 2nd Member States consultation, April 2017 in Sweden.
- Explore and start planning on: (i) AMR surveillance in invasive fungal infections and (ii) the application of molecular methods for AMR surveillance
- Create new modules in the GLASS IT platform for ESBL-*E.coli* Tricycle surveillance, Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP), and antimicrobial consumption.
- 1st GLASS report in the fourth quarter of 2017.

1.2 Surveillance of AMR in the food chain

Surveillance of AMR in the food chain is moving from a medical view of bacterial resistance to a more holistic, integrated, multisectoral, “One Health” approach. A revised list of critically important antibiotics and revised WHO AGISAR Guidance on Integrated Surveillance of AMR will be published in early 2017. Between 2010 and 2014, more than 26 AGISAR research projects and country pilot projects were carried out. Current activities include:

- The AGISAR ESBL-*E.coli* "Tricycle" project that aims to develop a global harmonized protocol on integrated surveillance of ESBL-producing *E.coli* in humans, the food chain and the environment
- Participation in Codex Alimentarius Scientific Advice on Antimicrobial Resistance to provide guidance on the design and implementation of a system for integrated surveillance of AMR
• A global sewage surveillance project in 63 countries.

1.3 Surveillance of antimicrobial use

Activities include:

• Ongoing integration of the antimicrobial consumption module into the GLASS IT platform
• Ongoing development of protocols for surveys to collect antimicrobial use (prescription and patient purchase) data
• Updating the Essential Medicines List with considerable revision to the chapter on antibiotics. A syndrome-based review of antibiotic treatments for 16 of the most common and/or severe infectious syndromes and five paediatric syndromes is being carried out. For each syndrome, there will be a proposed three-level antibiotic listing. The updated lists will be published in May 2017.
• The priority pathogens list to inform R&D will be available in February 2017.
• Development of policy recommendations on:
  o how AMR information is made available and used in Clinical Practice Guidelines
  o Key messages in antibiotic awareness campaigns
• Support to development of antimicrobial stewardship programmes to ensure appropriate and responsible use of antimicrobials
• The WHO Medicines Price and Availability Monitoring methodology: a mobile app, Survey123 Platform, is in development and there is an antibiotics survey plan for 2017

1.4 Surveillance of AMR in gonococci: update from the Gonococcal Antimicrobial Surveillance Programme (GASP)

The magnitude of the gonococcal AMR problem is not completely known due to lack of data in many countries. A recent survey of 108 countries found that less than half (46) countries conducted AMR testing for *Neisseria gonorrhoeae* in the past five years. The lack of information is particularly acute in countries with the highest *N. gonorrhoeae* burden and the greatest need for AMR monitoring. Challenges include: the increasing number of countries participating in GASP; achieving standardized and comparable data; improving on time, ad hoc reporting; information sharing; and sustaining regular monitoring instead of ad hoc surveys.

GASP is in the process of being integrated into GLASS – this will enable both national and regional focal points to access data via GLASS. As it is difficult to culture *N. gonorrhoeae*, molecular approaches are now being considered for monitoring and surveillance. GASP recommends that countries develop their national treatment guidelines based on national surveillance data. With increasing levels of resistance to azithromycin and ciprofloxacin/quinolones, countries must accord funds to surveillance systems – at the moment, budgets are being spent on drugs that are ineffective due to resistance.

1.5 Fostering the development of new diagnostic tests

An overview of the process for the development of new diagnostic tests for AMR was presented using the example of a consensus Target Product Profile for a tool to differentiate between bacterial and non-bacterial infections.
1.6 Global AMR Surveillance: Update from Food and Agriculture Organization (FAO)

The FAO AMR multidisciplinary working group and FAO AMR action plan were described. The complex nature of surveillance in this area, which includes meat, aquatic products, agriculture and fisheries was highlighted. Due to poor surveillance and data collection in many countries, estimates of antibiotic consumption in global agriculture vary, ranging from around 63 000 tonnes/year to over 240 000 tonnes/year. Between 70%-80% of antibiotics given to fish are excreted into water and spread through water systems. Between 75%-90% of tested antibiotics are excreted from animals un-metabolized and enter sewage systems and water sources. Information on the health and economic impacts of AMR on livestock and fisheries is lacking in developing countries.

FAO’s action plan focuses on four main areas of work
1. Increasing awareness and advocacy on AMR and related threats.
2. Promoting good practices in food and agriculture systems and the cautious use of antimicrobials.
3. Strengthening governance structures, i.e. policies and regulations, related to antimicrobial use in food and agriculture.
4. Developing capacity for surveillance and monitoring of antimicrobial resistance and antimicrobial usage in food and agriculture.

Discussion points
Discussions related to monitoring the quality of antibiotics; surveillance of ESBL –E.coli in the non-meat food chain; why there is surveillance of ESBL-E.coli and not carbapenemase; who has the lead in setting global standards for surveillance in the food and animal sectors; and how to manage the risk that traditional sources of surveillance information could be diminished/lost through the increased use of rapid point-of-care diagnostics.

2. Support to global surveillance

2.1 The Fleming Fund initiative

This is a £265 million one health programme, funded by UK Government, to support low- and middle-income countries (LMICs) in tackling AMR and to contribute to implementation of the Global Action Plan on AMR. The majority of the investment will be in the form of grants to countries and regions, and through a fellowship scheme, to be implemented by Mott MacDonald. Evaluation of the work will be carried out by ITAD and the University of Sussex, U.K.

An outline of the roadmap to grant making at country and regional level was presented. There will be a strong focus on country ownership. Country assessments to identify candidates for early investment will start in early 2017. These countries will receive support to develop proposals in line with GAP NAPs. There will be two streams: capacity building and surveillance.

Capacity building will cover human resources through the Fleming Fund Fellowship scheme. Laboratory infrastructure capacity building will also be supported.

Surveillance: the focus will use a one health approach where possible. The protocol developed by LSHTM is designed to assist countries to graduate into the lowest tier of GLASS. While many low-income countries (LICs) are still a long way from this step, the
aim of the Fleming Fund is to get these countries to a stage where they can practically implement GLASS. AMR in animal health, in the environment, antimicrobial use and antimicrobial drug quality will also fall under surveillance.

An 8-month inception period is envisaged, during which time the following activities will be carried out:

• Country assessments: desk based
• Early investment and piloting: four early investment countries
• Allocation models: where is the money best spent?
• Develop call for proposals
• Decide and develop funding streams

Following the assessments, countries will be invited to apply for the first wave of grants in 2017-2018 based on geographical spread and readiness of NAPs. This process will then be repeated in the 2018-2019 and 2019-2020 fiscal years. Fleming Fellowships will be available to all countries that are accessing grants. The Fellowships will provide support through mentorship from competent institutions, secondment, training, support for travel and collaborative projects. Expected outcomes include: better surveillance, improved stewardship, improved treatment, and averting the economic and social burden of AMR.

Discussion points:
There was discussion on the need for good coordination between the Fleming Fund and others with similar projects and approaches. The Fleming Fund representative emphasized that they want to complement and synergise investments already made – it is not the intention to complicate the situation, displace funding, or replicate activities. The focus will be on basic microbiology: more complex areas will not be in their purview.

It was noted that there is a concentration of donor support on some geographical areas but not on others. The Fleming Fund acknowledged that this was a challenge as a good coordination forum is lacking. The Fleming Fund representative called on FAO/WHO/OIE or a UN coordinating mechanism for support with this to ensure transparency.

2.2 Presentation of the newly established WHO CC Network and work plan

The newly established WHO AMR Surveillance and Quality Assessment Collaborating Centres Network to support global AMR surveillance capacity building was presented. Network members, drawn from 19 WHO Collaborating Centres, undertake to assist the GLASS Secretariat in the implementation of the 2017-2019 work plan. Four priority areas of work have been identified: capacity building and technical support to microbiology laboratories; capacity building and technical support to surveillance systems; GLASS development; and increasing the understanding of the impact of AMR. Target products for each area of work were defined and Lead CCs assigned. The WHO Collaborating Centre for antimicrobial resistance containment, Sweden (SWE-66) will assist with coordination of the Network for two years on a rotational basis. The report of the meeting, including the terms of reference for the CC Network and the work plan, can be accessed here.

3. AMR technical challenges and results from group work outlining next steps to address these challenges
Presentations were made on key AMR technical challenges following which participants were divided into working groups. Each group was asked to consider questions related to an AMR technical challenge and to produce specific outputs.

## 3.1 Emerging AMR in fungi causing invasive infections in humans

### 3.1.1 The top three fungi of concern are: Candida, Aspergillus, and fungi causing mucormycosis.

**Candida auris:** This yeast usually misidentified as other Candida species or Saccharomyces, when using biochemical methods (API strips or VITEK-2). *Candida auris* causes outbreaks and is transmitted in healthcare settings. Unlike other Candida species, it seems to colonize healthcare environments and skin and poses major infection control challenges. It was noted that with fungi, the higher the minimum inhibitory concentration (MIC) level, the poorer the patient outcome. *Candida auris* is often multi-drug resistant (41%) and associated with higher mortality in Intensive Care Unit in patients with immunosuppression. *C. auris* bloodstream infections are associated with nearly 70% mortality. Whole genome sequencing has produced puzzling findings indicating large genetic differences between continents while highly related within geographic regions. Findings suggest recent independent emergence in at least four places.

**Aspergillus** and the emergence of triazole-resistant *A. fumigatus*. First identified in the Netherlands, 2002-2006, resistance is now found worldwide. Clinical azole resistance rates in the Netherlands are very high at 16%. Azoles are used in crop protection with five azoles being the main drivers for resistance. However, the best antifungal drugs are azoles and as azoles are the only group available, there is a real problem. Invasive aspergillosis is very difficult to diagnose with resistant infections being even more difficult to diagnose. There are limited treatment options and mortality is 50%-100% (median 88%).

**Mucormycosis:** highly resistant organisms that are on the increase.

The Way Forward will require good antifungal stewardship and this should be included in programmes. New rapid diagnostic tests can be used to rule out infection and reduce the use of antifungals. There are several new antifungal drugs in development/early trials.

The presentation concluded with an overview of objectives and potential benefits of a global antifungal resistance surveillance system, as well as the potential benefits and risks of including antifungal resistance surveillance in GLASS. These were then discussed in the designated working group.

### 3.1.2 Feedback from the working group: AMR in invasive fungi

The group did not consider it appropriate to include AMR in invasive fungi in GLASS at the moment, principally due to the difficulties in some testing systems. However, *Candida* and *Aspergillus* should be considered by GLASS in next the evaluation phase. The group requested the entire meeting (fungal working group, other meeting participants and the GLASS Secretariat) develop antifungal resistance alert criteria and to discuss if *Candida* resistance can be included in the rapid alert component or not.

A situational analysis is required which should include the following: population based resistance rates, burden of disease and laboratory capacity throughout the world. The group agreed to try and put this together.
There is an arrangement for CDC to second a person to WHO as a focal point for fungal infections. Currently, worldwide, there are only three WHO CCs that address antifungal resistance. There is also a need to increase general regional participation to get better laboratory data sets.

A variety of research needs were listed, including PPS and cohort studies, and the group asked if a first burden of disease estimate could be generated by WHO (that includes resistant Candida). Given that seven new antifungals are in clinical development, the group wanted to know at what point new treatments become part of the portfolio of work. It was noted that the field is generally under-resourced with public health mycology currently a non-existent discipline that needs support and expertise. The group posed a general question for WHO and others on how to upscale this area.

Discussion points
With regards to submission of additional pathogens for surveillance reporting, attention was drawn to the “additional status” within GLASS reporting, where any extra information can be added. Therefore, GLASS will eventually allow reporting of antifungal resistance, but criteria need to be developed.

It was noted that a rapid alert applies to any pathogen and not just the eight priority pathogens. The alert system covers both changes in epidemiology as well as new emerging resistance.

In terms of criteria for reporting an unacceptable rate for a life-threatening infection, it was suggested that rates of 3%-5% be used. This may apply in cases of a lethal infection e.g. Aspergillosis or candidemia.

3.2 Detection of colistin resistance among Enterobacteriaceae

3.2.1 Colistin resistance in humans is still uncommon, but is becoming more common among isolates from animals (this may be related to greater use in animals). Susceptibility testing faces a number of complicating factors:

• Colistin diffuses poorly through agar, hence any agar diffusion test (disk or concentration gradient strip) has compromised performance
• The type of microtitre tray used, the type of broth used and the presence of surfactant in the broth can significantly affect the MIC result
• The methanesulfonate form of colistin administered to patients is an inactive prodrug

Few laboratories are able to perform broth microdilution tests and no other drug susceptibility testing requires this method. Therefore, the problem is how to generate and collect reliable data. A number of questions were presented for consideration in the working group.

3.2.2 Feedback from the working group: Detection of colistin resistance

Guidance for the detection of colistin resistance should not conflict with statements from CLSI/EUCAST. Given the practical issues related to laboratory capacity, colistin resistance testing will be purely for surveillance, not clinical management. The group considered different types of screening approaches. It was emphasized that these should only be used as screening techniques. If resistance is found, it should then be confirmed with a validated MIC assay. The group felt that support should be made available to those countries that wish to acquire the ability to perform assays. The group will prepare more concrete guidance in the coming months.
Discussion points
Optimism was expressed about the availability of well-performing tests coming to market in the very near future due to recent, new legislation in the U.S.

Some meeting participants wondered about the connection of this topic to GLASS – was the point to implement worldwide testing for colistin resistance? It was clarified that GLASS wants to make countries aware of the limitations of disk diffusion and provide further advice. Most countries use disk diffusion but if they want to be clearer about the result, GLASS will need to guide them. WHO will develop an online document explaining how to detect colistin resistance and how WHO can provide support to countries willing to undertake this testing.

3.3 Application of molecular methods (MM) to support AMR surveillance

3.3.1 The 22-year history of the WHO Global Surveillance of anti-TB Drug Resistance programme was recounted to illustrate the difference that molecular methods can make to surveillance. Molecular methods are particularly pertinent in countries where there is little or no surveillance activity due to weak laboratory capacity. The Democratic Republic of the Congo was cited as an example where, with molecular testing, it has been possible to do a representative national anti-TB drug resistance survey in one year. The advantages of molecular methods are that they: require far fewer cultures e.g. 100 cultures as opposed to 1200-1500 cultures required in a conventional survey; present reduced logistical challenges for sample transport; and reduced demand on laboratories (in terms of both expertise and time). However, it was noted that the test used in this example, Xpert MTB/RIF, alone cannot investigate resistance to anti-TB drugs other than rifampicin and needs to be combined with genome sequencing to explore resistance to additional anti-TB drugs.

Molecular assays have been conducted in 18 countries that have limited culture capacity.

It was noted that the WHO TB Supranational Reference Laboratory Network, with 36 laboratories worldwide, is moving towards surveillance entirely based on molecular technologies, including next-generation sequencing (NGS) which will offer many possibilities in low-resource settings. There is an opportunity for surveillance of AMR in common bacteria to build on existing networks. The presenter urged listeners to give serious consideration to the adoption of molecular testing in LMICs.

3.3.2 Feedback from working group: application of molecular methods

The objective of this working group was to develop the outline of a road map to provide guidance on molecular testing in GLASS. This will be a document to help the Collaborative Platform decide if MM could be used in GLASS and, if so, how to operationalize the use of these tools in GLASS. The contents will build on work already done by others and will likely be structured into five sections: background; priority pathogens to target; laboratory methods and minimum requirements for laboratory networks; data dissemination; and, operationalization and piloting.

Discussion points
The great gulf between the reality of poorly resourced LMICs and the world of MM was acknowledged as was the need to explore avenues for GLASS to incorporate MM in the future. All were agreed on the importance of having molecular testing acknowledged within GLASS but as a complement to core GLASS work.
The importance of epidemiological methods as part of comprehensive AMR surveillance was raised. It was suggested that GLASS needs a group to expand epidemiological methods. While not disagreeing with this, it was noted that support to AMR surveillance is still the backbone of GLASS. While too few countries are enrolled in GLASS to support a global point prevalence survey on AMR (as proposed by the Strategic Technical & Advisory Group) it could be possible to put in place surveillance strategies in low-resource settings that can be conducted in a short space of time to inform local efforts to contain AMR. Results from these can in turn inform the global picture. Some CCs have volunteered to assist GLASS to develop protocols for this and enhance epidemiological design for meaningful information.

Other comments noted that it might be easier, better and more cost effective to do genotypic testing for particular types of resistance rather than building laboratory capacity. In terms of data management, it was pointed out that if genetic data is to be part of the data set, GLASS must find a way to take this into account and record the relevant data. It was suggested that a relationship be made between GLASS data and databases that handle whole genome sequences to avoid duplication.

### 3.4 GLASS rapid alert component

#### 3.4.1 Participants were provided with the first draft of the document, “AMR Rapid Alert Framework and Risk Assessment” that had been prepared by the WHO GLASS Secretariat, and were invited to review, discuss and provide feedback on the document and on the general approach to the question of rapid alert in AMR surveillance.

#### 3.4.2 Feedback from working group: GLASS rapid alert component

It was agreed that the terms “emergency”, “rapid” and “alert” were misleading in this context. Terminology should be consistent with emerging infectious disease rather than with public health emergencies. It was also agreed that clarification was needed early in the document on the distinction and relationship between IHR reporting and AMR reporting. It was stressed that AMR reporting should reach all constituencies that might discover new resistance, not just public health.

It was noted that not all emerging AMR occurs as an outbreak and not all AMR outbreaks represent new AMR. The Risk Assessment should guide this process.

The provisional watch list will need to be regularly and easily updated (possibly as an annex). Additional resistance to consider including: antifungal resistance; change in epidemiology; change in ecology; increase in occurrence of life-threatening infections.

Reporting of new AMR will be via the GLASS IT platform and should be verified/confirmed by a laboratory with the necessary expertise and capacity. Reporting should be through the Ministry of Health, or if reported directly to WHO from a non-government laboratory, then WHO should inform the Ministry of Health concerned.

It was noted that there is an inherent tension in reporting new AMR and withholding information for later publication purposes. To address this, journals should be encouraged to consider AMR as a public health threat and recognize reporting to GLASS of a new AMR as a citable event.

The group recommended that the risk assessment be condensed and simplified. The risk assessment process should take into account the processes for non-human health sectors,
such as animals and environment, and that a more complete risk assessment may need to be published as a separate document.

The section on risk communication should be expanded and should include clarification of all constituencies that should receive notification of new AMR.

The WHO GLASS secretariat will revise the document ready to be provided to Member States by April 2017. Members of the working group volunteered to help with finalization of the document, although it was noted that no representative from the environment sector was able to participate. IT capacity to report emerging AMR would be in place once Member States had approved the process as outlined in the framework.

Discussion points
Discussions considered the challenge and necessity of assembling a risk assessment team with representatives from human and animal health and the environment, i.e. multisectoral, at the national level to ensure real progress on the ground. Another challenge will be the need for countries to implement protocols for reporting new types of AMR: Experience from other rapid alert systems indicate that while complete compliance will not happen immediately, over time, buy-in from all sectors should take place. The risk assessment section of the framework document will help in assessing if a newly-identified resistance should be reported or not.

The need for a dissemination strategy to make the rapid alert system more visible among the scientific community, and more broadly, was agreed. An editorial in the Lancet or other prestigious publication was suggested.

4. Concluding remarks

It was emphasized that GLASS does not intend to duplicate efforts; rather, it will align with existing initiatives and the CC Network will assist with this. The challenge is to make GLASS grow and enhance technical support capacity. The Secretariat is very grateful to the 19 CCs who comprise the Network. CCs have a formal contract with WHO and will lead in the technical areas discussed during this meeting. The CC leads will be contacting partners for contributions in developing target products from the work plan.

While the volume of work is huge, the focus must now be on implementation. Representatives from all six WHO Regional Offices have attended this meeting as they need help with implementation and this must be provided. Special thanks were extended to the Fleming Fund for providing resources to countries for implementation and for participation in GLASS.

On behalf of the GLASS Secretariat, Dr Pessoa-Silva thanked all present for their input as did the Chair Dr Perovic who formally closed the meeting.
Background:
The World Health Organisation (WHO) developed the Global Antimicrobial Resistance Surveillance System (GLASS) in accordance with the World Health Assembly (WHA) Resolution WHA68.7 to support the implementation of the global action plan on antimicrobial resistance (AMR).

GLASS collects data on AMR and on the implementation status of national AMR surveillance systems in order to enhance understanding of the extent and impact of AMR on populations and provide evidence for interventions and advocacy.

A call for country enrolment in GLASS was released in March 2016. As at 13 December 2016, 29 countries had enrolled and a further nine had expressed an interest in joining GLASS. Following the first technical meeting of the GLASS Collaborative Platform on early implementation in October 2015, much work has been done to support the process of GLASS early implementation.

In April 2016, a Collaborative Platform working group meeting took place in Geneva, to help develop (i) a proposed guide for implementation of diagnostic stewardship, (ii) a proposed guide for national AMR surveillance system implementation and participation in GLASS, including sample indicators for M&E at national level, and (iii) a core components checklist and questionnaire to assess the national AMR surveillance situation and capacities.

Further support is required to improve GLASS and address the surveillance challenges ahead. The focus of the second meeting of the GLASS Collaborative Platform was on (i) bacterial resistance relevant to human health and (ii) consolidating understanding among and engagement of partners in the work being done.

The purpose of the meeting was to support development, dissemination and implementation of global AMR surveillance.

Objectives of the meeting:

• To update participants on progress achieved over 2016

• To share information on AMR surveillance initiatives

• To outline the next steps to address some specific surveillance challenges:
  ➢ AMR in invasive fungi
  ➢ Detection of emerging colistin resistance in Enterobacteriaceae and application of molecular methods for AMR surveillance
  ➢ Rapid alert component for emerging/new types of AMR within GLASS

• To discuss dissemination strategies

Expected outputs from the meeting:
• A common understanding of the status of GLASS development and support to global AMR surveillance efforts
• Defined next steps to address some AMR surveillance challenges
  ➢ AMR in invasive fungi
  ➢ Detection of emerging colistin resistance in Enterobacteriaceae and use of molecular methods for AMR surveillance
  ➢ Rapid alert component for emerging/new types of AMR within GLASS

**Organization and process of the meeting**
On 15-16 December 2016 WHO hosted a meeting with WHO Collaborating Centres, partner technical institutions and international AMR surveillance networks on the implementation of the WHO global antimicrobial resistance surveillance system (GLASS). The purpose of the meeting is to support development, dissemination and implementation of global AMR surveillance.

The list of participants in the meeting is provided in Annex 1.

The agenda is provided in Annex 2.

The meeting was chaired by Professor Olga Perovic, assisted by Dr Sirenda Vong.

Dr Vong covered some administrative details:
(i) The meeting will be recorded to help the rapporteur.
(ii) The record of the meeting will be shared with all participants before being released for public dissemination.
(iii) He confirmed that all participants completed the WHO standard form for declarations of interest prior to the meeting. Each Declaration received from meeting participants was reviewed in the context of the objectives of the meeting and no conflicts were identified.

**Welcome and Opening Remarks**

The Chair welcomed everyone and introduced Dr Hajime Inoue, Assistant Director-General WHO Special Representative for Antimicrobial Resistance.

Dr Inoue introduced himself as the successor to Dr Keiji Fukuda who has recently been appointed as Professor of Public Health at Hong Kong University School of Public Health. He wished Dr Fukuda well in his new position. Dr Inoue related how struck he has been since taking up his position by the range of challenges presented by AMR. He recognizes the central role of WHO in combating AMR and stated that the first priority must be GLASS and ensuring good evidence.

Dr Inoue is involved in preparing for the next G20 meeting in Berlin: WHO proposed that AMR be addressed at the Summit meeting and this has been agreed. Dr Inoue finished by thanking everyone for their commitment and stated that AMR will be his first priority for the next coming years.

At the Chairperson's request, all those present introduced themselves.
Day One. Session One: Global surveillance of AMR and antimicrobial use: an update from WHO and partners

1.1 Overview of GLASS and update on early implementation.

Dr Carmem Pessoa-Silva, Acting Coordinator, GLASS Secretariat

1.1.1 Activities in 2016:

- Completed activities include: development of the IT platform for aggregated and individual data; adapting WHONET \(^1\) for GLASS; development of an implementation package, with a focus on low-income countries; development of guidelines on integrated surveillance in the food chain (led by WHO/FOS); and the (just established) WHO AMR Surveillance Collaborating Centre Network.

- Links to other AMR data: These include: the Gonococcal Antimicrobial Surveillance Programme (GASP); the ESBL-E.coli “Tricycle” programme; the HIV/TB/Malaria cluster within WHO which is working towards creating a common portal; antimicrobial use and consumption data from the Essential Medicines Programme; environmental surveillance data (still in the development stage); as well as collaborating with regional surveillance networks e.g. CAESAR, ReLAVRA and EARS-Net.

Countries enrolled in GLASS since March 2016. 38 have expressed interest and 29 have completed procedures for enrolment. Dr Pessoa-Silva acknowledged and thanked the CAESAR network and colleagues in ECDC, who lead EARS-Net, for their tremendous support motivating countries to join GLASS.

1.1.2 Next steps:

- Development of a rapid detection and alert framework
- 2\(^{nd}\) Member States consultation, April 2017 in Sweden. This will be an opportunity to get feedback from Member States on the difficulties they encounter and the feasibility of what GLASS proposes.
- Explore and start planning on: (i) AMR surveillance in invasive fungal infections and (ii) the application of molecular methods for AMR surveillance
- Create new modules in the GLASS IT platform for ESBL-E.coli Tricycle surveillance, EGASP, and antimicrobial use and consumption.
- There will be a data call in the second quarter of 2017 with the 1\(^{st}\) GLASS report due in the fourth quarter of 2017.

GLASS early implementation road map: GLASS is on track

Dr Pessoa-Silva concluded her presentation by thanking the GLASS Secretariat team for all their hard work.

1.2 Surveillance of AMR in the food chain

Dr Jorge Matheu-Alvarez, Project Officer, Food Safety & Zoonotic Diseases Dept, WHO

\(^1\) WHO microbiology laboratory database software
Dr Matheu opened his presentation by explaining that the approach to surveillance of AMR in the food chain is moving from a medical view of bacterial resistance to a more holistic, integrated, multisectoral, “One Health” approach.

1.5.1 Revised guidance and tools from the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR):

(i) List of critically important antibiotics (CIA) to support countries in prudent use of antimicrobials. The 5th revision and guidelines will be published in early 2017.
(ii) WHO AGISAR Guidance on Integrated Surveillance of AMR in the food chain: this assists countries and other stakeholders across the one health continuum to establish and develop integrated programmes for surveillance of AMR and antimicrobial use. It includes
   - Minimum requirements for integrated AMR surveillance in the food chain
   - Guidance on sampling strategies
   - Standards for laboratory methods and quality assurance
   - Proposes data analyses and reporting methods
Revised guidance will be published in early 2017.

1.5.2 How is the guidance promoted and used?

- Regional training workshops delivered through the Global Foodborne Disease Network (GFN). This has included laboratory capacity strengthening for integrated surveillance of foodborne diseases and AMR using a “One Health” approach and training of microbiologists and epidemiologists from the human, food and animal sectors. Three workshops were carried out in 2016.
- External Quality Assurance System for food borne pathogens
- Mentoring
- Reference service and laboratory protocols supported by Collaborating Centres (CCs)
- Focused research projects and country pilot projects
Between 2010 and 2014, more than 26 AGISAR projects were carried out.

1.5.3 AGISAR’s pilot project approach

- Supporting pilot research projects at the national level
  - Focused research projects that are smaller scale
  - Country projects on a larger scale
- Call for project proposals every two years, advertised on WHO website for a duration of one month
- Evaluation and selection by a panel of evaluators (AGISAR members)
- A mentor is assigned to each project to support implementation
- Mid-term and final technical and financial reports

1.5.4 Specific objectives of AGISAR projects

- Increased awareness or/and commitment to prevention and control of foodborne diseases and containment of AMR
- Better prevention and control of foodborne diseases including AMR along the food chain
- More synergies with existing initiatives in the country
- Integrated surveillance implementation, better detection and early warning
- An ability to identify trends in AMR
• Identification of associations between AMR and drug usage in human or animal sectors

1.5.5 Current activities

• AGISAR ESBL-E.coli "Tricycle" project. AGISAR have decided to focus on the development of a global harmonized protocol on integrated surveillance of ESBL-producing E.coli in humans, the food chain and the environment ("Tricycle Surveillance"). This project can be done with minimal resources in-country.
• Codex Alimentarius Scientific Advice on Antimicrobial Resistance. For the 2016 Codex Alimentarius Commission, an Intergovernmental Task Force on Antimicrobial Resistance was established to review the Code of Practice to Minimize and Contain Antimicrobial Resistance, and to provide guidance on the design and implementation of a system for integrated surveillance of AMR.
• Global sewage surveillance project. 80 samples have been collected from 63 countries: initial findings indicate varying levels of resistance in different regions.

1.3 Surveillance of antimicrobial use
Arno Muller, Dept. of Essential Medicines and Health Products, WHO

Dr Muller provided an overview of AMR–related activities of the Essential Medicines and Health Products Department.

1.3.1 Monitoring antimicrobial consumption

Objective: To provide an estimate of the level (quantity) and type of antimicrobials used at country level

Methods: Data collection of national aggregated sales of antimicrobials using the ATC/DDD methodology developed by CCs in 2016

Ongoing integration of the antimicrobial consumption module into the GLASS IT platform so that countries can submit consumption data in the same way that they will submit surveillance data. WHO is supporting 40–50 countries (2016-2017) at country level.

1.3.2 Antimicrobial use surveys

Ongoing development of protocols for surveys to collect antimicrobial use (prescription and patient purchase) data:

• In hospitals: Point prevalence surveys (similar to EU, US methodologies) adapted to LMIC contexts
• In community settings: General practitioners, dentists, Community Health Workers, outpatient clinics, hospital emergency departments. Data collection will be at both prescriber and dispenser level.

It is anticipated that the protocols will be published in the 1st quarter of 2017 with piloting of the surveys to be conducted in 2017/2018.

1.3.3 Essential Medicines List and Priority Pathogens List

The Essential Medicines List is being updated with considerable revision to the chapter on antibiotics, namely:

• Syndrome-based review of antibiotic treatments for 16 of the most common and/or severe infectious syndromes and five paediatric syndromes
• Review of Systematic Reviews and Clinical Practice Guidelines
  • For each syndrome, there will be a proposed antibiotic listing: three levels are proposed (see table below)

<table>
<thead>
<tr>
<th>Level 1 Core/Standard</th>
<th>Antibiotics that are first line choice for empirical therapy (e.g., penicillin G, amoxicillin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 2 Targeted/Specific</td>
<td>Antibiotics whose use should be limited to specific subgroups or target populations: patients with penicillin allergy, more severe disease, defined resistance, ...</td>
</tr>
<tr>
<td>Level 3 Restricted access</td>
<td>“Niche” - Antibiotics whose use should be limited to special “niche” indications (e.g., azithromycin in sexually transmitted diseases)</td>
</tr>
<tr>
<td></td>
<td>“Preserved” - Antibiotics that should be preserved and recommended only in limited, specific circumstances (e.g., linezolid for hospital-acquired MRSA pneumonia)</td>
</tr>
<tr>
<td></td>
<td>“Last resort” - Antibiotics whose use should be strictly preserved and “last resort”, (e.g., colistin for multi-drug resistant hospital-acquired infections)</td>
</tr>
</tbody>
</table>

WHO will endeavour to get an agreement with countries on antibiotics of “last resort” and therefore ensure their protection. This will be discussed in March with the Expert Committee.

• For publication in May 2017

Priority Pathogens List:

• Identification of priority pathogens for AMR and thus aid the development of research priorities
• Creation of a transparent approach for identifying priority pathogens
• The aim is to adapt the WHO R&D blueprint methodology for prioritizing diseases for use in identifying priority pathogens for AMR
• Work is ongoing and a final draft should be available in February 2017

1.3.4 Policy recommendations

Clinical Practice Guidelines: The team has surveyed how AMR information is made available and used in Clinical Practice Guidelines (CPGs) as information on resistance is essential to guide choice of antibiotics. A review was conducted of 150 CPGs for five common infectious conditions (respiratory tract infections, urinary tract infections). It was concluded that there is a lack of standards and key information on resistance on which to base antibiotic selection. WHO will provide some solutions and guidance for using AMR information in CPGs.

Key messages in antibiotic awareness campaigns: A comprehensive review of the most recent antibiotic awareness campaigns was conducted. This found messages that are most appropriate for national and local awareness campaign messages. It also identified messages that were not based on evidence and that might be harmful. The strengths and weaknesses of campaigns targeting different stakeholders (prescrbers, patients and health systems) were made clear.

1.3.4 Appropriate and responsible use of antimicrobials

Support to development of antimicrobial stewardship programmes is ongoing. The 2017 work plan will:
• Review existing antimicrobial stewardship programmes and activities in hospitals and community settings
• Provide guidance for implementation of antimicrobial stewardship programmes in LMICs
• Pilot antimicrobial stewardship programmes in some countries/hospitals/communities

1.3.5 Price and availability of antibiotics

The WHO Medicines Price and Availability Monitoring methodology will monitor price and medicines availability in pharmacies, hospitals and Central Drug Stores. Initially, a general basket of medicines (including some antibiotics) was considered but it was decided to have specific medicines in class-based baskets with one specifically for antibiotics. A mobile app called Survey123 Platform is in development and there is an antibiotics survey plan for 2017.

1.4 Surveillance of AMR in gonococci: update from the Gonococcal Antimicrobial Surveillance Programme (GASP)
Dr Teodora Wi, Medical Officer, Family, Women and Child Health Dept, WHO

Established in 1993, GASP is a global laboratory network covering over 60 countries in six regions, each with focal points and regional coordinating centres, that monitors the antimicrobial susceptibility of gonorrhoea in participating countries. Dr Wi acknowledged and thanked GASP’s regional collaborators and partners. GASP is currently trying to find more partners to provide greater support in the African and Eastern Mediterranean regions.

1.4.1 GASP objectives

• Ensure adequate sentinel AMR surveillance to inform treatment guidelines in all countries
• Establish a strategy to rapidly detect patients with gonococcal infections, with clinical and/or microbiological treatment failure following treatment with recommended cephalosporin therapy
• Ensure effective clinical management of infected patients and their sexual partners

1.4.2 GASP data 2009-2013

• 46 countries reported decreased susceptibility to extended spectrum cephalosporins. This means that guidelines will need to be changed again in the near future
• 10 countries reporting treatment failure to extended spectrum cephalosporins
• 17 countries reported resistance to azithromycin in > 5% of gonorrhoea isolates
• Majority of countries reported high level of resistance to quinolones

WHO is working to make GASP as effective as possible and address its many challenges, including limited national leadership, commitment and funding in many countries. The magnitude of the gonococcal AMR problem is not completely known due to lack of data in many countries. A recent survey of 108 countries found that less than half (46) conducted AMR testing for gonorrhoea in the past five years. The lack of information is particularly acute in countries with the highest gonorrhoea burden and the greatest need for AMR
monitoring. These are often countries with suboptimal diagnosis and surveillance capacity, freely available antibiotics (including counterfeit drugs), and lack of drug quality control contributing to the rapid development of resistance. They are also the countries most likely to rely on syndromic management of sexually transmitted infections (STIs), leading to a shortage of samples for AMR monitoring and lack of capacity and supplies for specimen collection, culture and sensitivity testing. Cultures are also carried out less frequently in more developed countries as diagnosis is improved by the use of molecular methods. Countries in Africa, South-east Asia and the Eastern Mediterranean region (as well as Eastern Europe and Central Asia) particularly, do not have functioning programmes in place to assess gonococcal antimicrobial susceptibility.

1.4.3 Challenges

- Number of countries participating in GASP – more are needed
- Standardized and comparable data (Threshold > 5% resistance)
  - Sample size is small
  - Variable antibacterial susceptibility testing (AST) methodologies
  - Quality data (laboratory capacity)
- Delayed, ad hoc reporting, therefore an early warning system does not exist. There is a need for:
  - Timely release of data
  - Collection of enhanced epidemiological and clinical information linked to microbiological information
  - Monitoring of antimicrobial treatment
- Sharing of information
- Sustaining regular monitoring instead of ad hoc surveys

The need now is to integrate GASP into GLASS and thus enable regional focal points to access data via GLASS – the integration process is in progress.

Molecular approaches: As it is difficult to culture gonorrhoea, molecular approaches are now being considered to monitor AMR and enhance surveillance. A number of countries have started work on this.

1.4.4 GASP recommendations

- Countries should develop their national treatment guidelines based on national surveillance data.
- With increasing levels of resistance to azithromycin and ciprofloxacin/quinolones, countries must accord funds to surveillance systems – at the moment, budgets are being spent on drugs that are ineffective due to resistance.

1.5 Fostering the development of new diagnostic tests
Dr Francis Moussy, Essential Medicines and Health Products Dept., WHO

Dr Moussy opened his presentation with a disclaimer that not as much progress as WHO would have liked to have made has been made due to lack of resources.

1.5.1 What is needed to facilitate the development of diagnostic tests for AMR?

- Coordinate the mapping of existing diagnostic tools for AMR
• Assess the needs and develop consensus Target Product Profiles (TPPs), with very clear definitions of point of care
• With various organizations e.g. Foundation for Innovative New Diagnostics (FIND), initiate (a) partnership(s) to develop priority AMR diagnostic tests based on the TPPs
• De-risk the development of new AMR diagnostics for companies (e.g, coordinate market analysis for diagnostics in response to the TPPs, advance market commitments, prizes, grant, etc).

The issues of access and use of the newly developed tests will also need to be addressed.

1.5.2 Why develop Target Product Profiles for diagnostics?

• To clearly inform the diagnostic industry and other R&D groups about the types of diagnostics that are needed (e.g. TB, Ebola, Zika, viral vs bacterial differentiation).
• To specify the desired (or acceptable) characteristics of the needed diagnostics.
• Funders, procurers and regulatory agencies also use such TPPs to focus their activities.

1.5.3 What is included in a diagnostic TPP?

• Scope: intended use, setting, user, target population
• Performance and operational characteristics
• Price
• Usually includes two categories: "acceptable" versus "desired"

1.5.4 Example of a consensus TPP in an expert driven approach. Steps taken to develop a tool to differentiate between bacterial and non-bacterial infections

1. Identification of the need during a biomarker workshop (September 2015)
2. Draft TPPs based on available literature
3. Circulated to 16 experts from academia, nongovernment organizations (NGOs) and WHO
4. Refined draft circulated prior to a face-to-face meeting
5. Consensus meeting with all experts
   – Ranking of priorities for in-depth discussion based on agreement/dissension in commenting round
   – All discussions noted and consensus achieved by majority vote
6. Publication of final TPPs in public domain

Dr Moussy described some selected characteristics of the TPP i.e. scope and test performance (acceptable and desired data). Following this, the TPP was published on the WHO and FIND websites as well as in peer reviewed journals, disseminated at conferences and actively to industry partners. In the future, AMR will be included in the new list of essential diagnostics, which may be an incentive for companies to become involved.

Dr Moussy provided a list of TPP working group partners and funders and gave special thanks to Sabine Dittrich (FIND) for providing several slides for the presentation.
1.6 Global AMR Surveillance: Update from the Food and Agriculture Organization of the United Nations. Julio Pinto, FAO AMR Working Group

1.6.1 Overview

The presenter acknowledged that the animal and food sectors were behind the health sector in terms of the design of surveillance strategies and platforms to collect surveillance data. Mr Pinto presented the FAO AMR action plan that is based on three pillars: practice, governance and evidence and described the AMR multidisciplinary working group. He also highlighted the recent publication "Drivers, dynamics and epidemiology of antimicrobial resistance in animal production".

It was noted that antibiotics are used three times more frequently in animals than in humans. Mr Pinto provided an overview of the global demand for meat and eggs (2005 v 2050) highlighting the ever-increasing demand. In the animal sector, the use of antimicrobials in animal husbandry can be seen to have an economic justification in the need to feed an increasing human population. The presenter indicated that the use of antimicrobials for increased productivity will probably continue in many regions. LMICs are recording increased meat demands. It will be important to send the right message about the need for responsible use of antimicrobials but it is likely that they will continue to be used for productivity in the food animal sector.

Surveillance in this area is complex – including meat, aquatic products, agriculture and fisheries - and it needs to be more integrated. There is a need to define what "one health" means and where surveillance is needed.

Mr Pinto presented an AMR risk map drawn from the results of a recent study by Van Boeckel et al. (2015) that used statistical models based on data from a limited number (32) of countries to estimate the extent of antimicrobial usage in food-producing animals at global level. Due to poor surveillance and data collection in many countries, estimates of antibiotic consumption in global agriculture vary, ranging from around 63 000 tonnes/year to over 240 000 tonnes/year. Between 70%-80% of antibiotics given to fish are excreted into water and spread through water systems. Antibiotics used for crops are relatively low in comparison to the quantities used in livestock, with estimates ranging from 0.2%-0.4% of total agricultural antibiotic consumption. Between 75%-90% of tested antibiotics are excreted from animals un-metabolized and enter sewage systems and water sources. Information on the health and economic impacts of AMR on livestock and fisheries is lacking in developing countries.

1.6.2 Issues:

- Complex due to different sectors, vested interests, a complex interface, farmer incentives etc.
- The message exists on how the problem can be reduced: what are needed are political will and technical capacities
- Need for evidence. Surveillance of antimicrobial use and resistance can help to provide the evidence base for plans and policy formulation
- How do we implement One Health surveillance effectively for AMR in this complex livestock/human/ecosystem interface? Surveillance needs to be prioritized and cost-effective - it is not feasible to conduct surveillance in all sectors and it will be context dependent. Risk assessment must be used in the prioritization process.

1.6.3 Objectives of a surveillance programme for AMR
to monitor food animal and human consumption of antimicrobial agents
• to monitor occurrence of AMR in bacteria isolated from food animals, food and humans (e.g. salmonella, campylobacter)
• to study and provide evidence on the association between antimicrobial consumption and antimicrobial resistance
• to support decision making by identifying routes of transmission (critical control points) and high risk interfaces or production systems for further research.

1.6.4 AMR surveillance plan

• Engagement of livestock farmers, value chain actors, communities.
• Need good information. How much is used and what is being prescribed/used
• How to collect information. If global standards are available from WHO, these can be used by FAO.
• Risk analysis/risk communication for supporting risk-based surveillance.
• AI and Recording systems: origin/destination, movement, treatment, history of a group or individual
• Setting up priorities and defining targets and evaluation

1.6.5 Key messages

• AMR is a bidirectional zoonosis. It also has multidirectional links to other environmental compartments, including aquaculture, food plants and water.
• Major gaps exist in surveillance of antimicrobial use and resistance and there needs to be more open data sharing in all sectors
• Integrated surveillance systems would enable data comparison from food-producing animals, food products and humans
• Surveillance is hampered by a lack of implemented global standards
• Multisectoral/One Health approach is required
• The food and agriculture sector including the livestock sector is part of the problem but also part of the solution

1.6.6 FAO’s Action Plan focuses on four main areas of work to tackle AMR.

1. Increasing awareness and advocacy on AMR and related threats.
2. Promoting good practices in food and agriculture systems and the cautious use of antimicrobials.
3. Strengthening governance structures, i.e. policies and regulations, related to antimicrobial use in food and agriculture.
4. Developing capacity for surveillance and monitoring of antimicrobial resistance and antimicrobial usage in food and agriculture.

1.7. Discussion points following the presentations

1.7.1 Surveillance of antimicrobial use: are you looking at the quality of antibiotics? It is difficult to measure in a questionnaire but it is important. Yes, WHO has a team that monitors substandard, falsified and counterfeit medical products which includes antibiotics.

1.7.2 Clinical Guidelines – is this a framework or a local activity? This will be a national-level activity.
1.7.3 There was a comment on the fact that there can be many different antibiotic surveillance systems within one country. GLASS is ideal as one system and will help to avoid this in the future. Countries should be advised to have one system only and learn from the mistakes of others.

1.7.4 Surveillance of AMR in the food chain: the impressive progress made by WHO in this area and others was acknowledged. Will there be surveillance of ESBL – *E.coli* in the non-meat food chain? For now the focus will be on food animals and will not include vegetables or fruit.

1.7.5 Rapid diagnostic tests: as we increase the use of rapid point-of-care (POC) diagnostics, there is a risk that traditional sources of surveillance information will be lost. Does GLASS have a plan to capture information from the use and results of POC tests? There is a need to keep both nucleic acid-based and culture tests.

How do we capture data if conducted outside of a surveillance system? When we promote the use of decentralized tests, we must make sure that those tests have the ability to send data out, and this must then be collected and analysed. Acknowledged that simple tests such as malaria RDTs will be difficult to capture but more sophisticated ones e.g. GeneXpert have the ability to send data.

1.7.6 How do we make data timely enough to become cutting edge? Acknowledgement that timeliness is an issue: a new system in needed hence it is sensible to integrate into GLASS.

1.7.7 There was a discussion about the merits of more basic versus more sophisticated tests – the conclusion being that one method doesn’t fit all organisms.

1.7.8 There was discussion that while the ESBL work is good, there should also be surveillance of carbapenemase. As the focus of GLASS is on LMICs and the need to carry out surveillance in all sectors, ESBL was chosen as it fits the criteria and can be followed to assess effectiveness of interventions. Carbapenemase is not easy to detect and is mostly found in the food chain and not in humans.

1.7.9 It was clarified that there is a technical support mechanism within FAO, similar to a WHO CC (but not specifically for AMR), that provides support for laboratory diagnostics, surveillance, risk analysis, epidemiological training in country. FAO also plans to establish centres in South-East Asia and Africa.

1.7.10 The question was posed regarding who has the lead in setting global standards for surveillance in the food and animal sectors? Many are working in this area but the lead is unclear. In response, the FAO representative noted the complex and multisectoral nature of surveillance in this area. OIE has plans for a global database on antimicrobial use and it is hoped they will develop global standards. A global set of minimum standards that can be shared with different platforms must be defined.

*Day One. Session Two: Support to global surveillance*
2.1 Fleming Fund initiative

Dr Charles Penn and Dr Toby Leslie

2.1.1 What is the Fleming Fund?

A £265 million “one health” programme to support LMICs in tackling AMR and to contribute to implementation of the Global Action Plan on Antimicrobial Resistance. It is part of the UK Government's Official Development Assistance strategy to promote the economic development and welfare of developing countries. The aim is to ensure that Fleming Funds add value given other global actors in the field of AMR and not duplicate efforts. The geographical focus for Fleming Funds is sub-Saharan Africa, south Asia and South-East Asia.

2.1.2 Aim of the Fund

To strengthen surveillance for AMR and collection of data on antimicrobial use in LMICs through grants to countries for:

- Implementation of standard, quality driven protocols for sample and data collection, analysis and reporting that take into account the need for clinical information and epidemiology. This includes data on the sale and use of antimicrobial medicines, particularly antibiotics.
- Laboratory capacity for bacterial diagnosis and AST
- Enabling the sharing of drug resistance data locally, regionally and internationally (e.g. WHO GLASS, Burden of Disease)
- Advocating the application of these data to promote the rational use of antimicrobials for human health, animal health and agriculture

2.1.3 Core Principles

- A one health approach to human and animal health and AMR in agriculture and the environment.
- In-country ownership through national action plans
- Sustainability.
- Alignment of activities and systems – from national to regional to international.

At the heart of the programme will be commitments to evaluation, continued improvement and value for money.

2.1.4 Programme structure
Scoping studies have been undertaken to document what has been done and these studies will be made publically available.

- An Analysis Of Approaches To Laboratory Capacity Strengthening For Drug Resistant Infections In Low And Middle Income Countries. Imelda Bates et al LSTM, Anthony Scott et al LSHTM
- An Analysis Of Networks And Education Resources Supporting Drug Resistant Infection Surveillance In Low And Middle Income Countries. Elizabeth Ashley et al MORU; Imelda Bates et al LSTM

A surveillance protocol has been developed with LSHTM to help countries participate in GLASS.

The majority of the investment will be in the form of grants to countries and regions, and a fellowship scheme, to be implemented by Mott MacDonald. Evaluation of the work will be carried out by Itad and the University of Sussex, UK.

2.1.5 The Roadmap

An outline of the Roadmap to grant making at country and regional level was presented with a strong focus on country ownership. Country assessments to identify countries for early investment will start in early 2017. These countries will receive support to develop proposals in line with GAP NAPs. There will be two streams: surveillance and capacity building.
Capacity building will cover human resources through the Fleming Fund Fellowship scheme – a process of medium-to-long term mentorship. More information on this will be released over the next 3-4 months. Laboratory infrastructure capacity building will also be supported.

Surveillance: the focus will use a one health approach where possible. The protocol developed by LSHTM is designed to assist countries to graduate into the lowest tier of GLASS. While many LICs are still a long way from this step, the aim of the Fleming Funds is to get these countries to a stage where they can practically implement GLASS. Animal health, the environment and antimicrobial use and drug quality will also fall under surveillance.

The outcomes will be: better surveillance, improved stewardship, improved treatment, and averting the economic and social burden of AMR.

Getting political will across the different sectors via the Prime Minister or President will be essential. There is a lot to do over 4.5 years in approximately 30 countries. Activity will concentrate on doing relatively small building-block work initially and trying to do it well. The process will begin in-country with the AMR coordination committee. Significant technical assistance will be required to achieve practicable, executable plans and generous technical assistance support monies exist for countries to develop their NAPs.

2.1.7 **Preparatory Work**

8-month inception period:
Country assessments: desk based
➢ Early investment and piloting: four early investment countries
➢ Allocation models: where is the money best spent?
➢ Develop call for proposals
➢ Decide and develop funding streams

Funding applications. The Fleming Fund will implement a phased approach. Following the assessments, countries will be invited to apply for the first wave of grants in 2017-2018 based on geographical spread and readiness of NAPs. This process will then be repeated in the 2018-2019 and 2019-2020 fiscal years. The number of countries in Wave 1 has not been decided yet. It is anticipated that between 9 and 16 countries will be in the first round.

Fleming Fellowships: These will be available to all countries who are accessing grants. The Fellowships will provide support through mentorship from competent institutions, secondment, training, support for travel and collaborative projects.

2.1.8 Discussion points:
1. Coordination: The presenters were asked about coordination between the Fleming Fund and others with similar projects and approaches. The Fleming Fund wants to complement and synergise investments already made. It is true that it is a very busy space at the moment but laboratory capacity support encompasses many different disciplines and diseases. The Fleming Fund is working strongly and building relationships with UN and US agencies and using UK Government diplomatic networks also. It does not want to complicate the situation, displace funding, or replicate activities. The focus will be on basic bacteriology and microbiology: more complex areas will not be in their purview.

   It was noted that WHO has a group working on corporate fund raising and coordination of donors. This group indicates gaps to donors but cannot oblige donors to fill those gaps. In addition to coordination, implementation can also be a challenge especially in limited resource settings or where there are many partners with little coordination in-country.

2. Geographical area support: It was noted that there is a concentration of donor support on some geographical areas but not on others. The Fleming Fund acknowledged that this was a challenge as a good coordination forum is lacking. The Fleming Fund representative called on FAO/WHO/OIE or a UN coordinating mechanism for support with this to ensure transparency. He assured listeners that the Fleming Fund is will coordinate well with others in the countries where they will be working.


2.2 Presentation of the newly established WHO CC network and work plan

Dr Malin Grape provided an update on the establishment of the WHO AMR Surveillance and Quality Assessment Collaborating Centres Network to support global AMR surveillance capacity building. It was established in response to a Member States’ request in Resolution WHA68.7. Network members, drawn from 19 WHO Collaborating Centres, undertook to assist the GLASS Secretariat in the implementation of the 2017-2019 work plan. Four priority areas of work were identified: capacity building and technical support...
to microbiology laboratories; capacity building and technical support to surveillance systems; GLASS development; and increase understanding of impact of AMR. Target products for each area of work were defined and Lead CCs assigned. The WHO Collaborating Centre for antimicrobial resistance containment, Sweden (SWE-66), will assist with coordination of the Network for two years on a rotational basis. Dr Grape noted that the role of CCs is mandated by WHO and the Network can act as a platform to collaborate with other partners, many of whom are present at this meeting. More details can be found in the meeting report available here.

The Chair thanked all and closed the session.

**Day One. Session Three: AMR technical challenges**

Chair: Dr Sirenda Vong

3.1 **Emerging AMR in fungi causing invasive infections in humans**

Dr Tom Chiller, Chief, Mycotic Diseases Branch, CDC, USA.

3.1.1 **Overview**

The top three fungi of concern are *Candida*, *Aspergillus*, and fungi causing Mucormycosis

**Candida**

- Risk factors: ANTIBIOTICS, immunosuppression, neutropenia, Intensive Care Unit care, abdominal surgery
- Humans are colonized
- Infected by our own strains or by acquisition in a healthcare setting
- Bloodstream infections (BSI) and invasive abdominal candidiasis
- #1 cause of healthcare-associated BSI in the U.S; 6% of all HAIs

**Molds**

- Risk factors: neutropenia, immunosuppression, diabetes
- The whole world is colonized
- Infected by inhaling airborne spores
- Lungs and sinuses, and can disseminate
- Aspergillosis is #1; Mucormycosis is #2

Different species carry different resistances: therefore as more species come into play, more treatments are used and resistance increases. Drug resistance is becoming more evident – 12% and 10% levels have been recorded at U.S. surveillance sites. It should be noted also that with fungi, the higher the minimum inhibitory concentration (MIC) level, the poorer the patient outcome. In multiple centres, resistance has been associated with death after adjustment for Intensive Care Unit (ICU) status and degree of immunosuppression.

3.1.2 **Candida auris: A global emerging multidrug-resistant yeast**

Dr Chiller gave an overview of the global emergence of this resistance 2009 – 2015. Starting as an ear infection in Japan, the first report of a *C. auris* BSI was recorded in 2011.
The US Centers for Disease Control and Prevention (CDC) launched its international C. auris work in Pakistan 2014-2015. C. auris is concerning as

- It is multi-drug resistant, exhibiting resistance to fluconazole, variable susceptibility to other azoles, amphotericin B, and echinocandins; and
- It is challenging to identify and is often mis-identified as C. haemulonii.

**C. auris early epidemiology**

- Patients of all age ranges (Neonatal ICU infants → elderly)
- Similar risk factors as for other Candida species
  - Diabetes
  - Antibiotic use
  - Recent surgery
  - Presence of a central venous catheter
- May occur in conjunction with other Candida species
- Patients on antifungal treatment when C. auris isolated
- Median time from admission to infections: 17 days
- Mortality ~70%;
  - 100% in Venezuela in NICU infants

**Antifungal susceptibility**

- 93% resistant to fluconazole
- 54% resistant to voriconazole
- 35% resistant to amphotericin B
- 7% resistant to echinocandins
- 41% multi-drug resistant isolates
- 4% resistant to all three major antifungal classes

**Why is there concern about C. auris?**

- Is multi-drug resistant
  - Some isolates resistant to all three major antifungal classes
- Can be misidentified
  - Usually misidentified as other *Candida* species or *Saccharomyces*
  - MALDI-TOF2 can detect *C. auris*
- Causes outbreaks and is transmitted in healthcare settings
  - Unlike other *Candida* species, it seems to colonize healthcare environments and skin
  - Major infection control challenges
- Puzzling findings from Whole Genome Sequencing (WGS):
  - Large genetic differences between continents
  - Highly related within geographic regions
  - Suggests recent independent emergence in at least four places

**3.1.3 Aspergillus: Emergence of Triazole-Resistant A. fumigatus**

Triazole-Resistant *A. fumigatus* was first identified in nine patients in Netherlands, 2002-2006 and is now found worldwide. Clinical azole resistance rates are currently very high in the Netherlands at 16%. Resistance has also been found in people's homes. In the Netherlands, resistance is thought to be driven by the tulip bulb industry while in Britain, resistance spreads via the onion crop but is also found in other settings. Azoles are used

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2 MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) is an automated mass spectrometry and software system designed for rapid microbial identification
in crop protection with five azoles being the main drivers for resistance. However, the best drugs are azoles and as azoles are the only group available, there is a real problem.

**Challenges Posed by Triazole-Resistant A. fumigatus**

- Invasive aspergillosis is very hard to diagnose
- Resistant infections are even harder to diagnose
  - Susceptibility testing is not routinely performed
  - Resistance is missed due to co-infection with susceptible strains
  - New polymerase chain reaction (PCR) assay, but concerns of limited sensitivity
- Limited treatment options
- Mortality 50-100\% (median 88\%)
- Fungicides are needed to feed the world

3.1.4 *Mucormycosis*: highly resistant organisms on the increase

3.1.5 *The Way Forward*

**Stewardship**

- Anti-bacterial use is the greatest factor associated with Candidemia
- Antifungal stewardship needs to be included in programs
- New rapid diagnostic tests can be used to rule out infection
- Less use of antifungals

**Pipeline of new, better drugs**

- Several new antifungal drugs in development/early trials
- New mechanisms of action
- New delivery

**AMR in fungi and GLASS**

1. *Candida*
   a. Easy to culture - start with blood (although other sites)
   b. Species level data an important association with resistance
      i. *C. glabrata, C parapsilosis, C auris*
   c. Antifungal susceptibility testing (AFST) more difficult but it is done globally
   d. Sentinel systems are present globally
   e. Most concerning for resistance and burden
2. *Aspergillus*: just *fumigatus* –azole
3. Mucormycosis: Challenging but on the increase

Dr Chiller concluded his presentation with an overview of objectives and potential benefits of a global antifungal resistance surveillance system, as well as the potential benefits and risks of including antifungal resistance surveillance in GLASS.
3.2 Detection of colistin resistance among *Enterobacteriaceae*

Christopher Oxenford, Health Emergencies Programme, WHO

3.1.1 Overview

Colistin is a polymyxin, discovered in the 1950s. Initially, there was little systemic use of colistin owing to neural and renal toxicity but interest has grown in it as an agent of last resort in recent years.

Colistin resistance: Resistance among isolates from humans is still uncommon, but is becoming more common among isolates from animals (related to greater use?)

3.1.2 Susceptibility testing - Complicating factors

- Colistin diffuses poorly through agar, hence any agar diffusion test (disk or concentration gradient strip) has compromised performance
- The type of microtitre tray used, the type of broth used and the presence of surfactant in the broth can significantly affect the MIC result
- The methanesulfonate form of colistin administered to patients is an inactive prodrug

3.1.3 Current recommendations

Colistin (polymyxin E) MIC determination is associated with several methodological issues. These have been investigated by the CLSI-EUCAST joint Polymyxin Breakpoints Working Group and the following method for determination of colistin MIC was agreed:

- Reference testing of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. is by the ISO-standard broth microdilution method (20776-1).
  - **Note:**
    - Cation-adjusted Mueller-Hinton Broth is used
    - No additives may be included in any part of the testing process (in particular, no polysorbate-80 or other surfactants)
    - Trays must be made of plain polystyrene and not treated in any way before use
    - Sulphate salts of polymyxins must be used (the methanesulfonate derivative of colistin must not be used - it is an inactive pro-drug that breaks down slowly in solution)
- Susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion, cannot be recommended until historical data have been reviewed or new study data have been generated. Work on these methods is ongoing.

3.1.4 Challenges

- Few laboratories are able to perform broth microdilution tests
  - Lack the training and supplies
- No other drug susceptibility testing requires this method
- How to generate and collect reliable data?

3.1.5 Questions to consider

1. What is a sustainable model for colistin resistance surveillance.
   - Ensuring a NRL has the capability to perform these tests
   - Establishment of a referral mechanism in-country
Recommendations on which strains should be tested, (only multi-resistant strains, carbapenemase and/or ESBL producers?) or only strains from invasive disease or as many strains as possible

2. What technical support can be provided to countries that wish to monitor colistin resistance
   - Training to perform and interpret the tests
   - Provision of reagents and control
   - International referral of isolates

3. What about monitoring for resistance genes?

3.3 Application of molecular methods to support AMR surveillance
Matteo Zignol, Scientist & Chris Gilpin, Scientist, Laboratories, Diagnostics and Drug-Resistance Unit, WHO

3.3.1 Global surveillance of anti-TB drug resistance and experiences with molecular methods (MM)

The 22-year history of this WHO programme was recounted to illustrate the difference that molecular methods can make to surveillance. The laboratory component is the backbone of drug resistant-TB surveillance and the WHO TB Supranational Reference Laboratory Network now has 36 laboratories worldwide. It is moving towards surveillance entirely based on molecular technologies, including next generation genome sequencing (NGS) which will offer many possibilities in low-resource settings. There is an opportunity for AMR surveillance to build on existing networks. It was noted that the adoption of molecular testing should be viewed in the same way that mobile phones were adopted in LMICs.

There are still countries where there is little or no surveillance activity due to weak laboratory capacity e.g. the Democratic Republic of the Congo (DRC). However, with molecular testing, it has been possible to do a representative national anti-TB drug resistance survey in one year. The advantage of molecular methods is that they require 100 cultures as opposed to 1200-1500 cultures required in a conventional survey. The survey design is given below.

TB laboratory capacity in DRC:
- Good capacity of sputum-smear microscopy
- Limited capacity for culture
- No capacity for drug susceptibility testing
- Availability of Xpert MTB/RIF3 in multiple sites

3.3.2 Xpert MTB/RIF for surveillance
- Reduces logistic challenges for sample transport
- Reduces demand on laboratories (expertise and time)

3 The Xpert MTB/RIF assay is a test that contributes to the rapid diagnosis of TB disease and rifampicin resistance
• Xpert MTB/RIF alone cannot investigate resistance to anti-TB drugs other than rifampicin
• Needs to be combined with genome sequencing to explore resistance to additional anti-TB drugs

3.3.3 Characteristics of sequencing

• Sequencing is the most accurate molecular test available
• High throughput: up to ~ 200 strains per run/3-4 days
• Cheaper than standard phenotypic testing (as low as 50 USD per strain and it is going down)
• Test accuracy:
  o RIF: possibly equivalent to phenotypic test
  o PZA: possibly equivalent to phenotypic test
  o INH: low sensitivity compared to phenotypic test
  o FQL: low sensitivity compared to phenotypic test
  o AGL: low sensitivity compared to phenotypic test
  o New drugs (BQL, DLM): to be studied

3.3.4 DRC Survey design

Molecular assays used in 18 surveys:
Surveys can now be conducted in countries with limited culture capacity
- Xpert MTB/RIF: already used in: Burkina Faso, Cote d'Ivoire, DR Congo, Djibouti, Lao PDR, Pakistan, Papua New Guinea, Senegal, Zimbabwe
planned to be used in 2016 in: Eritrea, Ethiopia, Indonesia, Malawi, Swaziland
- Line Probe Assays: already used in: Lesotho, Nigeria, Rwanda, Sudan, Tanzania.

A point of information was given: a company in Oxford, U.K. is producing a matchbox-sized sequencer that can be plugged into the computer/mobile phone and will be on the market soon. Molecular technology needs to be discussed now and we need to move with it.

3.4 GLASS rapid alert component: draft protocol

The draft protocol for a rapid alert component within GLASS was distributed for discussion in the group work session.

3.5 Group work: Outlining the next steps to address surveillance challenges

Participants were divided into working groups (see table) and a list of question guides and expected outputs were allocated. Following discussions, the meeting adjourned for the evening. Feedback was given on the morning of day two.

<table>
<thead>
<tr>
<th>Group work: Outlining the next steps to address the surveillance challenges</th>
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<tr>
<td><strong>Group I:</strong> Rapid Alert</td>
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Question guides and expected outputs for working groups

3.5.1 Group I. Emerging AMR: rapid alert and risk assessment framework

Participants were provided with the first draft of document, “AMR Rapid Alert Framework and Risk Assessment” that had been prepared by the WHO GLASS Secretariat, and were invited to review, discuss and provide feedback on the document and on the general approach to the question of rapid alert in AMR surveillance. Activities of the working group for this 2nd meeting were to:

- Discuss and advance the draft
- Define next steps for finalizing the document by Feb 2017
- Present document to Member States consultation in April 2017

3.5.2 Group II.

AMR in fungi: questions

1. Objectives and potential benefits of global antifungal resistance (AFR) surveillance

1. Which would be the specific objectives of AFR surveillance – local and global level?
   - Estimate the burden of resistant invasive fungal diseases
   - Monitor trends in antifungal resistance
   - Inform clinical guidelines for antifungal treatment
• Improve good clinical practices: for diagnosis, treatment and clinical care and infection prevention and control
II. Potential benefits of implementing AFR surveillance
• Improve understanding of impact of AFR
• Strengthen the performance of the clinical laboratories for detection, identification and antifungal susceptibility testing (AFST)
• Promote standardization/definition of diagnostic tools, AFST and standard operating procedures
II. AFR surveillance in GLASS
• Rationale for including AFR surveillance in GLASS
• Potential benefits and risks of including AFR surveillance in GLASS
• Challenges for implementing AFR surveillance at country level
• Steps and capacity required before starting AFR surveillance
• Selection of pathogens and antimicrobials under surveillance
• Development of standards for internal quality assurance
• Requirements for developing a network of reference laboratories providing support in identification and AFST

AMR in fungi: outputs
• Initial discussion
• Prioritize and define the way forward
• Working group?
• Resources and support needed

3.5.3 Group III.

A. Detection of colistin resistance: questions

1. Sustainable models for colistin resistance surveillance
• Establishment of a referral mechanism in-country
• Recommendations on which strains should be tested, (only multi-resistant strains, carbapenemase and/or ESBL producers?) or only strains from invasive disease or as many strains as possible

II. What technical support can be provided to countries that wish to monitor colistin resistance
• Training to perform and interpret the tests
• Provision of reagents and control
• International referral of isolates

Detection of colistin resistance: expected outputs
• Discussion
• Outline a WHO statement
• Define next steps to develop an advice document
• Working group?

B. Application of molecular methods in AMR surveillance: questions

1. Surveillance on AMR and conventional molecular testing
• How and which molecular tests can support the surveillance (epidemiologic, early detection, outbreaks) on AMR?
• What is the best approach to include and promote molecular testing in countries participating in GLASS?
II. What are the current approaches and experiences on molecular testing?
- TB and Rifampicin R
- Carbapenemases in Enterobacteriaceae
- MRSA
- Neisseria gonorrhoeae

III. What is in the pipeline on molecular testing and next-generation sequencing (NGS)?
- Status and future of whole genome sequencing (WGS)
- WGS for GLASS (long term view)

Application of molecular methods in AMR surveillance: outputs
- Broad discussion
- Way forward
- Working group?
- Road map to develop the guidance on molecular testing for GLASS

Day Two. Session Three: Group work reports

3.6 Group I. Emerging AMR: rapid alert and risk assessment framework
Rapporteur: Jean Patel

The rapporteur thanked the Chair, Dr Malin Grape, for a well-organized group discussion.

Key points

1. Terminology and clarification
   - It was agreed that the terms "emergency", "rapid" and "alert" were misleading in this context. Terminology should be consistent with emerging infectious disease rather than with public health emergencies. It was also recommended to avoid terms with legal or statutory implications such as notification.
   - It was also agreed that clarification was needed early in the document on the distinction and relationship between IHR reporting and AMR reporting, including a clear explanation on when each mechanism should be used.
   - It was stressed that AMR reporting should reach all constituencies that might discover new resistance, not just public health, recognizing that while some new AMR findings could represent a significant public health risk, others would not warrant an immediate response but would still be important to know.

2. Scope and target audience
   - It was noted that not all emerging AMR occurs as an outbreak and not all AMR outbreaks represent new AMR. The Risk Assessment should guide this process.
   - Response to new AMR may or may not warrant public health action, but options for action lay beyond the scope of this document.
   - The group recommended including specific examples, particularly those involving several different sectors to indicate how links should be made.

3. Provisional watch list
   - It was noted that the provisional watch list will need to be regularly and easily updated (possibly as an annex). It should not be presented as exhaustive and
should aim to be dynamic. Additional resistance to consider including: antifungal resistance; change in epidemiology; change in ecology; increase in occurrence of life-threatening infections.

4. Reporting of new AMR to GLASS

- Reporting will be via the GLASS IT platform and should be verified/confirmed by a laboratory with the necessary expertise and capacity. Reporting should be through the Ministry of Health, or if reported directly to WHO from a non-government laboratory, then WHO should inform the Ministry of Health concerned.

5. Question of incentivizing reporting

- It was noted that there is an inherent tension in reporting new AMR and withholding information for later publication purposes. To address this, journals should be encouraged to consider AMR as a public health threat and recognize reporting to GLASS of a new AMR as a citable event.
- It was also recommended to use the strongest language possible to encourage compliance without resorting to mandatory terms that implied obligation.

6. Risk Assessment

- The group recommended that the risk assessment be condensed and simplified. References to “hazards” are not relevant and should be removed, and more details specific to AMR should be included such as:
  o Frequency of resistance being reported
  o Risk of spread
  o Risk of increased mortality due to limited treatment options
  o Response needed (technical or public health intervention)
- The risk assessment process should take into account the processes for non-human health sectors, such as animals and environment, and that a more complete risk assessment may be needed to be published as a separate document.

7. Risk communication

- The section on risk communication should be expanded and should include clarification of all constituencies that should receive notification of new AMR. Support, mechanisms and tools may be needed for countries to help them develop risk communication messages. Input should be requested from FAO and OIE on the specific challenges of risk communication in their sectors (e.g. trade concerns).
- It was also noted that the document should be transparent on who has access to what levels of reported information.

Next steps:

The WHO GLASS secretariat will revise the document ready to be provided to Member States by April 2017. Members of the working group volunteered to help with finalization of the document, although it was noted that no representative from the environment sector was able to participate. IT capacity to report emerging AMR would be in place once Member States had approved the process as outlined in the framework.
Discussion points

1. There was discussion on how to make horizontal intersectoral risk assessment possible in real situations when sectors work in isolation. The document recommends assembling a risk assessment team with representatives from human and animal health and the environment, i.e., multisectoral, at the national level. This will be a challenge, not just in LMICs but in all countries, but it is what needs to happen.

2. The group will identify a process in early January for completion of the draft framework by end of February/early March so that Member States will have time to consider it prior to the Member States consultation meeting in April.

3. A question was raised with regards to the level of alert for notification: Countries are expected to implement protocols for reporting new types of AMR. It will be challenging at the beginning to get everyone involved in this communication process. Experience from other rapid alert systems exist, for example influenza, indicate that buy-in within the first year was not perfect but it happened and participation was good. Thus, one can anticipate the same for AMR: there won’t be complete compliance immediately but it will happen in time, with buy-in from all sectors.

4. There was discussion about the level of detail to be reported. It was noted that if something new is identified, the risk assessment section of the document should help in assessing if it should be reported or not. Dr Patel felt (personally) that it is important to report if routine detection assays or phenotypic tests do not pick up the new type. But if it is just a base change that is academic rather than functional, then it does not need to be reported.

5. Information/data will be reported on the GLASS website in the form of trends and signals captured.

6. The need for a strategy to promote the GLASS rapid alert system within the scientific community and make it more visible generally was discussed. An editorial in the Lancet or other prestigious publication, written by a technical person outside of WHO, was suggested as an example. As this is a very sensitive issue, it will require feedback from Member States. It was agreed that Member States should produce the editorial that could then be signed during the April consultation and published.

3.7 Group II: AMR in invasive fungi
Rapporteur Dr David Denning

Dr Denning opened by stating that the group did not consider it appropriate to include AMR in invasive fungi in GLASS at the moment.

Key points

1. Overview of the disease burden and current surveillance.

   A. Burden of disease categories
   (i) Hospitalized sick ICU patients – 750,000 people worldwide with invasive candidiasis, but the problem is likely to be under-estimated.
(ii) AIDS patients – very many fungal infections among AIDS patients but AMR testing is not appropriate for most. The first cases of antifungal resistance were noted in AIDS patients about 20 years ago. There is no estimate of how common it is.

(iii) Respiratory infections – e.g. TB and chronic Aspergillus conditions e.g. severe asthma with severe fungal infections. Just under 500,000 severe asthma deaths per year with some deaths related to fungal sensitization.

B. Surveillance: Fluconazole resistance in all Candida species. Rates range from <10%–40% reflecting species mix.

2014: AMR Surveillance report indicated from 3%-5% up to >50% resistance
2015: Canada had different priorities for AMR disease threats and assessed azole resistance rates
2013: The U.S. assessed fluconazole resistance in Candida (all species) as a mid-tier threat

2. Need for a stepwise approach

The group agreed that Candida and Aspergillus should be considered by GLASS. Given the difficulties in some testing systems a stepwise approach should be taken and lessons learned from the first experiences of GLASS with bacterial pathogens.

3. Need for a situational analysis

A situational analysis is required: the analysis should be a combination of resistance rates and disease burden. The group agreed to try and put this together. It was noted that rates vary a lot in different countries particularly for BSI with Candida as species distribution is a big factor. There is also a need to understand better the situation around the clinical laboratory and epidemiology and training time implications.

The specimen for Candida will be blood: Aspergillus is primarily a respiratory specimen and therefore not within GLASS. There is a concern around testing for Candida resistance in the U.S. due to overlap in breakpoints between different drugs.

There is a need for more environmental surveys to assess azole resistance in Aspergillus. Clinical azole resistance rates are very high in the Netherlands at 16%. Resistance has also been found in people’s homes. In the Netherlands, resistance is thought to be driven by the tulip bulb industry while in Britain, resistance spreads via the onion crop but is also found in other settings. Azoles are used in crop protection with five azoles being the drivers for resistance.

4. Approaches

Discussions have taken place between the TB/HIV/NTD Department of WHO and CDC for a focal point secondment from CDC for fungal diseases (to include AMR).

Currently, there are only three WHO CCs worldwide that address this issue: CDC in Atlanta, U.S.; an excellent laboratory in Chandigarh, India and the mycetoma centre in Khartoum, Sudan. AMR provides an opportunity for centres to collaborate with WHO and grow this area.
There is a need to increase general regional participation – CDC and perhaps the Chandigarh India centre can facilitate training to get better laboratory data sets together.

For discussion: Can *Candida* resistance rates be included in the rapid alert component? Group II requested that antifungal resistance alert criteria be developed within the group and GLASS.

5. Research needs

- Point prevalence studies (PPS) (like EPIC1 and EPIC2).
- Short-term cohort studies e.g. 1-month blood stream resistance study covering all pathogens in multiple centres to include *Candida*. It was clarified that these could be a complement to normal GLASS surveillance and could also complement areas of work discussed in the CC Network. They are relatively easy to do and could provide a first estimate of the burden.

There was a request for a first burden of disease estimate generated by WHO (that includes resistant *Candida*)?

- There is a need to look at oral thrush in AIDS - fluconazole is drug of choice in Africa and if there is resistance, then that is a difficult problem.
- More work on environmental *Aspergillus* is needed; this is not technically difficult but it does require training and support.
- There is a need for some fast-track incidence studies with trend analysis for *C. auris*

For the future: seven new antifungals are in clinical development. Assuming most of them get approval, at what point do they become part of the portfolio of work?

6. Resources

While CDC runs many prospective, multi-centre studies, national studies, some retrospective studies, lots of mapping, and will potentially provide a secondment to WHO, it was noted that the field is generally under-resourced. How can capacity in the laboratory for clinical work and for epidemiology be increased? Public health mycology is a non-existent discipline currently and needs support and expertise. It was suggested that there be a reference laboratory in each country.

Group II posed a general question for WHO and others on how to upscale this area?

Discussion points

1. The submission of additional pathogens for surveillance reporting was discussed. Routine reporting on the eight priority pathogens is the requirement to achieve minimum global data. Despite this, some countries will struggle to provide this data while others will be doing much more than the minimum. Therefore, the expectation is that a range of data and types of feedback from GLASS-enrolled countries will be received. There will be a need to review what is provided and how to focus on priorities and acknowledge other data. Types of data will include rates and the status of development of national surveillance systems to be
reported annually (this last was requested by Member States). It is likely that some countries will not be able to report on rates at the beginning and it may take a number of years before they have the ability to do this. Attention was drawn to the "additional status" within GLASS reporting, where any extra information can be added.

It was highlighted that a rapid alert applies to any pathogen and not just the eight priority pathogens. The alert system covers both changes in epidemiology as well as new emerging resistance.

2. Criteria for reporting: It was suggested that rates of 3%-5% be used as a criteria that will trigger additional action e.g. in the case of a case of a lethal infection such as Apergillosis candidemia.

3.8 Group III: Detection of colistin resistance
Rapporteur: Chris Oxenford

Key points
1. Guidance should not conflict with statements from CLSI/EUCAST in relation to colistin testing
2. Practically speaking, the only viable mechanism in LMICs will be via a centralized laboratory. Therefore, colistin resistance testing will be purely for surveillance, not clinical management.
3. The only validated assay is a broth microdilution so the numbers that a centralized laboratory will be able to cope with will not be large. Given this fact, the group considered different types of screening approaches. Tools that are being validated now and that could potentially be used include: selective media using disk diffusion of an as-yet-undetermined zone size; and polymerase chain reaction (PCR). It was emphasized that these should only be used as screening techniques. If resistance is found, it should then be confirmed with a validated MIC assay.
4. There was much discussion that clinical breakpoints currently in use are not of great utility. In future, epidemiological cut-offs will be used. This means that determining the zone size is still "up in the air": interpretation is still an issue for discussion.
5. Group III felt that support should be made available to those countries that wish to acquire the ability to perform assays e.g. resources such as reference strains, and support from training institutions. This should be pursued and offered to countries who will ultimately decide if this is a priority for them or not given their very limited resources. There should be a careful discussion at country level if this is going to be taken on.

Group III will prepare more concrete guidance in the coming months.

Discussion points
1. It was acknowledged that the breakpoints used for gram-negative colistin are essentially the same as epidemiological cut-offs.
2. The meeting was urged to only recommend well-validated methods. Screening before using a validated method is a good approach but then an appropriate method should be used to avoid a false susceptibility report— the most serious error that can occur in susceptibility testing.

3. Optimism was expressed about the availability of well-performing tests coming to market in the very near future due to recent, new legislation in the U.S. that allows any breakpoint in tests for colistin susceptibility. The Microscan panel, already on the market but up to now, disallowed from advertising itself as suitable for colistin resistance testing, works well and should be more available.

4. Potential problems with the sensitivity of molecular screens were raised. There is an increasing but still inadequate understanding of the genetic basis for resistance.

5. Some meeting participants wondered about the connection of this topic to GLASS – was the point to implement worldwide testing for colistin resistance? It was clarified that one of the indicators countries are asked to report on is resistance against colistin. However, the manual does not alert countries to the difficulties of confirming one result as a true result. GLASS needs to clarify that a finding cannot be considered a final result if it is only based on disk diffusion. There is no intention to replace/duplicate what CLSI/EUCAST are doing but GLASS wants to make countries aware of the limitations of disk diffusion and give further advice. Most countries use disk diffusion but if they want to be clearer about the result, GLASS will need to guide them.

3.9 Group III: Application of molecular methods (MM)
Rapporteur: Matteo Zignol

Dr Zignol started by thanking the Chair, Co-Chair, note taker and all members of the working group for their lively participation and contributions to the discussion.

The objective of this working group was to develop the outline of a road map to provide guidance on molecular testing in GLASS. This would be a document to help the Collaborative Platform decide if MM could be used in GLASS and, if so, how to operationalize the use of these tools in GLASS. The contents will build on work already done by others and will likely be structured into five sections:

1. Background
Why use molecular or genetic tests in surveillance? It is important to note that genotypic tests at this stage are complementary to cultures/AST and are not going to replace these methods. The (many) limitations of phenotypic tests need to be discussed and ways of bypassing those limitations explored. The pros and cons of the use of genotypic tests in AMR surveillance will be considered. The document will seek to capture and make sense of what is available and translate this into information that is useful for countries enrolled in GLASS. It is also important to make a distinction between molecular tests for surveillance purposes and those for diagnostic purposes.

2. What GLASS priority pathogens to target? The intention is to explore the use of MM for surveillance, starting with the easier ones, where the science is clear and to describe resistance markers and mechanisms.
3. Laboratory methods – minimum requirements for laboratory networks: e.g. nucleotide emphasisation assays such as NAATS, NGS, WGS. The aim here is to capture not just data on resistance but also information on transmission dynamics. It will be necessary to work on data interpretation: much work has already been done on this so it is not necessary to start from the beginning. What is required is to make it more usable/"digestible" so that it is useful in GLASS-supported countries. A description of bioinformatic needs and a description of the minimum requirements for laboratory networks to embark on this type of work are also needed. One laboratory in one country may not have the capacity to do everything and will need to link with other laboratories.

4. Data dissemination: this section will describe options for data reporting e.g. genotypic data, metadata. In addition, it will discuss data sharing issues. These will depend on the type of data reported with a different legal framework required for genome sequencing.

5. Operationalization and piloting: This section will include information on sourcing funds for different components e.g. consumables, maintenance, data management; how to design capacity building efforts such as education and training; IT support; developing a sustainability plan; web material; and, development of generic protocols for piloting.

Discussion points

1. The great gulf between the reality of poorly resourced LMICs and the world of MM was acknowledged as was the need to explore avenues for GLASS to incorporate MM in the future. The application of MM is speeding up data acquisition and management of AMR surveillance. The purpose of the working group on MM is to explore the issues that will need addressing even before it will be tested in GLASS. Once that has been done, approaches to operationalization will need to be considered. The use of MM is already happening in other areas e.g. in gonorrhoea, so consideration of MM for GLASS is not so far away.

2. MM must be presented as complementary to routine GLASS activity. Otherwise, countries may devote resources to MM and neglect other methods.

3. There was a request to include chronic Pseudomonas within GLASS: the list of priority pathogens will be reviewed in 2019.

4. Operationalization: capacity building often translates as training but more is needed than just education and training.

5. Data dissemination is not enough; there is a need to include recommendations for action based on the data.

6. It was remarked that there is a need to consider how to package GLASS to make it more attractive and less daunting to countries. Footnotes in manuals and short explanatory attachments were recommended.

7. Epidemiological methods. Comprehensive AMR surveillance must include epidemiological methods that cover timely detection, assessment of burden, transmission dynamics, and the potential implications of AMR in healthcare. To achieve these requires representativeness to minimize bias; good coverage of populations and settings, good assessment of the context, and the ability to predict dynamics. It was suggested that GLASS needs a group to expand epidemiological methods. While not disagreeing with this, it was noted that
support to AMR surveillance is still the backbone of GLASS. The Secretariat is conscious of the need to define sentinel sites in countries and assign at least one NRL and a NCC. When it comes to the design of the surveillance strategy, generic protocols e.g. short cohorts can be used to get a sense of what is happening. It was highlighted that the Strategic Technical & Advisory Group has formally proposed that GLASS consider a global point prevalence survey on AMR. However, only 30 countries are enrolled and this is too few for a representative study. On the other hand, it could be possible to put in place surveillance strategies in low-resource settings that can be conducted in a short space of time to inform local efforts to contain AMR and that can in turn inform the global picture. Some CCs have volunteered to assist GLASS to develop protocols for this and enhance epidemiological design for meaningful information.

8. Other comments noted that it could be easier, better and more cost effective to do genotypic testing for particular types of resistance rather than building laboratory capacity. Some commercial genotypic testing systems are coming online that require relatively little hands-on training; these could potentially fit into an epidemiologically structured survey, one that can then automatically report to the cloud. This system would provide an overarching view of the data that lends itself to working out rates of resistance in particular organisms.

9. The Fleming Fund perspective on capacity development: in many low-resource settings, the key problem is lack or complete absence of basic microbiology laboratory systems. For the Fleming Fund, the priority is to help countries put in place that fundamental capacity but also that laboratory surveillance comes with clinical and epidemiological information. The priority is to help countries generate good samples/organisms with the required pedigree who can then pass these samples on to those who have the higher capacity to study the data. This is not to state that MM are not important but rather that the Fleming Fund will try and support countries to feed into MM.

10. Data management: If genetic data is to be part of the data set, GLASS must find a way to take into account and record the relevant data. It is not clear how GLASS is proposing to handle whole genome sequences. If GLASS does not have line listing, then how will WGS work? There are a number of projects in the world in progress at the moment, and there are sure to be more, that will be global repositories for genome data. It was suggested that a relationship be made between GLASS data and these other databases and avoid duplication.

11. Clarification about new modules in GLASS: the GLASS manual, published in September 2015, committed to working on the need to capture trends and the emergence of new pathogens; to pursue the development of a module for rapid detection; and to explore the application of other technologies to enhance capacities for surveillance. The present meeting is part of the follow up to these commitments. There was acknowledgement that new GLASS modules should be identified and named clearly to avoid confusion.

Summary and Conclusions

Dr Vong summarized the outcomes and next steps
Rapid alert protocol

- The deadline for sharing the framework and risk assessment protocol with Member States is the end of February 2017. Dr Jean Patel, Prof Neil Woodford and Professor Roman Kozlov, with the help of Julio Pinto from FAO will do this. They will finalize the document following feedback in time for the Member States consultation at the end of April 2017.

- To inform the scientific community, the GLASS alert system will be promoted via a Lancet editorial.

- Changes to the document: the title will be changed; IHR will be differentiated from AMR and emergencies differentiated from emerging AMR; the need to share AMR information for mid-term and long-term actions will be noted.

Emerging AMR in fungi

- It is too early for inclusion of this in GLASS, mostly to do with difficulties in testing. It was advised that a stepwise approach be taken and additional activities be carried out, such as a situational analysis focusing on assessing resistance rates, burden of disease, and assessing laboratory capacity throughout the world.

- There is an arrangement for CDC to second a person to WHO as a focal point for fungal infections. The limited number (3) of CCs working in this area was noted.

- GLASS will allow reporting of antifungal resistance, but criteria need to be developed. Research needs in this area include PPS, cohort studies, developing antifungal treatments. The need for funding and resources was acknowledged.

Detection of colistin resistance

- There is a need for clarification on the rationale for doing this. Commercial kits are available for colistin susceptibility. Regarding issues around the difficulties of detecting colistin resistance, WHO will develop an online document explaining how to detect colistin resistance and how WHO can provide support to countries willing to undertake this testing.

Roadmap for guidance on MM

- All were agreed on the importance of having molecular testing acknowledged within GLASS but as a complement to core GLASS work. The roadmap document will likely comprise five sections covering: background; the GLASS priority pathogens to target; laboratory methods and minimum requirements for laboratory networks; data dissemination; and, operationalization and piloting.

- It was noted that this was only the beginning: there is lots of work to be done to map results. There is also a need for more epidemiological analysis, better distribution and more research such as global PPS. It is also important to consider the potential impact of MM on surveillance in terms of ease, quality and cost-effectiveness versus laboratory capacity building for particular types of resistance.

Concluding remarks

The development of GLASS is a shared initiative using a collaborative, consultative approach that belongs to all, not just WHO. Partners and groups will be acknowledged for their contributions. GLASS does not intend to duplicate; rather, it will align with existing initiatives and the CC Network will assist with this. Last year the Collaborative Platform recommended that GLASS improve communication via SharePoint and this has been established. The SharePoint will continue to develop as will the webpage.
The challenge now is to make GLASS grow and enhance technical support capacity. The Secretariat is very grateful to the 19 CCs who comprise the Network. The report of the Network’s first meeting and the work plan for the next three years will be available on the web. CCs have a formal contract with WHO and will lead in the technical areas discussed here. The CC leads will be contacting partners for contributions in developing target products from the work plan.

While the volume of work is huge, the focus must now be on implementation. Representatives from all six WHO Regional Offices have attended this meeting as they need help with implementation and we must provide it. Special thanks were extended to the Fleming Fund for providing resources to countries for implementation and for participation in GLASS.

On behalf of the GLASS Secretariat, Dr Pessoa-Silva thanked all present for their input as did the Chair Dr Perovic who formally closed the meeting.
## Annex 1: List of Participants

### World Health Organization

2nd Meeting of the Global AMR Surveillance System (GLASS) Collaborative Platform  
**15-16 December 2016**,  
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<td>Specialist</td>
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<td>Antimicrobial Resistance Surveillance Washington DC USA</td>
<td>Telephone No. : Email: <a href="mailto:galasmar@paho.org">galasmar@paho.org</a></td>
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<td>EMRO</td>
<td>Dr Ali R MAFI</td>
<td>Medical Officer</td>
<td>Cairo, Egypt</td>
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<td>EURO</td>
<td>Dr Danilo LO FO WONG</td>
<td>Programme Manager</td>
<td>Copenhagen, Denmark</td>
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<td>SEARO</td>
<td>Dr Sirenda VONG</td>
<td>Medical Officer</td>
<td>Delhi, India</td>
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<td>International Health and Regulations (IHR)</td>
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<tr>
<td>WPRO</td>
<td>Dr Babatunde OLOWOKURE</td>
<td>Team lead</td>
<td>Manila, The Philippines</td>
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<td>Emerging Disease Surveillance and</td>
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<td>Dr Jorge MATHEU-ALVAREZ Project Officer</td>
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<tr>
<td>Foodborne and Zoonotic Diseases</td>
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<td>Food Safety and Zoonotic Diseases Department</td>
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<tr>
<th>Health Systems and Innovation</th>
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<tr>
<td>Dr Arno MULLER Consultant Policy, access and Use</td>
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<tr>
<th>HIV/AIDS, TB and Neglected Tropical Diseases (HTM)</th>
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<tr>
<td>Dr Dennis FALZON Scientist Laboratories, Diagnostics and Drug-Resistance Unit</td>
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<td>Dr Alexei KOROBITSYN Technical Officer Laboratories, Diagnostics and Drug-Resistance Unit</td>
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<td>Telephone No.:</td>
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<td>Email: <a href="mailto:zignolm@who.int">zignolm@who.int</a></td>
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<td><strong>WHE</strong></td>
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<td>Mr Christopher OXENFORD</td>
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<td>Technical Officer</td>
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<td>IHR National Capacity Development Unit</td>
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<td>Global Capacities, Alert &amp; Response Department</td>
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<th><strong>AMR/ DGO</strong></th>
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<tr>
<td>Dr Marcus SPRENGER</td>
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<tr>
<td>Director</td>
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<td>Antimicrobial Resistance (AMR)</td>
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<td>Dr Carmem Lucia PESSOA-SILVA</td>
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<td>Acting Coordinator</td>
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<td>Dr Sergey EREMIN</td>
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<td>Dr Jolanta GRISKEVICIENE</td>
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<td>Technical Officer</td>
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<tr>
<td>Mr Tejinder CHOWDHARY</td>
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<td>Technical Officer</td>
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<td>Antimicrobial Resistance (AMR)</td>
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<td>Dr Elizabeth TAYLER</td>
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<td>Technical Officer</td>
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<td>Antimicrobial Resistance (AMR)</td>
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<td>Telephone No.: +41 22 791 4536 Email: <a href="mailto:taylere@who.int">taylere@who.int</a></td>
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<td>Dr Jung Kyu LEE</td>
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<td>Technical Officer</td>
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<td>Dr Muna ABU SIN</td>
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<tr>
<td>Consultant</td>
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<td>Telephone No.: 41 22 79 15403 Email: <a href="mailto:abusinm@who.int">abusinm@who.int</a></td>
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<tr>
<th><strong>ADMINISTRATIVE SUPPORT</strong></th>
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<tbody>
<tr>
<td>Mrs Mawuto FIWOO-MARKHAM</td>
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<tr>
<td>Antimicrobial Drug Resistance (AMR)</td>
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<tr>
<td>Telephone No.: +41 22 791 5403 Email: <a href="mailto:fiawoomarkhamm@who.int">fiawoomarkhamm@who.int</a></td>
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Annex 2: Meeting agenda

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>08:30-09:00</td>
<td>Registration</td>
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<tr>
<td>09:00-09:10</td>
<td>Welcome and introductions</td>
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<td>09:10-09:30</td>
<td>Meeting format, objectives and desired outcomes</td>
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<td>• Declarations of Interest</td>
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<td>• Meeting rules and procedures</td>
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<td>• Selection of chair</td>
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<td>• Format and desired outcomes</td>
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<tr>
<td>09:30-09:40</td>
<td>Overview of GLASS and update on early implementation</td>
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<td><em>Carmen L. Pessoa-Silva (WHO)</em></td>
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<td>09:40-09:50</td>
<td>Surveillance of AMR in the food chain</td>
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<td><em>Jorge Raul Matheu Alvarez (WHO)</em></td>
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<td>09:50-10:00</td>
<td>Surveillance of AMU</td>
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<td><em>Arno Muller (WHO)</em></td>
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<tr>
<td>10:00-10:10</td>
<td>Surveillance of AMR in gonococci: update from GASP</td>
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<td><em>Theodora WI (WHO)</em></td>
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<td>10:10-10:20</td>
<td>WHO initiative to foster development of new diagnostics</td>
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<td><em>Francis Moussy (WHO)</em></td>
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<td>10:20-10:30</td>
<td>Discussions</td>
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<td>10:30-10:45</td>
<td>Coffee Break</td>
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<td>10:45-11:05</td>
<td>Global AMR surveillance: update from FAO</td>
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<td><em>Julio Pinto (FAO)</em></td>
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<td>(15 mins presentation)</td>
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<td>5 min discussion</td>
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SESSION 2: Support to global AMR surveillance
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<tr>
<td>11:05-11:25</td>
<td><strong>Fleming Fund initiative</strong>&lt;br&gt;<em>(Fleming Fund representative)</em>&lt;br&gt;<em>(15 min presentation 5 min discussion)</em></td>
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<td>11:25-11:45</td>
<td><strong>Presentation of the newly established WHO CC network and its work plan</strong>&lt;br&gt;<em>TBD</em></td>
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<td>11:45-12:00</td>
<td><strong>Presentation of initiating group work</strong>&lt;br&gt;<em>Sergey Eremin (WHO)</em></td>
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<td>12:00-13:00</td>
<td><strong>Lunch</strong></td>
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<td><strong>SESSION 3: AMR technical challenges</strong></td>
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<td>13:00-13:20</td>
<td><strong>GLASS Rapid Alert Component: presentation of draft protocol</strong>&lt;br&gt;<em>Sergey Eremin (WHO)</em></td>
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<td>13:20-13:40</td>
<td><strong>Emerging AMR in fungi causing invasive infections in humans</strong>&lt;br&gt;<em>Tom Chiller (WHO Collaborating Centre)</em></td>
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<td>13:40-13:55</td>
<td><strong>Detection of colistin resistance among enterobacteriaceae</strong>&lt;br&gt;<em>Sebastien Cognat/Christopher Oxenford/Jorge Matheu (WHO)</em></td>
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<td>13:55-14:15</td>
<td><strong>Application of molecular methods to support AMR surveillance</strong>&lt;br&gt;<em>Matteo Zignol (WHO)</em></td>
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<td>14:15-14:30</td>
<td><strong>Coffee break</strong></td>
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<td>14:30-17:00</td>
<td><strong>Group work: Outlining the next steps to address the surveillance challenges</strong></td>
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<td><strong>Group I:</strong>&lt;br&gt;Rapid Alert&lt;br&gt;<strong>Group II:</strong>&lt;br&gt;AMR in invasive fungi&lt;br&gt;<strong>Group III:</strong>&lt;br&gt;Detection of colistin resistance &amp; Application of molecular methods</td>
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<tr>
<td>17:00</td>
<td><strong>Meeting adjourns</strong></td>
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Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America

Thomas F. Patterson,1,a George R. Thompson III,2 David W. Denning,3 Jay A. Fishman,4 Susan Hadley,5 Raoul Herbrecht,6 Dimitrios P. Kontoyiannis,7 Kieren A. Marr,3 Vicki A. Morrison,4 M. Hong Nguyen,9 Brahm H. Segal,10 William J. Steinbach,12 David A. Stevens,13 Thomas J. Walsh,14 John R. Wingard,15 Jo-Anne H. Young,16 and John E. Bennett17,a

1University of Texas Health Science Center at San Antonio and South Texas Veterans Health Care System; 2University of California, Davis; 3National Aspergillosis Centre, University Hospital of South Manchester, University of Manchester, United Kingdom; 4Massachusetts General Hospital and Harvard Medical School; and 5Tufts Medical Center, Boston, Massachusetts; 6University of Strasbourg, France; 7University of Texas MD Anderson Cancer Center, Houston; 8Johns Hopkins University School of Medicine and the Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland; 9Hennepin County Medical Center and University of Minnesota, Minneapolis; 10University of Pittsburgh, Pennsylvania; 11University at Buffalo Jacobs School of Medicine and Biomedical Sciences, and Roswell Park Cancer Institute, New York; 12Duke University Medical Center, Durham, North Carolina; 13California Institute for Medical Research, San Jose; 14New York–Presbyterian Hospital/Weill Cornell Medical Center, New York; 15University of Florida, Gainesville; 16University of Minnesota, Minneapolis; 17Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland

It is important to realize that guidelines cannot always account for individual variation among patients. They are not intended to supplant physician judgment with respect to particular patients or special clinical situations. IDSA considers adherence to these guidelines to be voluntary, with the ultimate determination regarding their application to be made by the physician in the light of each patient's individual circumstances.

Keywords: aspergillosis; invasive aspergillosis; allergic aspergillosis; chronic aspergillosis; fungal diagnostics; azoles; echinocandins; amphotericin.

EXECUTIVE SUMMARY

Background
Aspergillus species continue to be an important cause of life-threatening infection in immunocompromised patients. This at-risk population is comprised of patients with prolonged neutropenia, allogeneic hematopoietic stem cell transplant (HSCT), solid organ transplant (SOT), inherited or acquired immunodeficiencies, corticosteroid use, and others. This document constitutes the guidelines of the Infectious Diseases Society of America (IDSA) for treatment of aspergillosis and replaces the practice guidelines for Aspergillus published in 2008. Since that publication, clinical studies evaluating new and existing therapies including combination therapy for the management of Aspergillus infection have been conducted and the data on use of non-culture-based biomarkers for diagnosing infection have been expanded. The objective of these guidelines is to summarize the current evidence for treatment of different forms of aspergillosis. This document reviews guidelines for management of the 3 major forms of aspergillosis: invasive aspergillosis (IA); chronic (and saprophytic) forms of aspergillosis; and allergic forms of aspergillosis. Given the clinical importance of IA, emphasis is placed upon the diagnosis, treatment, and prevention of the different forms of IA, including invasive pulmonary aspergillosis (IPA), Aspergillus sinusitis, disseminated aspergillosis, and several types of single-organ IA.

Summarized below are the 2016 recommendations for the management of aspergillosis. Due to the guidelines' relevance to pediatrics, the guideline has been reviewed and endorsed by the Pediatric Infectious Diseases Society (PIDS). The panel followed a guideline development process that has been adopted by IDSA, which includes use of the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system, a systematic method of grading both the strength of the recommendation (weak or strong) and the quality of evidence (very low, low, moderate, and high) (Figure 1). The guidelines are not intended to replace clinical judgment in the management of individual patients. A detailed description of the methods, background, and evidence summaries that support each recommendation can be found in the full text of the guideline.

EPIDEMIOLOGY AND RISK FACTORS FOR INFECTION

I. How Can the Most Susceptible Patients Be Protected From Aspergillosis, and Which Patients Are Most Susceptible?

What Are Sources of Exposure to Aspergillus, and How Can Exposure Be Decreased? Is Environmental Surveillance Useful?
Recommendations.

1. Hospitalized allogeneic HSCT recipients should be placed in a protected environment to reduce mold exposure (strong recommendation; low-quality evidence).

2. These precautions can be reasonably applied to other highly immunocompromised patients at increased risk for IA, such as patients receiving induction/reinduction regimens for acute leukemia (strong recommendation; low-quality evidence).

3. In hospitals in which a protected environment is not available, we recommend admission to a private room, no connection to construction sites, and not allowing plants or cut flowers to be brought into the patient’s room (strong recommendation; low-quality evidence).

4. We recommend reasonable precautions to reduce mold exposure among outpatients at high risk for IA, including avoidance of gardening, spreading mulch (compost), or close exposure to construction or renovation (strong recommendation; low-quality evidence).

5. Leukemia and transplant centers should perform regular surveillance of cases of invasive mold infection. An increase in incidence over baseline or the occurrence of invasive mold infections in patients who are not at high risk for such infections should prompt evaluation for a hospital source (strong recommendation; low-quality evidence).

DIAGNOSIS OF ASPERGILLOSIS

II. How Can a Diagnosis of Invasive Aspergillosis Be Established?

How Should Aspergillus Be Identified, and How Does This Influence Management?

Recommendation.

6. Until molecular tools are more widely used in clinical laboratories, we recommend that tissue and fluid specimens be submitted in adequate quantities for simultaneous histopathologic/cytologic and culture examination. In the case of isolates with atypical growth or concerns for resistance, species
identification by molecular methods should be employed (strong recommendation; high-quality evidence).

What Is the Diagnostic Value of Nucleic Acid Testing in Clinical Specimens?

Recommendations.

7. There was debate among the committee members regarding the clinical utility of blood-based polymerase chain reaction (PCR) in diagnosing IA, and experts were not in agreement. One group favored recommendations for PCR testing, based on publications validating its role when used in conjunction with other tests such as antigen detection assays to diagnose IA and/or reduce preemptive antifungal usage. The other group thought that PCR assays are promising but could not be recommended for routine use in clinical practice at present due to the lack of conclusive validation for commercially available assays, the variety of methodologies in the literature, and questions about the extent to which results assisted diagnosis.

8. As research in the area continues, we recommend that clinicians choosing to use PCR assays employ them carefully in the management of individual patients on a case-by-case basis. Clinicians should be aware of the methodologies and performance characteristics of the specific assay used, and interpret results accordingly. When PCR assays are used, results should be considered in conjunction with other diagnostic tests and the clinical context (strong recommendation; moderate-quality evidence).

How Should Galactomannan and \((1 \rightarrow 3)\)-\(\beta\)-D-Glucan Be Used for the Diagnosis of Aspergillosis?

Recommendations.

9. Serum and BAL galactomannan (GM) is recommended as an accurate marker for the diagnosis of IA in adult and pediatric patients when used in certain patient subpopulations (hematologic malignancy, HSCT) (strong recommendation; high-quality evidence).

10. GM is not recommended for routine blood screening in patients receiving mold-active antifungal therapy or prophylaxis, but can be applied to bronchoscopy specimens from those patients (strong recommendation; high-quality evidence).

11. GM is not recommended for screening in SOT recipients or patients with chronic granulomatous disease (CGD) (strong recommendation; high-quality evidence).

12. Serum assays for \((1 \rightarrow 3)\)-\(\beta\)-D-glucan are recommended for diagnosing IA in high-risk patients (hematologic malignancy, allogeneic HSCT), but are not specific for Aspergillus (strong recommendation; moderate-quality evidence).

What Is the Approach to the Radiographic Diagnosis of Invasive Pulmonary Aspergillosis?

Recommendations.

13. We recommend performing a chest computed tomographic (CT) scan whenever there is a clinical suspicion for IPA regardless of chest radiograph results (strong recommendation; high-quality evidence).

14. Routine use of contrast during a chest CT scan for a suspicion of IPA is not recommended (strong recommendation; moderate-quality evidence). Contrast is recommended when a nodule or a mass is close to a large vessel (strong recommendation; moderate-quality evidence).

15. We suggest a follow-up chest CT scan to assess the response of IPA to treatment after a minimum of 2 weeks of treatment; earlier assessment is indicated if the patient clinically deteriorates (weak recommendation; low-quality evidence). When a nodule is close to a large vessel, more frequent monitoring may be required (weak recommendation; low-quality evidence).

What Is the Role of Bronchoscopy in the Diagnosis of Invasive Pulmonary Aspergillosis?

Recommendation.

16. We recommend performing a bronchoscopy with bronchoalveolar lavage (BAL) in patients with a suspicion of IPA (strong recommendation; moderate-quality evidence). Significant comorbidities such as severe hypoxemia, bleeding, and platelet transfusion refractory thrombocytopenia may preclude BAL. The yield of BAL is low for peripheral nodular lesions, so percutaneous or endobronchial lung biopsy should be considered. We recommend the use of a standardized BAL procedure and sending the BAL sample for routine culture and cytology as well as non-culture-based methods (eg, GM) (strong recommendation; moderate-quality evidence).

III. What Antifungal Agents Are Available for the Treatment and Prophylaxis of Invasive Aspergillosis, Including Pharmacologic Considerations, and What Is the Role for Susceptibility Testing?

Amphotericin B

Recommendations.

17. Amphotericin B (AmB) deoxycholate and its lipid derivatives are appropriate options for initial and salvage therapy of Aspergillus infections when voriconazole cannot be administered. However, AmB deoxycholate should be reserved for use in resource-limited settings in which no alternative agents are available. Lipid formulations of AmB should be considered in settings in which azoles are contraindicated or not tolerated (strong recommendation; moderate-quality evidence).

18. Aerosolized formulations of AmB may be considered as prophylaxis in patients with prolonged neutropenia (patients receiving induction/reinduction therapy for acute leukemia and allogeneic HSCT recipients following conditioning or during treatment of graft-vs-host disease [GVHD]) and in lung transplant recipients (weak recommendation; low-quality evidence).

Echinocandins

Recommendation.

19. Echinocandins are effective in salvage therapy (either alone or in combination) against IA, but we do not...
recommend their routine use as monotherapy for the primary treatment of IA (strong recommendation; moderate-quality evidence).

**Triazoles Recommendations.**

20. Triazoles are preferred agents for treatment and prevention of IA in most patients (strong recommendation; high-quality evidence).

21. For patients receiving triazole-based therapy for IA, prolonged azole prophylaxis, or other therapies for which drug interactions with azoles are anticipated, the committee recommends therapeutic drug monitoring (TDM) once the steady state has been reached. A moderate amount of data for itraconazole, voriconazole, and posaconazole suspension suggests this approach may be valuable in enhancing therapeutic efficacy, in evaluating therapeutic failures attributable to suboptimal drug exposures, and to minimize toxicities potentially attributable to the azoles (strong recommendation; moderate-quality evidence). Further studies are needed to address whether TDM is helpful or necessary with the extended-release or intravenous formulations of posaconazole or for isavuconazole.

22. Clinicians should obtain serum trough drug levels for azole antifungal agents (itraconazole, voriconazole, posaconazole, and possibly isavuconazole) and for potentially interacting drugs such as cyclosporine, tacrolimus, and sirolimus (and other CYP3A4 substrates such as tyrosine kinase inhibitors) to optimize therapeutic efficacy and to avoid potential toxicities of both groups of agents (strong recommendation; moderate-quality evidence).

**Preclinical and Laboratory Assessment of Combination Antifungal Therapy**

23. Combinations of polyenes or azoles with echinocandins suggest additive or synergistic effects in some preclinical studies. However, variable test designs and conflicting results of preclinical and in vitro testing have led to uncertainty as to how to interpret the findings (weak recommendation; low-quality evidence).

**When Should Antifungal Susceptibility Testing Be Performed, and How Should Results Be Interpreted and Affect Management? Recommendation.**

24. Routine antifungal susceptibility testing (AFST) of isolates recovered during initial infection is not recommended. AFST of *Aspergillus* isolates using a reference method is reserved for patients suspected to have an azole-resistant isolate or who are unresponsive to antifungal agents, or for epidemiological purposes (strong recommendation; moderate-quality evidence).

**INVASIVE SYNDROMES OF ASPERGILLUS**

IV. What Are the Recommended Treatment Regimens and Adjunctive Treatment Measures for the Various Clinical Presentation of Invasive Aspergillosis?

**How Should IPA Be Treated? Recommendations.**

25. We recommend primary treatment with voriconazole (strong recommendation; high-quality evidence).

26. Early initiation of antifungal therapy in patients with strongly suspected IPA is warranted while a diagnostic evaluation is conducted (strong recommendation; high-quality evidence).

27. Alternative therapies include liposomal AmB (strong recommendation; moderate-quality evidence), isavuconazole (strong recommendation; moderate-quality evidence), or other lipid formulations of AmB (weak recommendation; low-quality evidence).

28. Combination antifungal therapy with voriconazole and an echinocandin may be considered in select patients with documented IPA (weak recommendation; moderate-quality evidence).

29. Primary therapy with an echinocandin is not recommended (strong recommendation; moderate-quality evidence). Echinocandins (micafungin or caspofungin) can be used in settings in which azole and polyene antifungals are contraindicated (weak recommendation; moderate-quality evidence).

30. We recommend that treatment of IPA be continued for a minimum of 6–12 weeks, largely dependent on the degree and duration of immunosuppression, site of disease, and evidence of disease improvement (strong recommendation; low-quality evidence).

31. For patients with successfully treated IPA who require subsequent immunosuppression, secondary prophylaxis should be initiated to prevent recurrence (strong recommendation; moderate-quality evidence).

**Adjunctive Measures and Immunomodulation: When Should Withdrawal of Immunosuppressive Agents, or Addition of Colony-Stimulating Factors or Granulocyte Transfusions, Be Considered in the Treatment of Invasive Aspergillosis? Recommendations.**

32. Reducing doses of, or eliminating altogether, immuno- suppressive agents, when feasible, is advised as a component of anti-Aspergillus therapy (strong recommendation; low-quality evidence).

33. Colony-stimulating factors may be considered in neutropenic patients with diagnosed or suspected IA (weak recommendation; low-quality evidence). There is insufficient evidence regarding the value of granulocyte colony-stimulating factor vs granulocyte macrophage colony-stimulating factor (GM-CSF) in this setting.

34. Granulocyte transfusions can be considered for neutropenic patients with IA that is refractory or unlikely to respond to standard therapy, and for an anticipated duration of more than one week (weak recommendation; low-quality evidence).
35. Recombinant interferon-γ is recommended as prophylaxis in CGD patients (strong recommendation; high-quality evidence). Its benefit as adjunctive therapy for IA is unknown.

36. Surgery for aspergillosis should be considered for localized disease that is easily accessible to debridement (eg, invasive fungal sinusitis or localized cutaneous disease) (strong recommendation; low-quality evidence). The benefit for IA in other settings such as in the treatment of endocarditis, osteomyelitis, or focal central nervous system (CNS) disease appears rational. Other indications are less clear and require consideration of the patient’s immune status, comorbidities, confirmation of a single focus, and the risks of surgery.

When Is It Safe to Proceed With Chemotherapy or Transplantation in a Patient With Invasive Aspergillosis? Recommendations

37. IA is not an absolute contraindication to additional chemotherapy or HSCT (strong recommendation; moderate-quality evidence).

38. Decisions about when to proceed with additional chemotherapy or HSCT following the diagnosis of aspergillosis should involve both infectious diseases specialists and hematologists/oncologists. These decisions must consider the risk of progressive aspergillosis during periods of subsequent antineoplastic treatment vs the risk of death from the underlying malignancy if this treatment is delayed (strong recommendation; low-quality evidence).

What Approaches Are Needed for Refractory or Progressive Aspergillosis (Salvage Therapy)? Recommendations

39. We recommend an individualized approach that takes into consideration the rapidity, severity, and extent of infection, patient comorbidities, and to exclude the emergence of a new pathogen (strong recommendation; low-quality evidence). The general strategies for salvage therapy typically include (i) changing the class of antifungal, (ii) tapering or reversal of underlying immunosuppression when feasible, and (iii) surgical resection of necrotic lesions in selected cases.

40. In the context of salvage therapy, an additional antifungal agent may be added to current therapy, or combination antifungal drugs from different classes other than those in the initial regimen may be used (weak recommendation; moderate-quality evidence).

41. In patients currently receiving an antifungal and exhibiting an adverse event attributable to this agent, we recommend changing to an alternative class of antifungal, or the use of an alternative agent with a nonoverlapping side-effect profile (strong recommendation; low-quality evidence).

42. For salvage therapy, agents include lipid formulations of AmB, micafungin, caspofungin, posaconazole, or itraconazole. The use of a triazole as salvage therapy should take into account prior antifungal therapy, host factors, pharmacokinetic considerations, and possible antifungal resistance (strong recommendation; moderate-quality evidence).

How Can Biomarkers Be Used to Assess Patient Response to Therapy? Recommendations

43. Serial monitoring of serum GM can be used in the appropriate patient subpopulations (hematologic malignancy, HSCT) who have an elevated GM at baseline to monitor disease progression and therapeutic response, and predict outcome (strong recommendation; moderate-quality evidence).

44. (1 → 3)-β-D-glucan has not been extensively studied in IA to predict outcome (weak recommendation; low-quality evidence).

What Are the Recommended Treatments for Pediatric Patients With Aspergillosis? Recommendation

45. Treatment of aspergillosis in children uses the same recommended therapies as in adult patients; however, the dosing is different and for some antifungals is unknown (strong recommendation; high-quality evidence).

What Are Treatment Options for Aspergillosis of the Airways in Transplant and Nontransplant Recipients, and How Does It Differ From Invasive Pulmonary Aspergillosis? Recommendations

46. Saprophytic forms of tracheobronchial aspergillosis (TBA) do not require antifungal treatment except for symptoms or immunosuppressed patients. Treatment includes bronchoscopic removal of mucoid impaction. Mold-active triazole agents are recommended for immunocompromised patients in whom the possibility of invasive disease cannot be eliminated (strong recommendation; moderate-quality evidence).

47. Bronchocentric granulomatosis is treated in the same fashion as allergic bronchopulmonary aspergillosis (ABPA) (strong recommendation; low-quality evidence).

48. Invasive forms of TBA are treated with a mold-active triazole or intravenous lipid formulations of AmB (strong recommendation; moderate-quality evidence). We also recommend minimization or reversal of underlying immunosuppression when feasible, and bronchoscopic debridement of airway lesions in selected cases (strong recommendation; low-quality evidence).

49. In lung transplant recipients, we recommend treatment with a systemic antimold antifungal for TBA, including saprophytic forms. We also recommend adjunctive inhaled AmB in the setting of TBA associated with anastomotic endobronchial ischemia or ischemic reperfusion injury due to airway ischemia associated with lung transplant (strong recommendation; moderate-quality evidence). Duration of antifungal therapy is at least 3 months or until TBA is completely resolved, whichever is longer.
What Are the Treatment Considerations for Central Nervous System Aspergillosis?

Recommendation.

50. We recommend voriconazole as primary therapy for CNS aspergillosis (strong recommendation; moderate-quality evidence). Lipid formulations of AmB are reserved for those intolerant or refractory to voriconazole (strong recommendation; moderate-quality evidence).

How Is Aspergillus Endophthalmitis Treated?

Recommendation.

51. We recommend that Aspergillus endophthalmitis be treated with systemic oral or intravenous voriconazole plus intravitreal voriconazole or intravitreal AmB deoxycholate (strong recommendation; weak-quality evidence).

What Is the Role of Surgery in Aspergillosis of the Paranasal Sinuses?

Recommendation.

52. We recommend that both surgery and either systemic voriconazole or a lipid formulation of AmB be used in invasive Aspergillus fungal sinusitis but that surgical removal alone can be used to treat Aspergillus fungal ball of the paranasal sinus. Enlargement of the sinus ostomy may be needed to improve drainage and prevent recurrence (strong recommendation; moderate-quality evidence).

What Are the Treatment Recommendations for Aspergillus Endocarditis, Pericarditis, and Myocarditis?

Recommendation.

53. In Aspergillus endocarditis, we recommend early surgical intervention combined with antifungal therapy in attempts to prevent embolic complications and valvular decompensation (strong recommendation; moderate-quality evidence). Voriconazole or a lipid formulation of AmB is recommended as initial therapy (strong recommendation; low-quality evidence). Following surgical replacement of an infected valve, lifelong antifungal therapy should be considered (strong recommendation; low-quality evidence).

What Are the Treatment Recommendations for Aspergillus Osteomyelitis and Septic Arthritis?

Recommendation.

54. Surgical intervention is recommended, where feasible, for management of Aspergillus osteomyelitis and arthritis, combined with voriconazole (strong recommendation; moderate-quality evidence).

What Are the Treatment Recommendations for Cutaneous Aspergillosis?

Recommendations.

55. As cutaneous lesions may reflect disseminated infection, we recommend treatment with voriconazole in addition to evaluation for a primary focus of infection (strong recommendation; low-quality evidence).

56. In cases of aspergillosis in burns or massive soft tissue wounds, surgical debridement is recommended, in addition to antifungal therapy (strong recommendation; moderate-quality evidence).

What Are the Treatment Recommendations for Aspergillus Peritonitis?

Recommendation.

57. We recommend prompt peritoneal dialysis catheter removal accompanied by systemic antifungal therapy with voriconazole (strong recommendation; low-quality evidence).

What Are the Treatment Recommendations for Esophageal, Gastrointestinal, and Hepatic Aspergillosis?

Recommendations.

58. We suggest voriconazole and surgical consultation in attempts to prevent complications of hemorrhage, perforation, obstruction, or infarction (weak recommendation; low-quality evidence).

59. We suggest antifungal therapy with voriconazole or a lipid formulation of AmB as initial therapy for hepatic aspergillosis. For extrahepatic or perihepatic biliary obstruction, or localized lesions that are refractory to medical therapy, surgical intervention should be considered (weak recommendation; low-quality evidence).

What Are the Treatment Recommendations for Renal Aspergillosis?

Recommendation.

60. We suggest a combined approach of medical and urologic management for renal aspergillosis. Obstruction of one or both ureters should be managed with decompression if possible and local instillation of AmB deoxycholate. Parenchymal disease is best treated with voriconazole (weak recommendation; low-quality evidence).

What Are the Treatment Regimens for Aspergillus Ear Infections?

Recommendations.

61. Noninvasive Aspergillus otitis externa, also called otomycosis, is treated by thorough mechanical cleansing of the external auditory canal followed by topical antifungals or boric acid (strong recommendation; moderate-quality evidence).

62. We recommend that clinicians treat IA of the ear with a prolonged course of systemic voriconazole, usually combined with surgery (strong recommendation; low-quality evidence).
What Are the Treatment Recommendations for Aspergillus Keratitis?
Recommendation.
63. We recommend that clinicians treat Aspergillus keratitis with topical natamycin 5% ophthalmic suspension or topical voriconazole (strong recommendation; moderate-quality evidence).

How Should Aspergillus Bronchitis Be Diagnosed and Treated in the Nontransplant Population?
Recommendations.
64. We suggest the diagnosis of Aspergillus bronchitis in non-transplant patients be confirmed by detection of Aspergillus spp in respiratory secretions, usually sputum, with both PCR and GM on respiratory samples being much more sensitive than culture (weak recommendation; low-quality evidence).
65. We suggest treatment with oral itraconazole or voriconazole with TDM (weak recommendation; low-quality evidence).

PROPHYLAXIS OF INVASIVE ASPERGILLOSIS
V. What Are the Recommended Prophylactic Regimens, Who Should Receive Them, and How Should Breakthrough Infection Be Managed?
In Which Patients Should Antifungal Prophylaxis Against Aspergillus Be Used?
Recommendation.
66. We recommend prophylaxis with posaconazole (strong recommendation; high-quality evidence), voriconazole (strong recommendation; moderate-quality evidence), and/or micafungin (weak recommendation; low-quality evidence) during prolonged neutropenia for those who are at high risk for IA (strong recommendation; high-quality evidence). Prophylaxis with caspofungin is also probably effective (weak recommendation; low-quality evidence). Triazoles should not be coadministered with other agents known to have potentially toxic levels with concurrent triazole coadministration (eg, vinca alkaloids, and others) (strong recommendation; moderate-quality evidence).

What Are the Recommended Prophylactic Regimens for Patients With Graft-Versus-Host Disease?
Recommendations.
67. We recommend prophylaxis with posaconazole for allogeneic HSCT recipients with GVHD who are at high risk for IA (strong recommendation; high-quality evidence). Prophylaxis with other mold-active azoles is also effective. Voriconazole is commonly used for prophylaxis against IA in high-risk patients but did not show improved survival in clinical trials (strong recommendation; moderate-quality evidence). Prophylaxis with itraconazole is limited by tolerability and absorption (strong recommendation; high-quality evidence).
68. We recommend continuation of antifungal prophylaxis throughout the duration of immunosuppression in patients with chronic immunosuppression associated with GVHD (corticosteroid equivalent of >1 mg/kg/day of prednisone for >2 weeks and/or the use of other anti-GVHD therapies, such as lymphocyte-depleting agents, or tumor necrosis factor α (TNF-α) inhibition, for refractory GVHD) (strong recommendation; high-quality evidence).

What Are the Recommendations for Antifungal Prophylaxis in Lung Transplant Patients?
Recommendations.
69. We recommend antifungal prophylaxis with either a systemic triazole such as voriconazole or itraconazole or an inhaled AmB product for 3 to 4 months after lung transplant (strong recommendation; moderate-quality evidence).
70. Systemic voriconazole or itraconazole is suggested over inhaled AmB for lung transplant recipients with mold colonization pre- or post-lung transplant, mold infections found in explanted lungs, fungal infections of the sinus, and single-lung transplant recipients (weak recommendation; low-quality evidence).
71. We recommend reinitiating antifungal prophylaxis for lung transplant recipients receiving immunosuppression augmentation with either thymoglobulin, alemtuzumab, or high-dose corticosteroids (strong recommendation; moderate-quality evidence).

What Are the Recommendations for Antifungal Prophylaxis in Nonlung Solid Organ Transplant Recipients?
Recommendation.
72. We recommend prophylactic strategies in SOT recipients based on the institutional epidemiology of infection and assessment of individual risk factors (strong recommendation; low-quality evidence). Prospective trials are lacking to address the need for routine anti-Aspergillus prophylaxis other than for lung transplant recipients. Individual risk factors have been identified in cardiac (pretransplant colonization, reoperation, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, and single-lung transplant recipients, for >2 weeks and/or the use of other anti-GVHD therapies, such as lymphocyte-depleting agents, or tumor necrosis factor α (TNF-α) inhibition, for refractory GVHD) (strong recommendation; high-quality evidence).

MANAGEMENT OF BREAKTHROUGH INFECTION
How Should Breakthrough Aspergillosis Be Managed?
Recommendation.
73. We suggest an individualized approach that takes into consideration the rapidity and severity of infection and local...
epidemiology. As principles, we recommend an aggressive and prompt attempt to establish a specific diagnosis with bronchoscopy and/or CT-guided biopsy for peripheral lung lesions. Documentation of serum azole levels should be verified if TDM is available for patients receiving mold-active triazoles. Antifungal therapy should be empirically changed to an alternative class of antifungal with Aspergillus activity. Other considerations include reduction of underlying immunosuppression if feasible, and susceptibility testing of any Aspergillus isolates recovered from the patient (weak recommendation; moderate-quality evidence).

VI. When Should Patients Be Treated Empirically?
What Strategies Are Recommended for Empiric and Preemptive Strategies in Allogeneic Hematopoietic Stem Cell Transplant Recipients and Patients Treated for Acute Myelogenous Leukemia?
Recommendations.

74. Empiric antifungal therapy is recommended for high-risk patients with prolonged neutropenia who remain persistently febrile despite broad-spectrum antibiotic therapy. Antifungal options include a lipid formulation of AmB (strong recommendation; high-quality evidence), an echinocandin (caspofungin or micafungin) (strong recommendation; high-quality evidence), or voriconazole (strong recommendation; moderate-quality evidence).

75. Empiric antifungal therapy is not recommended for patients who are anticipated to have short durations of neutropenia (duration of neutropenia <10 days), unless other findings indicate a suspected invasive fungal infection (IFI) (strong recommendation; moderate-quality evidence).

76. The use of serum or BAL fungal biomarkers such as GM or (1 → 3)-β-D-glucan to guide antifungal therapy in asymptomatic or febrile high-risk patients (often referred to as preemptive or biomarker-driven antifungal therapy) can reduce unnecessary antifungal therapy. The preemptive approach can result in more documented cases of IA without compromise in survival and can be used as an alternative to empiric antifungal therapy (strong recommendation; moderate-quality evidence).

77. Early initiation of antifungal therapy in patients with strongly suspected IPA is warranted while a diagnostic evaluation is conducted (strong recommendation; moderate-quality evidence).

78. Management of suspected or documented breakthrough IPA in the context of mold-active azole prophylaxis or empiric suppressive therapy is not defined by clinical trial data, but a switch to another drug class is suggested (weak recommendation; low-quality evidence).

How Do Lung Transplant Recipients Differ From Other Immunosuppressed Patients in Management of Suspected Invasive Pulmonary Aspergillosis?
Recommendations.

79. In lung transplant recipients not on antimold prophylaxis, we suggest preemptive therapy with an antimold antifungal for asymptomatic patients with Aspergillus colonization of the airways within 6 months of lung transplant or within 3 months of receiving immunosuppression augmentation for rejection (weak recommendation; moderate-quality evidence).

80. Six months after lung transplant and in the absence of recent immunosuppression augmentation for rejection, it may be prudent to withhold antifungal therapy for Aspergillus airway colonization (ie, Aspergillus respiratory cultures in the absence of clinical features that suggest disease, such as compatible symptoms, or bronchoscopic, histopathologic, and/or radiographic findings) (weak recommendation; low-quality evidence).

CHRONIC AND SAPROPHYTIC SYNDROMES OF ASPERGILLUS

VII. How Should Chronic Aspergillosis, Allergic Syndromes, or Noninvasive Syndromes Be Managed?
How Can Chronic Cavitary Pulmonary Aspergillosis Be Diagnosed and Treated?
Recommendations.

81. The diagnosis of chronic cavitary pulmonary aspergillosis (CCPA) requires: (i) 3 months of chronic pulmonary symptoms or chronic illness or progressive radiographic abnormalities, with cavitation, pleural thickening, pericavitary infiltrates, and sometimes a fungal ball; (ii) Aspergillus IgG antibody elevated or other microbiological data; and (iii) no or minimal immunocompromise, usually with one or more underlying pulmonary disorders. The Aspergillus IgG antibody test is the most sensitive microbiological test (strong recommendation; moderate-quality evidence). Sputum Aspergillus PCR testing is more sensitive than culture (strong recommendation; moderate-quality evidence).

82. Patients with CCPA without pulmonary symptoms, weight loss, or significant fatigue, and those without major impairment of pulmonary function or gradual loss of pulmonary function may be observed without antifungal therapy and followed every 3–6 months (weak recommendation; low-quality evidence).

83. Patients with CCPA and either pulmonary or general symptoms or progressive loss of lung function or radiographic progression should be treated with a minimum of 6 months of antifungal therapy (strong recommendation; low-quality evidence).

84. Oral itraconazole and voriconazole are the preferred oral antifungal agents (strong recommendation; high-quality evidence); posaconazole is a useful third-line agent for those with adverse events or clinical failure (strong recommendation; moderate-quality evidence).

85. Hemoptysis may be managed with oral tranexamic acid (weak recommendation; low-quality evidence), bronchial
artery embolization (strong recommendation; moderate-quality evidence), or antifungal therapy to prevent recurrence (strong recommendation; low-quality evidence). Patients failing these measures may require surgical resection (weak recommendation; moderate-quality evidence). 86. In those who fail therapy, develop triazole resistance, and/or have adverse events, intravenous micafungin (weak recommendation; low-quality evidence), caspofungin (weak recommendation; low-quality evidence), or AmB (weak recommendation; low-quality evidence) yield some responses. Treatment may need to be prolonged. 87. Surgical resection is an option for some patients with localized disease, unresponsive to medical therapy, including those with pan-azole-resistant Aspergillus fumigatus infection or persistent hemoptysis despite bronchial artery embolization (strong recommendation; moderate-quality evidence). The outcomes from surgery are less favorable than those with single aspergilloma, and a careful risk assessment prior to surgical intervention is required. 88. In those with progressive disease, long-term, even lifelong antifungal therapy may be required to control disease (weak recommendation; low-quality evidence), with continual monitoring for toxicity and resistance.

What Are the Management Options for an Aspergillus Fungal Ball of the Lung (Aspergilloma)?

Recommendations.

89. Asymptomatic patients with a single aspergilloma and no progression of the cavity size over 6–24 months should continue to be observed (strong recommendation; moderate-quality evidence). 90. Patients with symptoms, especially significant hemoptysis, with a single aspergilloma, should have it resected, assuming that there are no contraindications (strong recommendation; moderate-quality evidence). 91. Peri-/postoperative antifungal therapy is not routinely required, but if the risk of surgical spillage of the aspergilloma is moderate (related to location and morphology of the cavity), antifungal therapy with voriconazole (or another mold-activeazole) or an echinocandin is suggested to prevent Aspergillus empyema (weak recommendation; low-quality evidence).

ALLERGIC SYNDROMES OF ASPERGILLUS

How Is Allergic Bronchopulmonary Aspergillosis Identified and Managed in Patients With Asthma and Cystic Fibrosis?

Recommendations.

92. Elevated Aspergillus immunoglobulin E (IgE) and total IgE are recommended to establish the diagnosis and are useful for screening (strong recommendation; high-quality evidence). 93. We suggest treating symptomatic asthmatic patients with bronchiectasis or mucoid impaction, despite oral or inhaled corticosteroid therapy, with oral itraconazole therapy with TDM (weak recommendation; low-quality evidence). 94. In CF patients with frequent exacerbations and/or falling forced expiratory volume 1 (FEV1), we suggest treating with oral itraconazole to minimize corticosteroid use with TDM, and consideration of other mold-active azole therapy if therapeutic levels cannot be achieved (weak recommendation; low-quality evidence).

What Is the Medical Management of Allergic Fungal Rhinosinusitis Caused by Aspergillus Species?

Recommendations.

95. We recommend establishing the diagnosis of allergic fungal rhinosinusitis in patients with nasal polyposis and thick eosinophilic mucin by visualizing hyphae in mucus, which is supported by a positive anti-Aspergillus IgE serum antibody assay or skin-prick test (where available) (strong recommendation; moderate-quality evidence). 96. We recommend polypectomy and sinus washout as the optimal means of symptom control and inducing remission; however, relapse is frequent (strong recommendation; moderate-quality evidence). 97. We recommend the use of topical nasal steroids to reduce symptoms and increase time to relapse, especially if given after surgery (strong recommendation; moderate-quality evidence). 98. We suggest oral antifungal therapy using mold-active triazoles for refractory infection and/or rapidly relapsing disease, although this approach is only partially effective (weak recommendation; low-quality evidence).

INTRODUCTION

IA remains a major cause of morbidity and mortality in high-risk immunocompromised patients. Additionally, chronic and allergic syndromes due to Aspergillus are recognized to affect an even greater number of additional patients. In recent years, the clinical evidence for the diagnosis and management of patients with Aspergillus syndrome has vastly increased. New agents and formulations along with studies for the use of older agents are available for treating patients with these infections, and new diagnostic tools have increased the ability to diagnose these infections in a timely manner. This document constitutes the guidelines of the Infectious Diseases Society of America (IDSA) for treatment of aspergillosis. These guidelines replace the practice guidelines for Aspergillus published in 2008 [1] and incorporate new clinical evidence in the recommendations. The objective of these guidelines is to summarize the current evidence for treatment of different forms of aspergillosis and treatment recommendations are summarized in Table 1. The panel followed the GRADE framework as adopted by the IDSA.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Primary</th>
<th>Alternative</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invasive syndromes of Aspergillosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPA</td>
<td>Voriconazole 6 mg/kg IV every 12 h for 1 d, followed by 4 mg/kg IV every 12 h; oral therapy can be used at 200–300 mg every 12 h or weight-based dosing (see text for pediatric dosing)</td>
<td>Primary: Liposomal AmB (3–5 mg/kg/day IV), isavuconazole 200 mg every 8 h for 6 doses, then 200 mg daily. Salvage: ABLC (5 mg/kg/day IV), caspofungin (70 mg/day IV x 1, then 50 mg/day IV thereafter), micafungin (100–150 mg/day IV), posaconazole (oral suspension: 200 mg TID; tablet: 200 mg BID on day 1, then 300 mg daily; IV: 300 mg BID on day 1, then 300 mg daily, itraconazole suspension (200 mg PO every 12 h)</td>
<td>Primary combination therapy is not routinely recommended; addition of another agent or switch to another drug class for salvage therapy may be considered in individual patients; dosage in pediatric patients for voriconazole and for caspofungin is different than that of adults; limited clinical experience is reported with anidulafungin; dosage of posaconazole in pediatric patients has not been defined</td>
</tr>
<tr>
<td>Invasive sinus aspergillosis</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Surgical debridement as an adjunct to medical therapy</td>
</tr>
<tr>
<td>Tracheobronchial aspergillosis</td>
<td>Similar to IPA</td>
<td>Adjunctive inhaled AmB may be useful</td>
<td>Similar to IPA</td>
</tr>
<tr>
<td>Aspergillosis of the CNS</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>This infection is associated with the highest mortality among all of the different patterns of IA; drug interactions with anticonvulsant therapy</td>
</tr>
<tr>
<td>Aspergillus infections of the heart (endocarditis, pericarditis, and myocarditis)</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Endocardial lesions caused by Aspergillus species require surgical resection; Aspergillus pericarditis usually requires pericardiectomy</td>
</tr>
<tr>
<td>Aspergillus osteomyelitis and septic arthritis</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Surgical resection of devitalized bone and cartilage is important for curative intent</td>
</tr>
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<td>Aspergillus infections of the eye (endophthalmitis and keratitis)</td>
<td>Systemic IV or oral voriconazole plus intravitreal AmB or voriconazole indicated with partial vitrectomy</td>
<td>Similar to invasive pulmonary aspergillosis; limited data with echinocandins and poor ocular penetration by this class</td>
<td>Systemic therapy may be beneficial in management of Aspergillus endophthalmitis; ophthalmologic intervention and management is recommended for all forms of ocular infection; topical therapy for keratitis is indicated</td>
</tr>
<tr>
<td>Cutaneous aspergillosis</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Surgical resection is indicated where feasible</td>
</tr>
<tr>
<td>Aspergillus peritonitis</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Removal of peritoneal dialysis catheter is essential</td>
</tr>
<tr>
<td>Empiric and preemptive antifungal therapy</td>
<td>For empiric antifungal therapy, Liposomal AmB 3 mg/kg/day IV, caspofungin (70 mg day 1 IV and 50 mg/day IV thereafter), micafungin (100 mg day), voriconazole 6 mg/kg IV every 12 h for 1 day, followed by 4 mg/kg every 12 h; oral therapy can be used at 200–300 mg every 12 h or 3–4 mg/kg q 12 h</td>
<td>Voriconazole (200 mg PO BID), itraconazole suspension (200 mg PO every 12 h); micafungin (50–100 mg/day), caspofungin (50 mg/day)</td>
<td>Preemptive therapy is a logical extension of empiric antifungal therapy in defining a high-risk population with evidence of invasive fungal infection (eg, pulmonary infiltrate or positive GM assay result)</td>
</tr>
<tr>
<td>Prophylaxis against IA</td>
<td>Posaconazole: Oral suspension: 200 mg TID Tablet: 300 mg BID on day 1, then 300 mg daily IV: 300 mg BID on day 1, then 300 mg daily</td>
<td>Vucconazole (200 mg PO BID), itraconazole suspension (200 mg PO every 12 h); micafungin (50–100 mg/day), caspofungin (50 mg/day)</td>
<td>Efficacy of posaconazole prophylaxis demonstrated in high-risk patients (patients with GVHD and neutropenic patients with AML or MDS)</td>
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<tr>
<td><strong>Saprophytic or colonizing syndromes of Aspergillosis</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Aspergillosis</td>
<td>No therapy or surgical resection</td>
<td>Itraconazole or voriconazole; similar to IPA</td>
<td>The role of medical therapy in the treatment of aspergillosis is uncertain; penetration into preexisting cavities may be minimal for AmB</td>
</tr>
<tr>
<td>Chronic cavitary pulmonary aspergillosis</td>
<td>Similar to IPA</td>
<td>Similar to IPA</td>
<td>Innate immune defects demonstrated in most of these patients; long-term therapy may be needed; surgical resection may lead to significant complications; anecdotal response to IFN-γ. Tranexamic acid may be helpful in management of hemoptysis</td>
</tr>
<tr>
<td>Allergic syndromes of Aspergillosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABPA</td>
<td>Itraconazole</td>
<td>Oral voriconazole (200 mg PO every 12 h) or posaconazole (dosage depends on formulation)</td>
<td>Corticosteroids are a cornerstone of therapy for exacerbations; itraconazole has a demonstrable corticosteroid-sparing effect</td>
</tr>
<tr>
<td>Allergic rhinosinusitis caused by Aspergillosis</td>
<td>Polypectomy and sinus washout with intranasal corticosteroids</td>
<td>Antifungal therapy reserved for refractory or relapsing cases</td>
<td></td>
</tr>
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Abbreviations: ABLC, amphotericin B lipid complex; ABPA, allergic bronchopulmonary aspergillosis; AmB, amphotericin B; AML, acute myelogenous leukemia; BID, twice daily; CNS, central nervous system; GM, galactomannan; GVHD, graft-vs-host disease; IA, invasive aspergillosis; IFN-γ, interferon gamma; IPA, invasive pulmonary aspergillosis; IV, intravenous; MDS, myelodysplastic syndrome; PO, oral; TID, 3 times daily.
In the recommendation section that follows, the panel answered a series of broad questions for managing syndromes of aspergillosis, and the background and evidence for the recommendations are presented:

I. How can the most susceptible patients be protected from aspergillosis, and which patients are most susceptible?

II. How can a diagnosis of IA be established?

III. What antifungal agents are available for the treatment and prophylaxis of IA, including pharmacologic considerations, and what is the role for susceptibility testing?

IV. What are the recommended treatment regimens and adjunctive treatment measures for the various clinical presentations of IA?

V. What are the recommended prophylactic regimens, who should receive them, and how should breakthrough infection be managed?

VI. When should patients be treated empirically?

VII. How should chronic aspergillosis, allergic syndromes, or noninvasive syndromes be managed?

METHODOLOGY

Panel Composition

The most recent version of the IDSA guidelines on the management of patients with aspergillosis was published in 2008 [1]. For this update, the IDSA Standards and Practice Guideline Committee (SPGC) convened a multidisciplinary panel of 17 experts in the management of patients with aspergillosis. The panel consisted of 17 members of the IDSA, and included 16 adult infectious diseases physicians and 1 pediatric infectious diseases physician. All panel members were selected on the basis of their expertise in clinical and/or laboratory mycology with a focus on aspergillosis.

Evidence Review: The GRADE Method

GRADE is a systematic approach to guideline development that has been described in detail elsewhere [2, 3]. The IDSA/HIV Medicine Association adopted GRADE in 2008. In the GRADE system, the guideline panel assigns each recommendation with separate ratings for the underlying quality of evidence supporting the recommendation and for the strength with which the recommendation is made (Figure 1) [4]. Data from randomized controlled trials begin as “high” quality, and data from observational studies begin as “low” quality. However, the panel may judge that specific features of the data warrant decreasing or increasing the quality of evidence rating, and GRADE provides guidance on how such factors should be weighed [3]. The strength assigned to a recommendation reflects the panel’s confidence that the benefits of following the recommendation are likely to outweigh potential harms. While the quality of evidence is an important factor in choosing recommendation strength, it is not prescriptive.

Process Overview

Panel members were each assigned to review the recent literature for at least one topic, evaluate the evidence, determine the strength of recommendations, and develop written evidence in support of these recommendations. The panel met face-to-face once and conducted a series of conference calls over a 10-month period. The panel reviewed and discussed all recommendations, their strength, and the quality of evidence. Discrepancies were discussed and resolved, and all final recommendations represent a consensus opinion of the entire panel. For the final version of these guidelines, the panel as a group reviewed all individual sections.

Panel subgroups generated a list of keywords that were used by librarians at the Health Sciences Library, University of Pittsburg (with grateful acknowledgement to Michele Klein-Fedyshin and Charles B. Wessel), to develop PICO (population, intervention, comparison, outcomes) search strings for use in PubMed, and results were returned to each primary author and the chairs for review. Searches were restricted to English-language publications and covered the period of January 2008 (when the last guideline was published) through December 2014. Abstracts presented at international conferences within the past 2 years were also reviewed for inclusion. Systematic reviews of relevant topics were identified using PubMed and the Cochrane library. Each primary topic author was responsible for reviewing the literature relevant to their section and for drafting recommendations and evidence summaries for review and discussion by the full panel.

Conflicts of Interests

The expert panel complied with the IDSA policy on conflicts of interest, which requires disclosure of any financial or other interest that may be construed as constituting an actual, potential, or apparent conflict. Panel members were provided IDSA’s conflicts of interest disclosure statement and were asked to identify ties to companies developing products that may be affected by promulgation of the guideline. Information was requested regarding employment, consultancies, stock ownership, honoraria, research funding, expert testimony, and membership on company advisory committees. Decisions were made on a case-by-case basis as to whether an individual’s role should be limited as a result of a conflict. Potential conflicts of interest are listed in the Notes section.

Review and Approval Process

The panel obtained feedback from 2 external peer reviewers. The guidelines were reviewed and endorsed by the PIDS. The guideline was reviewed and approved by the IDSA Standards and Practice Guidelines Committee and the IDSA Board of Directors prior to dissemination.

Future Guideline Revisions

At annual intervals, the panel chairs will be asked for their input on the need to update the guideline based on an examination of the current literature. The SPGC of the IDSA will consider this
input and determine the necessity and timing of an update. If warranted, the entire panel or a subset thereof will be convened to discuss potential changes.

**EPIDEMIOLOGY AND RISK FACTORS FOR INFECTION**

I. How Can the Most Susceptible Patients Be Protected From Aspergillosis, and Which Patients Are Most Susceptible?

What Are Sources of Exposure to Aspergillus, and How Can Exposure Be Decreased? Is Environmental Surveillance Useful?

**Recommendations.**

1. Hospitalized allogeneic HSCT recipients should be placed in a protected environment to reduce mold exposure (strong recommendation; low-quality evidence).

2. These precautions can be reasonably applied to other highly immunocompromised patients at increased risk for IA, such as patients receiving induction/reinduction regimens for acute leukemia (strong recommendation; low-quality evidence).

3. In hospitals in which a protected environment is not available, we recommend admission to a private room, no connection to construction sites, and not allowing plants or cut flowers to be brought into the patient’s room (strong recommendation; low-quality evidence).

4. We recommend reasonable precautions to reduce mold exposure among outpatients at high risk for IA, including avoidance of gardening, spreading mulch (compost), or close exposure to construction or renovation (strong recommendation; low-quality evidence).

5. Leukemia and transplant centers should perform regular surveillance of cases of invasive mold infection. An increase in incidence over baseline or the occurrence of invasive mold infections in patients who are not at high risk for such infections should prompt evaluation for a hospital source (strong recommendation; low-quality evidence).

**Evidence Summary.** Aspergillus species and other filamentous fungi are ubiquitous in the environment. The risks of exposure vary both temporally and geographically and are dependent on precipitation patterns, humidity, temperature, and wind conditions [5]. Inhalation of fungal spores is the most common portal of entry, with sinopulmonary disease the most frequent clinical manifestation. Mold exposure also may occur following the consumption or inhalation of products contaminated with fungal spores [6, 7]. Primary cutaneous aspergillosis has been reported in patients with a breach in the normal protective barrier of the skin, such as in burn victims and near vascular sites in neonates [8–11]. Contamination of water systems has also been considered a source of nosocomial aspergillosis and other mold infections [12–17].

Because there are numerous sources of mold in the environment, reasonable efforts should be made to decrease exposure to fungal spores in highly immunocompromised patients. Detailed guidelines have been published regarding hospital room design and ventilation to reduce mold exposure among allogeneic HSCT recipients [18]. A “protected environment” is recommended, which includes high-efficiency particulate air (HEPA) filtration (and/or laminar airflow), maintenance of positive pressure rooms, and a minimum number of air exchanges per hour. Other at-risk groups such as SOT recipients and burn patients are often also placed in HEPA-filtered rooms. Additional guidelines are provided to minimize mold exposure during hospital construction, renovation, and building [19]. These guidelines can reasonably be applied to other highly immunocompromised patients, such as those receiving induction/reinduction chemotherapy for acute leukemia. We are in agreement with these guidelines, but note that they are consensus criteria based rather than evidence based.

We recognize that highly immunocompromised patients may be admitted to hospitals that lack the engineering standards providing for a “protected environment.” In these settings, reasonable standards include admission to a private room without connection to construction sites, and not allowing plants/cut flowers to be brought into the patient’s room.

Patients at risk for mold infections are commonly managed as outpatients where engineering standards are not comparable to the “protected” environment of inpatients. We advise reasonable precautions to reduce mold exposure, including the avoidance of gardening, spreading mulch, or close exposure to construction or renovation. The effectiveness of masks (surgical or N95) to protect against mold infections associated with these exposures is unknown.

The majority of cases of invasive mold infections are sporadic, although outbreaks are well recognized [20–23]. Cases of invasive mold disease with onset of symptoms ≥7 days after hospital admission are more likely to be nosocomial [24]. In the absence of an outbreak with an identified environmental source (eg, a contaminated air vent) or molecular analysis that demonstrates clustering of fungal isolates, it is not possible to reliably distinguish community-acquired from nosocomial aspergillosis. As a quality control measure, leukemia and transplant centers should perform regular surveillance (eg, every 3 months) documenting the number of invasive mold infections within their institution. Due to the paucity of culture-confirmed cases of IA and lack of autopsy data in most medical centers, surveillance using case definitions for molds including GM and radiographic evidence of infection such as the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria is reasonable. An increase in incidence over baseline or the occurrence of invasive mold infections in patients who are not considered high-risk for such infections should prompt evaluation for a hospital source.

Environmental sampling can provide important insight about sources of aspergillosis, including the spread of azole-resistant strains [17, 25], although there is debate whether such surveillance is of value for routine patient care [26, 27]. In the absence of an outbreak, there is insufficient evidence that environmental sampling of fungal spores is of value. In the setting of
a documented or suspected nosocomial outbreak, a number of measures should be undertaken, including evaluation of the ventilation system to ensure adequate filtration, air flow, maintenance of positive pressure, and consideration of environmental sampling (eg, air vents and water system).

**What Acquired or Inherited Defects in Host Immunity Increase the Risk of Aspergillosis?**

Patients at risk for IA include those with prolonged neutropenia, allogeneic HSCT recipients, SOT recipients, patients receiving corticosteroids, those with advanced AIDS, and those with CGD. In patients with hematologic malignancies, myelodysplastic syndrome (MDS), and other diseases associated with marrow failure (eg, aplastic anemia), the intensity and duration of neutropenia predict the risk of IA [28, 29]. Patients with refractory or relapsed acute leukemia treated with reinduction regimens are at particularly high risk for IA and other mold infections.

Allogeneic HSCT recipients have a significantly higher risk of IA and other opportunistic infections compared with autologous HSCT recipients [30]. In allogeneic HSCT recipients, 3 periods of risk for invasive mold disease occur: (1) neutropenia following the conditioning regimen; (2) exogenous immunosuppression for treatment of acute GVHD; and (3) exogenous immunosuppression for treatment of chronic GVHD (after day 100 of transplant). The level of allogeneic donor and recipient human leukocyte antigen disparity is the major determinant for GVHD severity and intensity of immunosuppression to control GVHD, which, in turn, is the major predisposing factor for opportunistic fungal infections [30–32]. T cell–depleted or CD34-selected stem cell products can also increase the risk of IA [32, 33]. Among allogeneic HSCT recipients, polymorphisms in specific host defense genes of the donor or recipient can also influence the risk of aspergillosis [34–37].

In SOT recipients, the intensity of immunosuppression to prevent or treat allograft rejection, colonization, and coinfection with CMV drive the risk of IA. As in allogeneic HSCT recipients, polymorphisms in specific host defense genes in SOT recipients can also influence the risk of aspergillosis [38, 39]. Lung transplant recipients have the highest risk of IA [40–42]. In a multicenter surveillance study, approximately one-half of cases of IA in lung transplant recipients were late-onset, occurring 1 year or more after transplantation [41]. CMV infection is a risk factor for aspergillosis, notably in heart and lung transplant recipients [43]. Pretransplant *Aspergillus* airway colonization is frequent among cystic fibrosis (CF) patients, and increases the risk of post–lung transplant IA [44]. IA in patients with autoimmune diseases is uncommon. Prolonged use of corticosteroids and other immunosuppressive agents and possibly preexisting lung disease are risk factors [45]. In the era of highly active antiretroviral therapy, IA is a rare complication of human immunodeficiency virus (HIV) infection. AIDS-associated aspergillosis is most frequently associated with advanced AIDS and additional risk factors, such as neutropenia, corticosteroid use, and concurrent opportunistic infections [46, 47]. CGD, an inherited disorder of the phagocyte NADPH oxidase, is characterized by recurrent bacterial and fungal infections including IA, and other molds, which can be life-threatening [48–51].

Several agents that target immune cell populations and signaling pathways, including malignancies and autoimmune disorders, have also been identified as risk factors for IA. For example, alemtuzumab (anti-CD52) can lead to neutropenia and prolonged suppression of cell-mediated immunity, potentially CMV reactivation [52], and subsequent IA [53, 54]. TNF-α inhibitors are widely used for autoimmune diseases and have been associated with an increased risk of infections and cancer [55]. An analysis of nonviral opportunistic infections in patients with autoimmune diseases documented that the overall risk was greater in patients receiving TNF-α antagonists compared with nonbiological disease-modifying antirheumatic drugs; however, IA was only observed in 1 of >30 000 patients receiving a TNF-α antagonist [56]. By contrast, the use of infliximab for severe GVHD is associated with high risk for the development of IA [57]. Therefore, in assessing the risk for aspergillosis from a specific drug or antibody, one must consider all relevant factors, including the underlying disease being treated, comorbidities (eg, preexisting lung disease), neutropenia, and the use of concurrent immunosuppressive agents.

IA has also been recognized in critically ill patients without traditional risk factors. The exact proportion of critically ill patients with IA in the absence of other risk factors is difficult to determine. In a retrospective analysis, Meersseman et al [58] identified 127 patients out of 1850 intensive care unit admissions (6.9%) with microbiological or histopathologic evidence of *Aspergillus* infection; however, only 5 of these patients had proven IA without predisposing host factors. Trials that evaluate clinical approaches to diagnose IA in critically ill patients include a substantial proportion with classic risk factors for IA and other risk factors including chronic obstructive pulmonary disease (COPD) and cirrhosis [59, 60]. IA has been observed in critically ill patients following other major infections, including influenza [61, 62]. Because critically ill patients are heterogeneous with regard to the underlying disease, comorbidities, and level of immunocompromise [63], it is difficult to delineate the specific role of nonclassic risk factors (eg, multiple organ failure, prolonged mechanical ventilation, bacterial and viral infections including influenza) in driving the risk for IA.

**DIAGNOSIS OF ASPERGILLOSIS**

**II. How Can a Diagnosis of Invasive Aspergillosis Be Established?**

*How Should Aspergillus Be Identified, and How Does This Influence Management?*

**Recommendation.**

6. Until molecular tools are more widely used in clinical laboratories, we recommend that tissue and fluid specimens be
submitted in adequate quantities for simultaneous histopathologic/cytologic and culture examination. In the case of isolates with atypical growth or concerns for resistance, species identification by molecular methods should be employed (strong recommendation; high-quality evidence).

**Evidence Summary.** The EORTC/MSG revised criteria for defining IFIs, including IA, require a microbiologic and/or histopathologic diagnosis to define proven infection [64]. However, specimen acquisition is challenging in many patients. Histopathologic evidence of fungi is crucial to determine the significance of *Aspergillus* growing in culture, yet diagnostic accuracy of histopathology is suboptimal [65–67]. Moreover, these methods are time-consuming and insensitive. The most common specimens obtained are lung tissue by transthoracic percutaneous needle aspiration or video-assisted thoracoscopic biopsy, and bronchial lavage/wash specimens. These specimens should be submitted in adequate quantities for both histopathologic/cytologic testing and culture with a brief clinical history to aid the pathologist and microbiologist in interpretation of findings [68–72]. Methods to optimize yield should be employed including adequate quantity of specimens, timely delivery of fresh specimen to the laboratory or refrigeration if delay is anticipated (although refrigeration may reduce the recovery of some organisms, eg, Mucorales), incubation of cultures for at least 5 days (and up to 3 weeks for other fungal pathogens), and communication of suspicion for fungal infection with pathology and microbiology laboratory personnel [73]. In the pathology laboratory, standard and special fungal stains on fluid or tissue should be performed simultaneously when a fungal infection is suspected and may reveal the characteristic acute angle branching septate hyphae of *Aspergillus* spp. Molecular assays targeting ribosomal DNA sequences can also be used for detection of *Aspergillus* in tissues, although these methods have not been standardized nor cleared by the US Food and Drug Administration (FDA) for clinical use. The optical brightener methods, Calcofluor or Blankophor, are rapid stains utilized for direct examination and have a high sensitivity and specificity for detecting *Aspergillus*-like features [74,75]. Special stains on fixed tissue include Gomori methenamine silver (GMS) stain (also referred to as Grocott-Gomori) and periodic acid-Schiff stains. However, no histopathologic finding can definitively diagnose the pathogen, and confirmation by culture or nonculture technique is necessary to distinguish *Aspergillus* from other filamentous fungi such as *Fusarium* spp and *Scedosporium* spp. Additionally, atypical appearances of the organism may be seen in tissue, particularly in patients receiving antifungal therapy. Increasingly, DNA sequencing is being used in reference laboratories to identify “cryptic” species that are misidentified by microscopic appearance or only identified to the complex level. Some of these species are more resistant toazole antifungal agents. *Aspergillus* spp grow well on most media at 37°C at 2–5 days, and methods should include fungal-specific media. Despite this, culture yield is low and a negative culture does not exclude the diagnosis of IA [76]. This low yield notwithstanding, culture is critical for species complex identification and susceptibility testing where feasible until molecular methods are more routinely performed in clinical laboratories.

**What Is the Diagnostic Value of Nucleic Acid Testing in Clinical Specimens?**

7. There was debate among the committee members regarding the clinical utility of blood-based PCR in diagnosing IA, and experts were not in agreement. One group favored recommendations for PCR testing, based on publications validating its role when used in conjunction with other tests such as antigen detection assays to diagnose IA and/or reduce preemptive antifungal usage. The other group thought that PCR assays are promising but could not be recommended for routine use in clinical practice at present due to the lack of conclusive validation for commercially available assays, the variety of methodologies in the literature, and questions about the extent to which results assisted diagnosis.

8. As research in the area continues, we recommend that clinicians choosing to use PCR assays employ them carefully in the management of individual patients on a case-by-case basis. Clinicians should be aware of the methodologies and performance characteristics of the specific assay used, and interpret results accordingly. When PCR assays are used, results should be considered in conjunction with other diagnostic tests and the clinical context (strong recommendation; moderate-quality evidence).

**Evidence Summary.** Since the last IDSA guidelines, there have been numerous publications assessing the performance of *Aspergillus* PCR in clinical samples. Overall, direct comparison studies have shown *Aspergillus* PCR to be substantially more sensitive than culture in blood and respiratory fluids. In a meta-analysis of clinical trials evaluating the accuracy of serum or whole-blood PCR assays for IA, sensitivity and specificity were 84% and 76%, respectively [77]. These values are promising, but PCR of blood or serum is unable on its own to confirm or exclude suspected IA in high-risk patients. The sensitivity of *Aspergillus* PCR on BAL fluid was higher than within blood, but in many instances its specificity was lower [78,79]. In a systematic review of 9 studies using reference IA definitions strictly adherent to the EORTC/MSG criteria, the sensitivity and specificity of PCR of BAL were 77% and 94%, respectively [78]. Data included large 95% confidence intervals (CIs) that were attributed to the use of different PCR assays and inclusion of heterogeneous patient populations [78,79]. The lower specificity in BAL has been attributed to the fact that lungs are often colonized by *Aspergillus* (particularly in many high-risk populations, such as lung transplant recipients), and that PCR is not able to
differentiate colonization from disease or to distinguish different *Aspergillus* spp. The high negative predictive value of BAL PCR (usually ≥95%) suggests a role in ruling out IPA. To date, data suggest that the diagnostic performance of blood or BAL PCR is comparable to that of serum and BAL GM index (GMI; ratio of the optical density [OD] of the patient samples to the mean OD of control samples) of ≥0.5, respectively, and that sensitivity for both tests is affected by antifungal use. Using both PCR and GM in serum resulted in improved sensitivity with no sacrifice of specificity [78].

Clinical trials incorporating biomarkers into the management of adults with hematologic malignancies or allogeneic HSCT have shown that combined GM and PCR reduced use of antifungal treatment [80], and was associated with an earlier diagnosis and lower incidence of IA [81].

There have been fewer PCR studies using nonblood and non-BAL samples. In several studies, PCR is superior to culture in differentiating colonization from disease or to distinguish different *Aspergillus* spp in sputum specimens from patients with CF and allergic or chronic pulmonary aspergillosis [82–86]. Small studies of *Aspergillus* spp and *Candida* spp in sputum specimens from patients with CGD and IA [112], and was also not sensitive (23%) in patients with nonmalignancy, allogeneic HSCT, but are not specific for *Aspergillus* (strong recommendation; moderate-quality evidence).

Evidence Summary. The Platelia GM enzyme immunoassay is a relatively *Aspergillus*-specific, noninvasive diagnostic assay, and several studies have demonstrated good sensitivity (approximately 70%) in serum of patients with hematological malignancy or allogeneic HSCT [90–95]. A GM-based diagnostic strategy can also result in less empirical antifungal therapy usage [80, 96]. However, the specific patient population tested is critical to optimizing GM usefulness. GM sensitivity in non-neutropenic patients appears to be lower than in other subgroups [97], and decreases to approximately 20% in SOT recipients [98–100]. The GM assay has been repeatedly negative in patients with CGD and IA [101, 102], potentially due to a lack of angioinvasion or immune complex formation with high levels of *Aspergillus* antibodies. Similarly, serum GM has also been reported to be higher in patients with angioinvasive IA vs non-invasive airway IA [103]. While earlier reports suggested that GM was not reliable in pediatric patients due to a high false-positive rate, several subsequent studies have shown its usefulness in children and similar operating characteristics to adult patients [104–111]. Serum GM was not sensitive (38%) in patients with aspergillosis, but improved in those with hemoptysis [112], and was also not sensitive (23%) in patients with chronic pulmonary aspergillosis (CPA) [113] or COPD [114]. GM in patients with CF colonized with *Aspergillus* species was consistently negative [115].

Several variables, including concurrent mold-active antifungal therapy or prophylaxis, significantly reduce levels of circulating GM [91, 94]. The GMI may be increased in the setting of neutropenia and decreases in response to antifungal agents. In one study, the GMI in patients with absolute neutrophil count (ANC) <100 cells/µL and not receiving antifungal therapy was statistically higher than those patients with an ANC >100 cells/µL; however, the GMI in patients with an ANC <100 cells/µL and receiving antifungal therapy was not statistically different than those patients with an ANC >100 cells/µL. Laboratory data and clinical observations indicate that this effect may be due to a higher fungal burden in neutropenic patients, or a more robust inflammatory process in nonneutropenic patients.

**How Should Galactomannan and (1 → 3)-ß-D-Glucan Be Used for the Diagnosis of Aspergillosis? Recommendations.**

9. Serum and BAL GM is recommended as an accurate marker for the diagnosis of IA in adult and pediatric patients when used in certain patient subpopulations (hematologic malignancy, HSCT) (strong recommendation; high-quality evidence).

10. GM is not recommended for routine blood screening in patients receiving mold-active antifungal therapy or prophylaxis, but can be applied to bronchoscopy specimens from those patients (strong recommendation; high-quality evidence).

11. GM is not recommended for screening in SOT recipients or patients with CGD (strong recommendation; high-quality evidence).

12. Serum assays for (1 → 3)-ß-D-glucan are recommended for diagnosing IA in high-risk patients (hematologic malignancy, allogeneic HSCT), but are not specific for *Aspergillus* (strong recommendation; moderate-quality evidence).
with a corresponding decrease in the burden of disease, rate of dissemination, and GM release [116, 117].

False-positive results have been reported in several contexts, including in patients who have received certain antibiotics (historically most notably piperacillin-tazobactam, which appears now to no longer be cross-reactive [118], and amoxicillin-clavulanate), neonatal colonization with Bifidobacterium, when Plasmalyte is used in BAL fluids, and in patients with other invasive mycoses (including penicilliosis, fusariosis, histoplasmosis, and blastomycosis) [119–122]. Despite these limitations, this assay is a useful adjunctive test to establish an early diagnosis, particularly when used in serial screening of patients at high risk of infection who are not receiving antimold prophylaxis. The optimal rationale for diagnosis in neutropenic patients may be a combined approach guided by clinical, radiographic, and biweekly screening of GM in serum [123], possibly combined with other biomarkers. In patients receiving mold-active antifungal prophylaxis, the use of serum GM as a screening tool results in a very poor predictive value, with most positive tests being false positive in this setting [124]. The detection of GM in BAL fluid has been shown to have a sensitivity that exceeds 70% in most studies and provides additional sensitivity compared with culture even in the setting of mold-active antifungal therapy as discussed below [125–128].

Other potential circulating markers for detection of aspergillosis include (1 → 3)-β-D-glucan detected by the Tachypleus or Limulus assay [129, 130]. The Tachypleus or Limulus assay used to detect the presence of (1 → 3)-β-D-glucan is a variation of the limulus assay used to detect endotoxin. The presence of (1 → 3)-β-D-glucan in serum signifies the presence of fungal invasion but is not specific for Aspergillus species; other fungal diseases, including candidiasis, fusariosis, and Pneumocystis jirovecii pneumonia can result in a positive test. False-positive results can occur in a variety of contexts, such as through glucan-contaminated blood collection tubes, gauze, depth-type membrane filters for blood processing, and various drugs (eg, antibiotics including some cephalosporins, carbapenems, and ampicillin-sulbactam, and possibly chemotherapeutics such as pegylated asparaginase) [131]. The Fungitell assay (Associates of Cape Cod) for detection of (1 → 3)-β-D-glucan is cleared by the FDA for the diagnosis of invasive mycoses, including aspergillosis, and has been evaluated in high-risk patients with hematological malignancy and allogeneic HSCT [129, 132].

Comparative studies have shown that the Fungitell assay can be slightly more sensitive than GM for IA, but is limited by its poor specificity [133], while others have found that Fungitell is not as helpful for IA [111]. However, another study in a large cancer center that compared GM and (1 → 3)-β-D-glucan assays prospectively over a 3-year period in 82 patients, each for 12 weeks, found that the (1 → 3)-β-D-glucan assay was more sensitive than the GM assays for detection of IA and other mold infections in patients with hematological malignancy [134]. One meta-analysis of (1 → 3)-β-D-glucan assays revealed limitations [130], while another found similar deficiencies yet improvement in diagnostic capabilities with the combination of both biomarkers [135]. Other organizations have recommended the GM over Fungitell for specifically diagnosing IA [136].

**What Is the Approach to the Radiographic Diagnosis of Invasive Pulmonary Aspergillosis?**

**Recommendations.**

13. We recommend performing a chest CT scan whenever there is a clinical suspicion for invasive pulmonary aspergillosis (IPA) regardless of chest radiograph results (strong recommendation; high-quality evidence).

14. Routine use of contrast during a chest CT scan for a suspicion of IPA is not recommended (strong recommendation; moderate-quality evidence). Contrast is recommended when a nodule or a mass is close to a large vessel (strong recommendation; moderate-quality evidence).

15. We suggest a follow-up chest CT scan to assess the response of IPA to treatment after a minimum of 2 weeks of treatment; earlier assessment is indicated if the patient clinically deteriorates (weak recommendation; low-quality evidence). When a nodule is close to a large vessel, more frequent monitoring may be required (weak recommendation; low-quality evidence).

**Evidence Summary.** As clinical signs and symptoms are not specific for the diagnosis of IPA, radiographic imaging is critical. The role of imaging is to identify the site of infection, to assess the type, number and size of lesions, and local extension. Imaging also helps to direct diagnostic procedures (eg, BAL or CT-guided biopsy) to the most appropriate area [137].

CT scan is more sensitive than chest radiograph to identify lesions of IPA, especially at their early stage [138], and high-resolution computed tomography (also called thin-section CT scanning with a thin collimation of 0.25–1 mm) is the preferred method. CT angiography may be a useful test pending further evaluation [139]. Chest CT scan performed early after onset of fever helps to identify the cause of fever, may be informative before Aspergillus GM is positive, and has been associated with an increased survival in febrile neutropenic patients who have received intensive chemotherapy for a hematologic malignancy [140–142].

Typical features of IPA on CT imaging include nodules, consolidative lesions, and wedge-shaped infarcts. Particularly in neutropenic patients, a halo sign, defined as a nodule (>1 cm in diameter) surrounded by a perimeter of ground-glass opacity reflecting hemorrhage, may be observed [143–147]. Pleural effusions are occasionally observed. Appearance of an air crescent or a cavity in a mass, nodule, or consolidation is also suggestive of invasive mold disease but is usually a later sign, often associated with recovery from neutropenia [145, 146]. The reverse
halo sign is more frequently associated with pulmonary mucormycosis than with IPA [148, 149]. Similar to the halo sign, the reverse halo sign can also present in various other pulmonary conditions including tuberculosis and noninfectious diseases [150, 151].

The presence of nodules and a halo sign are characteristic of angioinvasion, and this form of aspergillosis typically occurs in severely neutropenic patients. IPA can also affect the airways with bronchiolar wall destruction, presence of centrilobular micronodules, and tree-in-bud opacities [152]. Airway disease and angioinvasive lesions can be present in the same patient.

Magnetic resonance imaging (MRI) has no additional value compared to CT scanning for early diagnosis of IPA [153], but is the preferred imaging modality to identify and characterize osseous, paranasal sinus lesions, or CNS disease [154–158].

In neutropenic patients, pulmonary lesions usually increase in size during the first week following initiation of therapy and while the patient recovers from neutropenia [159]. The size of lesions can increase up to 4-fold during the first week and then remain stable for another week. Repetition of a CT scan before 2 weeks after the start of treatment is not usually recommended unless the patient experiences clinical deterioration. An exception is the presence of a nodule close to a large vessel because of the risk for massive hemoptysis if lesions continue to increase in size.

**What Is the Role of Bronchoscopy in the Diagnosis of Invasive Pulmonary Aspergillosis?**

**Recommendations.**

16. We recommend performing a bronchoscopy with BAL in patients with a suspicion of IPA (strong recommendation; moderate-quality evidence). Significant comorbidities such as severe hypoxemia, bleeding, and platelet transfusion-refractory thrombocytopenia may preclude BAL. The yield of BAL is low for peripheral nodular lesions, so percutaneous or endobronchial lung biopsy should be considered. We recommend the use of a standardized BAL procedure and sending the BAL sample for routine culture and cytology as well as non-culture-based methods (eg, GM) (strong recommendation; moderate-quality evidence).

**Evidence Summary.** Flexible bronchoscopy with BAL remains the cornerstone for microbiological identification in diffuse interstitial or alveolar lung infiltrates, infiltrates in immunosuppressed patients, nosocomial pneumonia, or pneumonia with treatment failure [160–163]. As radiographic signs and symptoms of IPA are nonspecific, BAL increases the likelihood of a diagnosis by direct or indirect identification of mold.

BAL fluid analysis is based on gross observation (hemorrhage, alveolar proteinosis), cell count, and differential count (macrophages, neutrophils, eosinophils, lymphocytes and subpopulation, erythrocytes, malignant cells), and on microbiologic tests (stains and immunohistochemistry, cultures, antigen or nucleic acid detection). Importantly, BAL allows in the same procedure a search for bacterial, parasitic, viral, and fungal pathogens as well as noninfectious causes of the pulmonary lesions.

There is no uniform agreement on the best timing for bronchoscopy. In a survey of infectious diseases specialists, pulmonologists, and hematologists/oncologists, there was consensus that HSCT recipients who are nonneutropenic and do not have cavitary infiltrates on chest CT scan should receive bronchoscopy only after a failure of empiric antimicrobial therapy. However, there was no agreement between the groups on when neutropenic patients or those with cavitary lesions should undergo bronchoscopy [164].

BAL is an invasive procedure that requires instruction and consent from the patient, sufficient respiratory capacity of the patient, and no major bleeding diathesis. The British Thoracic Society has established guidelines on diagnostic flexible bronchoscopy [165], and specific recommendations for the lavage procedure are also available [166, 167].

Samponas et al evaluated a standardized procedure for BAL in 284 consecutive cancer patients with new pulmonary infiltrates [160]. The majority of patients had a hematological malignancy. Thrombocytopenia was not considered a contraindication to bronchoscopy or BAL, but platelet transfusions were administered in patients who had platelet counts <20 000 platelets/µL. Only 10 BAL-related complications were observed, and only one was serious but not fatal. In large series, major bronchoscopy-related complications rates range between 0.08% and 0.5%, with mortality rates of 0%–0.04%.

Lavage is usually performed in the segmental or subsegmental bronchus of the most affected area of the lung based on a recent CT scan [160]. Saline is the most often used fluid. False-positive Aspergillus GM detection tests were reported when Plasmalyte was used as fluid for BAL [168]. There is considerable variation between practitioners in the volume instilled and the methods of lavage fluid collection, and no consensus has been reached. The instilled volume in nonpediatric patients should be at least 100 mL (most commonly 100–150 mL in aliquots of 20–50 mL, with the initial aliquot likely representing airway sampling) [169]. BAL samples should be sent for cytologic assessment, Gram staining, fungal staining (eg, Calcofluor white or GMS stain), culture, and GM. GM testing from BAL samples provides additional sensitivity compared to culture and exceeds 70% in most studies [125–128]. The optimal threshold for GM positivity has not been determined; an OD of 1.0 has been cleared by the FDA for clinical testing, although some experts consider positivity at OD > 0.5. A higher threshold OD index results in a lower sensitivity but a higher specificity [128].

The diagnostic yield of BAL also varies by the type of radiographic lesion [170]. In this study there was no difference in the diagnostic yield between focal and diffuse infiltrates.
Amphotericin B: Recommendations.

17. AmB deoxycholate and its lipid derivatives are appropriate options for initial and salvage therapy of Aspergillus infections when voriconazole cannot be administered. However, AmB deoxycholate should be reserved for use in resource-limited settings in which no alternative agents are available. Lipid formulations of AmB should be considered in settings in which azoles are contraindicated or not tolerated (strong recommendation; moderate-quality evidence).

18. Aerosolized formulations of AmB may be considered as prophylaxis in patients with prolonged neutropenia (patients receiving induction/reinduction therapy for acute leukemia and allogeneic HSCT recipients following conditioning or during treatment of GVHD) and in lung transplant recipients (weak recommendation; low-quality evidence).

Evidence Summary. AmB is a polyene with poor oral absorption and is thus solubilized with deoxycholate for intravenous administration. Alternative routes of administration are intraperitoneal, intravitreal, intrathecal, bladder irrigation, and aerosolization. The primary mechanism of action of AmB has historically been considered due to the formation of ion channels in the fungal cell membrane, but recent evidence suggests that amphotericin forms large extramembranous aggregates that extract ergosterol from lipid bilayers, resulting in cell death [171]. Binding to cholesterol in mammalian cell membranes results in end organ dysfunction. A second mechanism of action involves oxidative cell membrane damage. AmB is highly protein bound (95%) before distribution predominantly into reticuloendothelial tissues and kidney. Peak serum concentrations of 1–2 µg/mL are achieved following infusion of 30–50 mg. Penetration into intact and inflamed meninges is poor. No metabolites have been identified. Drug elimination is biphasic with a terminal half-life for AmB deoxycholate of up to 15 days, and the primary route of elimination is not known. Serum levels are not influenced by hepatic or renal dysfunction, and it is poorly dialyzed. Doses of deoxycholate AmB range from 0.1 to 1.5 mg/kg daily. With drug-related renal dysfunction, 50% dose reduction or alternate-day dosing may be considered. Adverse events include acute infusion reactions (nausea, chills, and rigors), administration-site phlebitis, and nephrotoxicity (azotemia, urinary potassium/magnesium wasting, renal tubular acidosis). Azotemia is exacerbated by concomitant administration of nephrotoxic agents, underlying renal impairment, and diabetes. Volume expansion with a salt load immediately prior to AmB dosing, and monitoring of potassium and magnesium, with repletion as needed, are warranted to prevent renal toxicity. Utility of 24-hour infusions is limited. AmB is active against most, but not all, Aspergillus species.

Lipid formulations of AmB were developed to reduce AmB-related nephrotoxicity. Available formulations are AmB lipid complex (ABLC; Abelcet), AmB colloidal dispersion (ABCD; Amphocil, Amphotec), and liposomal AmB (AmBisome). Their pharmacokinetic profiles differ from AmB deoxycholate, as well as between each formulation. All preferentially distribute to reticuloendothelial tissue. Infusion reactions of fever and chills occur commonly with ABLC. A characteristic infusion-related reaction syndrome of dyspnea, chest pain, back pain, and hypoxia also may occur, particularly with liposomal AmB [172]. In addition to hypokalemia and hypomagnesemia, mild bilirubin and alkaline phosphate elevations may occur. Idiosyncratic reactions to one preparation do not preclude use of other formulations [173]. Approved dosages for aspergillosis therapy are: 5 mg/kg/day, 3–6 mg/kg/day, and 3–5 mg/kg/day for ABLC, ABCD, and liposomal AmB, respectively [174]. Higher dose-response relationships have not been well studied, although no improvement in efficacy has been demonstrated to date [175].

Aerosolized formulations of AmB have been used as prophylaxis. Lipid formulations of AmB are generally better tolerated than those involving AmB deoxycholate. Serum drug levels are negligible. These formulations have been utilized as prophylaxis in patients with prolonged neutropenia (patients receiving induction/reinduction therapy for acute leukemia and allogeneic HSCT recipients following conditioning) and in lung (with or without heart) transplant recipients, and therapeutically in recalcitrant fungal lung infections [176–184].

Echinocandins: Recommendations.

19. Echinocandins are effective in salvage therapy (either alone or in combination) against IA, but we do not recommend their routine use as monotherapy for the primary treatment of IA (strong recommendation: moderate-quality evidence).

Evidence Summary. Echinocandins are semisynthetic amphiphilic lipopeptide antifungal agents. Each of these large molecules is composed of a cyclic hexapeptide core linked to a variably configured N-linked fatty acyl side chain [185]. The
Echinocandins act by noncompetitive inhibition of the synthesis of (1 → 3)-β-D-glucan, a polysaccharide in the cell wall of many pathogenic fungi. Together with chitin, these rope-like glucan fibrils are responsible for the cell wall’s strength and shape. They are important in maintaining the osmotic integrity of the fungal cell and play a key role in cell division and cell growth.

Each echinocandin has a half-life of >10 hours, which allows for once-daily dosing. They exhibit dose-proportional plasma pharmacokinetics. Echinocandins are highly (>95%) protein bound and distribute well into all major organ sites except for the eye, uninfected spinal fluid where concentrations are lower than other body tissues, and in urine where concentrations are also low. They are available for parenteral administration only. Anidulafungin undergoes spontaneous chemical degradation, with fragment elimination in bile. Caspofungin is metabolized by the liver with some additional spontaneous chemical degradation, with a recommendation for a dose reduction in cases of markedly reduced hepatic function. Micafungin is metabolized by the catechol-O-methyltransferase pathway.

Echinocandins are generally well tolerated, with few side effects and few drug interactions. Caspofungin administration in children and adolescents provides exposure that is comparable to that obtained in adults [186]. There is an inverse relationship between micafungin clearance and age [187], as well as between clearance and weight [188], so micafungin dosing is individualized in patients aged ≤8 years, and in extremely obese patients [187, 188]. Both caspofungin and micafungin maintain linear pharmacokinetics when dose-escalated in adult patients with IA [189, 190]. Among the 3 compounds, caspofungin has more extensive hepatic metabolism, leading to some interactions with other medications. For example, caspofungin can reduce the area under the curve of tacrolimus by approximately 20%, but has no effect on cyclosporine levels. In contrast, cyclosporine increases the area under the curve of caspofungin by approximately 35%. Inducers of drug clearance and/or mixed inducer/inhibitors, namely efavirenz, nevirapine, phenytoin, rifampin, dexamethasone, and carbamazepine, may reduce caspofungin concentrations.

All 3 agents have activity against Aspergillus species. Data are limited regarding their use for primary treatment of invasive infections, due to low accrual in clinical trials. Use of caspofungin to treat 24 allogeneic HSCT recipients with 12 weeks of therapy led to a 42% complete or partial infection response, with a 12-week survival of 50% [191]. However, in a second stratum of that study, primary therapy with caspofungin was successful in only 20 of 61 (33%) patients with hematological malignancy. Based on this limited database, echinocandin monotherapy is not routinely recommended as primary treatment for IA [192]. Use of micafungin to treat 50 patients with CPA led to a 60% treatment response [193]. As a result of the difficulty in enrolling patients at the point of needing primary treatment for aspergillosis, patients with *Aspergillus* infections were more frequently studied once their infections became refractory to or intolerant of other approved therapies (ie, salvage therapy) [194–196]. In a study where 326 patients were treated with micafungin as salvage therapy, there was a 44% survival rate by the end of 6 weeks of follow-up, with 59% of deaths attributable to the *Aspergillus* infection [194]. Among 83 patients who received caspofungin for salvage therapy, favorable response rates were seen for 45%, compared with 16% among historical controls [195]. Although anidulafungin has been studied in combination therapy, it has not been evaluated in monotherapy as primary or salvage therapy for IA. Because of their distinct mechanism of action, the echinocandins have the potential for use in combination regimens with antifungal agents of differing mechanisms of action [194, 196–198]. When patients are treated with combination therapy, the impact of the echinocandin agent is difficult to specifically define.

**Triazoles Recommendations.**

20. Triazoles are preferred agents for treatment and prevention of IA in most patients (strong recommendation; high-quality evidence).

**Evidence Summary.**

**TRIAZOLE PHARMACOLOGY**

Itraconazole

Itraconazole is formulated as capsules and an oral solution in hydroxypropyl-β-cyclodextrin (HPCD), and aparenter solution, which is no longer sold in the United States, that also uses HPCD as solubilizing agent. Accumulation of the cyclodextrin molecule in the intravenous preparation occurs with renal impairment, although the toxicity of accumulated cyclodextrin in humans is uncertain. Systemic absorption of oral cyclodextrin is minimal, thus the use of the oral solution is not impacted by renal insufficiency. Itraconazole is highly protein bound (>99%) and is extensively metabolized by the liver (cytochrome P450 [CYP] 3A4) and undergoes enterohepatic circulation. The hydroxyitraconazole metabolite has approximately equivalent antifungal activity but with variable plasma concentration as native drug. Both must be measured to assess drug bioavailability. Itraconazole is an inhibitor and substrate for CYP3A4 and inhibitor of the permeability glycoprotein (p-gp) membrane transporter. The metabolites are excreted in the urine (40%) and bile (55%) [199, 200]. Significant pharmacokinetic variation exists between patients in absorption and distribution [201–203].

Most observed reactions to itraconazole are transient and include nausea and vomiting, hypertriglyceridemia, hypokalemia, and elevated hepatic aminotransferase enzyme levels. Gastrointestinal intolerance appears to be more frequent with oral...
HPCD itraconazole solution. Peripheral neuropathy associated with itraconazole has been reported, in particular with prolonged therapy and excessive serum concentrations [204]. Negative inotropic effects have been observed uncommonly but may be important in patients with ventricular dysfunction. Itraconazole is a substrate of CYP3A4 but also interacts with the heme moiety of CYP3A4, resulting in noncompetitive inhibition of oxidative metabolism of many CYP3A4 substrates. Serious interactions with some chemotherapeutic agents (eg, cyclophosphamide and vincristine) may require additional monitoring to avoid toxicity [205] as well as other agents that prolong the QTc interval. Because of these limitations, itraconazole is rarely recommended in patients with acute IPA, with its use reserved for patients with less severe or less invasive disease presentations.

Voriconazole
Voriconazole is formulated as tablets, an oral suspension, and a sulfobutyl-ether cyclodextrin solution for intravenous administration. Sulfobutyl-ether cyclodextrin and voriconazole dissociate in plasma and the cyclodextrin molecule is renally cleared. Accumulation of the vehicle occurs with renal insufficiency. Renal toxicity of hydroxypropyl-β-cyclodextrin after parenteral administration has been demonstrated in animal models, although no deleterious effects on renal function have been observed in humans [206, 207]; for this reason, the consequences of cyclodextrin plasma accumulation are unclear. The relative benefits and uncertain risks of intravenous administration of voriconazole in the context of IA and renal failure should be determined on an individual patient basis. This concern does not apply to orally administered voriconazole. The oral formulation has good bioavailability in the fed or fasted state.

Voriconazole is hepatically metabolized, with only 5% of the drug appearing unchanged in the urine. This agent exhibits nonlinear pharmacokinetics in adults, with the maximum concentration in plasma and area under the curve increasing disproportionally with increasing dose. Voriconazole is both a substrate and an inhibitor of CYP2C19 primarily, as well as of CYP3A4 [208–210]. Allelic polymorphisms in CYP2C19 may result phenotypically in rapid or slow metabolism of voriconazole, possibly resulting in significant variation in plasma concentrations [211]. Single-nucleotide polymorphisms contributing to slow metabolism are represented in higher frequencies among non-Indian Asian populations than among other populations.

Factors affecting voriconazole pharmacokinetics include patient age, liver function, CYP2C19- and CYP3A-interacting medications, diet and antacids, proton pump inhibitors, and patient weight, as well as the drug dose and formulation [212]. Reduced voriconazole levels may be observed with oral administration of the drug (vs intravenous), and coadministration with rifampin or phenytoin [213, 214]. Measurement of serum levels is useful in the majority of patients, both to evaluate for potential toxicity or to document adequate drug exposure, especially in progressive infection [213–226]. Toxicity is more common with higher drug levels but is not predictable based solely on this criterion [216, 220, 227]. The profile of adverse reactions to voriconazole includes transient visual disturbances (characterized principally by photopsia); hepatotoxicity, which may be dose limiting (manifested by elevated serum bilirubin, alkaline phosphatase, and hepatic aminotransferase enzyme levels); skin rash, erythroderma, photosensitivity, and perioral excoriations; nausea, vomiting, and diarrhea; visual or auditory hallucinations; and cardiovascular events including tachyarrhythmias and QT interval prolongations on electrocardiography [209, 211, 213, 228]. There have also been rare cases of arrhythmia (including ventricular arrhythmias such as torsade de pointes and bradycardia), cardiac arrest, and sudden death in patients taking voriconazole. These cases usually involve patients with multiple confounding risk factors, such as history of cardiotoxic chemotherapy, cardiomyopathy, hypokalemia, and concomitant medications (eg, quinolones) that may be contributory. Visual side effects or photopsia are self-limited, reversible, and not clearly associated with absolute drug levels [227, 229]. Mild hepatotoxicity is common as for all azoles and related to drug concentration [227, 230, 231]. Severe hepatotoxicity is uncommon. Reversible central and peripheral neurologic symptoms and hallucinations may be observed in association with higher drug concentrations but with significant variability; these may be confused with other etiologies of CNS dysfunction including posterior reversible leukoencephalopathy syndrome or calcineurin inhibitor toxicity [217, 224, 227, 232, 233]. Voriconazole concentrations may be a predictor of CNS neurotoxicity, which is reversible [214]. The use of prolonged voriconazole therapy (as for osteomyelitis or meningitis) or prophylaxis has revealed newer toxicities including periostitis with severe pain in bones or joints in association with elevated serum fluoride levels [234–240]. The risk for squamous cell skin cancer or melanoma in sun-exposed areas is enhanced by concomitant immunosuppression and chronic voriconazole use, especially in fair-skinned persons [241–243].

Posaconazole
Posaconazole, which is structurally similar to itraconazole, is available as an oral suspension, delayed-release tablet, and intravenous formulation but has been studied for the treatment and prophylaxis of IA only in the oral suspension in efficacy studies. Posaconazole exhibits not only linear kinetics but also saturable absorption of the suspension; thus, oral loading doses are not possible. Steady-state levels may not be achieved for up to a week with posaconazole therapy, which impacts use in primary therapy. The newer delayed-release tablet formulation has improved bioavailability and is given once daily [244–246], as is the intravenous formulation in β-cyclodextrin. Bioavailability of the new tablet is not affected by food or gastric acid, but the oral suspension requires a fed state to maximize...
bioavailability. Posaconazole undergoes hepatic metabolism via glucuronidation and also has the capacity for drug–drug interactions through inhibition of CYP3A4 isoenzymes [247]. Posaconazole pharmacokinetics are variable between patients and TDM seems useful, although the posaconazole exposure in plasma from the oral solution appears to underestimate the clinical response to therapy [248–252]. Toxicities are generally mild, including diarrhea and nausea, and do not appear to be related to drug concentrations [253] but may be increased with the higher serum levels attained with the delayed-release tablets. Other toxicities including prolonged QTc interval have been reported with the increased drug levels associated with the extended-release tablets. TDM is recommended based on both preclinical and clinical trials with the oral solution, which documented variable absorption and the relationship of levels to efficacy [254–256], and is likely indicated with the extended-release tablets that may achieve high drug concentrations and be associated with increased toxicities.

**Isavuconazole**

Isavuconazonium sulfate (referred to in these guidelines as isavuconazole) is a prodrug containing the active antifungal agent isavuconazole, a broad-spectrum triazole agent with a 5-day half-life [257]. The intravenous formulation does not contain cyclodextrin as do other triazoles. Isavuconazole requires a loading dose. The toxicity profile is similar to that of other triazoles, with a similar rate of gastrointestinal disorders, but based on limited experience, a lower rate of photosensitivity, skin disorders, and hepatobiliary and visual disturbances compared with voriconazole [258, 259]. Significant interactions with drugs metabolized by CYP are expected to occur, especially with substrates and inducers of the CYP3A4 enzyme, although preclinical studies suggest that these drug interactions are less severe than with voriconazole. Coadministration of methotrexate with isavuconazole increases exposure to 7-OH methotrexate, a potentially toxic metabolite. Tacrolimus and sirolimus levels are likely to be increased by coadministration of isavuconazole, whereas interactions with cyclosporine and glucocorticoids appear modest. Interestingly, in contrast to other triazoles, isavuconazole could shorten the QTc interval; the clinical significance of this is unclear. There is no effect of the polymorphisms of CYP2C19, which contributes to considerable interpatient variability in serum concentrations of voriconazole.

**Triazole Drug Interactions and Therapeutic Drug Monitoring Recommendations.**

21. For patients receiving triazole-based therapy for IA, prolonged azole prophylaxis, or other therapies for which drug interactions with azoles are anticipated, the committee recommends TDM once the steady state has been reached. A moderate amount of data for itraconazole, voriconazole, and posaconazole suspension suggests this approach may be valuable in enhancing therapeutic efficacy, in evaluating therapeutic failures attributable to suboptimal drug exposures, and to minimize toxicities potentially attributable to the azoles (strong recommendation; moderate-quality evidence). Further studies are needed to address whether TDM is helpful or necessary with the extended-release or intravenous formulations of posaconazole or for isavuconazole. 22. Clinicians should obtain serum trough drug levels for azole antifungal agents (itraconazole, voriconazole, posaconazole, and possibly isavuconazole) and for potentially interacting drugs such as cyclosporine, tacrolimus, and sirolimus (and other CYP3A4 substrates such as tyrosine kinase inhibitors) to optimize therapeutic efficacy and to avoid potential toxicities of both groups of agents (strong recommendation; moderate-quality evidence).

**Evidence Summary.** Despite a lack of definitive data from large clinical studies, TDM is increasingly recognized as a useful tool for optimizing the safety and efficacy of azole antifungals. Generally, an antifungal agent must meet 3 general criteria for antifungal TDM to be clinically useful. First, a sensitive assay must be available locally or in a reference laboratory that will report results back in a timely fashion (within days), otherwise the impact of monitoring on clinical decision making will be limited. Second, the antifungal must have an established therapeutic range, such that treatment success can be improved or toxicity potentially reduced if patients are dosed to maintain concentrations within this therapeutic window. Finally, the drug must have significant intra- or interpatient pharmacokinetic variability, such that variations in serum levels may jeopardize the effectiveness of therapy with standard dosing guidelines.

Triazole antifungal agents contribute to various important toxicities and drug–drug interactions that may limit therapy (Table 2).

Many of the drug interactions are class-related while common toxicities are often specific to the dose or duration of therapy with individual agents [260, 261]. The triazoles are metabolic substrates for, and inhibitors of, several CYP enzymes and inhibitors of the p-gp membrane transporter [262]. Polymorphisms are common in the genes encoding these CYP isoenzymes, particularly CYP2C19, and others with less prominent roles in triazole pharmacokinetics [263]. The polymorphisms of CYP3A4 are not considered to contribute significantly to differences in human metabolism of antifungal triazoles [264]. The polymorphisms of CYP2C19 are a common cause for substantial interpatient variability in drug levels in patients receiving voriconazole. The triazole antifungal agents demonstrate significant drug–drug interactions that may adversely affect patient outcomes [261]. Each patient’s current medications should be reviewed for potentially deleterious drug interactions. As a class, these include altered serum levels of the azoles and of coadministered agents including calcineurin inhibitors and mammalian target of rapamycin inhibitor immunosuppressive agents, anticoagulants, psychiatric and neurotropic medications, barbiturates,
associated with higher probability of breakthrough infection, levels of itraconazole and posaconazole suspension have been necessary during primary triazole prophylaxis, but low plasma starting therapy (when a patient is at a pharmacokinetic steady state) is reached. Stopping of CNI or mTOR may provoke graft rejection.

Corticosteroids

Levels are increased by azoles

May exacerbate immunosuppression favorable for fungal growth. Prolonged coadministration may elicit signs of excessive steroid exposure.

Table 2. Commonly Encountered Drug–Drug Interactions During Treatment of Aspergillosis

<table>
<thead>
<tr>
<th>Agent/Class</th>
<th>Interaction</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNI and mTOR inhibitor immunosuppressive agents</td>
<td>Significant increase in CNI levels byazole</td>
<td>CNI and mTOR agents should be reduced (approximately 30%–50% for CNI and greater for rapamycin) at the time of initiating azole therapy and serum levels for both agents monitored until steady state is reached. Stopping of CNI or mTOR may provoke graft rejection.</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Levels are increased by azoles</td>
<td>May exacerbate immunosuppression favorable for fungal growth. Prolonged coadministration may elicit signs of excessive steroid exposure.</td>
</tr>
<tr>
<td>Antiretroviral agents for HIV</td>
<td>Variable effects</td>
<td>Frequently used in combination with other classes of agents; monitoring of azole levels recommended, and bidirectional drug–drug interactions common.</td>
</tr>
<tr>
<td>Rifampin/rifabutin</td>
<td>Decreased levels of azole agents while rifampin/rifabutin levels are increased</td>
<td>Combined use of voriconazole, posaconazole, isavuconazole, or itraconazole with rifampin/rifabutin should generally be avoided. Some combinations are considered contraindicated; others may be managed by TDM and dose adjustment.</td>
</tr>
<tr>
<td>Agents that cause QTc interval prolongation (fluoroquinolone and macrolide antimicrobials, quinine, quinidine, digoxin, amiodarone and other antiarrhythmic drugs, calcium channel blockers, psychiatric drugs, antihistamines, and other agents)</td>
<td>QT interval prolongation, torsades de points, and other cardiac arrhythmias have been observed with azoles in combination with other agents or preexisting conditions that have these effects</td>
<td>Assess risk benefit and administer with caution to patients with cardiac disorders that increase the risk of arrhythmias.</td>
</tr>
<tr>
<td>Vincristine and other vinca alkaloid agents</td>
<td>Neurotoxicity including peripheral neuropathy and seizures in combination with azoles; azole levels also increased</td>
<td>Given the potential for serious toxicity, vincristine and other vinca alkaloids should generally not be coadministered with mold-active azoles. Alternative antifungal therapy (eg, amphotericin B formulation or echinocandin) should be used.</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Increased levels with coadministration of some azoles</td>
<td>Increased renal, hepatic, or genitourinary dysfunction</td>
</tr>
</tbody>
</table>

Abbreviations: CNI, calcineurin inhibitor; HIV, human immunodeficiency virus; mTOR, mammalian target of rapamycin; TDM, therapeutic drug monitoring.

glucocorticoids, digoxin, vinca alkaloids (eg, vincristine) and cyclophosphamide, and antiretroviral agents [260, 265–280]. All of the azoles have important interactions via the CYP enzymes, notably CYP3A4, which can interact with a large number of concomitant medications including tyrosine kinase inhibitors, macrolides, and antiarrhythmics, among others. Active transporters including the p-gp and the breast cancer resistance protein regulate access of the azoles to the drug-metabolizing enzymes of enterocytes and the liver; the clinical importance of the transporters remains to be further defined [260, 281, 282].

Currently, 3 triazoles (itraconazole, voriconazole, and posaconazole) are considered to meet these criteria and have established indications for TDM in IA [283, 284]. There is general agreement that documentation of adequate (and in the case of voriconazole, nontoxic) serum levels in the first 4–7 days after starting therapy (when a patient is at a pharmacokinetic steady state) is preferable for any patient with suspected or documented aspergillosis. Less agreement exists whether TDM is necessary during primary triazole prophylaxis, but low plasma levels of itraconazole and posaconazole suspension have been associated with higher probability of breakthrough infection, and limited data suggest that high levels of posaconazole may be associated with toxicity [285].

The need for continued or repeat monitoring is a patient-specific decision influenced by the clinical status of the host (eg, specific organ function, comorbidities, and receipt of concomitant medications), severity of infection, concerns regarding nonadherence, cost, TDM assay availability, possibly the duration of therapy [286], and the overall treatment plan. Determination of a plasma drug level, in conjunction with other measures of clinical assessment, can help define factors that may have led to therapeutic failure with oral triazoles and re-open prospects for use of the same oral drug in the future provided pharmacokinetic issues are corrected.

Overviews of clinical scenarios that frequently justify TDM are presented in Table 3. The therapeutic range for voriconazole and posaconazole have been primarily defined from single-center, retrospective studies and can only be considered a general guide for dosing [284].

Itraconazole

Itraconazole capsules require low gastric pH for dissolution, and are therefore poorly absorbed in many patient populations with relative achlorhydria associated with their underlying disease or pharmacotherapy. Itraconazole suspension is better absorbed, but is associated with higher gastrointestinal adverse effects, which are especially problematic in populations who already have nausea, vomiting, or diarrhea. Although a variable rate of breakthrough IA has been reported in patients on itraconazole prophylaxis, relatively few studies have examined the
relationship of itraconazole plasma concentrations and treatment efficacy for aspergillosis. Based primarily on prophylaxis data, most experts recommend dosing itraconazole to achieve trough concentrations >0.5–1 µg/mL (combined itraconazole/hydroxyitraconazole troughs >1.5 µg/mL). There are limited data suggesting that higher trough concentrations of itraconazole (>3 µg/mL) may be associated with increased toxicity [287].

**Voriconazole**

Various target concentrations associated with voriconazole efficacy have been reported, mostly from single-institution retrospective studies [214, 283]. Most experts would aim for dosing to achieve a voriconazole trough of >1–1.5 µg/mL for efficacy but <5–6 µg/mL to minimize toxicity, primarily CNS toxicity. Visual changes can be related to elevated voriconazole concentration but generally resolve spontaneously and without long-term sequelae. Although voriconazole trough concentrations can be elevated in patients with hepatic dysfunction, available data do not support the concept of a threshold level that could adequately discriminate who will be at higher risk for hepatotoxicity [229].

In a prospective, randomized blinded single-center trial of TDM during voriconazole therapy in 100 patients, the proportion of voriconazole discontinuation due to adverse events was significantly lower in the TDM group than in the non-TDM group (4% vs 17%; \( P = 0.02 \)) [288]. More importantly, higher rates of complete or partial response were observed in patients managed with TDM (81% vs those without TDM 57%; \( P = 0.04 \)). This study and several others suggest that antifungal TDM may reduce drug discontinuation due to adverse events and improve the likelihood of a therapeutic response. There are no widely validated algorithms on how to dose voriconazole. Weight-based dosing is recommended to rapidly achieve therapeutic range, with incremental increases and monitoring (ie, 50% increase in daily dose) for the patient who has trough levels <1 µg/mL. Voriconazole concentrations often increase disproportionately to administered doses due to saturable metabolism in adults. For patients with very low voriconazole levels, coadministering omeprazole (a CYP2C19 inhibitor) has been reported to “boost” voriconazole area under the curve by 41% [289]. Fundamental pharmacokinetics of voriconazole are different in children (linear) than in adults (nonlinear) [290].

In pediatric patients weighing <50 kg, higher voriconazole doses are needed [291] and drug monitoring is paramount (see specific evidence discussion following Recommendation 45 below).

**Posaconazole**

Increasing evidence supports an exposure–response relationship for plasma posaconazole concentrations for prophylaxis and treatment of IFIs [250]. This, in conjunction with the fact that posaconazole levels (using the suspension formulation) are commonly low (<0.7 µg/mL) in patients with documented IA receiving salvage treatment [1], makes prudent a strategy of monitoring posaconazole serum concentrations in patients with IA who are on chronic posaconazole suspension. On the other hand, a clear relationship has not been identified between posaconazole concentrations and the risk of breakthrough IA in the pivotal posaconazole registration trials [254, 292] in which the event rate (breakthrough IA) was low. Therefore, TDM during posaconazole prophylaxis may be best used in evaluating potential breakthrough infections. There is limited evidence to suggest that peak or trough posaconazole concentrations are predictive of subsequent hepatic or other toxicities, although higher rates of toxicity have been anecdotally observed in some patients with high serum levels (>1.5 µg/mL) achieved with the delayed-release tablet formulation.

The introduction of posaconazole extended-release tablets and the intravenous formulation of posaconazole more easily achieve increased posaconazole serum drug levels, even in patients with risk factors for posaconazole malabsorption [244].

### Table 3. Clinical Scenarios Where Therapeutic Drug Monitoring Is Useful in Treatment of Aspergillosis

<table>
<thead>
<tr>
<th>Clinical Scenarios Where Antifungal Therapeutic Drug Monitoring Is Useful</th>
<th>Examples, Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populations with increased pharmacokinetic variability</td>
<td>Impaired gastrointestinal function; hepatic (voriconazole, posaconazole, itraconazole); pediatric patients, elderly patients, obese patients, critically ill patients</td>
</tr>
<tr>
<td>Changing pharmacokinetics</td>
<td>Intravenous-to-oral switch, changing Gl function, changing hepatic or renal function, physiological instability</td>
</tr>
<tr>
<td>Interacting medications</td>
<td>Patient receiving medication that induces CYP3A4, antacids, proton pump inhibitors (itraconazole capsules, posaconazole suspension), antiretroviral medications Possibly corticosteroids (voriconazole)</td>
</tr>
<tr>
<td>Severe disease</td>
<td>Extensive infection, lesions contiguous with critical structures, CNS infection, multifocal or disseminated infection</td>
</tr>
<tr>
<td>Compliance</td>
<td>Important issue with longer-term consolidation therapy or secondary prophylaxis</td>
</tr>
<tr>
<td>Suspected breakthrough infection</td>
<td>TDM can help to establish whether fungal disease progression occurred in the setting of inadequate antifungal exposure</td>
</tr>
<tr>
<td>Suspected drug toxicity, especially neurotoxicity (voriconazole)</td>
<td>Although exposure–response relationships are described for other toxicities (eg, hepatotoxicity, bone disease), the utility of TDM to prevent their occurrence is less well established</td>
</tr>
</tbody>
</table>

Table developed from Andes et al [283]; Ashbee et al [284]. Additional studies are needed to assess role of TDM for isavuconazole and for posaconazole extended-release tablet and intravenous formulations.

Abbreviations: CNS, central nervous system; CYP, cytochrome P450; GI, gastrointestinal; TDM, therapeutic drug monitoring.
Further studies are needed to address whether higher posaconazole levels are associated with toxicity and whether TDM is helpful or necessary with the extended-release or intravenous formulations. The value of TDM to guide therapy and to avoid toxicity for isavuconazole, a once-daily extended-spectrum triazole with anti-Aspergillus activity with good absorption kinetics, similarly remains to be assessed [258].

**Preclinical and Laboratory Assessment of Combination Antifungal Therapy**

23. Combinations of polyenes or azoles with echinocandins suggest additive or synergistic effects in some preclinical studies. However, variable test designs and conflicting results of preclinical and in vitro testing have led to uncertainty as to how to interpret the findings (weak recommendation; low-quality evidence).

**Evidence Summary.** The rationale for combination therapy is to maximize treatment by targeting multiple targets or metabolic pathways or different points in the same pathway to improve efficacy through achieving an additive or synergistic effect. Other potential benefits include lowering the risk for emergence of drug resistance and the potential for shorter courses of therapy or lower doses of therapy in an attempt to reduce toxicity.

Antifungal drug combinations have been evaluated in multiple in vitro studies and studied in animal models. Combinations of polyenes or azoles with echinocandins have been most studied, and additive or synergistic effects have been noted in the majority of (but not all) studies when compared to monotherapy (especially echinocandins alone) [295–299]. Unfortunately, there are no standardized or validated protocols for in vitro synergy testing, and there are substantive differences in study design, laboratory assay conditions, definitions of endpoints, species and strains tested, animal models, drug choice and concentrations/doses, drug monotherapy comparator, inoculation size, and portal of pathogen administration. Furthermore, correlations between in vitro findings and in vivo observations have not always been consistent, and differences in drug metabolism between animals and humans make comparisons difficult. Also of importance is the order of administration. Some studies have suggested that prior azole administration subsequently reduces polyene activity [300–307].

Antagonism during the use of combination therapy has also been suggested by some studies, especially between polyenes and certain azoles [308]. By comparison, the combination of triazole and echinocandin agents exhibit synergistic to additive interactions in the same systems [309]. However, a murine model demonstrated possible antagonism between itraconazole and micafungin [310]. In vitro studies demonstrate that the combination of triazole and polypene may be antagonistic [310] or that there may be synergy or antagonism depending on the dose used [309, 311]. In addition to reduced antifungal activity, other potential harmful effects may include increased risk for resistance, additive toxicity, cost, and deleterious drug interactions. Although the preclinical studies have been generally favorable to consideration of combinations of mold-active azoles or polyenes with echinocandins, the variable test designs and conflicting results of preclinical testing have led to uncertainty as to the applicability to clinical practice.

**When Should Antifungal Susceptibility Testing Be Performed, and How Should Results Be Interpreted and Affect Management?**

24. Routine AFST of isolates recovered during initial infection is not recommended. AFST of Aspergillus isolates using a reference method is reserved for patients suspected to have an azole-resistant isolate or who are unresponsive to antifungal agents, or for epidemiological purposes (strong recommendation; moderate-quality evidence).

**Evidence Summary.** The goal of AFST is to detect resistant isolates that are more likely to fail therapy [312, 313]. Considerable progress since the previous guideline has occurred toward achieving this goal. The European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) have published standardized but different AFST methodologies in recent years [314, 315]. Aspergillus minimum inhibitory concentrations (MICs) utilizing EUCAST and CLSI methodologies from more recent clinical studies and large surveys have been determined. Although clinical breakpoints are not yet defined by CLSI, epidemiological cutoff values—the upper limit of wild-type MIC distributions which aid in the determining the likelihood of resistance in Aspergillus spp—have been proposed by CLSI [316–319]. Establishing epidemiological cutoff values for azoles and Aspergillus fumigatus, utilizing in vitro pharmacokinetic/pharmacodynamic studies, in vivo correlation of mutations and failure, and clinical experience aided derivation of proposed azole clinical breakpoints by EUCAST [312, 320–324]. Taken together, these advances resulted in the recommendation by some experts in Europe to perform routine voriconazole AFST [323].

The advances of molecular techniques have led to important changes to Aspergillus taxonomy contributed to by the phylogenetic species recognition concept [325]. This method, based on sequencing of several targets for species recognition analysis, has identified new cryptic species, some of which are more resistant to current antifungal drugs [326]. Azole resistance in filamentous fungi primarily involves mutations in the CYP51A target enzyme or promoter that lead to specific or pan-azole resistance, and is described more frequently in A. fumigatus complex than other species [327–333]. Other azole resistance mechanisms are also described [334–339]. Resistance to the echinocandins is uncommon, as is resistance to AmB apart from Aspergillus terreus, Aspergillus nidulans, and Aspergillus lentulus...
In conclusion, AFST advances in the past decade are limited and require further study [341]. However, in the case of fungi are under investigation but not yet standardized or validated at this time.

At this time, AFST is not routinely performed in most clinical laboratories in the United States. Molecular methods to identify azole and echinocandin resistance in filamentous fungi are under investigation but not yet standardized or validated and require further study [341]. However, in the case of isolates with atypical growth or concerns for resistance when molecular methods are not available, AFST should be employed. In conclusion, AFST advances in the past decade are significant; however, worldwide Aspergillus resistance remains low, and routine AFST for clinical management is not recommended at this time.

INVASIVE SYNDROMES OF ASPERGILLUS

IV. What Are the Recommended Treatment Regimens and Adjunctive Treatment Measures for the Various Clinical Presentations of Invasive Aspergillosis?

How Should IPA Be Treated?

Recommendations.

25. We recommend primary treatment with voriconazole (strong recommendation; high-quality evidence).

26. Early initiation of antifungal therapy in patients with strongly suspected IPA is warranted while a diagnostic evaluation is conducted (strong recommendation; high-quality evidence).

27. Alternative therapies include liposomal AmB (strong recommendation; moderate-quality evidence), isavuconazole (strong recommendation; moderate-quality evidence), or other lipid formulations of AmB (weak recommendation; low-quality evidence).

28. Combination antifungal therapy with voriconazole and an echinocandin may be considered in select patients with documented IPA (weak recommendation; moderate-quality evidence).

29. Primary therapy with an echinocandin is not recommended (strong recommendation; moderate-quality evidence). Echinocandins (micafungin or caspofungin) can be used in settings in which azole and polyene antifungals are contraindicated (weak recommendation; moderate-quality evidence).

30. We recommend that treatment of IPA be continued for a minimum of 6–12 weeks, largely dependent on the degree and duration of immunosuppression, site of disease, and evidence of disease improvement (strong recommendation; low-quality evidence).

31. For patients with successfully treated IPA who require subsequent immunosuppression, secondary prophylaxis should be initiated to prevent recurrence (strong recommendation; moderate-quality evidence).

Evidence Summary. Early initiation of antifungal therapy in patients with strongly suspected IPA is warranted while a diagnostic evaluation is conducted, both because early therapy has been shown to limit progression of disease and because the performance of diagnostic testing remains limited [145, 175]. Availability of drugs that have differential activity for molds that cause similar syndromes, specifically, the lack of voriconazole activity against mucormycosis, emphasizes the importance of a specific microbiologic diagnosis and antimicrobial susceptibility testing. Evidence supporting appropriate primary therapy of IPA has been generated in a series of randomized controlled trials (Table 1).

The first pivotal treatment trial performed for IA demonstrated better survival in patients who received voriconazole compared with AmB deoxycholate [348], justifying a recommendation against AmB deoxycholate therapy. Since that original randomized trial, multiple cohort studies subsequently published support this recommendation with approximately 15% improved survival at 12 weeks in all patient types with voriconazole compared with other intravenous therapies. Thus, for primary treatment of IPA in adults, intravenous or oral voriconazole is recommended for most patients. For seriously ill patients, the parenteral formulation is recommended. A switch to oral therapy, with dosing maximized to achieve recommended target serum levels, can be considered in patients who are able to tolerate oral therapy.

A randomized trial compared voriconazole with isavuconazole, which demonstrated noninferiority in treatment of IPA [349]. This study showed noninferiority in terms of clinical efficacy, measured by survival and composite clinical responses in the intent-to-treat population of patients with possible, probable, and proven aspergillosis. There were fewer drug-related adverse effects in people who received isavuconazole. Based on these data, isavuconazole was approved by the FDA for first-line therapy of IA and is recommended as an alternative primary therapy for IPA.

Another alternative for primary therapy of IA is liposomal AmB. Although no randomized trial has been performed to evaluate effectiveness of this drug compared to voriconazole for primary therapy, a series of randomized trials suggest effectiveness in therapy. Randomized trials of variable quality evaluating primary treatment of IA using lipid formulations of AmB have been reported to generally favor outcomes with lipid formulations, especially with regard to minimizing toxicities. The most compelling effectiveness data have been generated from randomized trials evaluating liposomal AmB. Cornely et al [175] compared an initial dosage of liposomal AmB of 10 mg/kg/day for 2 weeks with a dosage of 3 mg/kg/day. In that study, among 201 patients, overall outcomes in the 2 arms were similar (46% in the high-dose arm vs 50% in the low-dose arm), but there was more toxicity (32% vs 20%) in the high-dose arm, suggesting that higher doses were
not beneficial. These results suggest that liposomal AmB be considered as alternative primary therapy in some patients, especially in situations in which hepatic toxicities or drug interactions warrant nonazole alternatives, and when voriconazole-resistant molds (eg, mucormycosis) remain of concern.

Another lipid AmB alternative is ABLC (5 mg/kg/day), which has not been studied in randomized trials for IA, but has been reported to be effective in observational studies, particularly in the setting of salvage therapy, and is generally well tolerated compared with AmB deoxycholate [350–353].

Finally, ABCD was compared to AmB deoxycholate in a randomized trial of 174 patients. Although therapeutic responses were similar (52% vs 51%), infusion-related reactions were more common in ABCD. Renal toxicity occurred less frequently with ABCD [174], but due to an increase in serious drug reactions, principally fever, chills, and hypoxia, use of ABCD is not recommended.

Combination therapy in the treatment of IPA has been supported by generally favorable in vitro and in vivo preclinical data in support of combinations of polyenes or mold-active azoles with echinocandins. Nonrandomized clinical trial data suggest the benefit of some forms of combination therapy against IA, usually an azole (most commonly voriconazole) with an echinocandin in aspergillosis [197, 198, 296, 299, 304, 354–360]. There are limited prospective randomized first-line combination therapy trials [361, 362]. In a pilot trial [361], 30 hematologic malignancy patients with proven or probable IA were randomized to either a standard dose of liposomal AmB (3 mg/kg/day) plus caspofungin or high-dose liposomal AmB alone (10 mg/kg/day). Responses were better at the end of therapy with combination therapy but overall survival was similar. A more recent randomized trial compared outcomes of voriconazole monotherapy to combination therapy with voriconazole plus anidulafungin [362]. The trial enrolled 454 patients with hematologic malignancy to evaluate hypothesized superiority in 6-week survival in combination therapy recipients. Mortality at 6 weeks was 19.3% for combination recipients and 27.5% for monotherapy recipients (\(P = .087; 95\% \text{ CI}, -19 \text{ to } 1.5\)). Secondary mortality benefits favored combination therapy. In post hoc analyses of the dominant subgroup of patients who were diagnosed as having “probable” aspergillosis based on radiographic abnormalities and positive GM assays, the difference in mortality was most notable (15.7% combination vs 27.3% monotherapy; \(P = .037; 95\% \text{ CI}, -22.7 \text{ to } -4\)). Global clinical responses at 6 weeks were lower in the combination group (33% vs 43%), which was attributed to more patients in the combination group being unavailable for this secondary endpoint due to missing data. There were no toxicity differences. This study adds to prior preclinical and observational clinical studies that suggest potential benefits for combination therapy with voriconazole and an echinocandin [198, 356, 363]. For this reason, the committee suggests consideration for an echinocandin with voriconazole for primary therapy in the setting of severe disease, especially in patients with hematologic malignancy and those with profound and persistent neutropenia.

While caspofungin has been reported to have efficacy in several small noncomparative studies of drug administered for both primary and “salvage” therapy, the committee does not support use of this agent as monotherapy based on lack of robustly powered comparative trials in which outcomes were not favorable compared to historical data [190–192, 195, 364–366].

Duration of antifungal therapy for IPA is not well defined. We generally recommend that treatment of IPA be continued for a minimum of 6–12 weeks, depending on the severity and continuation of immunosuppression, as well as the extent of resolution of clinical disease. Therapeutic monitoring of IPA includes serial clinical evaluation of all symptoms and signs, as well as performance of radiographic imaging, usually with CT, at regular intervals. The frequency with which CT should be performed cannot be universally defined and should be individualized on the basis of the rapidity of evolution of pulmonary infiltrates and the acuity of illness in the individual patient. The volume of pulmonary infiltrates may increase for the first 7–10 days of therapy, especially in the context of granulocyte recovery [159]. The use of serial serum GM assays for therapeutic monitoring is promising but remains investigational. Progressive increases in Aspergillus antigen levels over time signify a poor prognosis. However, resolution of GM antigenemia to a normal level is not sufficient as a sole criterion for discontinuation of antifungal therapy. Long-term therapy of IA is facilitated by the availability of oral azole drugs in stable patients. For patients with successfully treated IA who will require subsequent immunosuppression, resumption of antifungal therapy can prevent recurrent infection [367, 368].

Surgical resection of Aspergillus-infected tissue may be useful in patients who have lesions that are contiguous with the great vessels or other critical organs, lesions causing calcific granulomoptysis from a single focus, and in lesions eroding into bone. This decision should be mindful of the probability of structural adhesion eliciting spillage of organism into the pleural space.

As discussed in Section II, increasing evidence suggests that attention should be placed on antifungal drug resistance, either that innate to the infecting Aspergillus species (such as A. terreus, A. flavus, or “cryptic” Aspergillus spp such as A. lentulus) or that acquired by a typically susceptible species.

**Adjunctive Measures and Immunomodulation: When Should Withdrawal of Immunosuppressive Agents, or Addition of Colony-Stimulating Factors or Granulocyte Transfusions, Be Considered in the Treatment of Invasive Aspergillosis? Recommendations.**

32. Reducing doses of, or eliminating altogether, immunosuppressive agents, when feasible, is advised as a component of anti-Aspergillus therapy (strong recommendation; low-quality evidence).
33. Colony-stimulating factors may be considered in neutropenic patients with diagnosed or suspected IA (weak recommendation; low-quality evidence). There is insufficient evidence regarding the value of granulocyte colony-stimulating factor (G-CSF) vs GM-CSF in this setting.
34. Granulocyte transfusions can be considered for neutropenic patients with IA that is refractory or unlikely to respond to standard therapy, and for an anticipated duration of more than one week (weak recommendation; low-quality evidence).
35. Recombinant interferon-γ is recommended as prophylaxis in CGD patients (strong recommendation; high-quality evidence). Its benefit as adjunctive therapy for IA is unknown.
36. Surgery for aspergillosis should be considered for localized disease that is easily accessible to debridement (eg, invasive fungal sinusitis or localized cutaneous disease) (strong recommendation; low-quality evidence). The benefit for IA in other settings such as in the treatment of endocarditis, osteomyelitis, or focal CNS disease appears rational. Other indications are less clear and require consideration of the patient’s immune status, comorbidities, confirmation of a single focus, and the risks of surgery.

Evidence Summary. Because immune reconstitution is an important factor in survival from IA, immunosuppressive agents should be tapered or removed, when possible. However, it is frequently not feasible to do so, for example, in patients with severe GVHD or in SOT recipients with allograft rejection. Clinical judgment is required in these cases.

Colony-stimulating factors: Colony-stimulating factors administered prophylactically (prior to the onset of neutropenia) are commonly used to shorten the duration of neutropenia in patients receiving cytotoxic regimens. G-CSF influences survival, proliferation, and differentiation of all cells in the neutrophil lineage and augments the function of mature neutrophils. G-CSF also stimulates neutrophil recovery and various neutrophil effector functions and is a potent activator of monocytes and macrophages. Pegfilgrastim, a pegylated formulation of G-CSF with a long half-life, is used to reduce the duration of neutropenia in patients with nonmyeloid cancers.

A meta-analysis of prophylactic G-CSF showed a reduction in the incidence of neutropenic fever and early deaths, including infection-related mortality [369]. Another meta-analysis showed a survival benefit of prophylactic G-CSF in patients with MDS and acute myelogenous leukemia (AML) [370]. Authoritative guidelines have been published regarding the appropriate use of colony-stimulating factors in patients with cancer, with the main goal of reducing neutropenic fever [371, 372]. The value of adjunctive (as opposed to prophylactic) colony-stimulating factors for the treatment of major infections is unclear. Studies in vitro and in murine aspergillosis suggest that G-CSF and GM-CSF can enhance antifungal host defense [373–376]. If not initiated in the prophylactic setting, use of colony-stimulating factors should be considered in neutropenic patients with diagnosed or suspected IA. Although colony-stimulating factors can augment phagocyte function in addition to cell numbers, there are insufficient data to recommend their use in patients who are not neutropenic.

Granulocyte transfusions: The rationale for granulocyte transfusions is to increase the number of circulating neutrophils until neutrophil recovery occurs and is usually recommended as an adjunctive measure if granulocyte recovery is anticipated. Granulocyte transfusions have been used for decades as adjunctive treatment for severe infections in patients with neutropenia. The impetus to reevaluate granulocyte transfusions stems largely from improvements made in donor mobilization methods using therapy with G-CSF and corticosteroids [377]. In addition, the use of unrelated community donors for granulocyteapheresis was shown to be feasible, thus increasing the pool of potential donors [378, 379]. A randomized trial evaluating the safety and effectiveness of granulocyte transfusions in patients with neutropenia and severe bacterial and fungal infections has recently been published (NCT00627393). Those who received an average dose per transfusion of >0.6 × 10⁹ granulocytes/kg tended to have better outcomes than those receiving a lower dose [380].

The overall benefit vs risk of granulocyte transfusions is currently unknown. Granulocyte transfusions were of benefit in experimental pulmonary aspergillosis in neutropenic mice [381]. Granulocyte transfusions can be considered for neutropenic patients with severe infections, including IA and other mold infections, which have failed or are unlikely to respond to standard therapy. Acute lung injury is the major risk of granulocyte transfusions. AmB may increase lung injury associated with granulocyte transfusions [382]; therefore, separating AmB and granulocyte infusions by several hours is advised. Alloimmunization leading to graft failure after allogeneic HSCT is another potential risk of granulocyte transfusions. In allogeneic transplants in which the donor and recipient are seronegative for CMV, use of CMV-seronegative granulocyte donors is recommended.

Recombinant interferon gamma (IFN-γ): IFN-γ augments the antifungal activity of macrophages and neutrophils ex vivo against a variety of fungal pathogens, including Aspergillus species. A high proportion of patients with CPA are poor producers of IFN-γ [383]. In addition, a high ratio of ex vivo T-cell production of IFN-γ interleukin 10 is associated with improved responses to antifungal therapy in patients with IA [384].

Recombinant IFN-γ (rIFN-γ) is licensed as a prophylactic agent for patients with CGD on the basis of a randomized trial in which rIFN-γ reduced the number and severity of infections (mostly bacterial) in patients with CGD by approximately 70% [385]. Its use as adjunctive therapy for patients with IA is limited to case reports and small series. One concern related to
rIFN-γ use in allogeneic HSCT recipients is the potential to worsen GVHD. A single-center retrospective analysis suggested that rIFN-γ was safe in allogeneic HSCT recipients [386]. Currently, the data supporting the efficacy of adjunctive rIFN-γ for aspergillosis are weak; it can be considered in patients with severe or refractory aspergillosis.

Surgery: In general, surgical treatment of aspergillosis should be considered for localized disease that is accessible to debridement. Emergent debridement of sinus aspergillosis can be life-saving and limit extension to the orbit and brain. Localized cutaneous aspergillosis should also be debrided. CNS aspergillosis is a devastating complication; neurosurgical removal combined with antifungal therapy may be life-saving, although the expected postsurgical neurologic outcome should also be considered during the decision process. Surgical resection of pulmonary lesions due to *Aspergillus* species can provide a definitive diagnosis and can potentially completely eradicate a localized infection. Surgical therapy may be useful in patients with lesions that are contiguous with the great vessels or the pericardium, uncontrolled bleeding, or invasion of the pleural space and chest wall. Intervention should also be considered for localized pulmonary aspergillosis refractory to antifungal therapy [387].

Another consideration for surgery is the resection of a single pulmonary lesion prior to intensive chemotherapy or HSCT. However, the favorable experience of HSCT in patients with prior IA suggests that antifungal therapy alone may be effective [367, 388–391]. An acceptable approach in patients with pre-transplant aspergillosis is close CT monitoring without surgical resection in the absence of additional complications, such as uncontrolled bleeding or chest wall extension. Decisions concerning surgical therapy should be individualized to account for a number of variables, including the degree of resection (eg, wedge resection vs pneumonectomy), potential impact of delays in chemotherapy, comorbidities, performance status, the goal of antineoplastic therapy (eg, curative vs palliative), and unilateral vs bilateral lesions.

**Recommendations.**

37. IA is not an absolute contraindication to additional chemotherapy or HSCT (*strong recommendation; moderate-quality evidence)*.

38. Decisions about when to proceed with additional chemotherapy or HSCT following the diagnosis of aspergillosis should involve both infectious diseases specialists and hematologists/oncologists. These decisions must consider the risk of progressive aspergillosis during periods of subsequent antineoplastic treatment vs the risk of death from the underlying malignancy if this treatment is delayed (*strong recommendation; low-quality evidence)*.

**Evidence Summary.** Patients with malignancy and IA frequently require additional antineoplastic therapy and/or HSCT. The major concern is that aspergillosis will progress during subsequent periods of immunosuppression. Several studies have shown that IA is not a contraindication for additional treatment, including HSCT [367, 388–391]. It is important to administer mold-active antifungal treatment during subsequent periods of immunosuppression (referred to as secondary prophylaxis) to avoid recurrence or progression. In a multicenter retrospective survey of patients with pretransplant aspergillosis, 27 of 129 patients developed progressive fungal disease following allogeneic HSCT. The variables that increased the 2-year cumulative incidence of aspergillosis progression were longer duration of neutropenia after transplantation, refractory malignancy, and <6 weeks from start of antifungal therapy and HSCT [389]. In a prospective, multicenter trial of voriconazole as secondary prophylaxis in patients with pretransplant IFIs (the majority were aspergillosis), the one-year cumulative incidence of invasive fungal disease was 7% following allogeneic HSCT [367].

Decisions about when to proceed with additional chemotherapy or HSCT following the diagnosis of aspergillosis must consider the risks of progressive aspergillosis and the risks of delaying treatment of the underlying malignancy. These decisions require expertise from infectious diseases specialists and oncologists. From the infectious disease standpoint, a period of several weeks of antifungal treatment and clear evidence of response to therapy is ideal before administering additional chemotherapy or HSCT. However, there are situations when this approach is not feasible, for example, in patients with refractory or relapsed acute leukemia who require urgent reinduction therapy.

**What Approaches Are Needed for Refractory or Progressive Aspergillosis (Salvage Therapy)?**

**Recommendations.**

39. We recommend an individualized approach that takes into consideration the rapidity, severity, and extent of infection, patient comorbidities, and to exclude the emergence of a new pathogen (*strong recommendation; low-quality evidence*). The general strategies for salvage therapy typically include (i) changing the class of antifungal, (ii) tapering or reversal of underlying immunosuppression when feasible, and (iii) surgical resection of necrotic lesions in selected cases.

40. In the context of salvage therapy, an additional antifungal agent may be added to current therapy, or combination antifungal drugs from different classes other than those in the initial regimen may be used (*weak recommendation; moderate-quality evidence*).

41. In patients currently receiving an antifungal and exhibiting an adverse event attributable to this agent, we recommend changing to an alternative class of antifungal, or the use of an alternative agent with a nonoverlapping side-effect profile (*strong recommendation; low-quality evidence*).
For salvage therapy, agents include lipid formulations of AmB, micafungin, caspofungin, posaconazole or itraconazole. The use of a triazole as salvage therapy should take into account prior antifungal therapy, host factors, pharmacokinetic considerations, and possible antifungal resistance (strong recommendation; moderate-quality evidence).

Evidence Summary. Many issues confound the interpretation of current published evidence for salvage therapy for IA including publication bias, inadequate statistical power, and heterogeneity of studies. In salvage therapy studies, differentiating Aspergillus-attributable mortality vs the impact of underlying disease or coinfections is not possible [392, 393]. It is also unclear whether different therapeutic approaches are needed when breakthrough infection is detected by GM alone vs culture, the latter likely representing a more advanced stage of disease.

Studies in the area of salvage therapy for aspergillosis also lack uniform criteria of what constitutes a “response.” For example, the volume of lesions on chest CT increase during the first 7–10 days on therapy, and neutrophil recovery may lead to immune reconstitution inflammatory syndrome (IRIS) that presents as transitory clinical worsening [159]. Salvage therapy trials that enroll patients after only 7 days of antifungal therapy may not adequately account for this phenomenon. Antifungal therapy initiated at the time of neutrophil recovery is also biased by the salutatory effects of immune recovery.

In addition, there is confusion in some studies between sequential vs true salvage therapy as the action of the failing drug may interact with the action of the salvage drug. The first drug may inflict damage to Aspergillus that enhances the action of the second drug, or there may be neutral or possibly even antagonistic effect. Another issue relates to antifungal agents with prolonged half-lives such as AmB formulations [394]. Thus, in patients receiving AmB-based initial therapy, the combined action of both AmB and the “salvage” antifungal agent will be present for several days to a week after cessation of AmB therapy. Finally, most salvage studies do not provide a robust explanation for the lack of response (eg, failure due to drug resistance or coinfection, disadvantageous pharmacokinetics/pharmacodynamics, intolerance to a study drug, or lack of recovery from immunosuppression).

The principal antifungal considered for salvage therapy include lipid formulations of AmB, posaconazole, itraconazole, and the echinocandins, caspofungin and micafungin, which have both been evaluated in salvage settings [255, 356, 395–398]. Voriconazole can also be considered as a salvage agent if not used in primary therapy, as could presumably isavuconazole, although isavuconazole has limited evaluation in the salvage setting. In patients who fail initial triazole therapy, a change in class to an AmB formulation (usually liposomal AmB), with or without an echinocandin, should be considered. Azole-specific pharmacokinetic problems must also be considered, including TDM. Most of the prospective studies of second-line therapy have been conducted by replacing the compound to which the patient is intolerant or against which the infection is progressing. Whether both drugs should be administered simultaneously has seldom been prospectively studied [194]. The addition of a second antifungal agent to a first agent that is failing is usually practiced out of understandable lack of therapeutic options.

Other drug combinations have not been extensively studied [297]. Additional questions of optimal drug combinations, optimal drug dosing, pharmacokinetic interactions, potential toxic interactions, and cost–benefit ratios of primary combination antifungal therapy require further investigation.

The need for surgical resection should be evaluated in cases of pulmonary lesions contiguous with the heart or great vessels, invasion of the chest wall, massive hemoptysis, and other special circumstances. Restoration of or improvement in impaired host defenses is critical for improved outcome of IA. Correction of comorbidities using various adjunctive strategies (eg, correction of hyperglycemia, recovery from neutropenia, or reduction of immunosuppressive medication dosages) is expected to improve outcomes in progressive IA but may also be associated with IRIS.


Serial monitoring of serum GM can be used in the appropriate patient subpopulations (hematologic malignancy, HSCT) who have an elevated GM at baseline to monitor disease progression and therapeutic response, and predict outcome (strong recommendation; moderate-quality evidence).

(1 → 3)-β-D-glucan has not been extensively studied in IA to predict outcome (weak recommendation; low-quality evidence).

Evidence Summary. Multiple studies have evaluated serial serum GM for both therapeutic monitoring as well as predicting prognosis and found excellent correlations between GMI and outcomes. A review of 27 published studies, including both adult and pediatric allogeneic or autologous HSCT recipients, found an excellent correlation between GMI and survival, including autopsy findings [399]. A prospective study of 70 patients with prolonged neutropenia found good GMI concordance with clinical outcome at 6 weeks and excellent correlation at 12 weeks, including perfect concordance with autopsy findings and significantly better survival in patients who became GM negative by 12 weeks [400]. Another retrospective study found similar results, including significantly better survival in patients whose GMI normalized compared to patients with persistently positive GM, regardless of resolution of neutropenia [401]. In one study, an adjusted hazard ratio (HR) for
respiratory or all-cause mortality increased from 2.25 with a serum GMI ≥ 0.5 to a HR of 4.9 with a serum GMI ≥ 2.0 [402]. GMI-based assessment can also predict outcome sooner [403].

Several studies have compared the initial GMI and subsequent rate of daily decay of GM, defined as the change from the initial GMI divided by the number of days since that initial value. Both initial GMI and rate of decrease of GM in response to therapy at one week after initiation of therapy have been predictive of mortality [404]. The adjusted HR for initial GM for time to mortality was 1.25 per unit increase in GMI, as well as an HR of 0.78 per unit decrease for survival [405]. GMI is also predictive of outcome in nonneutropenic patients [406–408].

A retrospective evaluation of the global aspergillosis clinical trial comparing voriconazole to AmB deoxycholate followed by other licensed therapy [348] found that GMI at week 1 was significantly lower than baseline GM in the eventual 12-week responders compared with nonresponders. A GMI reduction of >35% between baseline and week 1 predicted a probability of a satisfactory clinical response, whereas during antifungal treatment every 0.1-unit increase in GMI between baseline and week 2 increased the likelihood of a poor response by 21.6% [409]. A different analysis of the same trial found that those patients who received voriconazole and had a successful week 12 response showed earlier decreases in GMI at week 1 and week 2 as compared to those who eventually failed treatment. However, for patients randomized to initially receive AmB deoxycholate, this early difference trend between week 12 responders and nonresponders was not evident until week 4 [410].

There have been fewer studies with BAL GMI and outcome. A retrospective study of 145 patients found a BAL GMI ≥ 2.0 was significantly lower than baseline GM in the eventual 12-week responders compared with a BAL GMI < 0.5 [411]. However, another retrospective study of 100 allogeneic HSCT recipients found that serum GMI positivity and magnitude, but not BAL GMI, correlated with both 6-week and 6-month mortality [402].

In a single-center retrospective study, initial (1 → 3)-β-D-glucan value and early kinetics of (1 → 3)-β-D-glucan were not predictive of 6- or 12-week clinical outcome or mortality in IA [412].

What Are the Recommended Treatments for Pediatric Patients With Aspergillosis?
Recommendation.

45. Treatment of aspergillosis in children uses the same recommended therapies as in adult patients; however, the dosing is different and for some antifungals is unknown (strong recommendation; high-quality evidence).

Evidence Summary. Treatment in children follows the recommendations used for adults, yet antifungal dosing in children is often significantly different. Underdosing in children is a common etiology of insufficient drug levels and possibly clinical failures. Voriconazole, while only FDA approved for children 12 years and older, is the mainstay of pediatric aspergillosis treatment in all ages due to substantial pharmacokinetic data and experience. Fundamental pharmacokinetics of voriconazole are different in children (linear) than in adults (nonlinear) [290]. While voriconazole in adults is loaded at 6 mg/kg/dose twice daily, followed by 4 mg/kg/dose twice daily, the preferred pediatric dosing is substantially higher. Population pharmacokinetic analyses of voriconazole reveal that children should be given an intravenous 9 mg/kg loading dose twice daily to be comparable to a 6 mg/kg/dose in adults [413]. Maintenance intravenous dosing in children at 8 mg/kg/dose was comparable to a 4 mg/kg/dose in adults, and the oral dosing of 9 mg/kg/dose was similar to adults receiving 200 mg oral voriconazole twice daily. The majority of adolescents can be dosed as adults, but in younger adolescents (ages 12–14), body weight is more important than age in predicting voriconazole pharmacokinetics. Therefore, younger adolescents should be dosed as children if their weight is <50 kg and as adults if their weight is ≥50 kg [413]. Additionally, the oral bioavailability of voriconazole, thought to be >95% in adults, is lower in children at approximately 50%–65% [414, 415]. As in adult patients, there are still suggestions of the need for higher voriconazole doses [291], and drug monitoring is paramount.

Posaconazole is FDA approved for children 13 years and older for both the oral suspension and tablet, and for 18 years and older for the intravenous formulation. As such, pediatric dosing has not yet been fully defined. Caspofungin is FDA approved for children 3 months and older and dosing is based on body surface area, with a loading dose of 70 mg/m², followed by daily maintenance dosing of 50 mg/m², not to exceed 70 mg [186]. Micafungin is FDA approved for children 4 months and older and clearance increases in younger age groups. Doses in children are 2–3 mg/kg/day, with higher doses for younger children, and patients >40 kg use the adult dose (100 mg) [187]. Anidulafungin is not FDA approved for children, and a single pharmacokinetic study in children suggested a loading dose of 1.5–3 mg/kg and maintenance dose of 0.75–1.5 mg/kg [416]. Dosing of lipid formulations of AmB does not differ in children.

What Are Treatment Options for Aspergillosis of the Airways in Transplant and Nontransplant Recipients, and How Does It Differ From Invasive Pulmonary Aspergillosis?
Recommendations.

46. Saprophytic forms of TBA do not require antifungal treatment except for symptomatic or immunosuppressed patients. Treatment includes bronchoscopic removal of mucoid impaction. Mold-active triazole agents are recommended for immunocompromised patients in whom the possibility of invasive
disease cannot be eliminated (strong recommendation; moderate-quality evidence).

47. Bronchocentric granulomatosis is treated in the same fashion as ABPA (strong recommendation; low-quality evidence).

48. Invasive forms of TBA are treated with a mold-active triazole or intravenous lipid formulations of AmB (strong recommendation; moderate-quality evidence). We also recommend minimization or reversal of underlying immunosuppression when feasible, and bronchoscopic debridement of airway lesions in selected cases (strong recommendation; low-quality evidence).

49. In lung transplant recipients, we recommend treatment with a systemic antifungal for TBA, including saprophytic forms. We also recommend adjunctive inhaled AmB in the setting of TBA associated with anastomotic endobronchial ischemia or ischemic reperfusion injury due to airway ischemia associated with lung transplant (strong recommendation; moderate-quality evidence). Duration of antifungal therapy is at least 3 months or until TBA is completely resolved, whichever is longer.

Evidence Summary. Airway aspergillosis (or TBA) is similar to pulmonary aspergillosis in that it can occur in saprophytic, allergic (ABPA), or invasive forms. There is also an emerging entity of Aspergillus bronchitis among patients with CF, and others with bronchiectasis. The diagnosis of TBA is suggested by bronchoscopic findings and confirmed by culture and histopathology. Due to the limited number of studies, optimal evidence-based therapy is not clear, and recommendations are extrapolated from experience in treating invasive lung parenchymal aspergillosis and TBA case series.

Saprophytic forms of TBA include obstructing bronchial aspergillosis, endobronchial aspergillosis, and mucoid impaction. Obstructing bronchial aspergillosis is characterized by thick mucous plugs with minimal or no airway inflammation [417, 418]. Patients commonly present with the subacute onset of cough, dyspnea, chest pain, hemoptysis, and expectoration of fungal casts. Management typically consists of bronchoscopic clearance usually followed by oral antifungal therapy.

Endobronchial aspergillosis is generally found among patients with lesions such as broncholiths, cancer, or granulation tissue or suture material at the anastomotic site after lung resection. It is manifested as endobronchial lesions or mucous plugs in or around the bronchial stumps or sutures. In general, these saprophytic forms do not require systemic antifungal therapy unless patients are immunocompromised and locally invasive disease cannot be ruled out [418]. In symptomatic patients, local debridement or suture removal can be performed. There is no consistent evidence that systemic, inhaled, or local injection with an antifungal agent is effective in treating these forms of disease.

Mucoid impaction is a clinical-radiographic syndrome characterized by inspissated mucus filling of the bronchi [417, 418]. Finger-in-glove sign, referring to branching tubular opacities that extend peripherally, is the classic chest radiograph finding. Patients can be asymptomatic, or present with cough and expectoration of mucous plugs. Mucoid impaction is commonly associated with inflammatory conditions of the airways (such as bronchiectasis and ABPA), benign processes (such as broncholithiasis, foreign body aspiration, endobronchial lipoma, hamartoma, or papilloma), and malignant processes (such as carcinoid tumor or lung malignancies) causing obstruction of large airways. Mucous plugging that may appear hyperattenuated on computed tomography seems to be a particularly distinctive feature of ABPA, probably more common in India, with a high propensity for early relapse and corticosteroid dependence. Mucoid impaction associated with bronchiectasis is treated with maneuvers to promote airway clearance (chest physiotherapy, positive expiratory pressure and vibration devices, mucolytics, nebulized hypertonic saline) and treatment of airway infection (antimicrobial agents). Mucoid impaction associated with features of asthma and hypersensitization to Aspergillus is treated as for ABPA [417].

Bronchocentric granulomatosis is a form of ABPA that is characterized histopathologically by necrotizing granulomas with airway obstruction that destroy the bronchioles, but there is no tissue invasion by Aspergillus. Bronchoscopic findings include impaction of airway lumen by mucin and cellular debris. Treatment is similar to that of ABPA (see Recommendations 92–94 below) [419].

Invasive TBA is an uncommon disease that originates in the airway but may invade more deeply [417, 420]. It has been described most commonly in immunosuppressed patients (patients with hematologic malignancies, lung transplant or HSCT recipients, and patients on high-dose steroids). However, invasive TBA among patients with no known immunosuppression or following influenza infection has also been described [421, 422]. Invasive TBA consists of 2 forms: ulcerative and pseudomembranous [417, 423]. These 2 forms may represent different states of the same disease process that involves Aspergillus invasion of the tracheal or bronchial mucosa, which can extend into the cartilage. The ulcerative form is characterized by discrete ulcerative plaquelike lesions in the bronchial wall. This form is most commonly observed in lung transplant recipients or patients with AIDS [417, 423]. The pseudomembranous form is characterized by extensive membranes overlying the tracheal or bronchial mucosal surface. It is most commonly reported in severely immunocompromised patients with hematologic malignancies or those HSCT recipients with GVHD. Rarely, it has been linked to postinfluenza syndrome. In general, the ulcerative form carries a better prognosis than the pseudomembranous form. Treatment includes systemic antifungal therapy with a mold-active triazole agent or a lipid formulation of AmB. Follow-up bronchoscopy might be necessary to follow progression. Repeated bronchoscopies might be indicated for clearance of pseudomembranes and/or mucous plugs. The procedure might be complicated by bleeding, especially in the setting of necrotizing pseudomembranes with extension
into pulmonary vessels, and should be performed by experienced interventional bronchoscopists.

TBA is most commonly described in lung transplant recipients, affecting 4%-6% of patients [423, 424]. Potential underlying factors include the high rate of Aspergillus colonization both pre- and post-lung transplant, the direct exposure of the allograft lung to the environment, reduced mucociliary clearance, pulmonary denervation, and higher degree of immunosuppression than other organ transplant [425]. TBA typically occurs within 3–6 months of lung transplant, presumably as a result of airway ischemia due to disruption of bronchial vasculature during the transplant procedure. Furthermore, ischemic reperfusion injury might lead to airway stricture and other abnormalities that predispose to Aspergillus colonization and disease. Most lesions are asymptomatic and diagnosed by surveillance bronchoscopy; they manifest as pseudomembranes, ulceration, black eschar, or plaques. Rare cases of obstructing bronchial aspergillosis and TBA with bronchopleural fistulae have also been described. These lesions can develop despite systemic antifungal prophylaxis. Although TBA can progress to involve the lungs and disseminate, the overall outcome is better than that of IPA. Improved outcomes might result from early diagnosis based on surveillance bronchoscopy that is routinely performed in lung transplant. We recommend a mold-active triazole or intravenous lipid formulation of AmB based on case series. If the lesion develops while the patient is on antifungal prophylaxis, optimization of antifungal dosing with TDM is indicated. We also recommend adjunctive aerosolized AmB because the anastomotic site is devascularized, making it difficult for parenteral therapies to achieve therapeutic concentrations. Pseudomembranous TBA might be adjunctively treated with bronchoscopic debridement. Airway stenosis resulting from TBA might require balloon dilation, laser treatment, or stent placement. Endobronchial TBA with anastomotic dehiscence might need stent placement or surgical repair [426]. Duration of therapy for TBA is not well studied, but we recommend at least 3 months of systemic antifungal therapy with or without aerosolized AmB or until TBA is completely resolved, whichever is longer.

MANAGEMENT OF EXTRAPULMONARY ASPERGILLOSIS

What Are the Treatment Considerations for Central Nervous System Aspergillosis?

Recommendation.

50. We recommend voriconazole as primary therapy for CNS aspergillosis (strong recommendation; moderate-quality evidence). Lipid formulations of AmB are reserved for those intolerant or refractory to voriconazole (strong recommendation; moderate-quality evidence).

Evidence Summary. CNS aspergillosis is a devastating complication with a poor prognosis in the vast majority of affected patients [427]. Tenets of management include attempts to establish an early diagnosis, administration of an appropriate antifungal agent, assessment of the need for surgical intervention, and attempts to mitigate immunologic impairment(s) that led to CNS aspergillosis [428].

Diagnosis is suggested by the presence of focal neurologic deficits or seizures in the immunocompromised host, while meningeal signs are uncommon. CT and MRI are essential for the detection of infection and monitoring response to therapy. The radiographic pattern is dependent on the source of infection with direct extension from the sinuses, eye, or middle ear often causing only a single abscess within the frontal or temporal lobe, and those developing from hematogenous dissemination causing solitary or multiple small abscesses most frequently at the gray-white junction. Vascular invasion may occur and rupture with the development of a hemorrhagic or ischemic stroke, subarachnoid hemorrhage, or empyema formation. Definitive diagnosis is dependent on recovery of the organism, or examination of biopsy findings. Biopsy of lesions within the CNS is not always practical and infection of the CNS is commonly inferred by recovery of Aspergillus spp from a pulmonary or sinus source coincident with a characteristic brain lesion. The value of screening patients with IPA for asymptomatic CNS disease has not been determined.

Detection of GM [429] or (1 → 3)-β-D-glucan from the cerebrospinal fluid [430] is helpful in the diagnosis of CNS aspergillosis; however, other fungal pathogens also have positive results with these assays (eg, Fusarium spp) [431]. PCR assays have been examined for CNS aspergillosis, but these have not been standardized for widespread use [87].

Surgical intervention is frequently discussed during the care of patients with CNS aspergillosis as resection of infected tissue or abscesses eliminates areas containing viable fungi. A mortality benefit of surgery for the management of cerebral lesions, in combination with antifungal therapy with voriconazole, has been shown in a retrospective study of 81 patients [432]. Although this study was subject to selection bias for those patients who were ultimately able to undergo surgical intervention, a benefit of voriconazole followed by surgical intervention was suggested (HR, 2.1; 95% CI, 1.1–3.9; P = .02). Surgical intervention is also a useful adjunct in the management of CNS aspergillosis with contiguous infections of the paranasal sinuses or vertebral bodies and should be pursued in these circumstances when feasible.

The reversal or reduction of immunosuppression is essential in attempts to improve outcomes and should be managed in the same fashion as discussed elsewhere in this document.

Recommendations for the treatment of CNS aspergillosis with voriconazole are based primarily on open-label studies. In a direct comparative trial between AmB deoxycholate and voriconazole, a trend toward improvement of CNS aspergillosis in patients was noted in those who were treated with
voriconazole [348]. The open-label studies of voriconazole in adult and pediatric patients also demonstrate activity of voriconazole in the treatment of CNS aspergillosis [216, 432]. It should be noted that voriconazole interacts with some antiseizure medications (phenytoin, phenobarbital) that may be coadministered in patients with CNS mass lesions, likely resulting in subtherapeutic concentrations.

Lipid formulations of AmB have demonstrated favorable responses in animal models and patients with CNS aspergillosis. Among lipid formulations of AmB formulations, favorable responses have been achieved in case reports with liposomal AmB, ABLC, and ABCD [433–435]. Itraconazole and posaconazole have also been successfully used in treatment of CNS aspergillosis [255, 436, 437], and case reports describe the efficacy of caspofungin and micafungin in the treatment of CNS aspergillosis [398, 438]. Combination therapy for CNS disease is initiated by some practitioners out of understandable lack of therapeutic options given the mortality associated with this form of dissemination, and a favorable response has been observed in animal models and some patients [197], yet there are no data suggesting better outcomes with this approach.

Progressive neurologic deficits have led to the use of corticosteroid therapy in patients with evolving CNS disease; however, this practice is deleterious and should be avoided. Intrathecal or intralesional antifungal therapy is also not recommended for the treatment of CNS aspergillosis due to a failure of AmB delivered intrathecally to penetrate beyond the pia mater. Delivery via this method also has the potential for AmB-induced chemical meningitis, arachnoiditis, seizures, headache, or altered mental status [439].

Epidural aspergillosis is an unusual manifestation of CNS aspergillosis that most often arises from extension into the epidural space from vertebral abscess [440]. Systemic antifungal therapy and surgical drainage are considered to be standards of practice for management of epidural aspergillosis; however, most of the experience in managing epidural aspergillosis is based on individual case reports and brief case series.

How Is Aspergillus Endophthalmitis Treated?

Recommendation.

51. We recommend that Aspergillus endophthalmitis be treated with systemic oral or intravenous voriconazole plus intravitreal voriconazole or intravitreal AmB deoxycholate (strong recommendation; weak-quality evidence).

Evidence Summary. Hematogenous endophthalmitis presents in immunocompromised and noncomprised patients as sudden loss of vision, usually in one eye, beginning with subretinal lesions that cause retinal necrosis and rapidly extend into the vitreous humor [441]. A dense vitritis forms over a few days. A vitreal aspirate or vitrectomy specimen yields Aspergillus, usually A. fumigatus, on culture and smear [442]. Visual loss is usually permanent and enucleation often required for pain relief. Intravitreal voriconazole 100 µg or intravitreal AmB deoxycholate 5–10 µg appear to be essential in treatment, combined with systemic voriconazole [443]. Local concentration of drug is lower if intravitreal drug is injected at the end of a pars plana vitrectomy, lessening concern about retinal toxicity of AmB deoxycholate when that drug is used. Although intracameral injection (injection into the anterior chamber) has no role in aspergillosis of the posterior chamber, it has been reported that intracameral injection of voriconazole 100 µg was useful for extension of Aspergillus keratitis into the anterior chamber [444].

What Is the Role of Surgery in Aspergillosis of the Paranasal Sinuses?

Recommendation.

52. We recommend that both surgery and either systemic voriconazole or a lipid formulation of AmB formulation be used in invasive Aspergillus fungal sinusitis but that surgical removal alone can be used to treat Aspergillus fungal ball of the paranasal sinus. Enlargement of the sinus ostomy may be needed to improve drainage and prevent recurrence (strong recommendation; moderate-quality evidence).

Evidence Summary. In an uncomplicated Aspergillus fungal ball of the sinus, >90% being in the maxillary sinus, clinicians should remove the fungal ball, preferably using endoscopic techniques as this is usually curative. A wide maxillary antrostomy is done to improve sinus drainage, and a biopsy of the sinus wall is sometimes done to rule out mucosal invasion [445–447]. Local or systemic antifungals have no role in the treatment of a maxillary sinus fungal ball. Aspergillus fungal balls of the sphenoid sinus differ in that invasion into the cavernous sinus can occur from fungal invasion or excessive surgical debridement [448]. Systemic antifungal therapy may be advisable if there is a question of mucosal involvement, mucosal breach of the sphenoid sinus, or spread into the cavernous sinus. Local irrigation of the paranasal sinuses with AmB is not considered useful because topical AmB does not penetrate into tissues.

In granulomatous or chronic invasive and granulomatous aspergillosis of the paranasal sinus in immunocompetent patients, often diagnosed because of proptosis or extension to the brain or orbit, and in acute invasive paranasal sinusitis of severely immunocompromised patients, surgical debridement and systemic antifungal therapy is recommended. Sometimes multiple surgical procedures are required, and extensive debridement is best done once thrombocytopenia has resolved, to reduce the risk of postoperative hemorrhage. Voriconazole is the preferred therapy, or a lipid formulation of AmB; morbidity and mortality is high [158, 449, 450]. Allergic fungal rhinosinusitis (AFRS) is discussed elsewhere.
What Are the Treatment Recommendations for Aspergillus Endocarditis, Pericarditis, and Myocarditis?

Recommendation.

53. In Aspergillus endocarditis, we recommend early surgical intervention combined with antifungal therapy in attempts to prevent embolic complications and valvular decompensation (strong recommendation; moderate-quality evidence). Voriconazole or a lipid formulation of AmB is recommended as initial therapy (strong recommendation; low-quality evidence). Following surgical replacement of an infected valve, lifelong antifungal therapy should be considered (strong recommendation; low-quality evidence).

Evidence Summary. The diagnosis of Aspergillus endocarditis is often difficult and almost always delayed with the diagnosis made postmortem in up to one-third of cases [451]. Fever, the presence of a new murmur, and stigmata of peripheral emboli such as new neurologic deficits, heart failure, or dyspnea are the most commonly encountered clinical features and no different from those observed in bacterial endocarditis. Blood cultures are almost always negative, and examination of resected valvular tissue or emboli is the most common means of confirming the diagnosis. The converse is not true; positive blood cultures are more likely to be contaminants than indicating endocarditis. Noninvasive markers such as GM may be positive, but are not specific for the site of disease [452].

The aortic and mitral valves are those most frequently infected. Prior valvular abnormalities and/or prior valvular surgery predisposes to infection, although intravenous drug use and other cardiac procedures have also been presented as predisposing factors. Vegetations secondary to Aspergillus spp are often large and/or penduculated and therefore embolic complications are common, particularly to large arteries. For this reason, imaging of the brain is prudent at the time of diagnosis in attempts to define the full spectrum of disease. Mortality rates are high (50%–96%). The mean survival period for Aspergillus endocarditis was 11 days in one study, further illustrating the rapid and frequently lethal course of this infection [453].

Combined medical therapy and valve replacement are essential in attempts to improve outcomes as neither alone has a significant influence on patient outcomes [419, 454], and attempts to manage patients with antifungal agents alone are rarely successful. Voriconazole or liposomal AmB (3–5 mg/kg/day) are recommended as first-line agents. Comparative data are not available; however, case reports [455], case series [451], and animal models [456] have suggested the efficacy of these agents in Aspergillus infective endocarditis (IE). Combination therapy may also be used, but no evidence regarding the superiority of this approach has been presented.

The overall poor survival of IE secondary to Aspergillus spp limits the available data on recurrence rates. In other causes of fungal endocarditis, recurrence may occur late and even years after the initial diagnosis. For this reason, long durations of therapy (>2 years) and consideration of lifelong therapy should be considered concomitant with frequent clinical and echocardiographic assessment for possible recurrence [451].

Aspergillus pericarditis arises as the result of direct extension from: a contiguous focus of IPA, from a myocardial lesion, or intraoperative contamination [457, 458]. Pericardial tamponade may rapidly ensue, leading to hemodynamic deterioration and cardiac arrest. Diagnosis is suggested by pericardiocentesis (with positive culture or antigen testing), pericardectomy, or pericardial biopsy. A combined medical and surgical approach, with pericardial resection or drainage, is necessary in attempts to optimize outcomes [458].

Aspergillus myocarditis may manifest as myocardial infarction, cardiac dysrhythmias, or myoepicarditis [457]. This infection generally occurs in the context of disseminated disease and requires systemic antifungal therapy. An intracardiac abscess may be seen on echocardiography, although in other cases no echocardiographic lesions are observed [459].

What Are the Treatment Recommendations for Aspergillus Osteomyelitis and Septic Arthritis?

Recommendation.

54. Surgical intervention is recommended, where feasible, for management of Aspergillus osteomyelitis and arthritis, combined with voriconazole (strong recommendation; moderate-quality evidence).

Evidence Summary. Aspergillus osteomyelitis occurs by one of 3 mechanisms: (1) direct inoculation secondary to trauma, surgery, or epidural injection; (2) contiguous spread from pleuropulmonary disease; or (3) hematogenous spread from either coexistent pulmonary infection or intravenous injection [460, 461]. Most patients have traditional risk factors for IA; however, up to 34% of patients have no obvious predisposing factor or immunosuppression [462]. Vertebral osteomyelitis with or without discitis is the most common form, and the predominance of cases involve the lumbar vertebrae. Back pain is the most common clinical manifestation, with neurologic deficits secondary to cord compromise, or kyphosis also observed. Diagnostic imaging with CT and/or MRI is essential for staging disease and for providing a guide for orthopedic and/or neurosurgical intervention. Diagnosis can be confirmed by isolation of the organism from bone specimens or an aspirate of an adjacent fluid collection.

In cases without significant instability or neural compression and no evidence of disease progression, antifungal treatment alone may be sufficient provided the underlying immunologic deficit can be corrected; however, it should be noted that favorable outcomes more frequently occur in those receiving combined medical and surgical therapy [460]. In cases with spinal instability or symptoms consistent with spinal cord or radicular compression or abscess formation, surgical decompression in
combination with antifungal therapy is recommended [462]. The type and extent of surgery should be individualized.

Voriconazole has been successfully used as salvage and primary therapy, either alone or in combination with surgical debridement [463, 464], and has been shown to be superior to AmB in cases of disseminated aspergillosis [348]. Historical experience has shown the efficacy of AmB formulations. Itraconazole has been used subsequent to a course of AmB. There is little reported experience in the use of posaconazole or echinocandins in the treatment of Aspergillus osteomyelitis [465]. Therapy should be continued for a minimum of 8 weeks, with longer courses (>6 months) frequently necessary [460, 461].

Aspergillus arthritis may develop from hematogenous dissemination in immunocompromised patients, via injection, or by direct traumatic inoculation in immunocompetent hosts [466]. In many cases, Aspergillus arthritis arises as an extension from a contiguous focus of Aspergillus osteomyelitis [466]. Most of the successfully treated cases of Aspergillus arthritis have responded to combined medical therapy and drainage of the joint and/or synovectomy [467]. Historically, AmB formulations have demonstrated efficacy in cases of arthritis [466], although more recent data have shown an improvement in response rates when voriconazole is administered, which is the recommended antifungal agent in this setting [468].

What Are the Treatment Recommendations for Cutaneous Aspergillosis?
Recommendations.

55. As cutaneous lesions may reflect disseminated infection, we recommend treatment with voriconazole in addition to evaluation for a primary focus of infection (strong recommendation; low-quality evidence).

56. In cases of aspergillosis in burns or massive soft tissue wounds, surgical debridement is recommended, in addition to antifungal therapy (strong recommendation; moderate-quality evidence).

Evidence Summary. Cutaneous aspergillosis may develop in the context of hematogenous dissemination in the immunocompromised host or can occur in the context of traumatic or nosocomial device-related infection or in burn victims, and represents a heterogeneous disease [11, 469, 470]. The initial lesions of cutaneous aspergillosis may appear as macules, papules, nodules, or plaques. Pustules or lesions with purulent discharge generally occur in neonates [9]. Unlike IPA, which requires thoracic surgery or thoracoscopy to remove foci of infection, the eradication of cutaneous aspergillosis may be accomplished with considerably less risk [471]. Therefore, surgical intervention, for primary cutaneous infection, may be a useful adjunct to antifungal therapy. Biopsy for confirmation of mycological diagnosis is essential to distinguish aspergillosis from other potential pathogens (eg, fusariosis, mucormycosis) [472]. Skin biopsy should be taken from the center of the lesion and reach the subcutaneous fat to visualize hyphae invading blood vessels of the dermis and subcutaneous tissues [9].

What Are the Treatment Recommendations for Aspergillus Peritonitis?
Recommendation.

57. We recommend prompt peritoneal dialysis catheter removal accompanied by systemic antifungal therapy with voriconazole (strong recommendation; low-quality evidence).

Evidence Summary. Aspergillus peritonitis may occur as a complication of chronic ambulatory peritoneal dialysis [473]. Although Candida species are the most common cause of fungal peritonitis complicating chronic ambulatory peritoneal dialysis and fungal peritonitis typically occurs following an episode of bacterial peritonitis, Aspergillus species are an additional and well-established cause of this infection [474]. The diagnosis can be suggested by detection of (1 → 3)-β-D-glucan and GM in the peritoneal fluid, or confirmed by culture of peritoneal fluid [475]. In rare cases, peritoneal biopsy is required, although this is typically accomplished concurrently with peritoneal dialysis catheter removal [476]. Removal of the dialysis catheter is essential in cases of fungal peritonitis and has been associated with improved survival. In cases where the catheter cannot be promptly removed, some practitioners use intraperitoneal AmB in conjunction with voriconazole, but it should be recognized that intraperitoneal AmB administration may cause a chemical peritonitis and is not recommended by this panel [477]. In most cases the catheter should be immediately removed.

Following catheter removal, systemic antifungal therapy is required. Intravenous AmB formulations result in suboptimal and, in many cases, undetectable peritoneal drug concentrations [478, 479]. Systemic therapy with voriconazole for 6–8 weeks is thus recommended based on successful reports and adequate peritoneal concentrations in conjunction with catheter removal [480, 481]. Posaconazole and the echinocandins have been successfully used in fungal peritonitis from other causes and may have utility as salvage therapy in Aspergillus peritonitis [482]. Following treatment, a minority of patients may successfully return to peritoneal dialysis.

What Are the Treatment Recommendations for Esophageal, Gastrointestinal, and Hepatic Aspergillosis?
Recommendations.

58. We suggest voriconazole and surgical consultation in attempts to prevent complications of hemorrhage, perforation, obstruction, or infarction (weak recommendation; low-quality evidence).

59. We suggest antifungal therapy with voriconazole or a lipid formulation of AmB as initial therapy for hepatic aspergillosis. For extrahepatic or perihepatic biliary obstruction, or
localized lesions that are refractory to medical therapy, surgical intervention should be considered (weak recommendation; low-quality evidence).

Evidence Summary. Aspergillosis of the esophagus and gastrointestinal tract is relatively common in advanced cases of disseminated IA [483]. In fact, in autopsy studies, esophageal and gastrointestinal tract involvement is the third most common site of infection [483]. Disease may occur through hematogenous dissemination or ingestion, and some authors have suggested the gastrointestinal tract as a potential portal of entry for dissemination or ingestion, and some authors have suggested the site of infection [483]. Disease may occur through hematogenous and gastrointestinal tract involvement is the third most common of disseminated IA [483]. In fact, in autopsy studies, esophageal gastrointestinal tract is relatively common in advanced cases [483].

Hepatic aspergillosis may occur as single or multiple hepatic lesions. Dissemination to the liver is thought to occur via the portal venous system from the gastrointestinal tract, or as a component of general and widespread systemic dissemination [487]. Cholangitis secondary to Aspergillus spp is exceedingly uncommon, but has been described following biliary surgery [488]. Reports of therapeutic interventions are limited. Medical therapy for hepatic abscesses may be effective and preclude the need for surgical resection.

What Are the Treatment Recommendations for Renal Aspergillosis? Recommendation.

60. We suggest a combined approach of medical and urologic management for renal aspergillosis. Obstruction of one or both ureters should be managed with decompression if possible and local instillation of AmB deoxycholate. Parenchymal disease is best treated with voriconazole (weak recommendation; low-quality evidence).

Evidence Summary. Renal aspergillosis may develop as single or multiple parenchymal abscesses, usually as a result of hematogenous dissemination, or may present as a fungal ball in the pelvis of the kidney [489, 490]. This form of aspergillosis may cause hematuria, ureteropelvic obstruction from a fungal ball, perinephric abscess with extension into surrounding tissues, or passing of fungal elements into the urine.

Reports of management are limited to individual cases. Medical management alone may be successful if abscesses are relatively small. Management of larger abscesses may require surgical drainage. Microwave ablation has been successfully used as an adjunct to antifungal therapy in a single patient deemed a poor surgical candidate [491]. Nephrectomy should be performed only as a last option. Voriconazole, posaconazole, itraconazole, AmB formulations, and the echinocandins all exhibit poor urinary concentrations [492]. Irrigation via a nephrostomy tube with AmB deoxycholate allows high local concentrations and when given by this route is not absorbed and is not nephrotoxic. It thus may be useful in aspergillosis of the renal pelvis, but has no role in the treatment of parenchymal disease [493].

What Are the Treatment Regimens for Aspergillus Ear Infections? Recommendations.

61. Noninvasive Aspergillus otitis externa, also called otomycosis, is treated by thorough mechanical cleansing of the external auditory canal followed by topical antifungals or boric acid (strong recommendation; moderate-quality evidence).

62. We recommend that clinicians treat IA of the ear with a prolonged course of systemic voriconazole, usually combined with surgery (strong recommendation; low-quality evidence).

Evidence Summary. It is important to distinguish otomycosis, a common entity in healthy persons, from IA of the ear, which is rare and occurs in immunosuppressed persons and diabetic individuals. In otomycosis, Aspergillus species, often Aspergillus niger, grows on cerumen and desquamated cells in an external auditory canal but does not invade the lining [494, 495]. IA can involve the external auditory canal, middle ear, mastoid, or petrous portion of the temporal bone. When invasion begins in the external auditory canal, infection has been called malignant otitis externa. Tissue-invasive Aspergillus otitis should be treated with prolonged systemic antifungals [448], preferably with voriconazole, usually preceded by surgical debridement [496–499]. Colonization of the middle ear and mastoid by Candida, Aspergillus, or other molds can occur in patients with chronic otitis media in the presence of a perforated tympanic membrane, usually following multiple surgical procedures and many courses of antibacterial agents. In the absence of evidence of tissue invasion, we do not recommend that colonization should be treated [500].

What Are the Treatment Recommendations for Aspergillus Keratitis? Recommendation.

63. We recommend that clinicians treat Aspergillus keratitis with topical natamycin 5% ophthalmic suspension or topical voriconazole (strong recommendation; moderate-quality evidence).

Evidence Summary. Clinicians should treat Aspergillus keratitis with topical natamycin 5% ophthalmic suspension. In case series and randomized clinical trials of fungal keratitis, topical voriconazole 1% was inferior to natamycin, but Fusarium keratitis appeared to account for most of the difference [501–504]. Voriconazole for infusion, reconstituted with water to 1%, is a reasonable alternative for Aspergillus keratitis. Diagnosis should be confirmed by smear and culture of corneal scrapings [505]. Confocal microscopy and anterior segment coherence tomography are useful to monitor therapeutic response [505].
Ophthalmologists should consider penetrating keratoplasty for patients who do not respond to topical therapy, though patients with lesions extending to the corneal limbus, with corneal perforation or hypopyon, are at high risk of recurrence [506].

**How Should Aspergillus Bronchitis Be Diagnosed and Treated in the Nontransplant Population?**

**Recommendations.**

64. We suggest the diagnosis of aspergillus bronchitis in non-transplant patients be confirmed by detection of *Aspergillus* spp in respiratory secretions, usually sputum, with both PCR and GM on respiratory samples being much more sensitive than culture (weak recommendation; low-quality evidence).

65. We suggest treatment with oral itraconazole or voriconazole with TDM (weak recommendation; low-quality evidence).

**Evidence Summary.** *Aspergillus* is a cause of acute or chronic bronchitis usually seen as a complication of CF or bronchiectasis [83, 507, 508]. Its clinical features are not distinctive in CF, but include a more rapid decline in FEV1 than those with bronchiectasis [83, 507, 508]. Its clinical features are not distinctive in CF, but include a more rapid decline in FEV1 than those with bronchiectasis [83, 507, 508]. Its clinical features are not distinctive in CF, but include a more rapid decline in FEV1 than those with bronchiectasis [83, 507, 508]. It affects up to approximately 30% of adults with CF [509]. Patients present with recurrent, frequently relapsing acute bronchitis with thick sputum plugging and shortness of breath. Occasional patients develop mucoid impaction, or so-called “plastic bronchitis,” requiring urgent bronchial toilet. Identification of *Aspergillus* in airway secretions with culture, PCR, or GM is essential for the diagnosis, and elevated *Aspergillus* IgG serology is supportive of the diagnosis [507, 508]. Several *Aspergillus* species may be implicated.

It is likely that antifungal therapy is helpful in both CF and bronchiectasis by reducing the burden of organisms and thus reducing the inflammatory immune response [508, 510], but this has not been systematically studied. Itraconazole or voriconazole are first-line agents. Patients who fail oneazole agent may respond to a different azole. Relapse after improvement during antifungal therapy is common; long-term suppressive therapy may be necessary for symptom control. Triazole antifungal resistance has been documented, and so susceptibility testing is valuable. The role of inhaled antifungal therapy is uncertain.

**PROPHYLAXIS OF INVASIVE ASPERGILLOSIS**

**V. What Are the Recommended Prophylactic Regimens, Who Should Receive Them, and How Should Breakthrough Infection Be Managed?**

**In Which Patients Should Antifungal Prophylaxis Against Aspergillosis Be Used?**

**Recommendation.**

66. We recommend prophylaxis with posaconazole (strong recommendation; high-quality evidence), voriconazole (strong recommendation; moderate-quality evidence), and/or micafungin (weak recommendation; low-quality evidence) during prolonged neutropenia for those who are at high risk for IA (strong recommendation; high-quality evidence). Prophylaxis with caspofungin is also probably effective (weak recommendation; low-quality evidence). Prophylaxis with itraconazole is effective, but therapy may be limited by absorption and tolerability (strong recommendation; moderate-quality evidence). Triazoles should not be coadministered with other agents known to have potentially toxic levels with concurrent triazole coadministration (eg, vinca alkaloids and others) (strong recommendation; moderate-quality evidence).

**Evidence Summary.** Hematologic disorders with poorly functioning neutrophils (eg, aplastic anemia and variants thereof, MDS), acute leukemia with repeated and/or prolonged neutropenia, [511], or a history of IA prior to transplantation [512] have been identified as significant risk factors for IA.

A 2007 large randomized clinical trial of oral posaconazole solution demonstrated its superiority vs fluconazole or itraconazole in the prevention of IA among patients with AML and MDS undergoing chemotherapy [292]. This study demonstrated higher survival for patients in the posaconazole arm, although there was greater toxicity among recipients of posaconazole, compared with the fluconazole/itraconazole arm. With the approval of an extended-release tablet form of posaconazole, as well as an intravenous form, dosing will be different compared to the randomized prophylaxis trials, which used a solution formulation, and needs further evaluation in HSCT patients.

A previous trial compared voriconazole or fluconazole prophylaxis in allogeneic HSCT recipients; both arms were monitored with GM measurements [513]. *Aspergillus* infections were less frequent with voriconazole than with fluconazole prophylaxis, but the 180-day fungal-free survival and overall survival were not different [513]. In another trial, voriconazole was used as prophylaxis for leukemia patients with about 3 weeks of neutropenia during a construction risk period; less aspergillosis was noted among patients receiving prophylaxis (*P* = .04) [514]. Voriconazole has also been used among children as prophylaxis, although children require different dosing [515]. Voriconazole requires careful monitoring in children [516]. Patients receiving voriconazole prophylaxis remain at risk for both *Aspergillus* and non-*Aspergillus* fungal pathogens that are intrinsically resistant to this agent [517, 518].

A 2004 large, randomized prophylaxis trial comparing micafungin or fluconazole prophylaxis found that the composite endpoint of treatment success was significantly better among those receiving micafungin prophylaxis (*P* = .03), as there was less empiric AmB treatment during neutropenia (15.1% vs 21.4%), fewer breakthrough fungal infections (1.6% vs 2.4%), and less yeast colonization among those receiving micafungin prophylaxis (*P* = .03) [519]. There was a trend toward reduced breakthrough aspergillosis infections (0.2% vs 1.5%; *P* = .07), but micafungin was not approved by the FDA for prophylaxis.
of aspergillosis [519]. In clinical practice, the requirement for daily intravenous therapy with echinocandins may lead to a change to oral azole therapy at a time not studied in clinical trials, but these agents may be useful for prophylaxis when drugs that are contraindicated with triazoles (such as cyclophosphamide or vincristine) are required.

Caspofungin has been studied in smaller settings. The efficacy and safety of caspofungin was similar to other prophylactic regimens, in the setting of a low incidence of IFI [520–523].

Itraconazole may be effective, but the conclusions of several prospective trials regarding efficacy are limited, because study designs did not include patients at significant risk for aspergillosis [523–527]. Itraconazole oral capsules have erratic bioavailability [528]. Because there was an increase in transplant-related mortality when itraconazole was used together with cyclophosphamide during the conditioning regimen for HSCT, azole dosing is now delayed until after the stem cell product infusion [529].

Earlier studies of antifungal prophylaxis in hematologic malignancies are summarized in several large meta-analyses [524, 530, 531]. Among the studies that investigated parenterally administered AmB deoxycholate or liposomal formulations of AmB for prophylaxis, most have been historically controlled, and some have suggested a reduction in IA. Several prospective, randomized trials using polyene therapy have demonstrated a reduction in the number of IFIs, but none have demonstrated a significant reduction of IA in a prospective, randomized study [532–534]. Aerosolized AmB formulations have been shown to reduce the incidence of IPA, notably in lung transplant recipients [177].

What Are the Recommended Prophylactic Regimens for Patients With Graft-Versus-Host Disease?

Recommendations.

67. We recommend prophylaxis with posaconazole for allogeneic HSCT recipients with GVHD who are at high risk for IA (strong recommendation; high-quality evidence). Prophylaxis with other mold-active azoles is also effective. Voriconazole is commonly used for prophylaxis against IA in high-risk patients but did not show improved survival in clinical trials (strong recommendation; moderate-quality evidence). Prophylaxis with itraconazole is limited by tolerability and absorption (strong recommendation; high-quality evidence).

68. We recommend continuation of antifungal prophylaxis throughout the duration of immunosuppression in patients with chronic immunosuppression associated with GVHD (corticosteroid equivalent of >1 mg/kg/day of prednisone for >2 weeks and/or the use of other anti-GVHD therapies, such as lymphocyte-depleting agents, or TNF-α inhibition, for refractory GVHD) (strong recommendation; high-quality evidence).

Evidence Summary. A randomized clinical trial of posaconazole prophylaxis during GVHD in HSCT recipients found a significant reduction in proven and probable IFIs and similar toxicity in posaconazole recipients, compared with those receiving fluconazole, which has no mold activity [254]. Since this time, posaconazole extended-release tablets have become available and have replaced the use of oral solution at many centers and may further improve serum posaconazole levels without clinically relevant hepatotoxicity [244].

A 2010 large, randomized clinical trial of voriconazole prophylaxis following allogeneic transplant continued the antifungal prophylaxis to day 180 for higher-risk patients such as those with GVHD [513]. Aspergillus infections were less frequent with voriconazole than with fluconazole, but fungal-free survival and overall survival were no different [513]. Voriconazole provided effective prophylaxis when added specifically during corticosteroid therapy for GVHD [535]. Voriconazole has also been assessed among children as prophylaxis starting from the time of transplant, and then continued for those patients with acute GVHD [515]. Acute GVHD is a risk factor for hepatotoxicity attributable to voriconazole that requires careful monitoring in this setting [536]. The use of itraconazole for prophylaxis against Aspergillus during GVHD as in other populations is complicated by erratic bioavailability and drug toxicity [528, 537]. Patients receiving voriconazole or itraconazole prophylaxis remain at risk for both Aspergillus and non-Aspergillus fungal pathogens that are intrinsically resistant to this agent [517, 518].

What Are the Recommendations for Antifungal Prophylaxis in Lung Transplant Patients?

Recommendations.

69. We recommend antifungal prophylaxis with either a systemic triazole such as voriconazole or itraconazole or an inhaled AmB product for 3 to 4 months after lung transplant (strong recommendation; moderate-quality evidence).

70. Systemic voriconazole or itraconazole is suggested over inhaled AmB for lung transplant recipients with mold colonization pre- or post–lung transplant, mold infections found in explanted lungs, fungal infections of the sinus, and single-lung transplant recipients (weak recommendation; low-quality evidence).

71. We recommend reinitiating antifungal prophylaxis for lung transplant recipients receiving immunosuppression augmentation with either thymoglobulin, alemtuzumab, or high-dose corticosteroids (strong recommendation; moderate-quality evidence).

Evidence Summary. Antifungal prophylaxis for lung transplant recipients is commonplace at many centers but is not employed universally [538]. Furthermore, the types of prophylaxis (inhaled or systemic), antifungal agents used, and duration of prophylaxis also vary [538, 539]. To date, there have been no prospective comparative trials evaluating the long-term benefit of antifungal prophylaxis among lung transplant recipients. Retrospective and observational studies with historical controls showed lower rates of IFIs among patients receiving antifungal
prophylaxis [540–543]. Given these data, the presence of damaged airways early after transplant (see TBA above), high levels of immunosuppression following lung transplant, and poor outcomes of IFIs, it is reasonable to consider antifungal prophylaxis in the early posttransplant period.

Aerosolized AmB formulations have been shown to protect lung transplant recipients from pulmonary fungal infections [540]. There is no evidence that one formulation of AmB is superior to others, but AmB deoxycholate is associated with more side effects than other formulations, including cough, bronchospasm, taste disturbance, and nausea as well as difficulty in administering the drug [176, 182, 183, 540, 544–546]. The longer tissue half-life of the lipid formulations of AmB also permits less frequent administration [183]. An advantage of inhaled AmB is the lack of systemic adverse effects and/or drug-drug interactions; a disadvantage is its inability to prevent extrapulmonary fungal infections. Systemic voriconazole and itraconazole are also effective in preventing IFI [425, 542]. To date, there is no evidence that one agent is superior to the other. Azole prophylaxis is complicated by drug interactions with the calcineurin inhibitors, as well as liver toxicity. It should be noted that antifungal prophylaxis might only delay the onset of IFI [547], as the allograft is exposed to the environment, and patients are maintained on relatively high doses of immunosuppression lifelong.

In the absence of a head-to-head comparative trial of inhaled AmB vs a systemic mold-active antifungal, we suggest that systemic voriconazole or itraconazole be considered for (1) patients colonized with *Aspergillus* or other pathogenic molds pre- or post–lung transplant [548, 549]; (2) patients with evidence of mold infections found in explanted lungs [550]; (3) patients with evidence of fungal infections in the sinuses; and (4) single-lung transplant recipients [551]. For the remaining patients, inhaled AmB or systemic voriconazole or itraconazole might be equally effective. Posaconazole solution may not be ideal for prophylaxis in the early period after lung transplant, as many patients have gastrointestinal or nutritional issues and are taking a proton pump inhibitor as routine posttransplant prophylaxis for gastroesophageal reflux. There are no data on the efficacy and safety of the intravenous or tablet formulations of posaconazole for prophylaxis early after transplant.

A benefit to continuing antifungal prophylaxis beyond 3–4 months after lung transplant has not been established. Beyond this period of high risk, we suggest antifungal prophylaxis only in the setting of severe rejection requiring thymoglobulin or alemtuzumab, or high-dose and prolonged use of corticosteroids.

**What Are the Recommendations for Antifungal Prophylaxis in Nonlung Solid Organ Transplant Recipients?**

**Recommendation.**

72. We recommend prophylactic strategies in SOT recipients based on the institutional epidemiology of infection and assessment of individual risk factors (strong recommendation; low-quality evidence). Prospective trials are lacking to address the need for routine anti-*Aspergillus* prophylaxis other than for lung transplant recipients. Individual risk factors have been identified in cardiac (pretransplant colonzation, reoperation, CMV infection, renal dysfunction, institutional outbreak), liver (fulminant hepatic failure, reoperation, retransplantation or renal failure), and others with institutional outbreaks or prolonged or high-dose corticosteroid use. In such patients, the optimal duration of prophylaxis is not known.

**Evidence Summary.** Invasive *Aspergillus* infection occurs in up to 19% of all SOT recipients (estimated 0.65% per year), with recent mortality estimates of approximately 22% [40, 43, 552–554]. The incidence of infection varies with the organ transplanted, including recipients of liver (1%–9.2%) [553, 555–557], heart (1%–14%) [558, 559], kidney (0.7%–4%) [553, 556, 560, 561], and pancreas 3.4% [40, 560, 562]. The risks for IFI in general, and for *Aspergillus* infections in particular, are increased by patient-specific factors including the need for organ retransplantation (liver), posttransplant renal or hepatic failure with renal replacement therapy (liver and kidney), reexploration (liver and heart), pretransplant colonization with *Aspergillus* spp (heart), concurrent CMV infection (liver and heart), hepatitis C infection (liver), and steroid-based regimens [43, 556, 563–566]. The overall intensity of immunosuppression and the chronicity of systemic illness (malnutrition, hypogammaglobulinemia, and leukopenia) in the organ recipient is a general risk for IFI [40, 562]. Pulse-dosed corticosteroid therapy with lymphocyte depletion is a notable risk in the *Aspergillus*-colonized individual [562]. Infections tend to occur both early after transplantation (first month) and late (mean approximately 184 days) [40, 43]. Targeted antifungal prophylaxis varies with the immunosuppressive regimen and local epidemiology of infections [567–570].

**MANAGEMENT OF BREAKTHROUGH INFECTION**

**How Should Breakthrough Aspergillosis Be Managed?**

**Recommendation.**

73. We suggest an individualized approach that takes into consideration the rapidity and severity of infection and local epidemiology. As principles, we recommend an aggressive and prompt attempt to establish a specific diagnosis with bronchoscopy and/or CT-guided biopsy for peripheral lung lesions. Documentation of serum azole levels should be verified if TDM is available for patients receiving mold-active triazoles. Antifungal therapy should be empirically changed to an alternative class of antifungal with *Aspergillus* activity. Other considerations include reduction of underlying immunosuppression if feasible, and susceptibility testing of any *Aspergillus* isolates recovered from the patient (weak recommendation; moderate-quality evidence).
Evidence Summary. Breakthrough aspergillosis typically occurs in the setting of antifungal prophylaxis. There is a paucity of organized experience on the best way to manage these patients [571]. Documented breakthrough aspergillosis occurs infrequently, in no more than 3% of patients in modern “real life” series of patients receiving mold-active prophylaxis [285]. If the patient develops breakthrough aspergillosis in the setting of non-mold-active prophylaxis (e.g., fluconazole), we recommend the same approach for treatment of IA in the absence of prophylaxis. In a patient who develops breakthrough aspergillosis in the setting of mold-active prophylaxis (posaconazole, voriconazole, itraconazole, echinocandins), a “salvage” treatment plan individualized to patient circumstances and comorbidities is required. A typical approach would be to administer broad-spectrum antifungal therapy until the diagnosis is established and a response to treatment can be documented. For patients with apparent breakthrough aspergillosis on voriconazole, a lipid formulation of Amb (3–5 mg/kg/day) is recommended, especially in centers where mucormycosis is seen [522]. Knowledge of local epidemiology is essential for the selection of antifungal regimens for breakthrough aspergillosis.

In patients with breakthrough aspergillosis while on voriconazole prophylaxis, there are limited data suggesting that posaconazole retains its activity [573]. In patients with breakthrough aspergillosis while on posaconazole prophylaxis, some data support the use of an alternate triazole as salvage therapy, such as voriconazole or isavuconazole [256]. The benefits of combination antifungal therapy for breakthrough aspergillosis are unknown. If a decision is made to use combination therapy, we favor the initial use of a combination of antifungal agents from different classes than the antifungal the patient was initially receiving when the breakthrough aspergillosis was diagnosed.

Documentation of serum trough antifungal levels, especially for triazole antifungals, which may be prone to wide pharmacokinetic variability, can aid in the evaluation of patients with breakthrough aspergillosis. Several case series have reported that breakthrough aspergillosis in the setting of “therapeutically adequate” voriconazole exposures (recent trough >1 μg/mL) may favor the diagnosis of breakthrough mucormycosis over aspergillosis [218]. In some countries, breakthrough aspergillosis with multitriazole-resistant Aspergillus species has been described, but the prevalence of these strains in many centers in the United States is unknown [574]. The replacement of posaconazole solution with intravenous and extended-release tablets may reduce the frequency of extremely low serum concentrations. Further studies are needed to address whether TDM is helpful or necessary with the extended-release or intravenous formulations of posaconazole or for isavuconazole.

Diagnosis requires the early use of chest/sinus CT and Aspergillus GM, although CT can show atypical lesions [143] and serum GM is frequently negative or “low positive” in patients receiving mold-active agents preexposure. Although the yield of bronchoscopy in these patients might be low, it is recommended, as coinfections simulating breakthrough aspergillosis are not uncommon [575]. Furthermore, recent data indicate that the yield of GM in BAL is not affected by the presence of a mold-active agent [576]. In case there is growth of Aspergillus in a patient with breakthrough Aspergillus pneumonia, it would be prudent to document the susceptibility of the cultured isolate (using a reference method) because the patient will need secondary prophylaxis with a triazole antifungal after the initial treatment phase is completed.

VI. When Should Patients Be Treated Empirically?

74. Empiric antifungal therapy is recommended for high-risk patients with prolonged neutropenia who remain persistently febrile despite broad-spectrum antibiotic therapy. Antifungal options include a lipid formulation of Amb (strong recommendation; high-quality evidence), an echinocandin (caspofungin or micafungin) (strong recommendation; high-quality evidence), or voriconazole (strong recommendation; moderate-quality evidence).

75. Empiric antifungal therapy is not recommended for patients who are anticipated to have short durations of neutropenia (duration of neutropenia <10 days), unless other findings indicate a suspected IFI (strong recommendation; moderate-quality evidence).

76. The use of serum or BAL fungal biomarkers such as GM or (1 → 3)-β-D-glucan to guide antifungal therapy in asymptomatic or febrile high-risk patients (often referred to as preemptive or biomarker-driven antifungal therapy) can reduce unnecessary antifungal therapy. The preemptive approach can result in more documented cases of IPA without compromise in survival and can be used as an alternative to empiric antifungal therapy (strong recommendation; moderate-quality evidence).

77. Early initiation of antifungal therapy in patients with strongly suspected IPA is warranted while a diagnostic evaluation is conducted (strong recommendation; moderate-quality evidence).

78. Management of suspected or documented breakthrough IPA in the context of mold-active azole prophylaxis or empiric suppressive therapy is not defined by clinical trial data, but a switch to another drug class is suggested (weak recommendation; low-quality evidence).

Evidence Summary. This area has been reviewed in a related 2010 guideline from the IDSA [577]. Early reports from the National Cancer Institute and the EORTC underscored the...
importance of early initiation of therapy for treatment of IA and other IFIs [145, 175, 577–579]. These small randomized, nonplacebo, open-label trials demonstrated that high-risk neutropenic patients with persistent fever despite broad-spectrum antibacterial therapy have an increased risk of developing an overt IFI and empiric antifungal therapy reduced the frequency of overt IFIs. Although all AmB formulations are efficacious, nephrotoxicity and infusion reactions occur and the risk varies by formulation, with the greatest risk with AmB deoxycholate and the least risk with liposomal AmB. Liposomal AmB and itraconazole were as efficacious as and less toxic than AmB deoxycholate, and caspofungin was as efficacious as liposomal AmB in randomized trials [580–582]. Although the other echinocandins have been less well studied for this indication, the committee regards all the echinocandins as therapeutically equivalent. A randomized trial of voriconazole vs liposomal AmB did not fulfill criteria for noninferiority for the overall population but was comparable to liposomal AmB in the high-risk neutropenic population, with a significant reduction in the rate of emergent IA [583].

Empiric antifungal therapy appears to be most beneficial in patients with prolonged neutropenia (duration of neutropenia >10 days) in contrast to low-risk neutropenic patients [584]. One randomized trial [585] compared antifungal therapy initiated at the onset of first neutropenic fever with that initiated after 96 hours of fever in leukemic and allogeneic HSCT patients; there was no difference in rates of IFI. The initiation of antifungal therapy is generally recommended for persistent unexplained fever after 4–7 days with a broad-spectrum antibiotic regimen. In one trial, initiation at 4 days was associated with a trend to higher response rates and shorter time to defervescence than initiation at 8 days [586]. The use of empiric antifungal therapy still warrants a comprehensive approach to establishing a microbiological diagnosis where feasible.

Persistent fever has poor specificity for the diagnosis of an IFI, and empiric antifungal therapy may thus expose patients where antifungal treatment is not indicated. The use of noninvasive diagnostics to detect incipient IFIs either in asymptomatic at-risk patients or in patients with unexplained neutropenic fever is sometimes known as preemptive or biomarker-driven antifungal therapy; the latter is a logical alternative to empiric antifungal therapy, in that it targets a high-risk subpopulation on the basis of a surrogate marker of infection, such as abnormal CT findings or a positive result for GM antigen, (1→3)-β-D-glucan, or Aspergillus PCR where available commercially or as a research tool. Biomarkers have been evaluated in 2 ways: serial screening of asymptomatic high-risk patients [96, 587, 588] and guiding targeted antifungal therapy for a subset of persistently febrile patients [589, 590]. Because approximately 40% of patients receiving empiric antifungal therapy have pulmonary infiltrates, there is considerable overlap between the approaches of empiric and biomarker-targeted therapy. In a feasibility study, Maertens et al used serum GM and chest CT to detect IPA in patients with leukemia who received fluconazole prophylaxis [96]. This strategy reduced the use of empiric antifungal therapy and successfully treated cases of IPA, in which treatment often was initiated early, before onset of fever. Randomized trials have compared biomarker-driven strategies using serum GM [589, 590], Aspergillus PCR, or both [80] to trigger antifungal therapy vs symptom-driven empiric antifungal therapy in leukemia and HSCT. Different design issues such as the lack of standardization of antifyeast prophylaxis [80, 589], timing of biomarker screening (asymptomatic vs febrile patients), types of patients studied, duration of study, and inadequate sample size [588, 590] hamper generalizations. However, in general, these studies suggest that biomarker-driven strategies are associated with less unnecessary antifungal use without a compromise in overall survival. As would be expected by more intensive testing, more IFIs were generally seen, but without an increase in fungal-related mortality, presumably due to early initiation of antifungal therapy made possible by the intensive screening. One concern with the use of PCR assays for screening patients is the lack of commercial assays and technical challenges of different methodologies [591, 592]. Although some experts believe there is sufficient evidence to support the use of PCR assays [593], the committee does not recommend routine use of PCR assays outside the context of clinical trials or clinical research at this time. These various studies suggest that biomarker-driven antifungal therapy is an acceptable alternative to fever-driven empiric antifungal therapy in patients who are receiving antifyeast prophylaxis. Further study is needed to clarify which biomarker or combination of biomarkers is optimal, which risk group should be given antymold prophylaxis vs biomarker screening, and if routine screening in asymptomatic patients is preferable to screening only febrile patients. Data on biomarkers to guide preemptive therapy are limited for pediatric patients.

For persistently febrile neutropenic patients who may be receiving anti-Aspergillus prophylaxis, the causes of persistent fever are less likely to be of a fungal origin [594]. Careful evaluation for nonfungal causes, as well as the possibility of breakthrough IFIs that are resistant to the prophylactic regimen, should be considered in this patient population. Thus, routine initiation of empiric antifungal therapy in this context merits reevaluation.

Management of breakthrough IPA in the context of mold-activeazole prophylaxis is not defined by clinical trial data. The approach to such patients should be individualized on the basis of clinical criteria, including host immunosuppression, underlying disease, and site of infection, as well as consideration of antifungal dosing, therapeutic monitoring of drug levels, a switch to intravenous therapy, and/or a switch to another drug class.

There are other high-risk patients, such as those with refractory leukemia, those with solid tumors, other SOT recipients, those receiving corticosteroid therapy, those with liver failure, those with COPD with progressive infiltrates despite antibiotics,
and critically ill patients in whom empiric therapy may be warranted on a case-by-case basis.

**How Do Lung Transplant Recipients Differ From Other Immunosuppressed Patients in Management of Suspected Invasive Pulmonary Aspergillosis?**

**Recommendations.**

79. In lung transplant recipients not on antimold prophylaxis, we suggest preemptive therapy with an antimold antifungal for asymptomatic patients with *Aspergillus* colonization of the airways within 6 months of lung transplant or within 3 months of receiving immunosuppression augmentation for rejection *(weak recommendation; moderate-quality evidence).*

80. Six months after lung transplant and in the absence of recent immunosuppression augmentation for rejection, it may be prudent to withhold antifungal therapy for *Aspergillus* airway colonization *(ie, Aspergillus* respiratory cultures in the absence of clinical features that suggest disease, such as compatible symptoms, or bronchoscopic, histopathologic, and/or radiographic findings) *(weak recommendation; low-quality evidence).*

**Evidence Summary.** Many lung transplant centers routinely perform scheduled bronchoscopies with transbronchial biopsies and BAL. These surveillance bronchoscopies allow inspection for airway complications, rejection monitoring, and detection of microbial colonization *(bacteria, fungi, and/or viruses)* before the onset of overt infection. Between 20% and 46% of lung transplant recipients are colonized in the airway with *Aspergillus* spp at some point after transplant [595, 596]. The risk of IA is increased 11-fold in patients with *Aspergillus* colonization of the airways, and mortality rates are high [595]. Furthermore, *Aspergillus*-colonized patients have an increased risk of chronic lung allograft dysfunction due to bronchiolitis obliterans and death [596, 597]. At present, it is not known whether asymptomatic patients with *Aspergillus* colonization should be treated with antifungal agents. Given the high rate of *Aspergillus* disease among colonized patients, we suggest a course of antifungalazole therapy within 6 months of transplant. Preemptive antifungal therapy based on culture has been successfully used in clearing *Aspergillus* from the airway [598–600]. In asymptomatic patients who are colonized with *Aspergillus* after 6 months, we suggest a thorough physical exam, to rule out signs of disseminated aspergillosis, and a chest CT. We also suggest a sinus CT for patients with signs or symptoms of sinus disease. If screening is negative, clinicians should consider factors such as immunosuppression augmentation for rejection within the previous 3–4 months *(especially with alemtuzumab, thymoglobulin, or high-dose and prolonged duration of corticosteroids),* the presence of recent CMV disease or uncontrolled CMV infection, and the presence of an airway stent or airway abnormalities at the time of positive culture. If physical findings or imaging abnormalities are suggestive of aspergillosis, or any of the aforementioned factors are present, we suggest a course of 1–3 months of preemptive antifungal therapy and conversely, if negative, a watchful waiting approach without antifungal therapy.

**CHRONIC AND SAPROPHYTIC SYNDROMES OF ASPERGILLUS**

**VII. How Should Chronic Aspergillosis, Allergic Syndromes, or Noninvasive Syndromes Be Managed?**

**How Can Chronic Cavitary Pulmonary Aspergillosis Be Diagnosed and Treated?**

**Recommendations.**

81. The diagnosis of CCPA requires: *(i)* 3 months of chronic pulmonary symptoms or chronic illness or progressive radiologic radiographic abnormalities, with cavitation, pleural thickening, pericavitary infiltrates, and sometimes a fungal ball; *(ii)* *Aspergillus* IgG antibody elevated or other microbiological data; and *(iii)* no or minimal immunocompromise, usually with one or more underlying pulmonary disorders. The *Aspergillus* IgG antibody test is the most sensitive microbiological test *(strong recommendation; moderate-quality evidence).* Sputum *Aspergillus* PCR testing is more sensitive than culture *(weak recommendation; moderate-quality evidence).*

82. Patients with CCPA without pulmonary symptoms, weight loss, or significant fatigue, and those without major impairment of pulmonary function or gradual loss of pulmonary function may be observed without antifungal therapy and followed every 3–6 months *(weak recommendation; low-quality evidence).*

83. Patients with CCPA and either pulmonary or general symptoms or progressive loss of lung function or radiographic progression should be treated with a minimum of 6 months of antifungal therapy *(strong recommendation; low-quality evidence).*

84. Oral itraconazole and voriconazole are the preferred oral antifungal agents *(strong recommendation; high-quality evidence);* posaconazole is a useful third-line agent for those with adverse events or clinical failure *(strong recommendation; moderate-quality evidence).*

85. Hemoptysis may be managed with oral tranexamic acid *(weak recommendation; low-quality evidence)*, bronchial artery embolization *(strong recommendation; moderate-quality evidence),* or antifungal therapy to prevent recurrence *(strong recommendation; low-quality evidence).* Patients failing these measures may require surgical resection *(weak recommendation; moderate-quality evidence).*

86. In those who fail therapy, develop triazole resistance, and/or have adverse events, intravenous micafungin *(weak recommendation; low-quality evidence)*, caspofungin *(weak recommendation; low-quality evidence),* or AmB *(weak recommendation; low-quality evidence)* yield some responses. Treatment may need to be prolonged.

87. Surgical resection is an option for some patients with localized disease, unresponsive to medical therapy, including

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those with pan-azole-resistant *Aspergillus fumigatus* infection or persistent hemoptysis despite bronchial artery embolization *(strong recommendation; moderate-quality evidence)*. The outcomes from surgery are less favorable than those with single aspergilloma, and a careful risk assessment prior to surgical intervention is required.

88. In those with progressive disease, long-term, even lifelong antifungal therapy may be required to control disease *(weak recommendation; low-quality evidence)*, with continual monitoring for toxicity and resistance.

**Evidence Summary.** Chronic cavitary pulmonary aspergillosis is defined as one or more pulmonary cavities that may or may not contain solid or liquid material or a fungal ball, with a positive *Aspergillus* IgG antibody test or microbiological evidence implicating *Aspergillus* spp with significant pulmonary or systemic symptoms and overt radiographic progression (new cavities, increasing pericavity infiltrates, or increasing pleural thickening) over at least 3 months [601, 602]. It is one manifestation of CPA [603, 604], single aspergilloma and *Aspergillus* nodule being others, and chronic fibrosing pulmonary aspergillosis (CFPA) an end-stage complication of CCPA [601].

CCPA complicates other pulmonary diseases, including tuberculosis, nontuberculous mycobacterial infection (both of which may occur concurrently, although they are usually antecedent), fibrocystic sarcoidosis, ABPA, asthma, prior pneumonia, pneumothorax or lobectomy, COPD, ankylosing spondylitis and rheumatoid arthritis, hyper IgE syndrome, and congenital bullous disease [603]. Patients with mild or moderate immunosuppression may develop what was termed chronic necrotizing pulmonary aspergillosis, but is better considered subacute IPA [602, 605]. Patients with CCPA and CFPA have numerous underlying immunological defects, probably mostly genetic [606, 607]. As these defects and their pulmonary damage from prior disease are irreversible, long-term suppressive antifungal therapy is the default mode of treatment, although patients with mild cases may be able to stop therapy, and others may be forced to stop if medication intolerance or side effects develop.

Patients present with primarily pulmonary or general symptoms, or both. Response to therapy should be assessed against each person’s symptom complex. Hemoptyisis, shortness of breath, and productive cough are usual, whereas fever and chest pain are uncommon. Weight loss and fatigue are the most common general symptoms and may be profound [581]. Patients are often mistakenly thought to have tuberculosis.

If a fungal ball is present on chest imaging, the diagnosis is almost certainly CPA, either a single aspergilloma or CCPA. Confirmation is with *Aspergillus* IgG testing [608, 609], and the distinction between these 2 entities is made on the basis of symptomology and radiologic appearance. However, the majority of CCPA patients do not have a fungal ball but either multiple empty cavities, or cavities with an irregular (bumpy) internal wall with associated pleural thickening, and pericavitary infiltrates. Mats of hyphae within the cavity become dislodged and eventually coalesce to form a fungal ball [610]. Diagnosis of CCPA is with *Aspergillus* IgG testing, excluding coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. Occasionally patients present with mycobacterial infection at the same time as CCPA. Rarely, a necrotizing lung cancer can be infected with *Aspergillus*, giving rise to a similar radiographic appearance. Multiple sputa (expectorated or induced) increase the probability of positive microscopy or fungal culture providing mycological support for the diagnosis. A majority of patients have negative sputum cultures; *Aspergillus* PCR is more sensitive [85]. If culture is positive and the patient has been receiving an azole, the isolate should be submitted for susceptibility testing. Hyphae may be seen on microscopy, and the culture is negative. Biopsy of the wall of a cavity in CCPA yields chronic inflammatory cells and fibrosis, sometimes with granulomata; hyphae consistent with *Aspergillus* spp are usually seen adjacent to the cavity wall, but are not truly invasive. Percutaneous aspiration of a cavity with a positive *Aspergillus* culture is an alternative means of establishing the diagnosis. More than 50% of patients have an increased total and *Aspergillus*-specific IgE titer; eosinophilia may be present [581].

The objectives of therapy of CCPA are to (1) improve symptoms; (2) reduce hemoptysis; (3) reduce progressive lung fibrosis, in particular preventing CFPA, which can occur rapidly; and (4) prolong survival. Oral therapy with itraconazole or voriconazole is a first-line therapy, depending on tolerance and affordability [602, 611–614]. Resistance to itraconazole during therapy has been reported more frequently than with voriconazole, so in patients with a large fungal load, voriconazole may be preferable, although clinical evidence to support this approach is lacking. Posaconazole is currently third-line therapy, because of the general lack of data and cost over long periods [615]. Treatment should be continued for a minimum of 6 months, and if well tolerated with a good response, may be continued for years [616]. Monitoring of therapy is critical and should be undertaken by physicians experienced with antifungal therapy. Toxicity may develop with long-term triazole therapy as previously discussed.

Occasional patients have a marked increase in shortness of breath shortly after starting antifungal therapy, which may respond to a short course of corticosteroids. Otherwise, all steroids should be avoided in CCPA, unless the patient is receiving adequate antifungal therapy and/or requires them for underlying disease, such as those with rheumatoid arthritis. Inhaled corticosteroids should be stopped in those with COPD and reduced in those with asthma, if possible.

Hemoptyisis can usually be controlled with oral tranexamic acid [617,618]. If hemoptyisis is significant, bronchial artery embolization is recommended, and should be performed by an experienced interventional radiologist [619–622]. It may be necessary to embolize abnormal vessels arising from the...
internal mammary, subclavian, and lateral thoracic arteries as well. Abnormal vessels arising close to the origin of both spinal and vertebral arteries should not be embolized. Recurrence of hemoptysis is common if antifungal therapy is not given and optimized, and may be a sign of antifungal failure.

Standard monitoring includes assessing radiographic change (every 3–12 months), preferably with low-dose CT without contrast or chest radiograph, inflammatory markers, Aspergillus IgG titers, and annual pulmonary function tests. Failure of therapy can be difficult to determine, but is based on a deteriorating clinical status, especially a new productive cough and/or weight loss, new or continuing hemoptysis, radiographic progression, or worsening respiratory function. Other causes of weight loss should be excluded, including celiac disease. Concurrent infection, including nontuberculous mycobacterial infection, is important to exclude, usually with multiple sputum cultures and occasionally bronchoscopy. Antifungal blood concentrations should be checked. Azole resistance should be sought.

On therapy, azole resistance may occur. Susceptibility testing of isolates obtained in patients on therapy may be extremely useful to guide therapeutic choices, and it is recommended that clinical laboratories not discard A. fumigatus isolates for 3 months, to allow clinicians to determine if patients are failing at their next outpatient appointment. Some isolates are only resistant to itraconazole or voriconazole, some to itraconazole and posaconazole, and others pan-azole resistant.

In patients who fail, are intolerant, or develop azole resistance or a combination of these circumstances, the clinician may need to resort to intravenous therapy. In addition, acutely ill patients may require an initial course of intravenous antifungal therapy. Both AmB deoxycholate and liposomal AmB and micafungin have been extensively used for CCPA, with modest response rates [193, 601, 623, 624]. In addition to its anti-Aspergillus activity, liposomal AmB has many TH1 upregulating effects, which are generally deficient in patients with CPA, and may contribute to a clinical response. It is better tolerated than AmB deoxycholate, but both may result in treatment-limiting renal dysfunction. Micafungin has been examined in the treatment of CCPA and found to be effective [193]. There are few data for caspofungin and none for ABLC, ABCD, anidulafungin, or isavuconazole [625].

A common cause of death in CCPA, and possibly a trigger for additional lung fibrosis, is intercurrent bacterial infection. Common infections include Streptococcus pneumoniae, Haemophilus influenzae, and occasionally Pseudomonas aeruginosa and Staphylococcus aureus. Pneumococcal and Haemophilus immunization may reduce infections. Some CCPA patients have overt hypogammaglobulinemia. Pseudomonas aeruginosa eradication or control with high-dose oral ciprofloxacin, intravenous therapy, or inhaled colistin or tobramycin is also recommended for these patients. Minimizing bacterial infections allows simpler decision making if patients deteriorate on antifungal therapy. Occasionally surgical resection is necessary for CCPA, typically for intractable hemoptysis, destroyed lung (CFPA) with poor quality of life, or azole resistance. Patients need to be fit enough (see section on simple aspergilloma for considerations, recommendations 89–91 below). A conventional lobectomy [626–629], video-assisted thoracic surgical procedure [630–632], or caverno-stomy with space reduction using a limited thoracoplasty may be required. The outcomes from surgery are acceptable, but both the risk of death and complications such as pleural space infection is higher in CCPA than for single aspergilloma. Relapse rates up to 25% are documented [633], which makes decision making difficult, especially in the knowledge that subtle immune deficits will persist after surgery. All CCPA patients undergoing resection surgery require active follow-up.

What Are the Management Options for an Aspergillus Fungal Ball of the Lung (Aspergilloma)?

Recommendations.

89. Asymptomatic patients with a single aspergilloma and no progression of the cavity size over 6–24 months should continue to be observed (strong recommendation; moderate-quality evidence).

90. Patients with symptoms, especially significant hemoptysis, with a single aspergilloma, should have it resected, assuming that there are no contraindications (strong recommendation; moderate-quality evidence).

91. Peri-/postoperative antifungal therapy is not routinely required, but if the risk of surgical spillage of the aspergilloma is moderate (related to location and morphology of the cavity), antifungal therapy with voriconazole (or another mold-active azole) or an echinocandin is suggested to prevent Aspergillus empyema (weak recommendation; low-quality evidence).

Evidence Summary. Single aspergilloma, previously often referred to as simple aspergilloma, may occur with CPA so that the evidence supporting management of a fungal ball due to Aspergillus should be considered in the context of CPA in that situation. These patients may be asymptomatic, present with hemoptysis, shortness of breath, or cough. “Single uncomplicated aspergilloma” is defined as a single pulmonary cavity containing a fungal ball in a nonimmunocompromised patient with microbiological or serological evidence of Aspergillus spp with minimal or no symptoms and no radiographic progression over at least 3 months [603]. An aspergilloma is described radiographically as an approximately spherical shadow with surrounding air, also called a fungal ball, in a pulmonary cavity, with evidence that Aspergillus spp is present in the material. Aspergillus fumigatus is the usual cause. Fungal balls of the lung may rarely be caused by other fungi, such as A. flavus, or other molds like Scedosporium spp. Single aspergilloma represents a manifestation of CPA with a favorable prognosis, and is usually not rapidly progressive so that management decisions are not usually acute, unless severe hemoptysis has occurred.

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The optimal management of a single aspergilloma is surgical resection, either by conventional lobectomy [626–629] or a video-assisted thoracic surgical procedure [630–632]. However, surgical planning requires the following considerations [633]: Respiratory reserve should be adequate, as based on FEV1 and especially exercise tolerance; patients who are taking antithrombotic medication should be able to have their medication suspended for at least 4 days, and preferably longer; and preoperative bronchial artery embolization allows more time for surgical assessment and planning, but has little impact on postoperative bleeding [634].

An evaluation of risk of spillage at surgery needs to be made based on the difficulty of separating the cavity containing the fungal ball from the chest wall [633]. Extrapleural dissection over the apex may be required but may be followed by bleeding from collateral arterial vessels crossing the pleura from the chest wall. If it is likely or possible that the cavity will be opened during the surgical procedure, leading to pleural contamination, then antifungal therapy with voriconazole (or another mold-active azole) or micafungin (or another echinocandin) should be given, starting preoperatively with voriconazole or perioperatively for micafungin. Use of voriconazole may alter the preferred anesthetic approach, as prolongation of benzodiazepine sedation is problematic with voriconazole. If no spillage occurs during surgery, antifungal therapy can be stopped. If spillage does occur, some clinicians advise washing out the pleural space with AmB or antifungal topical disinfectant such as tauroloidine 2%, although evidence to support either approach is minimal. Antifungal therapy should be continued postoperatively and an infectious diseases physician involved in care to monitor therapy and determine the length of treatment. If there is no evidence of infection following spillage during surgery, a minimum of 4 weeks of therapy is typically recommended.

Patients with 2 separate aspergillomas [635] may be considered for bilobar resections or pneumonectomy depending on locations and their respiratory reserve. If respiratory reserve does not allow resection, then medical therapy alone can be offered to minimize recurrent hemoptysis.

Relapse following resection does occur; 25% of patients in one CPA series had relapse of infection including some aspergilloma cases [633]. Most surgical series do not provide long-term follow-up. For patients with spillage, active follow-up (typically at 4- to 6-month intervals) assessing radiographic change, inflammatory markers, and Aspergillus IgG titers for 3 years is advised. If spillage has not occurred, then active follow-up is not advised, unless there is ongoing active pulmonary disease.

**ALLERGIC SYNDROMES OF ASPERGILLUS**

**How Is Allergic Bronchopulmonary Aspergillosis Identified and Managed in Patients With Asthma and Cystic Fibrosis?**

**Recommendations.**

92. Elevated Aspergillus IgE and total IgE are recommended to establish the diagnosis and are useful for screening (strong recommendation; high-quality evidence).

93. We suggest treating symptomatic asthmatic patients with bronchiectasis or mucoid impaction, despite oral or inhaled corticosteroid therapy, with oral itraconazole therapy with TDM (weak recommendation; low-quality evidence).

94. In CF patients with frequent exacerbations and/or falling FEV1, we suggest treating with oral itraconazole to minimize corticosteroid use with TDM, and consideration of other mold-active azole therapy if therapeutic levels cannot be achieved (weak recommendation; low-quality evidence).

**Evidence Summary.** ABPA complicates asthma and CF [83, 509, 636, 637]. In asthmatic patients it presents as poorly controlled asthma, “pneumonia” that represents mucoid impaction, persistent eosinophilia, and bronchiectasis or with CPA and lung fibrosis, the latter both late complications. Some patients are asymptomatic. In CF, it tends to present with difficult-to-control exacerbations, responsive to corticosteroids, although mucoid impaction is described.

The key criterion for diagnosis is an elevated Aspergillus-specific IgE, supported by an elevated total IgE, detectable Aspergillus-specific IgG, eosinophilia, and positive skin prick tests for Aspergillus (where available) [83, 637, 638]. Uncommonly, other fungi can produce a similar clinical picture. Patients with severe asthma, not fulfilling the criteria for ABPA, may have severe asthma with fungal sensitization, also responsive to antifungal therapy [636, 639]. There are some areas of overlap with these syndromes, and some experts consider all patients with these diagnoses under the term “fungal asthma.”

Screening for ABPA in patients with asthma and CF, probably on an annual basis, is recommended, particularly if patients are symptomatic with frequent asthma exacerbations. Asthma admitted to hospital, including intensive care, should be evaluated for fungal asthma [640].

The optimal management of ABPA in both asthma and CF depends on patient response, severity of disease and exacerbation frequency, drug adverse effects, and the emergence of antifungal resistance [637, 639, 641]. Treatment involves a 2-pronged approach: controlling the immune response (which is what makes the patient symptomatic), and decreasing the burden of organisms so that there is less of an immune response.

Oral corticosteroids reduce the inflammatory response in acute exacerbations of ABPA, but are associated with many adverse effects, some short-term, others long-term, such as diabetes in CF. Relapse is frequent after discontinuation. Inhaled corticosteroids control asthma in some patients. Anti-IgE (omalizumab) therapy might be helpful, but data are scant [642]. Cough and sputum production may be reduced by azithromycin or antifungal therapy or both. Nebulized hypertonic saline helps some patients clear sputum [643]. Prevention of exacerbations may be affected by pneumococcal and/or Haemophilus vaccination. Avoidance of substantial fungal exposures, as in composting, farming, and house renovation may also prevent exacerbations.
Antifungal therapy is helpful for many patients [639, 641, 644, 645]. Itraconazole is currently the first-line agent for symptomatic patients, CF patients with low FEV1, or those with complications such as bronchiectasis, mucoid impaction, or CPA. Itraconazole solution is preferred in CF patients because of poor absorption of capsules. Patients who fail itraconazole, or are intolerant to itraconazole, may respond to voriconazole, posaconazole, or inhaled AmB [646]. Relapse after improvement during antifungal therapy is common; long-term suppressive therapy may be necessary. Interactions of itraconazole with some inhaled corticosteroids can precipitate Cushing’s syndrome, so that reduction in inhaled steroid dose or a switch to ciclesonide may be useful for those patients. Triazole antifungal resistance has been documented in some geographic regions, so susceptibility testing may be valuable in areas where epidemiologic data indicate environmental resistance or isolates are cultured from patients on antifungal therapy.

**What Is the Medical Management of Allergic Fungal Rhinosinusitis Caused by Aspergillus Species? Recommendations.**

95. We recommend establishing the diagnosis of AFRS in patients with nasal polyposis and thick eosinophilic mucin by visualizing hyphae in mucus, which is supported by a positive Aspergillus IgE serum assay or skin-prick test (where available) *(strong recommendation; moderate-quality evidence).*

96. We recommend polypectomy and sinus washout as the optimal means of symptom control and inducing remission; however, relapse is frequent *(strong recommendation; moderate-quality evidence).*

97. We recommend the use of topical nasal steroids to reduce symptoms and increase time to relapse, especially if given after surgery *(strong recommendation; moderate-quality evidence).*

98. We suggest oral antifungal therapy using mold-active triazoles for refractory infection and/or rapidly relapsing disease, although this approach is only partially effective *(weak recommendation; low-quality evidence).*

**Evidence Summary.** AFRS is a small subset (<10%) of chronic rhinosinusitis occurring in adults and children [647]. AFRS is characterized by eosinophilic mucin and fungal hyphae in the paranasal sinuses, often associated with immediate hyper-sensitivity to various fungi. Fungal culture of nasal secretions is usually unhelpful as it reflects airborne fungi, so clarity about the specific fungus involved is usually inferential or unclear. The disease is commonly associated with nasal polyposis, and sometimes with ABPA [648]. Local complications of AFRS include ophthalmic involvement with oculomotor palsy, bony erosion, and cavernous venous thrombosis [649]. The disease course is long, with many patients having extended periods of remission with exacerbations often following viral and/or bacterial infections. Short courses of modest doses of oral corticosteroids may shrink polyps and allow drainage, but relapse is common, and not usually prevented by topical steroids. Surgical removal of polyps and mucus is the most important aspect of management, with postoperative systemic or topical nasal steroids recommended to reduce the time to relapse [650, 651]. Saline washes are often helpful. Omaluzimab has been reported to be helpful in studies of severe asthma with associated chronic rhinitis [652]. Oral antifungal therapy for AFRS, usually itraconazole, is helpful for refractory disease and to prevent relapse in patients with frequent recurrences [653–655].

**FUTURE DIRECTIONS**

There are many unanswered and unresolved epidemiological, laboratory, and clinical questions that need to be addressed and understood in the diagnosis, treatment, and prevention of aspergillosis. Better diagnostic tests and improved understanding of the optimal use of current methods are needed both to facilitate more accurate identification of patients with IA and to permit earlier initiation of therapy. The availability of more active and better tolerated antifungal agents has significantly improved therapy of patients at risk for serious *Aspergillus* infections, but even with optimal antifungal therapy the mortality rate remains high; therefore, further development of new antifungal agents is greatly needed. Critical gaps in knowledge remain regarding management of these infections including the optimal utility of combination therapy, tools for early detection of these infections, evaluation of response, therapy for patients with breakthrough or refractory infection, and the population of patients for whom prophylaxis would be most beneficial.

**Notes**

**Dedication.** The panel dedicates these guidelines to the memory of our dear friend Susan Hadley, MD, a core member of the Mycoses Study Group, caring physician, and wonderful colleague.

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recommendation of consideration). The reader of these guidelines should be mindful of this when the list of disclosures is reviewed. For activities outside the submitted work, T. F. P. received research grant support to the University of Texas Health Science Center San Antonio from Astellas, Merck, and Revolution Medicines and has been a consultant for or served on advisory boards to Amplyx, Astellas, Durata, Cidara Therapeutics, Gilead, Merck, Pfizer, Revolution Medicines, Scynexis, Toyama, Vical, and Viamet. For activities outside the submitted work, G. R. T. received research support to the University of California, Davis from Astellas, Merck, Pfizer, and Scynexis, and has been a consultant for Astellas. For activities outside the submitted work, D. W. D. holds Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company and in Novocyt, which markets the Myconostica real-time molecular assays; has current grant support from the National Institute of Health Research, Medical Research Council, Global Action Fund for Fungal Infections, and the Fungal Infection Trust; serves as a consultant to Astellas, Sigma Tau, Basilea, and Pulmocide; and has received honoraria from Astellas, Dynamiker, Gilead, Merck, and Pfizer. For activities outside the submitted work, J. A. F. served on scientific advisory boards for Revolution Medicines. For activities outside the submitted work, S. H. served as a consultant to Merck. For activities outside the submitted work, R. H. served on advisory boards for Astellas, Basilea, Gilead, and Pfizer and received research grants from Alsafe contre le Cancer and Pfizer. For activities outside the submitted work, D. P. K. served as a consultant to Astellas, Merck, and Pfizer; received research support from Astellas, Merck, Pfizer, and T2 Biosystems; and received honoraria from Astellas, Merck, Pfizer, T2 Biosystems, Gilead, and F2G, Inc. For activities outside the submitted work, K. A. M. received honoraria from Amplyx, Astellas, Cidara, F2G, Merck, Pfizer, Revolution Medicines, and Vical, and has a patent US No. 13/511 264 licensed. For activities outside the submitted work, V. A. M. served as a consultant for Celgene, Amgen, GSK, Merck, and Astellas, and served on the speaker’s bureaus for Genentech and Celgene. For activities outside the submitted work, M. H. N. received research grants from Astellas, Pfizer, Merck, ViraCor, and the National Institutes of Health (National Institute of Allergy and Infectious Diseases). For activities outside the submitted work, B. H. S. served on advisory boards for Merck and Astellas, and has contracts for laboratory research from Astellas and Assembly Biosciences. For activities outside the submitted work, W. J. S. served on scientific advisory boards from Merck and received research grants to Duke University from Merck and Astellas. For activities outside the submitted work, T. J. W. served as a consultant or scientific advisor for Astellas, Novartis, Pfizer, and Methylened and received research grants to Well Cornell Medical Center from Astellas, Merck, and Novartis. For activities outside the submitted work, J. R. W. served as consultant/scientific advisor for Gilead, Astellas, Pfizer, Merck, and Vical. For activities outside the submitted work, J. H. Y. received research support to the University of Minnesota from Astellas, Merck, and Pfizer. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology

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The burden of human disease related to medically important fungal pathogens is substantial. An improved understanding of antifungal pharmacology and antifungal pharmacokinetics–pharmacodynamics has resulted in therapeutic drug monitoring (TDM) becoming a valuable adjunct to the routine administration of some antifungal agents. TDM may increase the probability of a successful outcome, prevent drug-related toxicity and potentially prevent the emergence of antifungal drug resistance. Much of the evidence that supports TDM is circumstantial. This document reviews the available literature and provides a series of recommendations for TDM of antifungal agents.

Keywords: triazoles, fungal pathogens, fungal diseases

Introduction

Fungal diseases exact a significant toll on human health and compromise clinical outcomes of patients. There has been a progressive understanding of antifungal pharmacology and characterization of antifungal drug exposure–response relationships. There is increased recognition that therapeutic drug monitoring (TDM) of antifungal agents is important in a wide range of clinical settings.1–4 This document reviews the available literature and provides recommendations for antifungal TDM.

The three main classes of antifungal agents in clinical use are the polyenes, the triazoles and the echinocandins. The polyenes have a broad spectrum of activity that includes yeasts and moulds. For the triazoles, susceptibility is more variable and depends on the specific agent. Fluconazole has no activity against Aspergillus spp. and the mucoraceous moulds, while voriconazole lacks activity against the mucoraceous moulds. Posaconazole has the broadest spectrum of activity for all the triazoles, including activity against Aspergillus spp. and the mucoraceous moulds. The echinocandins are active against most medically important species of Aspergillus and Candida, but lack activity against Cryptococcus, Fusarium and the mucoraceous moulds. The key pharmacokinetic properties of each agent are summarized in Table 1 (available as Supplementary data at JAC Online).

Patients at risk of systemic fungal infections are varied and include those with neutropenia (caused by haematological malignancy or chemotherapy), bone marrow transplant recipients, solid organ transplant recipients and a range of critically ill patients. Other patient groups with more subtle immune dysfunction are also at heightened risk, including diabetic patients with poor glycaemic control and patients with chronic obstructive pulmonary disease receiving high-dose inhaled corticosteroids.

Methods

References for these guidelines were identified through searches of PubMed, Embase and Medline by use of the search terms ‘TDM’, ‘therapeutic drug monitoring’, ‘drug monitoring’, ‘drug concentrations’, ‘tissue concentrations’ and ‘serum levels’ and each term combined with the name of the antifungals: flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, amphotericin B, caspofungin, micafungin and anidulafungin. References were retrieved and collated. Secondary references embedded in papers that were not identified in the original search were retrieved and reviewed. Following a systematic review of the literature, a series of recommendations were developed. The GRADE system (Grades of Recommendations Assessment, Development and Evaluation5) was used to assess the strength of evidence for each recommendation (the GRADE system is summarized in Table 1). The GRADE system uses either ‘strong’ or ‘weak’ recommendations and generally high or moderate levels of evidence resulted in a...
strong recommendation, with low or very low quality evidence resulting in a weak recommendation. In areas where the quality of evidence was very variable or there was limited evidence, the recommendation was based not only on the available literature but also on the clinical judgement and experience of the authors. The recommendations for TDM for each compound are summarized in Tables 5–8. The evidence base that supports each recommendation is discussed in turn.

Overview

The importance of antifungal TDM is increasingly recognized. Nevertheless, there are no definitive data (and there are never likely to be any) from large clinical trials that address its use in every clinical context. Most evidence supporting TDM is circumstantial. Antifungal TDM is potentially expensive and time consuming, and the ultimate impact on clinical care may be difficult to estimate. There is debate as to whether TDM should be routine (as it is for some antimicrobial compounds, such as aminoglycosides) or used more selectively. This balance depends to some extent on the clinician, the patient case mix, the severity of infection, cost, and access to a TDM service. The indications for potentially recommending TDM for antifungal agents are summarized in Table 2.

There is an increased interest in the use of personalized medicines—TDM is completely consistent with this concept. Clinical input and judgement remain central to the process of TDM.Clinicians frequently forget that therapeutic concentration ranges cited by reference laboratories are derived from populations of patients. A therapeutic target that is appropriate for one patient may not necessarily be satisfactory for another. Therefore, TDM requires continuous clinical input to ensure appropriate targets are chosen rather than using a ‘one size fits all’ approach. The clinical circumstances that may favour the use of TDM are summarized in Table 3. The optimal frequency of TDM for patients on long-term antifungal therapy is unknown, but will largely depend upon clinical judgement. Once target concentrations have been achieved, consideration of the circumstances described in Table 3 (e.g. compliance, changing pharmacokinetics) should guide the frequency with which repeat TDM measurements are made, as well as the context in which the drug is being used.

Several methods have been used for measuring serum concentrations of antifungal agents, including bioassay, HPLC and mass spectrometry. Advantages, disadvantages and examples of each are summarized in Table 4. A key requirement for any TDM service is participation in a quality control programme and an international scheme is available for the triazole antifungals, whilst the UK National External Quality Assessment Service (NEQAS) runs a scheme for the triazoles and flucytosine in the UK. A further consideration is the turn-around time. While it may be ideal to have assays performed on site, the cost of developing and running

### Table 1. Quality of evidence and definitions according to the GRADE system

<table>
<thead>
<tr>
<th>Quality of evidence</th>
<th>Basis of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>High quality</td>
<td>further research is very unlikely to change our</td>
</tr>
<tr>
<td></td>
<td>confidence in the estimate of effect</td>
</tr>
<tr>
<td>Moderate quality</td>
<td>further research is likely to have an important impact on</td>
</tr>
<tr>
<td></td>
<td>our confidence in the estimate of effect and may change the</td>
</tr>
<tr>
<td>Low quality</td>
<td>further research is very likely to have an important</td>
</tr>
<tr>
<td></td>
<td>impact on our confidence in the estimate of effect and is</td>
</tr>
<tr>
<td></td>
<td>likely to change the estimate</td>
</tr>
<tr>
<td>Very low quality</td>
<td>any estimate of effect is very uncertain</td>
</tr>
</tbody>
</table>

### Table 2. Overall summary of the need for therapeutic drug monitoring when using antifungal agents (see individual tables for detailed recommendations in specific indications)

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>GRADE quality of evidence and strength of recommendation</th>
<th>Prophylaxis</th>
<th>Treatment</th>
<th>Toxicity</th>
<th>Table with specific details</th>
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</thead>
<tbody>
<tr>
<td>Itraconazole</td>
<td>evidence quality recommendation</td>
<td>moderate</td>
<td>moderate</td>
<td>moderate</td>
<td>Table 5</td>
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<tr>
<td>Voriconazole</td>
<td>evidence quality recommendation</td>
<td>strong</td>
<td>strong</td>
<td>weak</td>
<td>Table 6</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>evidence quality recommendation</td>
<td>moderate</td>
<td>strong</td>
<td>high</td>
<td>Table 7</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>evidence quality recommendation</td>
<td>high</td>
<td>strong</td>
<td>strong</td>
<td>see text</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>evidence quality recommendation</td>
<td>strong against</td>
<td>low</td>
<td>strong</td>
<td>Table 8</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>evidence quality recommendation</td>
<td>high</td>
<td>weak</td>
<td>high</td>
<td>see text</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>evidence quality recommendation</td>
<td>high</td>
<td>strong</td>
<td>high</td>
<td>see text</td>
</tr>
</tbody>
</table>

NA, not applicable.
assays may mean that many TDM services are only available in specialist centres. Commercially available assays are now available from at least two manufacturers (Recipe and Chromsystems), removing the need to develop in-house assays that could facilitate the implementation of TDM services in non-specialist centres where HPLC equipment is available.

Antifungal TDM

Antifungal TDM is generally indicated for the mould-active triazoles (itraconazole, voriconazole and posaconazole) and the nucleotide flucytosine (5-fluorocytosine). There may be limited clinical circumstances in which TDM of fluconazole is warranted (e.g. critically ill patients on haemofiltration), but there is inadequate evidence to recommend the routine use of TDM for this agent. There is no evidence or indication at the current time to support the routine use of TDM for polyenes (amphotericin B deoxycholate, liposomal amphotericin B and amphotericin B lipid complex) or the echinocandins (micafungin, caspofungin and anidulafungin). Nevertheless, a better understanding of antifungal exposure–response relationships may mean that TDM becomes an important adjunct to the routine administration of these compounds in the future.

### Fluconazole

#### Introduction

Fluconazole is a triazole antifungal that is active against most species of Candida (with the notable exceptions of C. krusei and C. glabrata—the latter often exhibits reduced susceptibility or overt resistance to fluconazole with MICs $\geq 32$ mg/L). Fluconazole is also active against Cryptococcus neoformans and various dimorphic fungi. Fluconazole is available as capsules, an oral suspension and an intravenous (iv) preparation. However, for systemic infections it is usually 400–800 mg/day. Higher dosages (1200–2000 mg/day) have been used for cryptococcal meningitis.

### Table 3. Clinical circumstances that may favour the use of TDM

<table>
<thead>
<tr>
<th>Context</th>
<th>Example</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic variability</td>
<td>children, neonates, elderly, obese, organ dysfunction, critical illness haemodialysis, haemofiltration, extracorporeal membrane oxygenation, cardiopulmonary bypass</td>
<td>pharmacokinetics of many antifungal agents very poorly defined in special populations</td>
</tr>
<tr>
<td>Changing pharmacokinetics</td>
<td>physiological instability, critical illness, diarrhoea, iv-to-oral switch</td>
<td>drug–drug interactions well defined and documented for many antifungal compounds</td>
</tr>
<tr>
<td>Interacting drugs</td>
<td>antacids, histamine antagonists, proton pump inhibitors and itraconazole capsules; agents known to decrease concentrations of triazoles</td>
<td>compliance may be a significant issue for longer-term consolidation therapy or secondary prophylaxis</td>
</tr>
<tr>
<td>Compliance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor prognosis disease</td>
<td>extensive or bulky infection, lesions contiguous with critical structures (mediastinum), CNS disease; multifocal or disseminated infection</td>
<td></td>
</tr>
<tr>
<td>Persistent and/or significant underlying immunological defects</td>
<td>prophylaxis versus established disease</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Advantages, disadvantages and examples of methods for determining drug levels in serum

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioassay</td>
<td>cheap; simple to perform</td>
<td>subject to interference from other drugs, including other antifungals; may measure combined activity of parent and metabolites (e.g. itraconazole)</td>
<td>32,132</td>
</tr>
<tr>
<td>HPLC with ultraviolet fluorescence detection</td>
<td>technology widely available; commercially available assays; can quantify multiple drugs in single sample</td>
<td>subject to interference from miscellaneous substances; run times maybe slow</td>
<td>133–135</td>
</tr>
<tr>
<td>Liquid chromatography–mass spectrometry</td>
<td>very sensitive and specific; can quantify multiple drugs in single sample</td>
<td>expensive; not widely available</td>
<td>136–139</td>
</tr>
</tbody>
</table>
Fluconazole is highly orally bioavailable and has linear pharmacokinetics. Most active drug is excreted renally, and downward dose adjustment is required for patients with renal failure. TDM of fluconazole is not routinely required. Nevertheless, there is increasing information related to drug exposure–response relationships. An AUC:MIC ratio of ~100 is associated with improved clinical outcomes (when the MIC is tested using EUCAST methodology). The measurement of fluconazole concentrations may be indicated in rare circumstances (e.g. CNS disease, unstable patient receiving renal supportive care, treatment of an organism with a high MIC). In this case, there is some uncertainty related to an appropriate target. One potential solution is to collect several samples throughout the dosing interval to estimate an AUC, and thereby an AUC:MIC. Sampling at 1, 4 and 24 h would enable a reasonable estimate of the AUC in the majority of patients. Dosages can be adjusted to ensure an AUC:MIC ratio of >100 is achieved.

See Table 2 for recommendations for TDM for fluconazole.

Itraconazole

Introduction

Itraconazole is a triazole antifungal with broad-spectrum antifungal activity. It is active against the commonest medically important fungal pathogens, such as Candida spp., C. neoformans and Aspergillus spp. Current formulations include capsules, an oral solution and an iv preparation; the last two are formulated with hydroxypropyl-β-cyclodextrin. The iv formulation is no longer available in the USA. A wide range of generic formulations are available in countries outside the European Union, and pharmacokinetics may differ significantly from the original formulations developed by Janssen Pharmaceuticals (Sporonox).

Itraconazole is used for the treatment of oral and oesophageal candidiasis, prevention of fungal infections in patients with profound and prolonged neutropenia, and treatment of invasive aspergillosis and cryptococcosis in patients who are refractory and unstable patient receiving renal supportive care, treatment of an organism with a high MIC. In this case, there is some uncertainty related to an appropriate target. One potential solution is to collect several samples throughout the dosing interval to estimate an AUC, and thereby an AUC:MIC. Sampling at 1, 4 and 24 h would enable a reasonable estimate of the AUC in the majority of patients. Dosages can be adjusted to ensure an AUC:MIC ratio of >100 is achieved.

See Table 2 for recommendations for TDM for fluconazole.

Recommendation 1: TDM should be performed in the majority of patients receiving itraconazole

The evidence for the potential clinical benefits of TDM for patients receiving itraconazole is strong, but largely circumstantial. TDM should be considered in the majority of patients receiving itraconazole for both invasive and allergic disease on the basis of: (i) considerable inherent pharmacokinetic variability, a portion of which is due to variable oral bioavailability that is affected by food intake and gastric pH; (ii) clinical and experimental evidence suggesting clinically relevant drug exposure–response relationships; (iii) potential problems with compliance, especially with use of the oral solution, which is unpalatable; and (iv) clinical evidence for drug exposure–toxicity relationships.

The strongest evidence to support TDM of itraconazole is for the prevention of invasive fungal infections in profoundly immunocompromised patients. Many early clinical studies analysing the efficacy of itraconazole were inconclusive, predominantly because they were underpowered. A meta-analysis of these studies suggests that higher itraconazole serum concentrations are protective against invasive fungal infections and decrease mortality. In addition, an early study of itraconazole for primary treatment of invasive aspergillosis also suggests patients with serum concentrations >8 mg/L (measured using bioassay) tend to have better clinical outcomes.

Recommendation 2: A lower target concentration for TDM is a trough of >0.5–1 mg/L measured using HPLC or mass spectrometry

Breakthrough infections are more common in neutropenic patients with trough itraconazole concentrations of <0.25–0.5 mg/L. Furthermore, mortality is significantly higher in patients with concentrations <0.5 mg/L. Patients with invasive
infections caused by Aspergillus spp.,38 C. neoformans39,40 and Histoplasma capsulatum41 all tend to have better clinical outcomes with higher itraconazole trough concentrations. Patients with oropharyngeal and oesophageal candidiasis also have better responses to itraconazole therapy if serum concentrations are >0.6–1 mg/L.42,43 Collectively, therefore, a target for the prevention and treatment of invasive fungal infections is a trough concentration of 0.5–1 mg/L when measured using HPLC/mass spectrometry. The precise target that is ultimately chosen by the clinician depends on the organism, its MIC, the site of infection and overall clinical context.

Therapeutic concentration targets to optimize the antifungal effect of itraconazole have been derived exclusively in the context of prevention or treatment of invasive disease. Itraconazole is used in the treatment of other fungal diseases, such as treatment of infections with dimorphic fungi (e.g. Blastomyces, Sporothrix and Histoplasma), cryptococcal meningitis, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis (ABPA) and in some cases of severe asthma with fungal sensitization (SAFS). There is no evidence that concentration targets derived from the prevention of invasive fungal infection are necessarily optimal for these other diseases, although in the absence of specific evidence to the contrary, use of these same targets is probably reasonable.

A potential limitation of using a standard trough concentration is that it does not incorporate the MIC of the fungal pathogen in question. Experimental models of aspergillosis44–46 and candidiasis47,48 have demonstrated that greater drug exposure is required for successful outcomes for infections caused by isolates with higher MICs. The identification of concentration targets for TDM occurred in an era when resistance to anti-Aspergillus triazoles was uncommon. The most appropriate target value for treatment of pathogens with elevated MICs is not known. Furthermore, the relationship between this target and the emergence of drug resistance is not known, and may be important for chronic and allergic forms of aspergillosis, both of which require long-term antifungal therapy. These areas require further research.

**Recommendation 3: Itraconazole TDM should be performed to minimize drug-related toxicity**

Adverse events associated with itraconazole include gastrointestinal disturbances, neurological problems and hepatitis.16 Some of the gastrointestinal intolerance may be primarily caused by the osmotic effects of the hydroxypropyl-β-cyclodextrin component of the oral or iv solution.59 Two studies have demonstrated an increased incidence of toxicity at higher concentrations. Both studies used a bioassay to quantify itraconazole concentrations;50,51 An average concentration of 17 mg/L (bioassay) is a reasonable upper concentration bound to minimize the probability of drug-related toxicity. The equivalent target using HPLC has not been specifically determined, but is ≏ 5-fold lower.32

**Recommendation 4: Itraconazole concentrations should be measured in the first week of therapy and regularly thereafter**

Because itraconazole exhibits non-linear pharmacokinetics, the time to steady state cannot be expressed in terms of half-life (i.e. itraconazole does not have a half-life). Itraconazole concentrations steadily increase and reach 0.5–1 mg/L in the first 2 weeks of therapy. One approach for TDM is to draw a pre-dose sample at the end of the first week of therapy and then at regular intervals that are appropriate to the clinical context. More frequent sampling may be required if there is great clinical urgency, or there is a change in dosage and/or formulation. Moreover, a change in other clinical

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<table>
<thead>
<tr>
<th>Patient group</th>
<th>Specific indication</th>
<th>Quality of evidence</th>
<th>Strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocompromised patients receiving itraconazole for prevention of invasive fungal infection</td>
<td>target trough concentration for prophylaxis is 0.5 mg/L, measurement of trough serum concentrations 5–7 days after initiation of therapy or dose adjustment</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>when interacting drugs start or stop (either inhibiting absorption or affecting metabolism)</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>uncertain compliance with oral therapy</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>concerns about gastrointestinal absorption</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>potential clinical or laboratory manifestations of toxicity occur</td>
<td>moderate</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>target trough concentration for treatment is &gt;0.5 mg/L, measurement of trough serum concentration 5–7 days after initiation of therapy or dose adjustment</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>when interacting drugs start or stop (either inhibiting absorption or affecting metabolism)</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>uncertain compliance for oral therapy</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>concerns about gastrointestinal absorption, especially for prolonged periods of time</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>potential clinical or laboratory manifestations of toxicity occur</td>
<td>low</td>
<td>weak</td>
</tr>
</tbody>
</table>

*aThe target concentration for treatment is inferred from prophylaxis data, although there are few treatment studies that have addressed this.
parameters, such as the development of achlorhydria, the addition of agents that decrease gastric acidity (e.g. concomitant use of itraconazole capsules with antacids, histamine antagonists or proton pump inhibitors) or the addition of agents that interact via hepatic oxidative mechanisms (e.g. rifampicin, carbamazepine, phenytoin) may also mandate more frequent sampling. The requirement for repeated sampling for a patient who is stable and on longer-term itraconazole therapy is less clear. Nevertheless, intermittent measurements may be helpful to exclude issues with compliance or unanticipated changes in pharmacokinetics.

Voriconazole

Introduction

Voriconazole is a broad-spectrum second-generation triazole antifungal agent that has activity against Candida (including fluconazole-resistant species), C. neoformans, Aspergillus, many dimorphic fungi and several other medically important fungi. Voriconazole is a structural congener of fluconazole, but has significantly diminished aqueous solubility. A number of formulations are available for clinical use, including an iv preparation (containing sulfobutyl ether β-cyclodextrin sodium) and oral capsules (available as 50 and 200 mg), as well as a suspension designed for oral use in children.

Voriconazole is a first-line agent for the treatment of invasive aspergillosis, invasive candidiasis caused by Candida spp, with reduced susceptibility to fluconazole, and serious infections caused by Scedosporium or Fusarium spp. Voriconazole is the drug of choice for CNS aspergillosis. Voriconazole may potentially be used in combination with other antifungal agents for the treatment of invasive aspergillosis. Despite several clinical studies demonstrating the safety and efficacy of voriconazole for the prevention of invasive fungal infections, it is not currently licensed for this indication. The currently licensed dose is 6 mg/kg iv twice daily for two dosages, followed by 4 mg/kg iv twice daily. If therapy is initiated with oral voriconazole, a loading dose of 400 mg twice daily for two doses is used (for individuals <40 kg), followed by 200 mg twice daily, and in individuals <40 kg the maintenance dose is 100 mg twice daily. The dosage can be increased to 300 mg twice daily if clinically indicated. Recent population-based pharmacokinetic studies have suggested that higher oral doses than those currently recommended may be needed to achieve optimal plasma concentrations and therapeutic responses. There has been considerable debate about appropriate paediatric regimens that produce equivalent drug exposures to those observed in adults, for which efficacy has been established in Phase II and III clinical trials. A loading dose of 9 mg/kg twice daily for two doses followed by 8 mg/kg twice daily is now recommended for the iv preparation, with oral dosing maintained at 9 mg/kg twice daily, and reflects the higher weight-adjusted clearance of voriconazole that is observed in paediatric patients.

Voriconazole exhibits classical Michaelis–Menten (non-linear) pharmacokinetics in adults that are related to saturable clearance mechanisms. This has important implications for dosage adjustment because of unanticipated and unpredictable changes in drug exposure (i.e. significantly greater or smaller than anticipated). Voriconazole is highly orally bioavailable, with current estimates of ~80%–86% in children and adults, although estimates as low as 60% have recently been reported. Oral bioavailability may also be lower in children, hence TDM is especially important in this setting.

Voriconazole is metabolized via oxidative mechanisms. The predominant cytochrome P450 isoenzymes involved in this process are CYP3A4, CYP2C19 and CYP2C9. CYP2C19 exhibits a number of clinically relevant polymorphisms that have been associated with differing rates of enzyme activity and therefore clearance of voriconazole. These polymorphisms account for a portion of the observed variance in serum concentrations, which is otherwise extensive (e.g. 100-fold in healthy volunteers). Voriconazole inhibits CYP3A4 activity (as well as CYP2C19 and 2C9), which results in a number of clinically relevant drug–drug interactions that have been extensively reviewed elsewhere.

See Table 6 for recommendations for TDM for voriconazole.

Recommendation 5: TDM should be performed in the majority of patients receiving voriconazole

There is an increasing evidence base that supports TDM for voriconazole. The British Society for Medical Mycology (BSMM) working party recognizes that it is possible to use voriconazole without TDM and that the definitive trials used for registration were all performed using a fixed regimen. Nevertheless, the case supporting TDM as a routine adjunct to the use of voriconazole is increasing and rests with the following arguments: (i) concentration–effect and concentration–toxicity relationships are consistently reported in both experimental and clinical contexts and, in patients, these relationships have been defined in both adults and children; (ii) the pharmacokinetic variability of voriconazole is extensive, and has been rigorously quantified using non-parametric population pharmacokinetic modelling techniques, and a consequence of this pharmacokinetic variability is that an unacceptably low proportion of patients receiving a fixed regimen have drug exposures associated with a high probability of success and low probability of toxicity; and (iii) dosage adjustment results in fewer cases of toxicity, and may improve clinical responses. More recently, a prospective, randomized controlled trial compared clinical outcomes in patients who had voriconazole dosages adjusted based on serum concentrations with the outcomes in those who received a fixed voriconazole regimen. Outcomes (complete or partial response) in patients undergoing TDM (who had plasma concentrations maintained between 1.0 and 5.5 mg/L) were significantly better (81%) than those in the non-TDM group (57%).

Recommendation 6: A minimum lower target concentration for TDM for treatment of established disease is a trough concentration of >1 mg/L or a trough:MIC ratio of 2–5

The potential relationship between voriconazole serum concentrations and clinical outcome was initially described in a Phase II clinical study of voriconazole for invasive aspergillosis. In that study, a serum concentration of <0.25 mg/L was associated with a higher probability of clinical failure. Subsequently, a number of retrospective studies from single centres also suggested a relationship between drug exposure and clinical outcome. These studies are all limited by difficulties in estimating voriconazole drug exposure in individual patients and controlling for the
myriad of clinical factors that also have an impact upon clinical outcome. Studies variously identified target concentrations of $\geq 1$ mg/L or $\geq 2$ mg/L as being associated with improved outcomes, whilst one large study found no relationship between exposure and clinical outcome. Recent experimental and retrospective clinical studies have incorporated the MIC into targets for TDM; both suggest that a trough concentration:MIC target of 2–5 (when the MIC is estimated using CLSI methodology) is tenable and this may be useful if the MIC of the invading pathogen is known.

The most appropriate concentration target for prevention of invasive fungal infections in immunocompromised patients is less clear. A study of allogeneic haematopoietic stem cell transplant recipients suggests breakthrough infections only occur in patients with serum concentrations $<2$ mg/L. Similarly, lung transplant recipients who are colonized (with various fungi) or who develop invasive fungal infections have lower median trough concentrations compared with patients without colonization or infection (0.92 versus 1.72 mg/L). More studies are required to further define these relationships.

Collectively, therefore, a trough concentration of $>1$ mg/L is required to maximize efficacy for patients with invasive fungal infections. The probability of a clinical response increases with higher concentrations, but only incrementally. The target that is chosen for dosage adjustment depends on the clinical context. A higher target (e.g. 2 mg/L) should be used if there is disease with a poor prognosis (e.g. CNS infection, bulky disease, multifocal infection; see Table 3).

**Recommendation 7: A trough concentration to minimize drug-related toxicity is $<4$–$6$ mg/L**

Concentration–toxicity relationships for voriconazole have been estimated in several key studies. Voriconazole toxicity may manifest as visual disturbances (photopsia), liver dysfunction, skin reactions and neurotoxicity (confusion and visual hallucinations). Trough concentrations that are associated with greater probability of toxicity vary from study to study, and include $\geq 4$, $\geq 5$ and $\geq 6$ mg/L. Some studies do not define a specific cut-off value, but note a progressively higher probability of toxicity with higher voriconazole concentrations. There is a statistically significant (albeit relatively weak) relationship between average voriconazole concentration and the probability of elevated bilirubin, alkaline phosphatase, aspartate transaminase and alanine transaminase. Furthermore, there is a relationship between the trough concentration and the probability of encephalopathy, which manifests as confusion and hallucinations. Active dosage adjustment to keep serum concentrations $<5.5$ mg/L prevents voriconazole-related toxicity.

**Recommendation 8: Voriconazole concentrations should be measured in the first 5 days of therapy and regularly thereafter**

At the current time, a trough concentration is the most readily interpretable measure of drug exposure. Voriconazole concentrations change faster than those of itraconazole and posaconazole, and initial sampling in the first 2–5 days of therapy is reasonable. Some patients sampled at this time may have progressively accumulating drug concentrations even though the initial concentration is ‘therapeutic’. This occurs if serum concentrations are $>K_m$ (the Michaelis constant for that individual), meaning that clearance mechanisms are saturated. Therefore, a second sample should be collected to ensure voriconazole concentrations are stable and in a desired therapeutic range. The same sampling strategy is required if there is a change in dosage, a change in clinical condition or an iv-to-oral switch.

### Table 6. Recommendations for TDM for voriconazole

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Specific indication</th>
<th>Quality of evidence</th>
<th>Strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocompromised patients receiving voriconazole for prophylaxis of invasive fungal disease</td>
<td>Target trough concentration for prophylaxis is $&gt;1$ mg/L</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>Measurement of trough serum concentration within the first 7 days after initiation of therapy, and regularly thereafter</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>When interacting drugs start or stop</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>Uncertain compliance for oral therapy</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>Concerns about gastrointestinal absorption, especially for prolonged periods of time</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>Potential clinical or laboratory manifestations of toxicity occur</td>
<td>high&lt;sup&gt;a&lt;/sup&gt;</td>
<td>strong</td>
</tr>
<tr>
<td>Patients receiving voriconazole for invasive fungal diseases</td>
<td>Target trough concentration for treatment is $&gt;1$ mg/L</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>Measurement of serum trough concentration within 7 days of initiation of therapy or following dose adjustment</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>When interacting drugs start or stop</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>Uncertain compliance for oral therapy</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>Concerns about gastrointestinal absorption, especially for prolonged periods of time</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>Potential clinical or laboratory manifestations of toxicity occur</td>
<td>high</td>
<td>strong</td>
</tr>
</tbody>
</table>

<sup>a</sup>This is inferred from treatment studies.
Posaconazole

Introduction

Posaconazole is a broad-spectrum triazole agent that is structurally similar to itraconazole. Posaconazole has activity against a large number of medically important fungal pathogens, including Candida, Aspergillus, Cryptococcus and the mucormaceous moulds. Posaconazole is currently only available as an oral suspension (40 mg/mL), although other orally bioavailable and IV formulations are under development.96,97

The current licensed indications for the use of posaconazole include salvage therapy for aspergillosis, treatment of coccioidiomycosis, chromoblastomycosis, mycetoma or Fusarium infections. Posaconazole is increasingly used for the prevention of infections in patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) who are expected to become neutropenic, and stem cell transplant recipients receiving immunosuppressive agents for graft-versus-host disease.15 The dose for treatment of established infection is 800 mg/day in two to four divided doses (i.e. 200 mg four times daily or 400 mg twice daily), with four divided doses providing the best exposure. A dose of 600 mg/day in three divided doses is used for the prevention of invasive fungal infections in immunocompromised patients (i.e. 200 mg every 8 h).

Posaconazole is primarily metabolized by glucuronidation, with little involvement of oxidative mechanisms. Metabolites are excreted in the faeces and urine.68 Posaconazole inhibits CYP3A4 activity and dosage adjustment of drugs metabolized via this pathway (most importantly ciclosporine and tacrolimus) is required. The oral absorption of posaconazole appears saturable and this may be affected by both the rate and the extent of absorption. Dosage escalation beyond 800 mg/day does not result in a proportional increase in systemic drug exposure, although some studies do suggest there may be some incremental benefit.98

There is a significant food effect (increased oral bioavailability with food),99 acid effect (increased absorption with an acidic environment)100 and fat effect (increased oral bioavailability with administration with fatty food or nutritional supplements99,101). All these characteristics have a potential impact on the ability to increase systemic drug exposure. Posaconazole has a long terminal half-life (~34 h) and does not achieve steady-state serum concentrations until the end of the first week of dosing. Because the dosing interval is significantly shorter than the half-life, the concentration-time profile is typically reasonably flat and there is a high degree of concordance between the average and trough concentrations. Posaconazole is generally well tolerated, but can cause nausea, vomiting and hepatotoxicity.102 To date, there are no data that suggest any correlation between toxicity and drug exposure, but with the newer formulations of posaconazole in development this may change.

See Table 7 for recommendations for TDM for posaconazole.

Recommendation 9: TDM should be performed in the majority of patients receiving posaconazole

There is an increasing evidence base that supports TDM for posaconazole. The BSMM working party recognizes that posaconazole has been extensively used without TDM, and that the efficacy of this compound for the prevention of invasive fungal infections was established without resorting to TDM. Posaconazole TDM should be considered in the majority of cases in which it is used and this is based on the following: (i) concentration–effect relationships are apparent and have been established in experimental models of invasive fungal infection103,104 and in clinical contexts;105,106 (ii) the pharmacokinetic variability is extensive and has been quantified using a variety of pharmacokinetic modelling approaches;106,107,108 and (iii) serum concentrations are potentially suboptimal in a relatively high proportion of patients receiving a fixed regimen. Many studies note the problems of achieving...

Table 7. Recommendations for TDM for posaconazole

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Specific indication</th>
<th>Quality of evidence</th>
<th>Strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunocompromised patients receiving posaconazole for prophylaxis of invasive fungal disease</td>
<td>target for prophylaxis is &gt;0.7 mg/L at steady state or 0.35 mg/L 48 h after initiation of therapy</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>measurement of trough serum concentration 7 days after initiation of therapy and following dose adjustment</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>when interacting drugs start or stop</td>
<td>low</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>uncertain compliance</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>concerns about gastrointestinal absorption, especially for prolonged periods of time</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td>Patients receiving posaconazole for salvage therapy of invasive fungal diseases</td>
<td>target for treatment is &gt;1 mg/L within 7 days of initiation of therapy or following dose adjustment</td>
<td>moderate</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>when interacting drugs start or stop</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>uncertain compliance</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>concerns about gastrointestinal absorption, especially for prolonged periods of time</td>
<td>high</td>
<td>strong</td>
</tr>
</tbody>
</table>

aThis is from a pharmacokinetic model.113

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nominal target concentrations in patients because of sub-optimal absorption that is compounded by mucositis and/or graft-versus-host disease of the gut. For example, ~50% of patients receiving posaconazole have serum concentrations <0.5 mg/L, which are potentially subtherapeutic (see Recommendation 10). Recent studies have shown that concentrations of posaconazole associated with the cellular membranes in the lung may be many times in excess of those levels found in the blood, which may in future influence the recommendations for monitoring of blood levels during prophylactic use.

Recommendation 10: A lower target concentration for TDM for patients receiving posaconazole for prophylaxis is a trough concentration of >0.7 mg/L

A target trough concentration of 0.7 mg/L for patients receiving posaconazole for prophylaxis is widely cited (see e.g. Bryant et al., Jang et al. and Tonini et al.). This target concentration is derived from analysis by the FDA of pharmacokinetic data from two Phase III prophylaxis studies that were originally used for the purposes of registration. There is a degree of uncertainty about the relevance of this target concentration because a composite endpoint for successful clinical outcome was used—there were simply too few patients with microbiologically documented breakthrough infection in these studies to rely solely on this as a criterion for success. Because posaconazole concentrations are not at steady state until after the first week of therapy, a target concentration of 0.35 mg/L after 48 h of treatment has also been proposed. Several other studies have reported a correlation between drug exposure and efficacy in a range of clinical contexts. Target concentrations vary in these studies from 0.5 to 0.7 mg/L. Although many of the studies are small, retrospective in design and generally underpowered, they all show a general trend towards an increased probability of response with greater drug exposure. In the absence of more definitive data, a concentration target of 0.7 mg/L is reasonable, although the evidence that supports this is relatively weak, and the BSMM working party have graded this recommendation accordingly (see Table 7).

Recommendation 11: A lower target concentration for TDM for patients with established infection is a trough concentration of >1.0 mg/L

Patients with invasive aspergillosis who are intolerant or refractory to other licensed antifungal agents receiving posaconazole have a progressively higher clinical response with higher posaconazole drug exposures. In that study, among patients with a C_{\text{max}} and C_{\text{avg}} of 0.142 and 0.134 mg/L, respectively, 24% had a successful clinical outcome. In contrast, patients with a C_{\text{max}} and C_{\text{avg}} of 1.48 and 1.25 mg/L, respectively, had a 75% response rate. Thus, there appears to be a progressive increase in the probability of a response with increasing drug exposure. A pragmatic approach for TDM is to attempt to obtain the highest possible concentration, although suboptimal and saturable absorption may mean this is simply not feasible even following progressive dosage escalation. A trough concentration of 1 mg/L can be used as a lower concentration target for TDM.

The use of a target concentration of 1 mg/L does not specifically incorporate the MIC of the invading pathogen (unlike voriconazole; see above). Experimental data suggest that the MIC and genotype of the invading pathogen are important determinants of exposure–response relationships.

The Antifungal Subcommittee of EUCAST has recently set breakpoints for posaconazole against Aspergillus spp. and specifically incorporated TDM into the classification of isolates into ‘susceptible’, ‘intermediate’ and ‘resistant’ categories. Hence, isolates with MIC <0.125 mg/L and >0.25 are deemed susceptible or intermediate only if adequate drug exposure has been documented with TDM, with a therapeutic target serum concentration of 1 mg/L at steady state being recommended.

Recommendation 12: Posaconazole concentrations should be measured in the first week of therapy and regularly thereafter

Posaconazole concentrations steadily increase in the first week and plateau thereafter. A steady-state trough concentration is not apparent until the end of the first week, and changes to dosage will take a further 7 days before a new steady state is established. Repeat testing is required if the clinical condition changes or following dosage adjustment. Serum samples can be collected earlier than 7 days (before the attainment of steady state), but the use of a lower therapeutic target of 0.35 mg/L after 48 h of therapy is appropriate.

Flucytosine

Introduction

Flucytosine is a pyrimidine analogue that acts as a subversive substrate within the pyrimidine salvage pathway of a number of clinically important fungal pathogens. Flucytosine is active against the majority of Candida spp. and C. neoformans, but also has activity against Aspergillus spp. and rare dematiaceous fungal pathogens causing chromoblastomycosis. Flucytosine should always be used in combination with other antifungal agents because of the significant risk of emergent drug resistance when used as monotherapy.

The advent of newer antifungal agents and classes has somewhat relegated the importance of flucytosine in many clinical settings. Nevertheless, it remains a cornerstone for induction therapy of cryptococcal meningitis, in combination with either a polyene (amphotericin B deoxycholate, liposomal amphotericin B) or fluconazole. Flucytosine is highly orally bioavailable, making it an especially attractive option for use in resource-poor healthcare settings, although the oral preparation is not available in all countries. Furthermore, it penetrates the CSF and cerebral parenchyma. Flucytosine may also be useful in some cases of refractory infections caused by Candida spp., especially if there is deep infection where poor drug penetration may compromise the therapeutic response. The standard dose is 100–150 mg/kg/day, and is usually administered in three or four divided dosages. A dosage reduction is required with renal impairment (creatinine clearance >50 mL/min, 150 mg/ kg/day; creatinine clearance 26–50 mL/min, 75 mg/kg/day; creatinine clearance 13–25 mL/min, 37 mg/kg/day; creatinine clearance...
<13 mL/min, avoid flucytosine\textsuperscript{125}). Alternatively, the normal dose can be given, but with an increased interval between doses.

Flucytosine is a small polar molecule that is cleared via renal mechanisms. Flucytosine is generally well tolerated, although there is well-documented associated toxicity that includes bone marrow suppression (primarily manifesting as neutropenia), gastrointestinal intolerance, hepatitis and rash. However, the more serious liver toxicity and myelosuppression are generally only seen with prolonged maintenance of high blood levels. Flucytosine has few (if any) direct drug–drug interactions. Flucytosine rapidly accumulates with the onset of renal impairment if there is not an appropriate downward revision of dosage. Historically, the most common agent that leads to renal impairment and subsequent accumulation of flucytosine is amphotericin B deoxycholate.

See Table 8 for recommendations for TDM for flucytosine.

**Recommendation 13: TDM should be performed in the majority of patients receiving flucytosine**

TDM for flucytosine has long been considered a standard of care.\textsuperscript{126} A requirement for TDM is predominantly based on the well-established concentration–toxicity relationships,\textsuperscript{125} the most important of which is myelosuppression (see below). There is also some evidence from reference centres that flucytosine concentrations are variable and frequently outside nominal concentration targets for TDM.\textsuperscript{127,128} There is also a theoretical concern for the emergence of drug resistance, which occurs rapidly when *Candida* is exposed to flucytosine in vitro. There are well-described drug exposure targets for flucytosine against *Candida albicans*, with a requirement for serum concentrations to exceed the MIC for \(\approx 45\%\) of the dosing interval.\textsuperscript{129,130} Nevertheless, the use of TDM to aid in the optimization of the antifungal efficacy (as opposed to prevention of toxicity) of flucytosine remains poorly elucidated.

**Recommendation 14: A lower target concentration for TDM is a trough concentration of \(>20–40\) mg/L**

A number of lower target concentrations have been used to direct flucytosine dosing. The use of this target concentration is primarily based on *in vitro* findings in which the emergence of drug resistance is observed when yeasts are exposed to lower concentrations.\textsuperscript{125,131} The clinical relevance of these concentrations for patients is less clear. Furthermore, the optimal concentration targets for flucytosine in combination with other antifungal agents—

### Table 8. Recommendations for TDM for flucytosine

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Specific indication</th>
<th>Quality of evidence</th>
<th>Strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients receiving flucytosine in combination with other antifungal agents for treatment of invasive fungal diseases</td>
<td>trough concentration of 20–40 mg/L; peak concentration should not exceed 100 mg/L within 72 h of initiation of therapy or following dose adjustment when interacting drugs start or stop uncertain compliance for oral therapy\textsuperscript{a} potential clinical or laboratory manifestations of toxicity occur</td>
<td>weak</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>high</td>
<td>strong</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>high</td>
<td>strong</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Oral therapy is not widely available.

### Table 9. Strategies for dose adjustments for patients with low serum concentrations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Upward dosage adjustment</th>
<th>Additional strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itraconazole</td>
<td>increase from 200 mg twice daily to 300 mg twice daily</td>
<td>• change capsules to solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• if using capsules, stop or reduce H2 antagonists or proton pump inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• if using solution check it is being given in the fasting state</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• check compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• stop interacting drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• check compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• stop interacting drugs</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>increase iv therapy by 50% to a maximum of 6 mg/kg twice daily (adults); increase oral therapy from 200 mg twice daily to 300 mg twice daily</td>
<td>• administer with food</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• administer with high-fat food (e.g. ice cream)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• remove acid suppression if possible (i.e. stop or reduce H2 antagonists or proton pump inhibitors)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• check compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• stop interacting drugs</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>increase from 600 mg/day to 800 mg/day; fractionate total daily dose and administer every 6 h</td>
<td>• administer with food</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• administer with high-fat food (e.g. ice cream)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• remove acid suppression if possible (i.e. stop or reduce H2 antagonists or proton pump inhibitors)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• check compliance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• stop interacting drugs</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>increase dose by 50%</td>
<td>• check compliance</td>
</tr>
</tbody>
</table>
which is the way fluycytosine is invariably administered—are not well defined.

**Recommendation 15: A concentration target to minimize fluycytosine drug-related toxicity is a peak concentration of 50–100 mg/L**

There is strong evidence that there is an increased risk of myelotoxicity with peak concentrations of fluycytosine >100 mg/L. This concentration target was defined 2 h after an oral dose of fluycytosine in a cohort of patients receiving 0.3 mg/kg/day amphotericin B deoxycholate and fluycytosine. A total of 23/37 patients with a peak concentration of >100 mg/L over a 2 week treatment period had fluycytosine-related toxicity, whereas only 15/48 patients with concentrations <100 mg/L had drug-related toxicity. The implications of these findings for the current BSMM recommendations are slightly difficult to interpret because a peak concentration was defined as the concentration 2 h after an oral dose of fluycytosine. Comparable concentration targets for peak samples taken 30 min after the dose in patients receiving iv fluycytosine are not known and require further study. Although the dosage of fluycytosine which produced these concentrations was higher than that in current use, toxicity is still seen with current dosages, especially in the setting of renal impairment.

**Recommendation 16: Fluycytosine concentrations should be measured in the first 72 h of therapy and regularly thereafter**

Fluycytosine has a short half-life. Serum concentrations can change quickly, especially if renal function changes. Regular measurements are required to prevent persistence or recurrence of potentially toxic concentrations. Serum concentrations should be re-measured following dosage adjustment.

**Strategies for dose adjustment**

Dosage adjustments may be required in patients with low serum concentrations or other measures may need to be taken, such as the cessation of interacting drugs. The strategy that is required varies between drugs and is detailed in Table 9.

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**Supplementary data**

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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*Candida auris* is a multidrug-resistant yeast that causes a wide spectrum of infections, especially in intensive care settings. We investigated *C. auris* prevalence among 102 clinical isolates previously identified as *Candida haemulonii* or *Candida famata* by the Vitek 2 system. Internal transcribed spacer region (ITS) sequencing confirmed 88.2% of the isolates as *C. auris*, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) easily separated all related species, viz., *C. auris* (n = 90), *C. haemulonii* (n = 6), *C. haemulonii* var. *vulnere* (n = 1), and *Candida duobushaemulonii* (n = 5). The *in vitro* antifungal susceptibility was determined using CLSI broth microdilution (CLSI-BMD), the Vitek 2 antifungal susceptibility test, and the Etest method. *C. auris* isolates revealed uniformly elevated fluconazole MICs (MIC_{50} 64 μg/ml), and an alarming percentage of isolates (37%) exhibited elevated caspofungin MICs by CLSI-BMD. Notably, 34% of *C. auris* isolates had coexisting elevated MICs (≥2 μg/ml) for both fluconazole and voriconazole, and 10% of the isolates had elevated coexisting MICs (≥2 μg/ml) to two additional azoles, i.e., posaconazole and isavuconazole. In contrast to reduced amphotericin B MICs by CLSI-BMD (MIC_{50} 90), elevated MICs were noted by Vitek 2 (MIC_{50} 8 μg/ml), which were statistically significant. *Candida auris* is an unnoticed pathogen in routine microbiology laboratories, as 90% of the isolates characterized by commercial identification systems are misidentified as *C. haemulonii*. MALDI-TOF MS proved to be a more robust diagnostic technique for rapid identification of *C. auris*. Considering that misleading elevated MICs of amphotericin B by the Vitek AST-Y07 card may lead to the selection of inappropriate therapy, a cautionary approach is recommended for laboratories relying on commercial systems for identification and antifungal susceptibility testing of rare yeasts.
and data obtained by CLSI were compared with those obtained by the commercial Vitek 2 system and the Etest method.

(Part of this study was presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy [54th ICAAC], M-1203, slide session, Washington, DC, USA [10].)

MATERIALS AND METHODS

Isolates. A total of 102 clinical isolates, from individual patients, previously identified as Candida haemulonii/C. famata by the Vitek 2 compact system (bioMérieux, Marcy l’Etoile, France) in 4 tertiary care hospitals in Delhi, North India, and a single center in Kochi, Kerala, South India, from 2010 to 2014 were included. Three centers were about 1,000-bed general hospitals, and the remaining 2 were a pediatric hospital and a transplant center. The pediatric hospital had neonatal intensive care units and surgical intensive care facilities. The isolates were mainly from patients with candidemia (blood; n = 78), and other specimens from invasive Candida infections included gancare tissue (n = 4), pleural fluid (n = 6), and peritoneal fluid (n = 7). Also, 7 isolates from urine (n = 4) and sputum (n = 3) specimens from immunocompromised patients were included. The control and type strains of 3 C. auris isolates from Korea (KCTC 17809, KCTC 17810) and Japan (DSM 21092T) and one isolate each of C. haemulonii strain CBS 7802 and C. duobushaemulonii strain CBS 7798T were also analyzed.

Phenotypic characterization. The isolates were identified by standard mycological procedures, including colony color on CHROMagar Candida medium (Difco, Becton Dickinson & Company, Baltimore, MD, USA) and morphology on rice Tween 80 agar. Growth patterns at different temperatures, 37°C, 42°C, and 45°C, were also observed (1). Additionally, the assimilation profile of all yeast isolates was done by commercially available API strips (ID32C; bioMérieux, Marcy l’Etoile, France), which were read and interpreted at 48 h.

Sequence of ITS region. Genomic DNA was extracted from all test isolates along with reference strains as described by Xu et al. (11). DNA was amplified and sequenced using the ITS-1 (5′-TCCGTAGTGTAACCGTGCCG-3′) and ITS-4 (5′-TCTCAGGATATGATGC-3′) primers, which amplify the ITS region of the ribosomal subunit (8). Sequences were aligned, and GenBank Basic Local Alignment Search Tool (BLAST) searches were performed for species identification. For phylogenetic analyses, the ITS gene sequences of the C. auris, C. haemulonii, and C. duobushaemulonii isolates were aligned with the ClustalW program (version 1.82), and the final alignments were edited manually. A neighbor-joining (NJ) tree based on ITS gene sequences using 2,000 bootstrap replications was generated using MEGA version 5 (12). The sequences of the reference/type strains of C. auris from Japan (JCM 15448T) and Korea (KCTC 17809 and KCTC 17810), along with C. haemulonii (CBS 5150, Portugal; CBS 7801, United States), C. haemulonii var. vulnera (CNMCL-7462, Spain), and C. duobushaemulonii (CBS 7799, USA), were retrieved from GenBank and included for the analysis.

MALDI-TOF MS. The ethanol–formic acid extraction procedure was followed according to the manufacturer’s protocol for the identification of yeast isolates (13). The spectra were analyzed using the Flex Control 3.1 software (Bruker Daltonics, Inc., Billerica, MA, USA) and MALDI Biotype OC version 3.1 (Bruker Daltonics, Bremen, Germany). Score values were analyzed as per manufacturer recommendations: a score of ≥2 indicated confidence to the species level, 1.7 to 1.99 indicated confidence to the genus level, and <1.7 indicated no identification.

MALDI data analysis. The MALDI Biotyper version 3 database contains spectra of 3 strains of C. auris, two from Korea (KCTC 17809 and KCTC 17810) and a type strain from Japan (DSM 21092T). For phylogenetic analysis, spectra of 90 C. auris isolates were added manually to the library for the creation of a score-oriented dendrogram in Biotyper as described previously for Aspergillus species (14). The mass spectra of each quadruplicate of the respective isolates with a score value of ≥2 were considered for dendrogram preparation. Additionally, available spectra of reference strains of C. auris from Japan (DSM 21092T) and Korea (KCTC 17809 and KCTC 17810) and of C. haemulonii (CBS 5149T and CBS 5150), C. duo-
bushaemulonii (CBS 7799 and CBS 7800), and C. pseudoahaemulonii (CBS 10004 and CBS 12453T) in the database were imported in the software for the analysis of the dendrogram. The dendrogram was generated using the respective functionality of the MALDI Biotyper 3.1 offline client. The spectra of all the isolates tested were analyzed by a score-oriented dendrogram using an arbitrary distance level of 1,000 as the cutoff.

AST. (i) CLSI-BMD method. Antifungal susceptibility testing (AST) was carried out using the Clinical and Laboratory Standards Institute broth microdilution method (CLSI-BMD), following the M27-A3 guidelines (15). Antifungals tested were amphotericin B (AMB; Sigma, St. Louis, MO, USA), fluconazole (FLU; Pfizer, Groton, CT, USA), itraconazole (ITC; Lee Pharma, Hyderabad, India), voriconazole (VRC; Pfizer), posaconazole (POS; Merck, Whitehouse Station, NJ, USA), isavuconazole (ISA; Basilea Pharmaceutica, Basel, Switzerland), ituclosyne (3-FC; Sigma), caspofungin (CAS; Merck), micafungin (MFG; Astellas, Toyama, Japan), and anidulafungin (AFG; Pfizer). RPMI 1640 medium with glutamine without bicarbonate (Sigma) buffered to pH 7 with 0.165 mol/liter 3-N-morpholinosulfonylic acid (MOPS; Sigma) was used. Drug-free and yeast-free controls were included, and microtiter plates were incubated at 35°C and read visually after 24 h, as validated recently by Pfäffler et al. (16, 17). CLSI-recommended Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used as quality control strains, and two reference strains of C. auris (KCTC 17809 and DSM 21092T) were also included. Excepting AMB, the MIC endpoints for all the antifungals were defined as the lowest drug concentration that caused 50% growth inhibition vis-à-vis the drug-free controls. The MIC for AMB was defined as the lowest concentration at which there was 100% inhibition of growth. The susceptibility for all the isolates was performed by two different personnel on two occasions, which revealed reproducible results.

(ii) Vitek 2 Compact system using an AST-Y507 card. Susceptibility was determined using an AST-Y507 card, which tests the MIC of 6 antifungals, i.e., FLU, 5-FC, VRC, AMB, CAS, and MFG. All the C. auris isolates were tested as per the manufacturer’s instructions. The time of incubation ranged from 18 to 27 h, based on the rate of growth in the drug-free control well, and the results were expressed as MICs in micrograms per milliliter.

(iii) Etest method. Further, the isolates which revealed >2-fold discrepancies in the antifungal MICs by the above-described two methods were also tested for susceptibility by Etest using Etest Technical Guide 4: Antifungal Susceptibility of Yeasts (AB Biodisk, bioMérieux, Solna, Sweden), as described previously (19, 20). The antifungals tested were AMB, CAS, and VRC. Briefly, the inoculum density of 0.5 × 10⁶ to 2.5 × 10⁶ cells/ml prepared for the CLSI-BMD test was used, and the test medium included RPMI 1640 with 1.5% agar supplemented with glucose (2%) and was buffered to pH 7.0 with MOPS. In addition, AMB was also tested on antibiotic medium 3 (AM3) agar plates. The plates were inoculated by dipping a sterile cotton swab into the inoculum and streaking it across the entire surface of the agar in three directions. The plates were dried for 15 min before the Etest strips (AB Biodisk) were applied and kept at 35°C and read visually after 24 h. The Etest MIC was defined as the drug concentration at which the border of the elliptical zone of complete inhibition intersected the scale on the antifungal test strip.

Statistical analysis. Statistical analyses were performed with SPSS version 20.0 (SPSS, Chicago, IL, USA). MIC values from the CLSI-BMD, Vitek 2, and Etest methods were assessed by using the Student t test (paired sample). The Etest MIC endpoints, which were in between the 2-fold dilution scale of the CLSI method, were rounded to the corresponding next upper 2-log dilution to simplify comparisons. The discrepancies among MIC endpoints of ≥2 dilutions (two wells) were used to calculate the essential agreement (EA).

FKS gene sequencing. Candida auris isolates with elevated CLSI-BMD MICs of CAS (MICs ≥1 μg/ml) were subjected to sequencing of the FKS1 and FKS2 genes. Considering that the genome sequence of C. auris is not yet available, the published mutations in the FKS gene of echinocandin-resistant Candida glabrata isolates were used to analyze the mutations in
the FKS gene of *C. auris* isolates by sequence homology (21, 22). Genomic DNA was amplified and sequenced for hot spot regions of both the genes. The primers were designed based on the *C. glabrata* FKS1 and FKS2 gene sequences (GenBank accession no. XM_446406 and XM_448401, respectively). The primers for FKS1 (F1SF: 5'-CATTGCTATTTTTCTCAG TCTAGC-3' and F1SR, 5'-CCACGAAAGACAGTTGTTGA-3') and FKS2 (F2SF: 5'-CATTGCTATTTTTCTCAG TCTAGC-3'; F2SR, 5'-TCCAGGGATAGTGGAAATCC-3') were designed using Primer3 version 4 (http://primer3.ut.ee/). DNA sequences were analyzed with Sequencing Analysis software version 5.3.1 (Applied Biosystems). Consensus sequences were made using EditFast software (version 7.0.5.3) and were aligned with hot spot FKS regions of reference *C. glabrata* (GenBank accession no. HM366439 for FKS1 and HM366442 for FKS2).

**Nucleotide sequence accession numbers.** The sequences determined in this study were deposited in GenBank under accession no. KF689009 to KF689022, KC692039 to KC692052, and KP862745 to KP862818.

**RESULTS**

Vitek 2 identified 100 isolates as *C. haemulonii* (88 with 91 to 98% identity and 12 with a low discrimination profile), whereas the remaining 2 isolates were identified as *C. famata* (93% identity). Of the 102 isolates, 88.2% (*n* = 90) were confirmed as *C. auris* by ITS sequencing. The remaining 12 isolates were identified as *C. haemulonii* (*n* = 6), *C. haemulonii* var. *vulnera* (*n* = 1), and *C. duobushaemulonii* (*n* = 5). All *C. auris* (*n* = 90) isolates showed smooth, white to cream-colored colonies on Sabouraud dextrose agar (SDA), whereas they developed a pink color on CHROMagar agar (SDA), whereas they differentiated in 2 separate clades. The other species of the *C. haemulonii* var. *vulnera* (n/11005) showed 100% homology with *C. duobushaemulonii* (n/11005). The dendrogram clearly revealed separation of members of the *Metchnikowia* clade in 4 phylogroups (Fig. 2). The mass spectra of the Indian *C. auris* isolates showed marked similarity, whereas the Japanese (*n* = 1) and Korean *C. auris* isolates (*n* = 2) exhibited variations in mass spectra among themselves and with those of Indian *C. auris* isolates, resulting in a separate cluster in *C. auris* (phylogroup 4). The dendrogram generated was in agreement with the phylogenetic NJ tree with ITS sequences.

**In vitro susceptibility and FKS mutation analysis.** The *in vitro* susceptibility data and the MIC distribution of *C. auris* isolates using different methods along with essential agreements between the tested methods are presented in Tables 1 and 2.

**CLSI-BMD.** FLU exhibited no activity against 89% (*n* = 80) of *C. auris* isolates (MIC of 16 to >64 µg/ml), whereas the remaining 10 isolates revealed a MIC of 4 µg/ml. Similarly, an elevated MIC<sub>iso</sub> i.e., 8 µg/ml, was noted for VRC. Notably, 58% of *C. auris* isolates (*n* = 52) showed VRC MICs of ≥1 µg/ml. In contrast, MIC<sub>iso</sub> values of POS (0.06 µg/ml) and ISA (0.25 µg/ml) were relatively low compared to that of VRC (Table 1). Also, 11% of *C. auris* isolates revealed MICs of ≥1 µg/ml for both POS and ISA, and a solitary isolate showed a MIC of ≥1 µg/ml only to ISA. All *C. auris* isolates showed reduced MICs to ITC (geometric mean [GM] MIC, 0.15 µg/ml). Furthermore, *C. auris* isolates had AMB MIC<sub>iso</sub> values of 1 µg/ml; however, 15.5% (*n* = 14) of the isolates revealed MICs of ≥2 µg/ml for AMB. Moreover, elevated GM MICs were observed for CAS (0.58 µg/ml) in comparison to MFG (0.11 µg/ml) and AFG (0.23 µg/ml). Notably, 37% (*n* = 33) of the *C. auris* isolates revealed MICs of ≥1 µg/ml to CAS. Also, the echinocandins had no activity in 8% (*n* = 7) of the isolates, with MICs ranging from 4 to >8 µg/ml (Table 2). Further, 88% of *C. auris* isolates had reduced MICs to 5-FC (GM MIC, 0.4 µg/ml), whereas 11 isolates showed highly elevated MICs (≥32 µg/ml). In contrast to *C. auris*, all the *C. haemulonii* (*n* = 7) and *C. duobushaemulonii* (*n* = 5) isolates had markedly elevated AMB MICs ranging from 4 to 16 µg/ml. Also, variable FLU MICs were observed for *C. haemulonii* (MIC range 2 to >64 µg/ml) and *C. duobushaemulonii* (MIC range, 1 to 16 µg/ml). However, reduced MICs of VRC (MIC range, 0.03 to 0.5 µg/ml) were noted for both the *C. haemulonii* and *C. duobushaemulonii* isolates, except a solitary isolate of *C. haemulonii*, which showed a MIC of 4 µg/ml. Moreover, both the *C. haemulonii* and *C. duobushaemulonii* isolates exhibited reduced GM MICs to ISA (0.027 µg/ml and 0.023 µg/ml), followed by POS (0.05 µg/ml and 0.11 µg/ml) and ITC (0.31 µg/ml). Also, in contrast to *C. auris* (GM MIC, 0.58 µg/ml), both *C. haemulonii* (GM MIC, 0.19 µg/ml) and *C. duobushaemulonii* (GM MIC, 0.14 µg/ml) showed reduced MICs to CAS. However, a wide MIC range (<0.125 to 64 µg/ml) for 5-FC was observed for *C. haemulonii*, while reduced MICs (GM MIC, 0.125 µg/ml) were found for *C. duobushaemulonii* (Table 1).
FKS gene sequencing of *C. auris* isolates with elevated caspofungin MICs (>1 μg/ml). Amplification of FKS1 and FKS2 regions generated amplicons of 391 bp and 460 bp, respectively. Mutations reported for caspofungin-resistant *C. glabrata* were not observed in the FKS1 and FKS2 regions of any of the tested *C. auris* strains.

**Vitek 2.** In contrast to low AMB MICs recorded by CLSI for *C. auris*, exceptionally elevated AMB MICs (CLSI MIC50 of 1 μg/ml compared to Vitek MIC 50 of 8 μg/ml) were noted, which was in concordance with CLSI. Vitek 2 MIC50 values of VRC (1 μg/ml), CAS (0.5 μg/ml), and MFG (0.125 μg/ml) of *C. auris* isolates were in 100% agreement with those by the CLSI method. Vitek 2 MIC50 values of FLU (32 μg/ml) and 5-FC (1 μg/ml) were within 2 dilutions by CLSI-BMD.

**Etest.** Similar to CLSI MICs, low MIC50 values of AMB for *C. auris* isolates were observed by Etest on AM3 medium (0.5 μg/ml) and on RPMI agar (1 μg/ml). Except a solitary isolate, all *C. auris* isolates showed MICs of ≤1 μg/ml for AMB. The MIC50 (1 μg/
ml) of VRC was similar to that by CLSI-BMD. In contrast, Etest CAS MICs were better differentiated than CLSI-BMD MICs, and Vitek showed a wide range from 0.002 to 4 μg/ml. Specifically, 33 C. auris isolates which showed MICs of ≥1 μg/ml for CAS by CLSI revealed highly variable MICs ranging from 0.064 to 4 μg/ml by Etest. Interestingly, 26 of these 33 isolates revealed MICs of 0.064–4 μg/ml by CLSI-BMD with Etest. AMB MICs were better differentiated than CLSI-BMD MICs, and Etest MICs of 0.5–1 μg/ml, which were in agreement with the CLSI-BMD MICs. Further, Etest MICs of C. haemulonii and C. duobushaemulonii for AMB, CAS, and VRC were within ±2 dilutions of CLSI MICs.

**Agreement between methods.** The essential agreement within ±2 dilutions for the comparison of 24-hour CLSI-BMD with Vitek 2 and Etest results showed 10% and 81% for AMB, 90% and 48% for CAS, and 91% and 79% for VRC, respectively.

**DISCUSSION**

The present study highlights that *Candida auris* remains an unnoticed pathogen in routine microbiology laboratories in India, as 90% of the isolates characterized by commercial identification systems misidentify this yeast as *C. haemulonii*. In the past 5 years, *Candida auris* has emerged as a significant pathogen in tertiary care general hospitals and a pediatric center in north and south India, representing 8.6% to 30% of cases of candidemia (7, 8). The actual prevalence of *C. auris* in varied clinical settings in India is unexplored, as the majority of centers do not perform molecular or MALDI-TOF MS-based identification. In this work, a large number of *C. auris* isolates were tested for antifungal susceptibility with three methods which showed uniform fluconazole resistance and an alarming percentage of isolates (37%) exhibiting elevated caspofungin MICs by CLSI-BMD. Taken together, 10% of isolates showed highly elevated MICs to 4 antifungals drugs (AMB, FLU, CAS, VRC) by the CLSI-BMD method. Notably, 34% of isolates had coexisting elevated MICs for two commonly used azoles, i.e., FLU and VRC (MICs of ≥2 μg/ml), and 10% of the isolates had elevated MICs of ≥1 μg/ml to two additional azoles, i.e., CAS and ISA. Considering the frequent prevalence of MDR strains of *C. auris* in the intensive care units and other wards of 5 different hospitals in the present series, the accurate identification and antifungal susceptibility testing of this yeast is pertinent for guiding therapy and determining the prognosis in such settings. Also, accurate identification of the cryptic species *C. auris* is important in assessing the epidemiology and pathogenicity of the disease.

**TABLE 1 In vitro antifungal susceptibility profile of *C. auris*, *C. haemulonii*, and *C. duobushaemulonii* strains by the CLSI M27-A3 broth microdilution method**

<table>
<thead>
<tr>
<th>Species tested</th>
<th>MIC parameter</th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>ISA</th>
<th>POS</th>
<th>FLU</th>
<th>5-FC</th>
<th>CAS</th>
<th>MFG</th>
<th>AFG</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. auris</em> (&lt;90 isolates)</td>
<td>GM</td>
<td>16</td>
<td>0.25</td>
<td>0.60</td>
<td>0.023</td>
<td>0.11</td>
<td>0.11</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>0.5</td>
<td>2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>0.25–0.5</td>
<td>&lt;0.03–4</td>
<td>&lt;0.015–0.5</td>
<td>2–64</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. haemulonii</em> (&lt;7 isolates)</td>
<td>GM</td>
<td>16</td>
<td>0.25</td>
<td>0.60</td>
<td>0.023</td>
<td>0.11</td>
<td>0.11</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>0.5</td>
<td>2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>0.25–0.5</td>
<td>&lt;0.03–4</td>
<td>&lt;0.015–0.5</td>
<td>2–64</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. duobushaemulonii</em> (&lt;5 isolates)</td>
<td>GM</td>
<td>16</td>
<td>0.25</td>
<td>0.60</td>
<td>0.023</td>
<td>0.11</td>
<td>0.11</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>0.5</td>
<td>2</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>16</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>0.25–0.5</td>
<td>&lt;0.03–125</td>
<td>&lt;0.015–0.5</td>
<td>2–64</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td>0.125–8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; ISA, isavuconazole; POS, posaconazole; FLU, fluconazole; 5-FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; AFG, anidulafungin.

**TABLE 2 Distribution of MICs of amphotericin B, caspofungin, and voriconazole obtained by 3 different methods for *Candida auris* (<90) strains**

<table>
<thead>
<tr>
<th>Drug tested&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Test method</th>
<th>No. of isolates at MIC (&lt;μg/ml)&gt;</th>
<th>MIC (&lt;μg/ml)&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>CLSI-BMD</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td>CAS</td>
<td>CLSI-BMD</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td>VRC</td>
<td>CLSI-BMD</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
<td>0.03 0.06 0.125 0.25 0.5 1 2 4 8 16</td>
</tr>
</tbody>
</table>

<sup>a</sup> AMB, amphotericin B; CAS, caspofungin; VRC, voriconazole.
caused by this underreported pathogen in different geographic areas. In the past 5 years, \textit{C. auris} fungemia has been reported from South Africa, South Korea, Japan, and India (3, 5-8). All of the reports from these countries confer the major issue of notable elevated MICs for azoles and caspofungin in \textit{C. auris} and its misidentification by phenotypic methods. The present study employed MALDI-TOF MS, a more robust diagnostic technique, for rapid identification. The strength of the present study is that it developed a comprehensive reference database built with a large number of molecularly characterized \textit{C. auris} strains from different geographical regions of India to supplement the Bruker Biotype library, which has a database of only 3 strains from Korea and Japan. Not only was interspecies differentiation well characterized, but also the mass spectra variation at the intraspecies level separated \textit{C. auris} isolates from India. It is pertinent to mention here that, previously, Indian \textit{C. auris} isolates have been reported to exhibit differences in biochemical profiles compared to the Japanese and the Korean \textit{C. auris} isolates (7, 8). Similarly, genotypic variation among \textit{C. auris} isolates from different geographical regions has also been observed with M13 fingerprinting and amplified fragment length polymorphism analysis (7). It is evident from the present study that the high resolution and discriminatory power of MALDI-TOF MS facilitate differentiation of closely related cryptic species within the Metschnikowiaceae clade (23), which has also been documented previously for Mucorales, particularly the \textit{Lichtheimia} species (24).

Another issue of concern is the mislabeling highly elevated MICs of AMB with Vitek automated readings in all \textit{C. auris} isolates tested. The overall EA between Vitek automated readings and the CLSI-BMD method for AMB was very low (10%). Notably, the reference CLSI-BMD method in the present series showed reduced AMB MICs in 84% of \textit{C. auris} isolates. Similarly, low AMB MICs (0.25 to 1 µg/ml) by CLSI-BMD were reported for 20 \textit{C. auris} isolates from South Korea by Shin et al. (25). However, these authors observed a high EA (100%) between the CLSI-BMD and Vitek method for AMB, which is in contrast with the observations in the present study. This deviation could be attributed to the low number of isolates tested (25). Major errors of azole susceptibility in 218 isolates of 5 \textit{Candida} species using another commercial automated reading system (ATB FUNGUS 3) have been reported recently from China, resulting in pseudohigh rates of antifungal resistance (26). In fact, the erroneously elevated MICs by the Vitek 2 automated reading method not only may lead to inappropriate selection of antifungal therapy but also depict false rates of high antifungal resistance in epidemiological studies. Further, the percentage of \textit{C. auris} isolates that showed elevated CAS MICs (≥ 1 µg/ml) by CLSI-BMD in the present series (37%) declined to 12% using the Etest. The lower Etest MIC values than CLSI-BMD MIC values for CAS have also been reported earlier for other \textit{Candida} species (27). Recently, the performance of the CAS Etest based on the recently revised CLSI breakpoints for \textit{Candida} isolates showed that 13.1% were misclassified as intermediate or resistant (28). Also, marked interlaboratory variation has been observed with both CLSI-BMD and the EUCAST method for CAS susceptibility (29). In order to investigate the resistance mechanism with respect to elevated CAS MICs, in the present series we attempted to sequence FKS hot spot regions in \textit{C. auris} isolates using known FKS \textit{C. glabrata} primers due to a lack of published genomic data for \textit{C. auris}. Although none of the isolates with elevated CAS MICs harbored mutations reported for echinocandin-resistant \textit{C. glabrata}, the possibility of other mutations not reported so far could not be ruled out. Future studies on complete genomic analysis are warranted to detect true antifungal resistance in this significant pathogen.

Finally, \textit{C. auris} is emerging as a serious multidrug nosocomial pathogen in many centers in India, which could be reliably and rapidly identified by MALDI-TOF MS. Notwithstanding the fact that routine laboratories heavily rely on commercial systems for identification and antifungal susceptibility testing for yeasts, a cautionary approach is recommended for isolates showing elevated MICs with these systems.

**ACKNOWLEDGMENTS**

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18. Reference deleted.


Candida infective endocarditis

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Abstract Candida infective endocarditis (IE) is uncommon but often fatal. Most epidemiologic data are derived from small case series or case reports. This study was conducted to explore the epidemiology, treatment patterns, and outcomes of patients with Candida IE. We compared 33 Candida IE cases to 2,716 patients with non-fungal IE in the International Collaboration on Endocarditis—Prospective Cohort Study (ICE-PCS). Patients were enrolled and the data collected from June 2000 until August 2005. We noted that patients with Candida IE were more likely to have prosthetic

ICE-PCS Group investigators are listed in the Appendix.

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valves ($p<0.001$), short-term indwelling catheters ($p<0.0001$), and have healthcare-associated infections ($p<0.001$). The reasons for surgery differed between the two groups: myocardial abscess (46.7% vs. 22.2%, $p=0.026$) and persistent positive blood cultures (33.3% vs. 9.9%, $p=0.003$) were more common among those with Candida IE. Mortality at discharge was higher in patients with Candida IE (30.3%) when compared to non-fungal cases (17%, $p=0.046$). Among Candida patients, mortality was similar in patients who received combination surgical and antifungal therapy versus antifungal therapy alone (33.3% vs. 27.8%, $p=0.26$). New antifungal drugs, particularly echinocandins, were used frequently. These multi-center data suggest distinct epidemiologic features of Candida IE when compared to non-fungal cases. Indications for surgical intervention are different and mortality is increased. Newer antifungal treatment options are increasingly used. Large, multi-center studies are needed to help better define Candida IE.

Because of the rarity of candidal IE at any single institution, the epidemiology, prognosis, and optimal therapy of Candida IE are poorly defined, and treatment guidelines are derived mostly from single-site case series and case reports [3–6]. The recommended treatment of Candida IE is an amphotericin B-based regimen plus surgical intervention, often followed by long-term fluconazole for suppression [5]. However, because of the availability of safe, effective drugs for invasive candidiasis, emerging azole resistance, and high mortality, alternative drugs are now being increasingly used for Candida IE [7–14].

In the current investigation, we used a contemporary, prospective, international, multi-center cohort of patients with definite endocarditis to better evaluate the clinical characteristics, current antifungal treatment practices, and outcome of patients with Candida IE. Moreover, we compare and contrast Candida IE cases with non-fungal cases in order to highlight differences in epidemiology and outcomes.

**Introduction**

*Candida* infective endocarditis (IE) is a rare and poorly understood complication of fungemia. Although *Candida* IE has been regarded traditionally as an uncommon infection, the rates of fungemia have increased by as much as 128% in recent years, leaving a growing number of patients at risk for this complication [1]. Despite aggressive antifungal and surgical therapy, mortality approaches 80% in some series and a better understanding of this infection is needed [2–4].

**Materials and methods**

**Study population**

The patient data are derived from the International Collaboration of Endocarditis–Prospective Cohort Study (ICE-PCS), a multi-national database of prospective cases of endocarditis. Details of the ICE-PCS have been described previously [15–17]. From June 2000 to August 2005, there were 2,760 cases of definite IE contributed by 61 centers in 28 countries. Of the 2,760 cases of definite IE, there were 33 cases due to *Candida* spp. All cases were classified as definite IE based on revised Duke criteria [18] and all cases were verified by the coordinating center (Duke University Medical Center). Fungal IE cases caused by organisms other than *Candida* (11 cases) were excluded from analysis. From each enrolled patient, data were collected from the index hospitalization and entered using an Internet-based system. The data collected included demographics, symptoms associated with IE, underlying medical conditions, predisposing factors, clinical signs and symptoms, antifungal therapy, echocardiographic findings, associated complications, and outcomes (stroke, embolic events, heart failure, intracardiac abscesses, persistently positive blood cultures, and death). Healthcare-associated IE was defined as either nosocomial infection or non-nosocomial healthcare-related infection. Nosocomial infection was defined as IE developing in a patient hospitalized for more than 48 h before the onset of signs/symptoms consistent with IE. Death was determined at the time of hospital discharge. Data on longer-term mortality was not collected.
Statistical methods

Categorical variables were represented as frequencies and percentages of the specified group. The associations between clinical characteristics and Candida IE were measured using the Wilcoxon rank sum test for continuous variables and Chi-square or Fisher's exact methods for categorical variables. For all tests, statistical significance was determined at the 0.05 level. All statistical analyses were performed using SAS software (version 9.1, SAS Institute, Cary, NC).

Results

Patient characteristics

Of the 2,749 patients with definite IE, 33 (1.2%) were Candida IE cases. The mean age of patients with Candida IE was 54.9 years. Patient characteristics including diabetes, renal disease, malignancy, intravenous drug use, and congenital heart disease were similar between the two groups (Table 1). Patients with Candida IE were less likely to be male (51.5% vs. 67.9%, p=0.04), more frequently had previous endocarditis (21.2% vs. 7.8%, p=0.005), and were more likely to have short-term indwelling catheters (21.2% vs. 4.4%, p<0.0001). Among patients who had an invasive procedure within 60 days prior to the onset of symptoms, coronary artery bypass grafting (CABG) was more common among Candida IE patients (22.2% vs. 3.7%, p=0.007). Prosthetic valve IE was more common in Candida patients (48.8% vs. 19.6%, p=0.005), and Candida IE patients were more likely to have the infection classified as being healthcare-related (51.5% vs. 25.8%, p=0.0009).

Clinical findings

Of patients with any IE etiology, most (75%; 2,068/2,749) experienced the first clinical manifestation less than one month before presentation, and the timing of IE manifestations was similar between the two groups. The most common clinical manifestations among all of the patients were fever (79.5%; 2,170/2,728), new murmur (47.9%; 1,053/2,198), hematuria (22.1%; 607/2,737), pulmonary edema (22.3%; 556/2,491), and evidence of a vascular embolic event (15.9%; 435/2,728). Overall, there was little difference in symptoms and signs at presentation between the Candida and non-fungal IE groups (Table 2). A total of 1,316 (47.9%) of 2,749 patients had surgery for endocarditis, and this was not different for the two groups. Candida IE patients were more likely to have surgery indicated because of embolization (40% vs. 19.8%, p=0.054), persistent fungemia (33% vs. 9.9%, p=0.003), and myocardial abscess (46.7% vs. 22.2%, p=0.026). By contrast, surgery for the indications of congestive heart failure (42.6% vs. 13.3%, p=0.02) and valvular regurgitation (68% vs. 40%, p=0.018) were more common in patients with non-fungal IE.

Complications

Congestive heart failure, systemic embolization after presentation, and stroke were common but had similar occurrence in the two groups. Candida IE was associated with persistently positive blood cultures (39.4% vs. 8.8%, p<0.001) (Table 3). Mortality at the time of discharge was higher among Candida IE patients than non-fungal IE patients (20.3% vs. 17%, p=0.046). This mortality difference was more pronounced among those patients who had surgery for this episode of IE (33.3% vs. 13.8%, p=0.03). Among 15 Candida IE patients who underwent surgical intervention for this episode of endocarditis, mortality at discharge was similar to Candida IE patients who did not have surgery (33.3% vs. 27.8%, p=0.26). Those patients who underwent surgical intervention were more likely to have previous IE (40% vs. 5.7%, p=0.016), previous surgery for IE (33.3% vs. 5.6%, p=0.009), paravalvular complications on ECHO (46.7% vs. 11.1%, p=0.015), and systemic embolization (46.7% vs. 16.7%, p=0.04) when compared with patients with Candida IE who were not treated with surgical intervention.

Organisms and antifungal treatment

Among the 33 patients with Candida IE, 16 (48%) were caused by C. albicans, 7 (21%) C. parapsilosis, 5 (15%) C. glabrata, and 3 (9%) C. tropicalis. Two (6%) isolates were not fully specified. Treatment data were available for 27 (82%) of 33 patients (Table 4). The most common antifungal agent used was amphotericin B (AmbB), either conventional AmbB (13/27; 48.1%) or a lipid formulation (3/27; 11.1%). Fluconazole was used in 12 (44.4%) of 27 patients. Primary therapy with fluconazole was used in 6 (54.5%) of 11 patients with complete fluconazole treatment data available. Ten patients (37%) received treatment with the newer antifungal agents caspofungin or voriconazole. Among the patients who received single-drug therapy, death occurred in 6 (40%) of 15 patients; death occurred in 2 (25%) of 8 who received sequential therapy. In only two cases, combination therapy was used and both patients were alive at discharge. Two (20%) of 10 people who received newer therapies (caspofungin or voriconazole) died.

Discussion

Candida IE is an uncommon but frequently fatal infection [3, 4, 6]. A better understanding of the epidemiology, associated risk factors, and treatment methods is needed.
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<th>Characteristic</th>
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<th>Non-fungal, n=2,716 (%)</th>
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a P-values were obtained by Chi-square or Fischer's exact methods
b Among patients who had an invasive procedure within 60 days prior to the onset of symptoms
c Refers to pacemakers, intracardiac defibrillators, or other SD=standard deviation; IVDA=intravenous drug abuse; IE=iefective endocarditis; CABG=coronary artery bypass grafting

but it is difficult to obtain because of the rarity of cases and the lack of large prospective cohorts. We compared contemporary clinically well-characterized cases of candidal IE to non-fungal IE cases registered as part of a large, multi-center, prospective dataset to better understand Candida IE. This analysis revealed several important observations regarding predisposing conditions, clinical findings, and treatment modalities.

Important risk factors or predisposing conditions for fungal endocarditis have been reported in recent, extensive
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<tr>
<th>Clinical finding</th>
<th>Level</th>
<th>Candida, r=33 (%)</th>
<th>Non-fungal, r=2,716 (%)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Fever &gt;38°C&lt;sup&gt;b&lt;/sup&gt;</td>
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reviews, and the most frequently reported are previous surgery, vascular lines, antibiotic use, underlying heart disease, prosthetic valves, and immunocompromising conditions [2–4, 6]. We found similar predisposing conditions and noted several distinct differences among Candida and non-fungal IE cases. First, CABG and prosthetic valve IE were significantly more common in Candida patients. An increase in previous CABG among Candida IE patients could be explained by CABG being performed in association with prosthetic valve surgery. Second, healthcare-associated IE was more common among patients with Candida IE. The increase in hospital-acquired Candida IE, in general, is consistent with recent data describing Candida as an emerging nosocomial bloodstream pathogen over the past decade [19].

The clinical findings and presentation of patients with Candida and non-fungal IE are very similar, as has been previously described [6]. The most important exceptions discovered in our review are related to indications for cardiac surgery. Of patients who had surgery during this episode of IE, those with Candida IE were more likely to have surgery based on the finding of myocardial abscess or persistently positive blood cultures. Non-fungal cases more commonly had heart failure or valvular insufficiency as a reason for surgery.

There were few differences in complications and outcomes in the two groups except mortality. Candida IE mortality has been reported to be up to 80% in previous reviews [2–4, 6], but variability in the data collection and description of individual cases makes it difficult to determine an appropriate risk of death. Ellis et al. [3], in a recent review, demonstrated that the crude survival of patients with fungal endocarditis had increased over the past 20 years, from 14% before 1970 to 41% in the period

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Level</th>
<th>Candida, ( n=33 ) (%)</th>
<th>Non-fungal, ( n=2,716 ) (%)</th>
<th>( P )-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke</td>
<td>Yes</td>
<td>4 (12.1)</td>
<td>450 (16.6)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28 (84.8)</td>
<td>2,213 (81.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>1 (3)</td>
<td>53 (2)</td>
<td></td>
</tr>
<tr>
<td>Embolization</td>
<td>Yes</td>
<td>10 (30.3)</td>
<td>592 (21.8)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>22 (66.7)</td>
<td>2,053 (75.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>1 (3)</td>
<td>71 (2.6)</td>
<td></td>
</tr>
<tr>
<td>CHF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes</td>
<td>8 (24.2)</td>
<td>856 (31.5)</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23 (69.7)</td>
<td>1,794 (66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>2 (6)</td>
<td>66 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Persistent positive blood ex&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>13 (39.4)</td>
<td>238 (8.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>19 (57.6)</td>
<td>2,297 (88.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>1 (3)</td>
<td>81 (3)</td>
<td></td>
</tr>
<tr>
<td>Mortality at discharge</td>
<td>Yes</td>
<td>10 (30.3)</td>
<td>464 (17)</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23 (66.7)</td>
<td>2,243 (82.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>0</td>
<td>9 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Mortality (with surgery)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Yes</td>
<td>5 (33.3)</td>
<td>179 (13.8)</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10 (66.7)</td>
<td>1,120 (86.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>0</td>
<td>2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Mortality (without surgery)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Yes</td>
<td>5 (27.8)</td>
<td>285 (20.3)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>13 (72.2)</td>
<td>1,117 (79.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>0</td>
<td>1 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> \( P \)-values were obtained by Chi-square and Fisher's exact methods

<sup>b</sup> Refers to cardiothoracic surgery. Mortality determined at the time of discharge

<sup>c</sup> CHF=congestive heart failure; ex=culture
Table 4 Treatment for 27 patients with Candida infective endocarditis (IE)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Organism</th>
<th>Therapy</th>
<th>Surgery</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>C. albicans</td>
<td>FLU then CASPO</td>
<td>No</td>
<td>Dead</td>
</tr>
<tr>
<td>3</td>
<td>C. albicans</td>
<td>CASPO then FLU</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>C. parapsilosis</td>
<td>FLU/CASPO^3</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>C. glabrata</td>
<td>FLU then CASPO</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>C. albicans</td>
<td>FLU</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>C. glabrata</td>
<td>AmB</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>C. tropicalis</td>
<td>AmB</td>
<td>Yes</td>
<td>Dead</td>
</tr>
<tr>
<td>9</td>
<td>C. albicans</td>
<td>AmB then FLU</td>
<td>No</td>
<td>Dead</td>
</tr>
<tr>
<td>10</td>
<td>C. glabrata</td>
<td>CASPO+lipid AmB followed by CASPO^4</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>C. parapsilosis</td>
<td>AmB/CASPO^3</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>12</td>
<td>C. glabrata</td>
<td>CASPO</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>13</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>14</td>
<td>C. albicans</td>
<td>Lipid AmB then FLU</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>15</td>
<td>C. albicans</td>
<td>FLU</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>16</td>
<td>C. albicans</td>
<td>CASPO</td>
<td>Yes</td>
<td>Dead</td>
</tr>
<tr>
<td>17</td>
<td>C. glabrata</td>
<td>AmB</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>18</td>
<td>C. albicans</td>
<td>AmB</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>19</td>
<td>C. albicans</td>
<td>AmB</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>20</td>
<td>C. parapsilosis</td>
<td>AmB then FLU</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>21</td>
<td>C. albicans</td>
<td>FLU+SFC</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>22</td>
<td>C. tropicalis</td>
<td>AmB</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>23</td>
<td>C. parapsilosis</td>
<td>CASPO then FLU</td>
<td>No</td>
<td>Alive</td>
</tr>
<tr>
<td>24</td>
<td>C. tropicalis</td>
<td>Lipid AmB</td>
<td>Yes</td>
<td>Dead</td>
</tr>
<tr>
<td>25</td>
<td>C. albicans</td>
<td>AmB then VORI</td>
<td>Yes</td>
<td>Alive</td>
</tr>
<tr>
<td>26</td>
<td>C. albicans</td>
<td>AmB</td>
<td>No</td>
<td>Dead</td>
</tr>
</tbody>
</table>

^1 Only 27 patients had treatment data available
^2 Outcome at the time of hospital discharge
^3 Treatment data other than the drugs received were unavailable
^4 Patient received 1 month of VORI for suppressive therapy after an initial 11 weeks of treatment with CASPO and lipid AmB. Because of toxicity with VORI, CASPO was administered for an additional 8 weeks
AmB=amphotericin B; Lipid AmB=liposomal AmB; CASPO=caspofungin; FLU=fluconazole; SFC=fluotosine; VORI=voriconazole

1991–1995. Possible reasons for this improved survival were attributed to better echocardiographic techniques, earlier diagnosis of endocarditis, or better supportive care of ill patients [3]. Nearly one-third of patients in our series died during hospitalization, with mortality significantly greater than non-fungal cases. The mortality among patients with Candida IE in our series is surprisingly less than that reported in previous reviews, but may be due to a multitude of factors. Diagnostic and treatment modalities have improved in the past decade, but, likely, cannot account for such a difference in survival. The inclusion of Candida cases only, which often have better survival compared to other fungal causes [3, 4], and the survival end-point defined at hospital discharge (compared to literature reviews, where follow-up data were available for up to several years) may reflect the lower mortality in this series [3]. Finally, the use of newer antifungal therapies, such as the echinocandins and lipid preparations of amphotericin B, not included in previous reviews because of the lack of availability, may have an impact on outcomes and warrant further evaluation.

The traditional antifungal treatment of Candida IE is amphotericin B (6–8 weeks), often followed by fluconazole as suppression because of frequent relapse [5, 6]. In addition, surgical intervention with valve replacement is generally recommended in most cases. The combination of antifungal and surgical therapy is purported to be more beneficial than antifungal therapy alone, although controlled studies have not been performed for confirmation [3, 4, 20]. In this cohort, surgical therapy was not associated with increased survival compared to antifungal therapy alone. It is encouraging that patients who did not receive surgical therapy fared relatively well; however, we speculate that the lack of a significant difference between the groups may reflect a combination of factors, including increased morbidity and complications at presentation.
among patients who underwent surgery. Patients who underwent surgical intervention were more likely to have previous IE, previous surgery for IE, paravalvular complications on ECHO, and systemic embolization. Although these may be important differences that influenced the risk of death, with the limited number of patients evaluated, it is difficult to draw conclusions with respect to the appropriate management.

In this cohort, an amphotericin B preparation was the most frequent drug used. Fluconazole was the second most common, and was used either for primary or sequential therapy. Sequential therapy was frequently employed, and mortality in this group was lower than in patients who received a single agent. This probably results from selecting a subset of patients that lived long enough to “step down” to azole therapy. The lengths of therapy and dosages were not captured, so appropriate comparisons cannot be made. An important obstacle in the successful antifungal therapy of Candida IE has been adverse events associated with prolonged amphotericin B administration. With the approval of new antifungal agents in the past several years, specifically echinocandins and newer azoles, questions have arisen about the role of these agents for the treatment of Candida IE. The echinocandins and voriconazole have shown efficacy and safety for the treatment of invasive candidiasis and candidemia [21, 22]; however, data on usage in endocarditis is limited to case reports [7–14]. Although some clinical success has been documented, selection bias may be present, and determinations of efficacy cannot be made. Our series reflects a shift in the treatment of Candida IE. More than one-third of patients received newer antifungal agents, particularly the echinocandin, caspofungin, and mortality among these patients (20%) was similar to the other groups. Adverse events from drug use and isolate susceptibilities were not captured in the database, so the reasons for the use of these drugs are unclear.

Although an important aspect of this dataset is its overall size, and this represents the largest reported number of definite Candida IE cases compared to non-fungal cases, there are important limitations. The data were collected prospectively, but analysis was conducted retrospectively. The number of Candida cases is not large enough to draw conclusions regarding treatment, and long-term mortality data were not collected.

These data represent a multi-center collaborative effort describing a large cohort of definite endocarditis cases. There appear to be distinct epidemiologic features of Candida IE when compared to non-fungal cases. Indications for surgical intervention are different, mortality is increased, and alternative antifungal treatment options are increasingly used for this devastating disease. Large datasets or series, despite their limitations, are needed to help better define Candida IE.

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JJM: None.

EA: None.

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EB: None.

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TE: None.

ZK: None.

JK: None.

SL: None.

DL: None.

DS: None.

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AJM: None.

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VHC: None.

CC: None.

Appendix

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References

ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients


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Abstract

This part of the EFISG guidelines focuses on non-neutropenic adult patients. Only a few of the numerous recommendations can be summarized in the abstract. Prophylactic usage of fluconazole is supported in patients with recent abdominal surgery and recurrent gastrointestinal perforations or anastomotic leakages. Candida isolation from respiratory secretions alone should never prompt treatment. For the targeted initial treatment of candidaemia, echinocandins are strongly recommended while liposomal amphotericin B and voriconazole are supported with moderate, and fluconazole with marginal strength. Treatment duration for candidaemia should be a minimum of 14 days after the end of candidaemia, which can be determined by one blood culture per day until negativity. Switching to oral treatment after 10 days of intravenous therapy has been safe in stable patients with susceptible Candida species. In candidaemia, removal of indwelling catheters is strongly recommended. If catheters cannot be removed, lipid-based amphotericin B or echinocandins should be preferred over azoles. Transoesophageal echocardiography and fundoscopy should be performed to detect organ involvement. Native valve endocarditis requires surgery within a week, while in prosthetic valve endocarditis, earlier surgery may be beneficial. The antifungal regimen of choice is liposomal amphotericin B +/- flucytosine. In ocular candidiasis, liposomal amphotericin B +/- flucytosine is recommended when the susceptibility of the isolate is unknown, and in susceptible isolates, fluconazole and voriconazole are alternatives. Amphotericin B deoxycholate is not recommended for any indication due to severe side effects.

Keywords: Candidiasis, Guideline, non-neutropenic, prophylaxis, treatment

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This guideline was presented in part at ECCMID 2011.

*European Society for Clinical Microbiology and Infectious Diseases.

*Members of the subgroup committee mainly responsible setting up this manuscript.
Introduction

Invasive candidiasis remains a challenging complication, which frequently occurs in patients with one or more underlying diseases or surgical interventions. In recent point prevalence studies, a candidaemia incidence of 6.9 per 1000 ICU patients was reported, and 7.5% of ICU patients received antifungal therapy [1,2]. Candidaemia increases mortality rates in the range of 20–49% [3,4], but still there are many open management questions.

The unmet medical needs surrounding candidaemia and invasive candidiasis are defined in general from diagnosis to prophylaxis, empiric and pre-emptive strategies to treatment. So far, the scientific community has not achieved to accurately predict invasive candidiasis and thus to define populations that benefit from prophylaxis or early treatment [5]. Although it is well known that treatment is being initiated too late in the majority of patients, identification of the optimal time point to commence antifungal therapy remains challenging [6,7]. Intertwined with this problem is insufficient support of reliable mycological assays preventing timely and diagnosis-driven early treatment initiation [173].

With the diversity of various groups of patients with organ involvement beyond the bloodstream, a body of diverse evidence on the best treatments and infectious diseases management decisions, for example, treatment duration is provided.

In the light of the medical need to analyse the scientific evidence in the field of invasive Candida diseases, the ESCMID European Fungal Infection Study Group (EFISG) developed comprehensive practical guidance for microbiologists and clinicians to facilitate evidence-based decision making.

This guideline follows the clinical events in a chronological order. Prophylaxis in patient populations at risk for invasive Candida disease is followed by fever- and diagnosis-driven approaches to early therapy and finally targeted therapy. Important clinical questions on catheter management to step-down strategies are being addressed. Specific situations in deep tissue candidiasis are cherished, and for each topic, a table lists the medical/scientific evidence.

Methods

An expert group (OAC, MB, TC, JG, BJK, OL and WM) was set up by EFISG and searched the literature. Documents and views were shared by email, teleconferences, and face-to-face meetings during 2010–2012. Once a first consensus was reached, the preliminary recommendations were presented to the whole group, that is, the other authors, discussed, developed further, and finalized as a group consensus. The methods to evaluate the quality of evidence and to reach group consensus recommendations are described in this issue of Clinical Microbiology and Infection [172]. Definition of the strength of recommendation is given in Table 1. The quality of the published evidence is defined in Table 2. Grouping quality of evidence into three levels only may lead to diverse types of published evidence being assigned specifically a level II. To increase transparency in the evaluation of the evidence, we added an index (Table 2) to the level II recommendations, where appropriate. Of note, the strength of recommendation and the quality of evidence were assigned in two separate evaluations, thus allowing, for example, a recommendation strongly supporting a procedure even if there is a lower level of evidence.

Results

Prophylaxis

Antifungal prophylaxis has been discussed as a promising approach in ICU patients. At this moment, the optimal target population for antifungal prophylaxis remains unknown, as this question has not been sufficiently addressed in clinical trials. Some special populations though have been enrolled in randomized clinical trials, and recommendations for these can be given.

<table>
<thead>
<tr>
<th>TABLE 1. Definition of the strength of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2. Definition of the quality of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESCMID EFISG</td>
</tr>
<tr>
<td>Level</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
</tbody>
</table>

Index (for quality of evidence II) |
- Meta-analysis or systematic review of randomized controlled trials |
- Transferred evidence, that is, results from different patients’ cohorts, or similar immune-status situation |
- Comparator group is a historical control |
- Uncontrolled trial |
- Published abstract (presented at an international symposium or meeting)
Evidence. Patients who had undergone abdominal surgery recently and who had recurrent gastrointestinal perforations or anastomotic leakages were treated either with fluconazole 400 mg/day or with placebo in order to prevent intraabdominal Candida infection. The rate of intraabdominal candidiasis was significantly lower in the fluconazole prophylaxis group. This clinical trial exhibited high technical quality, but was performed in a very high baseline incidence population and is limited by enrolling 43 evaluable patients only [8]. In a small non-comparative trial, standard dosed caspofungin was evaluated in the same indication, but no evidence can be derived [9]. In a large prophylaxis trial, critically ill surgical patients with an expected ICU stay of ≥3 days were randomized to receive either fluconazole 400 mg/day or placebo. The primary endpoint was the time to fungal infection, which was significantly delayed in the fluconazole prophylaxis group. The trial was well designed and enrolled 260 patients. A limitation of the study is the inclusion of presumed invasive fungal infection, defined for example, by repeatedly positive urine cultures and catheter tips with ≥15 yeast colonies, into the primary endpoint [10]. In another study, patients ventilated for 48 h and expected to remain ventilated for another ≥72 h received selective digestive decontamination with polymyxin B, neomycin and vancomycin and were randomized to receive fluconazole 100 mg/day or placebo. This trial was well designed, and 204 patients were randomized. Candidaemia was more successfully prevented in fluconazole recipients, but the selective digestive decontamination regimen used in this clinical trial is not a standard in most countries [11–13]. Meta-analyses of the clinical trials above and some other studies on highly selected populations found fluconazole 400 mg/day to be superior to placebo in preventing invasive fungal infection in critically ill surgical patients [14–18]. A more recent clinical trial compared caspofungin 50 mg/day with placebo for prophylaxis in a highly selected population of ventilated patients receiving antibiotics, having a central venous catheter and fulfilling at least one of the following criteria: parenteral nutrition, dialysis, major surgery, pancreatitis, systemic steroids or other immunosuppressant medication. The primary endpoint of this trial was the incidence of proven and probable invasive candidiasis according to EORTC/MSG definitions [19]. The investigators found a trend only towards a reduced incidence of invasive candidiasis [5]. Other antifungals have been evaluated in prophylactic indications [20–22]. For ketoconazole 200 mg/day, evidence of prophylactic benefit is weak while adverse events and drug interactions limit its use in general [22]. The same is true for itraconazole 400 mg/day [21]. Nystatin 4 Mio IU/day has been evaluated, but concept and patient setting are basically outdated [20]. Intravenous amphotericin B and the echinocandins have not been sufficiently evaluated in this indication [23]. Antifungal prophylaxis in solid organ transplant recipients is not part of this guideline.

Of note, none of the trials proved a reduction in overall or attributable mortality. All trials were lacking power to address the potential emergence of less azole-susceptible strains during prophylaxis. Apart from historical control studies in intensive care and abdominal surgical populations, this has been shown in prophylactic settings in haematology during substantially longer azole exposure periods [24–26]. Selection of less-susceptible strains remains a caveat against broadly using antifungals in populations where substantial benefit has not been proven.

Recommendations. Fluconazole prophylaxis against invasive candidiasis is recommended in patients who recently underwent abdominal surgery and had recurrent gastrointestinal perforations or anastomotic leakages. For further recommendations, refer to Table 3.

Fever-driven approach (empiric)

We defined empiric therapy as a fever-driven approach in the clinical situation of a patient at risk for invasive candidiasis who is persistently febrile with no microbiological evidence of infection.

Evidence. The value of initiating antifungal therapy in this situation has been addressed in a number of retrospective studies. Incubation time [27] and time from first positive blood culture drawn to initiation of empiric antifungal therapy correlated with mortality increases [6,28]. Similarly, in a population-based retrospective study, empiric antifungal treatment was associated with higher survival rates, if the isolate turned out to be susceptible to the empiric regimen [29]. Another retrospective study in patients with septic shock due to any cause found empiric antifungal therapy was given infrequently, and those with invasive fungal infection not receiving empiric antifungals had a statistically significantly higher mortality [7].

Although uncontrolled, all of these studies suggest that initiating empiric therapy may be beneficial to reduce overall mortality, but none could identify reliable triggers for antifungal treatment. They analysed patients with candidaemia but not the whole population of febrile patients.

One randomized double-blind placebo-controlled clinical trial evaluated fluconazole 800 mg/day in 270 adult ICU patients with an APACHE II score >16. Rates of invasive candidiasis were not statistically different between the two groups. The primary endpoint was driven by resolution of
fever, and empirical fluconazole treatment did not improve outcome when compared with placebo [30].

**Recommendations.** Early treatment of presumed fungaemia is presumably associated with higher survival rates, but the optimal time point for initiating empiric antifungal treatment remains undetermined. Due to lack of data, no recommendation can be given for choosing a specific drug for fever-driven therapy. In general, such choice should be based on local epidemiology and drug–drug interactions in the individual patient and should be made among the same drugs as recommended for candidaemia. Further recommendations are given in Table 4.

**TABLE 3. Recommendations on antifungal prophylaxis in ICU patients**

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent abdominal surgery AND recurrent gastrointestinal perforations or anastomotic leakages</td>
<td>To prevent intraabdominal Candida infection</td>
<td>Fluconazole 400 mg/day</td>
<td>B</td>
<td>I</td>
<td>[8]</td>
<td>Placebo N = 43 Single arm N = 19</td>
</tr>
<tr>
<td>Critically ill surgical patients with an expected length of ICU stay ≥3 day Ventilated for 48 h and expected to be ventilated for another ≥72 h</td>
<td>To delay the time to fungal infection</td>
<td>Fluconazole 100 mg/day</td>
<td>C</td>
<td>I</td>
<td>[162]</td>
<td>Placebo N = 204 SDD used</td>
</tr>
<tr>
<td>Critically ill patients with risk factors for invasive candidiasis/candidaemia</td>
<td>To prevent invasive candidiasis/candidaemia</td>
<td>Caspofungin 50 mg/day</td>
<td>C</td>
<td>I</td>
<td>[5]</td>
<td>Placebo N = 186 EORTC/MSG criteria used</td>
</tr>
<tr>
<td>Surgical ICU with catabolism</td>
<td>To prevent invasive candidiasis/candidaemia</td>
<td>Ketoconazole 200 mg/day</td>
<td>D</td>
<td>I</td>
<td>[22]</td>
<td>Placebo N = 27</td>
</tr>
<tr>
<td>ICU patients persistently febrile, but without microbiological evidence</td>
<td>To reduce overall mortality</td>
<td>Fluconazole or echinocandin</td>
<td>C</td>
<td>IIu</td>
<td>[163]</td>
<td>Open N = 147</td>
</tr>
<tr>
<td>ICU patients with candida isolated from respiratory secretions</td>
<td>To cure invasive candidiasis or candidaemia early</td>
<td>Any antifungal</td>
<td>D</td>
<td>IIu</td>
<td>[42]</td>
<td>Placebo N = 46</td>
</tr>
<tr>
<td>ICU patients with positive (1,3)-β-D-glucan test</td>
<td>To cure invasive candidiasis or candidaemia early</td>
<td>Any antifungal</td>
<td>C</td>
<td>IIu</td>
<td>[39]</td>
<td></td>
</tr>
<tr>
<td>Any patient with Candida isolated from a blood culture</td>
<td>To cure invasive candidiasis</td>
<td>Antifungal treatment</td>
<td>A</td>
<td>II</td>
<td>[46]</td>
<td></td>
</tr>
</tbody>
</table>

SoR, Strength of recommendation; QoE, Quality of evidence; ICU, intensive care unit; CVC, central venous catheter; IU, international units. The table displays the published evidence; therefore, other available antifungal agents are not mentioned here.

**TABLE 4. Recommendations on fever-driven and diagnosis-driven therapy of candidaemia and invasive candidiasis**

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult ICU patients with fever despite broad-spectrum antibiotics and APACHE II ≥16</td>
<td>To resolve fever</td>
<td>Fluconazole 800 mg/day</td>
<td>D</td>
<td>I</td>
<td>[30]</td>
<td></td>
</tr>
<tr>
<td>ICU patients persistently febrile, but without microbiological evidence</td>
<td>To reduce overall mortality</td>
<td>Fluconazole or echinocandin</td>
<td>C</td>
<td>IIu</td>
<td>[28]</td>
<td>[163]</td>
</tr>
<tr>
<td>ICU patients with candida isolated from respiratory secretions</td>
<td>To cure invasive candidiasis or candidaemia early</td>
<td>Any antifungal</td>
<td>D</td>
<td>IIu</td>
<td>[42]</td>
<td></td>
</tr>
<tr>
<td>ICU patients with positive (1,3)-β-D-glucan test</td>
<td>To cure invasive candidiasis or candidaemia early</td>
<td>Any antifungal</td>
<td>C</td>
<td>IIu</td>
<td>[39]</td>
<td>[31]</td>
</tr>
<tr>
<td>Any patient with Candida isolated from a blood culture</td>
<td>To cure invasive candidiasis</td>
<td>Antifungal treatment</td>
<td>A</td>
<td>II</td>
<td>[46]</td>
<td>[47]</td>
</tr>
</tbody>
</table>

APACHE, acute physiology and chronic health evaluation.

*The (1,3)-β-D-glucan tests have low specificity and sensitivity with false-positive results in the presence of haemodialysis, other fungal or bacterial infection, wound gauze, albumin or immunoglobulin infusion.

Diagnosis-driven approach (pre-emptive)

We defined pre-emptive therapy as therapy triggered by microbiological evidence of candidiasis without proof of invasive fungal infection.

Evidence. Several studies have addressed diagnosis-driven therapy on grounds of detecting (1,3)-β-D-glucan in serum or plasma. In a study on 46 ICU patients without infection or with confirmed bacterial or fungal infection, glucan test results (G-test; Associates of Cape Cod, East Falmouth, MA, USA) correlated with infection [31]. This was the key finding in a study using the Fungitell™ test.
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(Assoc. of Cape Cod) test, too [32]. Another group of investigators found glucan (FungitellTM; Seigakaku Kogyo, Tokyo, Japan) testing useful in predicting invasive fungal infection, but in a very small population of 32 patients only [33]. During twice weekly monitoring in long-term ICU patients, glucan concentrations (GlucatellTM; Cape Cod) were higher in individuals with proven fungal infection than in those without. As patients with invasive fungal infection had more bacterial infections and other intercurrent complications, the test result could still not clearly distinguish between both groups [34]. Similar results were found in a surgical ICU patient group (N = 57) and in a mixed ICU population (N = 95) where higher glucan concentrations (FungitellTM) were found in those with invasive candidiasis, but still the positive predictive value was limited [35,36]. Findings from a retrospective study on a larger number of patients (N = 871) were in favour of the test (FungitellTM), but documented generally higher glucan concentrations in patients on haemodialysis and in those receiving albumin or intravenous immunoglobulin infusions [37]. Other reasons for positive test results in the absence of invasive candidiasis have been described due to (1,3)-β-D-glucan-containing cell walls of a variety of fungi, for example, Aspergillus or Histoplasma [32,38]. Indeed, the Fungitell™ assay has been suggested useful in the diagnosis of pneumocystis pneumonia as well [39]. A discussion of glucan tests and their cut-offs to positivity can be found in the ESCMID Candida Guidelines on Diagnostic Procedures in this issue [173]. In some of the studies above, it has been stated that a negative glucan test practically rules out invasive candidiasis. Currently, the glucan tests cannot reliably confirm invasive candidiasis, although there may be a role as part of a set of diagnostic tools and patient characteristics.

Recommendations on mannan and anti-mannan antibody detection is part of the EFISG guideline on diagnosis of invasive candidiasis [173].

A controversial issue is the initiation of antifungal therapy upon Candida isolation from respiratory secretions. Two forms of pulmonary candidiasis have been distinguished, that is, pulmonary abscesses resulting from haematogenous spread during candidaemia, especially in febrile neutropenic patients, and direct invasion of bronchial and lung tissues. Most articles on the topic of pulmonary candidiasis were published in the 1970s and 1990s. There are hardly any data on ICU populations, but case series of patients with haematological malignancy and stem cell recipients [40,41]. While Candida can frequently be isolated from respiratory secretions, it appears that Candida invading the lung tissue is a very rare event. In a recent prospective autopsy study (N = 232) on ICU patients, a total of 58% had proven pneumonia. Regardless of whether Candida had been isolated pre-mortem or not, in neither case histopathological proof of Candida tissue invasion was found [42].

Recommendations. Candida isolation from respiratory secretions should never trigger treatment, but rather be interpreted as one site of colonization among others. (1,3)-β-D-glucan detection in serum or plasma prompting antifungal treatment is marginally supported. Detailed recommendations are given in Table 4.

Targeted treatment

Candida isolated from a single peripheral blood culture or a single central-line blood culture defines candidaemia [19,43,44]. Previous definitions may have described asymptomatic patients with a blood culture positive for Candida, and it has been debated whether there are patients who do not need antifungal treatment despite a positive blood culture [45]. This appears to be a very rare clinical situation, as usually blood cultures are triggered by a clinical sign, for example, fever. Each case of candidaemia, even from surveillance blood cultures in asymptomatic patients requires targeted treatment [46–49].

Evidence. A plenitude of well-designed clinical trials evaluated antifungals for the initial treatment of candidaemia and invasive candidiasis. Amphotericin B deoxycholate clearly is a very potent drug against Candida, but the well-documented significant toxicity justifies a recommendation against using this compound [50–55]. In the past, several approaches aimed at reducing toxicity, for example, continuous intravenous administration, but efficacy of this strategy in candidiasis remains unclear [56]. Amphotericin B lipid complex has been evaluated in candidaemia, but the single randomized trial to date has been published as abstract only. Amphotericin B lipid complex appeared to be less nephrotoxic than the deoxycholate formulation although not more effective [57], findings which were supported by a phase IV study [58]. As opposed to laboratory-confirmed adverse events, clinically defined side effects, such as infusion-related fever and chills, tend to be underestimated in uncontrolled post-marketing studies. When ABLC was compared to liposomal amphotericin B in persistently febrile neutropenic patients, infusion-related adverse events occurred very frequently [59]. Data on amphotericin B colloidal dispersion stem from a non-randomized, non-comparative study describing nephrotoxicity in the same range as found with amphotericin B lipid complex [60]. Liposomal amphotericin B and amphotericin B deoxycholate have not been compared directly in patients with candidaemia. But, liposomal amphotericin B appears at least as effective, but less toxic than the deoxycholate formulation when considering results from a large clinical trial on candidaemia and invasive candidiasis evaluating liposomal amphotericin B and micafungin [61]. Compared to micafungin,
efficacy was similar, but renal toxicity was higher with liposomal amphotericin B [61,62]. Caspofungin when compared to amphotericin B deoxycholate was as effective, but significantly less toxic [55]. A clinical strategy became feasible, which avoided amphotericin B toxicity without losing efficacy. Two doses of micafungin (100, 150 mg/day) were compared with caspofungin in a phase III trial. All three regimens were similarly effective and safe [63]. While all echinocandin trials above proved statistical non-inferiority of the experimental study drug as compared to standard regimens, anidulafungin was found to be superior over fluconazole [64]. In particular, the outcomes for patients with Candida albicans were significantly better with anidulafungin (81%) than with fluconazole (62%). The latter result remained valid in a subsequent subgroup analysis of ICU patients: global response for anidulafungin 67% vs. fluconazole 47% [65].

With regard to Candida, all three echinocandins exhibit a broad spectrum activity; acquired resistance is rare, although there has been a first large epidemiological evaluation describing acquisition of resistance genes in Candida glabrata [66]. There is an ongoing debate on whether echinocandins are appropriate for treating Candida parapsilosis, because minimal inhibitory concentrations are found to be higher than those of other Candida species. Overall, that is, clinical and microbiological, response rates in C. parapsilosis infection were not statistically significantly different throughout the echinocandin trials: for caspofungin/amphotericin B, the success rates were 70% and 65%, for micafungin/liposomal amphotericin B 89.2% and 86.7%, for caspofungin/micafungin 100/150 rates were 64.3%, 75.9% and 71.4%, and for anidulafungin/fluconazole, they were 64% and 83% [55,61,63]. However, there were numerically higher numbers of persistent fungaemia due to C. parapsilosis during caspofungin as compared to amphotericin B deoxycholate treatment [55], and during standard dose caspofungin as compared to high dose, that is, 150 mg/day, caspofungin [67], and the eradication rate in C. parapsilosis fungaemia was lower with anidulafungin than with fluconazole [64]. It is important to note that none of these trials were powered to detect such differences.

Two further aspects we considered important when interpreting the latter trial are (i) the microbiological eradication rate as well as the overall success rate in C. albicans infection was higher with anidulafungin than with fluconazole and (ii) Candida krusei infection was excluded from the anidulafungin trial, because of fluconazole being the comparator drug [64].

In the clinical trials, all three echinocandins were well tolerated and appeared very safe. Micafungin though carries a warning label against use unless other antifungals are not appropriate by the European Medicines Agency, which reflects results of rats developing liver tumours after very long and high-dosed exposure [68]. This statement has elicited some debate in terms of its relevance to humans, but has not been withdrawn or disproved so far.

An advantage of the echinocandin class is the low potential for drug–drug interactions. For anidulafungin, no interactions have been described, and for micafungin, very few relevant interactions need to be considered [68,69]. Co-administering caspofungin with rifampin lowers caspofungin exposure, and it has been recommended to increase the dose of caspofungin in the rare cases, where both drugs need to be administered concomitantly. In addition, caspofungin dose has to be increased in patients with a high body weight [70].

For many years, fluconazole was considered the drug of choice for candidaemia [71–73]. This was based on a great number of clinical trials evaluating fluconazole in this indication [52–54,64,74–76]. As anidulafungin was superior over fluconazole in patients with candidaemia, especially those infected with C. albicans, we do no longer consider fluconazole as the drug of choice [64]. Fluconazole was inferior in the subgroup of patients with high APACHE scores and is known to have a limited spectrum of activity, being inactive against C. krusei and being considered hardly active in C. glabrata infection. Microbiologically, it might though be the better drug against C. parapsilosis, which is supported by a trend towards better outcomes in the comparative trial [64], but clinical proof is not in support of this. There have been no trials with sufficient power to assess non-inferiority of echinocandins for C. parapsilosis. In a large clinical trial, voriconazole was non-inferior to amphotericin B deoxycholate followed by fluconazole [43], and voriconazole offers an important additional treatment option for first-line and salvage situations [77,78]. Still there are certain limitations, that is, the multiple drug–drug interactions [79], the limit of the intravenous use to 14 days duration [79] and the variable pharmacokinetics of the drug [80]. Itraconazole yielded negative results when compared to fluconazole [76]. There are no published data on posaconazole treatment of candidaemia.

Very few clinical trials used combination treatment. Lipid-based amphotericin B was supplemented with placebo or efungumab, a monoclonal antibody targeting heat shock protein 90 (HSP-90), in 139 patients. The study design and analysis drew substantial criticism for (i) enrolling an ill-defined patient population, for example, symptomatic candiduria, (ii) enrolling patients with negative fungal cultures and (iii) excluding patients from the efficacy population who died while on treatment [81]. Furthermore, the trial allowed extensive prior antifungal treatment, used a short, 10-day, treatment time until response evaluation and did not specify...
the proportion of patients receiving which type of lipid-based amphotericin B formulation.

The combination of amphotericin B deoxycholate and fluconazole has been as effective as fluconazole monotherapy in a randomized trial, but patients had an increased risk of toxicity and no survival benefit [74]. A small study (N = 72) comparing fluconazole with amphotericin B deoxycholate and 5-flucytosine showed no difference in overall response to treatment [75].

Recommendations. Targeted treatment of candidaemia with echinocandins is strongly recommended. The recommendation for liposomal amphotericin B or voriconazole is less stringent, and fluconazole is recommended with marginal strength only, except for C. parapsilosis. For detailed recommendations, refer to Table 5.

Duration of targeted treatment, step-down to oral treatment and diagnostics in candidaemia

Evidence. The duration of treatment depends on the extent of organ involvement. In a population without documented organ involvement, treatment aims to clear the infection and at the same time to avoid deep-organ involvement. This can be achieved by treating for 14 days after the end of candidaemia [82]. To determine the end of candidaemia, at least one blood culture per day should be taken until culture results come back negative. Treatment can probably be simplified by stepping down to oral fluconazole after 10 days of intravenous treatment, if the patient is stable, tolerates the oral route and if the species is susceptible [55,63,64].

The diagnostic procedures to detect organ involvement comprise transoesophageal echocardiography, fundoscopy and search for a thrombus. A recent observational study found infectious endocarditis in 8.3% of patients with candidaemia; the majority of these patients had no well-established risk factors, that is, vascular prosthesis or persistent candidaemia [83].

Some prospective studies addressed ocular candidiasis as complication of candidaemia. The diagnostic approach was usually based on weekly eye examinations. Immunosuppression and repeatedly positive blood cultures are risk factors

### Table 5. Recommendations on initial targeted treatment of candidaemia and invasive candidiasis in adult patients

<table>
<thead>
<tr>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin 200/100 mg</td>
<td>A</td>
<td>I</td>
<td>[64]</td>
<td>Consider local epidemiology (Candida parapsilosis, Candida krusei), less drug–drug interactions than caspofungin</td>
</tr>
<tr>
<td>Caspofungin 70/50 mg</td>
<td>A</td>
<td>I</td>
<td>[67]</td>
<td>Consider local epidemiology (C parapsilosis)</td>
</tr>
<tr>
<td>Micafungin 100 mg</td>
<td>A</td>
<td>I</td>
<td>[61]</td>
<td>Consider local epidemiology (C parapsilosis), less drug–drug interactions than caspofungin, consider EMA warning label</td>
</tr>
<tr>
<td>Amphotericin B liposomal 3 mg/kg</td>
<td>B</td>
<td>I</td>
<td>[61]</td>
<td>Similar efficacy as micafungin, higher renal toxicity than micafungin</td>
</tr>
<tr>
<td>Voriconazole 6/3 mg/kg/day&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>B</td>
<td>I</td>
<td>[43]</td>
<td>Limited spectrum compared to echinocandins, drug–drug interactions, limitation of IV formulation in renal impairment, consider therapeutic drug monitoring</td>
</tr>
<tr>
<td>Fluconazole 400–800 mg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>C</td>
<td>I</td>
<td>[165]</td>
<td>Limited spectrum, inferiority to anidulafungin (especially in the subgroup with high APACHE scores), may be better than echinocandins against C. parapsilosis</td>
</tr>
</tbody>
</table>

<sup>a</sup>Not all experts agreed, SoR results from a majority vote.
<sup>b</sup>The licensed maintenance dosing is 4 mg/kg/day.
<sup>c</sup>The licensed maintenance dosing is 4 mg/kg/day.

EMA, European Medicines Agency.

Comparative clinical trials did not prove a survival benefit of one treatment over another. Primary intention of treating candidaemia is clearing the blood stream.

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for eye involvement and should prompt fundoscopic evaluation [84,85]. Other risk factors coincided with those for candidaemia [86]. In a large clinical trial, fundoscopy revealed ocular candidiasis in 16% of patients with candidaemia, the majority had eye involvement upon diagnosis of candidaemia and additional cases were detected during treatment. Most of the patients had chorioretinitis while endophthalmitis was uncommon (1.6%) [43,87].

In patients with a central venous catheter or a peripherally inserted central catheter, the possibility of a thrombus should be taken into account.

**Recommendations.** For uncomplicated candidaemia, treatment duration of 14 days after the end of candidaemia is recommended. The end of candidaemia should be determined by at least one blood culture per day until negativity. Transoesophageal echocardiography and fundoscopy should be performed to detect organ involvement. Switching to oral treatment can be considered after 10 days of intravenous therapy. For detailed recommendations, refer to Table 6.

**Catheter-related blood stream infection**

In general, indwelling lines need to be removed early after diagnosing catheter-related candidaemia; however, removal or exchange is not always possible. As the predominant mode of device-related infections is likely biofilm formation [88], certain differences in antifungal activity on Candida grown in biofilms vs. planktonic cells may help decision making. Liposomal amphotericin B, amphotericin B lipid complex, caspofungin and micafungin were active against Candida cells in biofilms, while cells were resistant towards amphotericin B deoxycholate, fluconazole, ravuconazole and voriconazole [89]. In animal models, amphoterin B lipid complex and anidulafungin reduced candida cell numbers in biofilms, while fluconazole did not [90,91].

**Evidence. Duration of candidaemia:** In a prospective randomized clinical trial comparing fluconazole with amphotericin B deoxycholate for candidaemia in non-neutropenic patients [53], the exchange of catheters – not over a guidewire – within the first 24 h was associated with a shorter duration of candidaemia [92]. A post hoc analysis of two pooled phase III trials comparing micafungin to caspofungin or liposomal amphotericin B (N = 842) did not find an improved time to mycological eradication, if central venous catheters were removed within 24 or 48 h [61,63,93].

**Impact of catheter removal on mortality:** Catheter removal was identified as a protective factor in a prospective study on 272 episodes of candidaemia [94]. A population-based study analysing 345 cases of candidaemia concluded that catheter removal was associated with an improved probability of survival [95,96]. In a retrospective analysis on 92 patients with cancer, removal of non-tunnelled central venous catheters ≥72 h after diagnosis of candidaemia was associated with a significantly decreased survival rate, [97] and in a univariate analysis on 244 ICU patients with candidaemia, catheter removal within 24 h was associated with better survival [73]. Early removal of central venous catheters, that is, within 24 or 48 h, had no impact on survival at 28 or 42 days in the post hoc analysis of the two pooled micafungin phase III trials [93]. However, in a recent individual patient level (n = 1915) pooled analysis of seven prospective randomized controlled trials for treatment of invasive candidiasis and candidaemia, the removal of a central venous catheter was associated with decreased mortality (OR, 0.50; 95% CI, 0.35–0.72, p = 0.0001) [98].

**Recommendations.** In candidaemia, removal of indwelling intravascular catheters is strongly recommended. When catheter removal is not possible, lipid-based amphotericin B formulation or an echinocandin is preferable. For detailed recommendations, refer to Table 7.

**TABLE 6. Recommendations on the duration of targeted treatment, step-down to oral treatment and diagnostics in candidaemia**

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidaemia with no organ involvement detected</td>
<td>To avoid organ involvement</td>
<td>Treat for 14 days after the end of candidaemia</td>
<td>B</td>
<td>II</td>
<td>[82]</td>
</tr>
<tr>
<td>Candidaemia with no organ involvement detected</td>
<td>To detect organ involvement</td>
<td>Take at least one blood culture per day until negative</td>
<td>B</td>
<td>III</td>
<td>No reference found</td>
</tr>
<tr>
<td>Candidaemia with no organ involvement detected</td>
<td>To detect organ involvement</td>
<td>Transoesophageal echocardiography</td>
<td>B</td>
<td>II</td>
<td>[83]</td>
</tr>
<tr>
<td>Candidaemia with no organ involvement detected</td>
<td></td>
<td>Fundoscopy</td>
<td>B</td>
<td>II</td>
<td>[87]</td>
</tr>
<tr>
<td>Any</td>
<td>To simplify treatment</td>
<td>If CVC, PICC or intravascular devices, search for thrombus</td>
<td>B</td>
<td>III</td>
<td>No reference found</td>
</tr>
<tr>
<td>Any</td>
<td></td>
<td>*Step-down to fluconazole after 10 days of IV, if species is susceptible, patient tolerates PO, and patient is stable</td>
<td>B</td>
<td>II</td>
<td>[64]</td>
</tr>
</tbody>
</table>

CVC, central venous catheter; PICC, peripherally inserted central catheter.

*If C. parapsilosis is identified, step-down to fluconazole may occur earlier.
Urinary tract infection
Candiduria is commonly encountered in hospitalized patients, particularly those with a urinary catheter. Candiduria is indicative for a wide spectrum of conditions which may or may not require treatment.

Evidence. Asymptomatic candiduria has been followed long term, but no adverse consequences have been described [99]. Funguria resolved without specific treatment in 76% of a large (N = 861) clinical cohort [100]. In a well-designed trial, fluconazole was superior over placebo in clearing candiduria, but at 2-week follow-up candiduria rates were similar between both groups. Removal of the urinary catheter was the most promising intervention [101]. Bladder irrigation appeared as a rarely used alternative, if treatment is judged necessary [100,102]. In symptomatic candida cystitis, fluconazole has been advocated as well as amphotericin B deoxycholate with or without 5-flucytosine, but clinical data are sparse for all these approaches [100,103]. In the rare cases of fungus balls, surgical intervention is the only promising treatment option [104,105]. Echinocandins do not achieve high urine concentrations and are thus rarely considered in urinary tract infection. Some cases though have successfully been treated with caspofungin. These were partly candidaemia with concomitant candiduria and partly infections limited to the urinary tract [106]. For candida pyelonephritis, fluconazole and amphotericin B deoxycholate each with or without flucytosine may be used, but clinical trials have not been performed.

Recommendations. Asymptomatic candiduria should not be treated, while symptomatic cystitis should be treated with fluconazole, if the isolate is susceptible. Fungus balls or casts in the pyelum or urinary bladder need surgical intervention. To cure pyelonephritis fluconazole as well as lipid-based amphotericin B are recommended either alone or in combination with flucytosine. For detailed recommendations, refer to Table 8.

Ocular candidiasis
Ocular candidiasis may cause pain or disturbed vision, but should rather be diagnosed prior to becoming clinically symptomatic [86,107]. There are two forms of ocular candidiasis. Chorioretinitis is the inflammation of the choroid and the retina, while endophthalmitis is the inflammation of the vitreous body. Fungal endophthalmitis may develop from chorioretinitis as advanced disease and is associated with poor visual outcomes [108]. Most publications in this field report on individual cases or small series, and not all clearly differentiate between the two forms of ocular involvement.

Evidence. Amphotericin B deoxycholate has been advocated for ocular candidiasis, but dosing information was not always disclosed in the early reports [107,109,110]. Amphotericin B deoxycholate followed by fluconazole has been used successfully to treat ocular involvement in the voriconazole phase III trial [43,87]. Information on amphotericin B lipid complex use in ocular candidiasis is sparse. One case of breakthrough ocular candidiasis during amphotericin B lipid complex treatment has been described [111], and another case in which amphotericin B lipid complex was successfully used with concomitant flucytosine [112]. In a rabbit model evaluating the penetration of amphotericin B deoxycholate, liposomal amphotericin B and amphotericin B lipid complex, the highest penetration into the eye was achieved with the liposomal formulation [113,114]. Intravitreal injection of amphotericin B deoxycholate 5–10 μg dissolved in 0.1 mL sterile water is part of standard approaches and frequently combined with systemic antifungals and surgery [110,115].

All three echinocandins appear to have limited penetration into the eye [116–118]. With caspofungin treatment, varying outcomes have been reported, some patients failed treatment [116,119], while only two patients have been described who responded successfully [120,121]. Successful use of fluconazole has been reported in case series, where it was used at doses varying from 100 to 400 mg

### Table 7. Recommendations on catheter management in candidaemia

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central venous catheter can be removed&lt;br&gt;Central venous catheter cannot be removed</td>
<td>Remove indwelling lines (not over a guidewire)&lt;br&gt;Echinocandin, liposomal amphotericin B or amphotericin B lipid complex&lt;br&gt;Azole or amphotericin B deoxycholate</td>
<td>A, B, D</td>
<td>II,</td>
<td>[98], [99], [96], [90], [91], [93], [92], [95], [98], [73], [97], [96], [94]</td>
</tr>
</tbody>
</table>

Interventions are intended to clear candidaemia and to improve survival.

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for at least two and up to 8 weeks. A number of these patients were treated with concomitant systemic amphotericin B deoxycholate [122–125]. Overall fluconazole 400 mg alone appeared to be effective in less-advanced disease [126].

In advanced disease, a combined strategy of surgical intervention with intraocular amphotericin B deoxycholate, and systemic fluconazole has successfully been applied [110]. Systemic antifungal treatment duration varied between 2 and 12 weeks [110,127]; an individual decision will usually take reduction of immunosuppression and the extent of ocular candidiasis into consideration.

More recently, intravitreal voriconazole has been evaluated, and in animal models, doses of 25 mg/L vitreous, that is, 100 μg absolute in an adult human eye, were found to be safe [126,128]. Published cases were frequently treated with combined approaches, so that the efficacy of voriconazole monotherapy has not yet been defined [126,129,130]. In the post hoc analysis of eye involvement in the voriconazole phase III trial on candidaemia, treatment was successful in most cases, but endophthalmitis was rare [87].

Recommendations. In ocular candidiasis, liposomal amphotericin B either alone or combined with fluconazole is recommended when the susceptibility of the isolate is unknown. In susceptible isolates fluconazole or voriconazole are the drugs of choice. In the case of vitreal involvement, vitrectomy and intravitreal injection of amphotericin B are recommended in addition to systemic therapy. For details, refer to Table 9.

Candida meningitis

Candida meningitis is a rare disease, and only very few reports have been published. Prognosis is generally poor [131].

Evidence. Liposomal amphotericin B has been combined with fluconazole for 10 weeks, followed by fluconazole for 5 weeks in a neonate [132]. In another neonate, a Candida isolate was resistant to fluconazole, and liposomal amphotericin B was combined with fluconazole for a total of 4 weeks [133]. Amphotericin B deoxycholate/fluconazole treatment had failed in the latter patient [133]. However, it is unclear to what extent these experiences can be extrapolated applied to adults. In a series of HIV-infected patients with candida meningitis, amphotericin B deoxycholate was frequently combined with fluconazole, and four of five patients were treated successfully [131]. In two other series, 27 of 34 patients survived after similar treatments [134,135]. In some cases, individualized maintenance regimens were given [131,134]. In the more recent case reports, amphotericin B deoxycholate toxicity frequently forced to replace it with the liposomal amphotericin B.

Fluconazole has been used in higher doses to treat Candida meningitis, when lower doses proved insufficient [136]. Published data on voriconazole use in Candida meningitis are sparse. In central nervous system, aspergillosis voriconazole is the drug of choice [137]. Brain tissue levels of voriconazole are satisfactory, but concentrations in cerebrospinal fluid are variable [138].

With caspofungin, a patient was cured from Candida meningitis refractory to amphotericin B deoxycholate and fluconazole [139], but poor penetration of echinocandins limit their use in central nervous system infection.

Recommendations. Due to lack of data, no strong recommendation can be given. Treatment should build on liposomal amphotericin B combined with fluconazole or with fluconazole if isolate is susceptible. For detailed recommendations, refer to Table 10.
Candida endocarditis

Candida endocarditis may manifest as native valve endocarditis, prosthetic valve endocarditis or infection in the presence of pacemaker or other implanted material prone to biofilm formation. In general, prognosis is poor with 1-year mortality >50% and substantial relapse rates [140–142].

Evidence. In native valve Candida endocarditis, primary intention is to decrease mortality [140]. Retrospective data suggest that patients should undergo surgery within the first week [140,141,143]. Treatment regimens published are liposomal amphotericin B or caspofungin, either one has been combined with fluconazole [140,141]. In prosthetic valve Candida endocarditis, valve replacement surgery needs be performed as soon as possible [142,143]. In single cases where comorbidities prevented surgery, caspofungin and liposomal amphotericin B were used successfully with or without subsequent life-long suppressive therapy with fluconazole [142,144,145]. In patients with pacemakers, implantable defibrillators or assist devices, removal of the device appears mandatory [146].

Recommendations. In native valve Candida endocarditis, surgery within a week is recommended, and in prosthetic valve Candida endocarditis, even earlier surgery may be beneficial. The antifungal regimen of choice is liposomal amphotericin B, which can be combined with fluconazole. For detailed recommendations, refer to Table 11.

TABLE 9. Recommendations on Candida chorioretinitis and endophthalmitis

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility of isolate unknown</td>
<td>Liposomal amphotericin B 5 mg/kg</td>
<td>B</td>
<td>III</td>
<td>[113]</td>
</tr>
<tr>
<td></td>
<td>Liposomal amphotericin B plus fluconosine</td>
<td>B</td>
<td>III</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B lipid complex plus fluconosine</td>
<td>B</td>
<td>III</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B deoxycholate 0.7–1.0 mg/kg (for 3–7 days), followed by</td>
<td>C</td>
<td>II</td>
<td>[87]</td>
</tr>
<tr>
<td></td>
<td>fluconazole 400 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphotericin B deoxycholate 0.6–1.0 mg/kg</td>
<td>C</td>
<td>II</td>
<td>[107]</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B lipid complex 5 mg/kg</td>
<td>C</td>
<td>III</td>
<td>No reference found</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B deoxycholate plus fluconosine</td>
<td>C</td>
<td>III</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td>Caspofungin 50–100 mg</td>
<td>D</td>
<td>II</td>
<td>[116]</td>
</tr>
<tr>
<td>Susceptible isolate</td>
<td>Fluconazole 400–800 mg</td>
<td>A</td>
<td>II</td>
<td>[122]</td>
</tr>
<tr>
<td></td>
<td>Voriconazole 12½ mg/kg IV, followed by 400 mg PO</td>
<td>A</td>
<td>II</td>
<td>[129]</td>
</tr>
<tr>
<td>Vitreal involvement*</td>
<td>Amphotericin B deoxycholate 5–10 µg intravitreal injection</td>
<td>B</td>
<td>II</td>
<td>[110]</td>
</tr>
<tr>
<td></td>
<td>Voriconazole 12½ mg/kg IV, followed by 400 mg PO</td>
<td>A</td>
<td>II</td>
<td>[129]</td>
</tr>
<tr>
<td></td>
<td>Voriconazole 100 µg intravitreal injection</td>
<td>B</td>
<td>III</td>
<td>[110]</td>
</tr>
</tbody>
</table>

Frequent eye examinations are needed to detect disease progression.
*Endophthalmitis requires local and systemic treatment plus surgery.

**TABLE 10. Recommendations on Candida meningitis**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomal amphotericin B 3 mg/kg for 10 weeks + fluconazole 150 mg/kg for 10 weeks, followed by fluconazole 3 mg/kg for 5 weeks</td>
<td>B</td>
<td>III</td>
<td>[132]</td>
</tr>
<tr>
<td>Liposomal amphotericin B 3 mg/kg for 4 weeks + fluconazole 6 mg/kg for 4 weeks</td>
<td>B</td>
<td>III</td>
<td>[133]</td>
</tr>
<tr>
<td>Voriconazole 12½ mg/kg*</td>
<td>C</td>
<td>III</td>
<td>[137]</td>
</tr>
<tr>
<td>Fluconazole 800 mg</td>
<td>C</td>
<td>III</td>
<td>[136]</td>
</tr>
<tr>
<td>Amphotericin B deoxycholate 0.5–1.0 mg/kg for &gt;2 weeks +/- fluconazole 30–120 mg/kg for &gt;2 weeks</td>
<td>D</td>
<td>IL</td>
<td>[130]</td>
</tr>
<tr>
<td>Caspofungin 70/50 mg for 4 weeks, followed by fluconazole 400 mg for 2 weeks</td>
<td>D</td>
<td>III</td>
<td>[139]</td>
</tr>
</tbody>
</table>

Interventions are intended to cure Candida meningitis.
*Therapeutic drug monitoring recommended.
Bone and joint candidiasis

*Candida* infections of bones and joints are grouped into osteomyelitis/spondylodiscitis, arthritis and prosthetic joint infection. No randomized clinical trials have been conducted, so that evidence for the best therapeutic approach is somewhat limited.

**Evidence.** Typical indications for surgical debridement in osteomyelitis or spondylodiscitis are instability or large abscesses. Usually, cases of *Candida* osteomyelitis are diagnosed by biopsy. Over the years, most experience has been gathered with amphotericin B formulations, sometimes combined with fluconosine, sometimes followed by fluconazole [147]. Today, in patients with osteomyelitis as well as spondylodiscitis due to a susceptible isolate, treatment can commence with liposomal or lipid complex amphotericin B to be followed by fluconazole [147], or – if isolate is susceptible – fluconazole monotherapy may be used from the beginning [148–149]. Posaconazole has been successfully used in a single case as add-on during unsuccessful caspofungin treatment [150]. Voriconazole treatment has been reported in three patients with *Candida* osteomyelitis [78]. In addition, in *Aspergillus* osteomyelitis, voriconazole was used either as the only antifungal or as maintenance following liposomal amphotericin B [151]. Use of echinocandins has not been reported, with the exception of four patients with osteomyelitis and/or septic arthritis successfully treated with caspofungin [120].

### TABLE 11. Recommendations on *Candida* endocarditis

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native valve</td>
<td>To cure</td>
<td>Liposomal ampho B + flucytosine for 6–8 weeks, followed by fluconazole</td>
<td>B</td>
<td>II</td>
<td>[171]</td>
</tr>
<tr>
<td>Prosthetic valve</td>
<td>To cure</td>
<td>Surgery within days</td>
<td>A</td>
<td>III</td>
<td>[142]</td>
</tr>
<tr>
<td>Prosthetic valve, if surgery not possible</td>
<td>To cure</td>
<td>Liposomal amphotericin B 5 mg/kg</td>
<td>B</td>
<td>III</td>
<td>[142]</td>
</tr>
<tr>
<td>Pacemaker, ICD, VAD</td>
<td>To cure</td>
<td>Removal</td>
<td>A</td>
<td>II</td>
<td>[146]</td>
</tr>
</tbody>
</table>

ICD, implantable cardioverter defibrillator; VAD, ventricular assist device.

Surgery – even if restricted to removal of hardware – always needs to be combined with systemic antifungal treatment.

### TABLE 12. Recommendations on bone and joint candidiasis

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteomyelitis/spondylodiscitis</td>
<td>To cure</td>
<td>Surgical debridement + flucytosine for 6–12 months</td>
<td>C</td>
<td>III</td>
<td>[147]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liposomal amphotericin B 3 mg/kg or amphotericin B lipid complex 5 mg/kg for 2–6 weeks followed by fluconazole 400 mg for 5–11 months</td>
<td>A</td>
<td>I</td>
<td>[149]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posaconazole 800 mg for ≥6 weeks</td>
<td>C</td>
<td>III</td>
<td>[150]</td>
</tr>
<tr>
<td>Arthritis</td>
<td>To cure</td>
<td>Liposomal Amphi B 3 mg/kg/ABLC 5 mg/kg 2 weeks, followed by fluconazole 400 mg for ≥6 weeks</td>
<td>A</td>
<td>I</td>
<td>[154]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluconazole 400 mg for ≥4 weeks</td>
<td>A</td>
<td>I</td>
<td>[155]</td>
</tr>
<tr>
<td>Prosthetic joint infection</td>
<td>To cure</td>
<td>Prosthesis removal</td>
<td>A</td>
<td>III</td>
<td>[154]</td>
</tr>
<tr>
<td>Prosthetic joint infection</td>
<td>To suppress infection</td>
<td>Fluconazole 400 mg, life long</td>
<td>A</td>
<td>III</td>
<td>[160]</td>
</tr>
</tbody>
</table>

Indications for surgery are, for example, instability or large abscess.

Surgery needs to be combined with antifungal treatment.

Treat longer if erythrocyte sedimentation rate or C-reactive protein not returned to normal.
A case of Candida shoulder arthritis was cured with a 3-week course of caspofungin [152], and a knee arthritis was treated with 7 weeks of caspofungin added on to a failing fluconazole therapy [153]. The most prevalent joint prone to Candida infection is the knee. Standard treatment of knee Candida arthritis due to Candida was an amphotericin B-based approach, which may have been supplemented with flucytosine [154]. More recently, fluconazole and voriconazole were used with success [78,155,156].

Joint prosthesis is an important risk factor for Candida arthritis, and prosthesis is mandatory [154,157,158]. If the prosthesis must be retained, lifelong suppressive treatment should be tried. In some patients, surgery was considered not possible, and knee or hip prosthetic joint arthritis was cured with use of fluconazole alone [157,159–161]. Bias towards publishing the unusual and successful cases can be assumed, so that the standard approach remains prosthesis removal and an intensive course of systemic antifungals.

Recommendations. Treating osteomyelitis, spondylodiscitis or arthritis with fluconazole is strongly recommended if species is susceptible. Fluconazole may be preceded by an induction phase with lipid-based amphotericin B. If joint prosthesis cannot be removed, lifelong fluconazole suppressive therapy is indicated. For details, refer to Table 12.

Transparency Declarations

O.A.C. is supported by the German Federal Ministry of Research and Education (BMBF grant 01KN1106) and has received research grants from, is an advisor to or received lecture honoraria from 3M, Actelion, Astellas, Basilea, Bayer, Biocryst, Cubist, Celgene, F2G, Genzyme, Gilead, GSK, Merck/Schering, Miltenyi, Optimer, Pfizer, Sanofi Pasteur, Quintiles and Viropharma.

M.B. has received research grants from Pfizer, MSD and Astellas and is/was an advisor or received lecture honoraria from Astellas, Astra Zeneca, Angelini Farmaceutici, Aventis, Bayer, Cephalon, Cubist, Gilead, MSD, Novartis, Shionogi, Pfizer, Teva and Vifor. He is also a board member for Pfizer, Angelini Farmaceutici, Cubist, MSD, Astellas, Novartis and Astra Zeneca.

T.C. is member of the Speaker bureau and is advisor or consultant for Astellas, Baxter, bioMérieux, Elsai, Evolva, Novartis, Merck Sharp and Dohme-Chibret AG, Immunexpress, Eli Lilly Suisse and Pfizer and received grant support from Baxter, bioMérieux, Merck Sharp and Dohme-Chibret AG and Roche Diagnostic. He has also received speaker’s fees from MSD, Institut Pasteur and Gilead Sciences, travel support from Astellas, Pfizer and MSD.

J.G. has nothing to declare.

B.J.K. has received research grants from Bio-Mérieux and Cephalon. He is a consultant to Pfizer and is a member of the Gilead, MSD and Pfizer speaker’s bureaus.

O.L. is a member of the MSD board, is a consultant for Astellas and Gilead Sciences and received grants or speaker’s fees from MSD, Astellas, Gilead Sciences and Pfizer.

WM has received grant support from MSD and Pfizer. He had been an advisor to MSD and Pfizer. He has received honoraria for presentations on behalf of MSD/Schering Plough and Pfizer.

M.A. received research grants and honoraria for talks and consultancy from Merck, Pfizer and Gilead.

M.C.A. has received grant support from Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. She has been a consultant or at the advisory board for Gilead Sciences, Merck Sharp and Dohme, Pfizer, Pcovery and Schering Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough.

S.A.A. has received investigator-initiated research grant support from Pfizer and speaker honoraria from Merck and Pfizer. She has been at the advisory board for Pfizer-Turkey.

J.B. has nothing to declare.

E.C. has participated as invited speaker to symposia organized by Gilead, Pfizer, Astellas, Merck ans Novartis, and he has been member of advisory boards for Astellas and Pfizer.

M.C.E. has received in the past 5 years grant support from Astellas Pharma, bioMérieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA, Ferrer International, the European Union, the ALBAN program, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, The Spanish Health Research Fund, The Instituto de Salud Carlos III, The Ramon Areces Foundation and The Mutua Madrileña Foundation. He has been an advisor/consultant to the Panamerican Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough.
J.P.D. has received grant support from Astellas, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. He has been a consultant or on an advisory board for Astellas, Gilead Sciences, Merck Sharp and Dohme and Pfizer. He has received remuneration for giving lectures on behalf of Gilead Sciences, Merck and Pfizer.

A.H.G. has received research support from Gilead, Merck and Schering. He has acted as speaker and/or consultant for Astellas, Cephalon, Gilead, Merck, Pfizer, Schering and Vicuron.

R.H. has been a consultant or at the advisory board for Astellas Pharma, Basilea, Gilead Sciences, Merck Sharp and Dohme, Novartis, Pfizer and Schering-Plough. He has been paid for talks on behalf of Astellas, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. He has received research support from and been paid investigator fees for a clinical trial by Pfizer.

W.W.H. has received grant support from National Institute of Health Research (NIHR), Medical Research Council, National Institute for the Replacement, Refinement and Reduction, of Animals in Research, Pfizer, Gilead, Schering-Plough, Merck and Astellas, and has served as a consultant for Pfizer, Astellas, Gilead, F2G, Vectura, and Schering-Plough. He has also received speaker’s fees from Pfizer, Astellas and Gilead and travel support from ESCMID.

H.E.J. has nothing to declare.

C.L.-F. has received grant support in the past 5 years from Astellas Pharma, Gilead Sciences, Pfizer, Schering-Plough and Merck Sharp and Dohme. She has been an advisor/consultant to Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering-Plough. Her travel and accommodation expenses have been covered by Astellas Pharma, Pfizer, Gilead Sciences, MSD and Schering-Plough.

G.P. has received research grants from Gilead, Pfizer, Astra Zeneca, Novartis, GSK, Astellas and MSD, has acted as paid consultant to Janssen Cilag, Gilead, Astellas and MSD and is a member of the Gilead, Astellas and MSD speaker’s bureaus. He has also received travel support from ESCMID, Gilead, Astellas and Pfizer.

M.D.R. has received grants, speaker’s honoraria and travel support from ESCMID, Pfizer, Astellas, MSD and Gilead Sciences. He has also received book royalties from Blackwell Publishing and conference support from Astellas Pharma, as well as consulted for Gilead Sciences and MSD.

E.R. has received research support from Pfizer, Gilead and Merck, and he has made contributions in advisory boards of Gilead, Astellas and Pfizer. He has also been paid for talks on behalf of Gilead, Cephalon, Pfizer, Wyeth, Schering, Merck, Aventis and Astellas.

P.E.V. has received research grants from Pfizer, Astellas, Cephalon, Gilead Sciences, Merck and Schering-Plough. He has also received travel support from Gilead Sciences.

C.V. received grants as speaker/moderator in meetings sponsored by Pfizer, Gilead, MSD, Astellas, Abbott and BMS and received grants for participation in advisory boards by Gilead, Astellas, MSD and Pfizer. Further, he obtained research grants for his institution from Pfizer, MSD, Gilead, Abbott, Jansen, BMS, and Novartis. He is a member of the SAG (Scientific Advisory Group) for antibacterials and antifungals of CHMP-EMA and consultant for Italian Medical Drug Agency Member of various levels of local Infection Control, Antibiotic Stewardship, Vaccine and HIV Committees (Genoa, Liguria, Italy). He has also received payment for educational presentations from Nadirex International (Pavia, Italy).

A.J.U. has received research grants from MSD (Schering-Plough) and is/was an advisor or received lecture honorarium from Astellas, Aicuris, Basilea, Gilead, MSD and Pfizer.

References


ESCMI D and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi


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Abstract

The aetiological agents of many invasive fungal infections are saprobes and opportunistic pathogens. Some of these fungi are darkly pigmented due to melanin production and traditionally have been named ‘dematiaceous’. The melanized fungi cause a wide array of clinical syndromes ranging from superficial to deep-seated infections. Diagnosis relies on histopathological examination of clinical specimens and on examination of cultures. Sequencing is recommended for accurate species identification, especially for unusual or newly described pathogens. In cases of mycetoma and chromoblastomycosis, pathognomonic histological findings are useful and the Fontana–Masson stain, specific for melanin, usually confirms the diagnosis. There are no standardized therapies but voriconazole, posaconazole and itraconazole demonstrate the most consistent in vitro activity against this group of fungi. Oral itraconazole has been considered the drug of choice, given the extensive clinical experience with this drug. However, voriconazole may presumably be superior for central nervous system infections
because of its ability to achieve good levels in the cerebrospinal fluid. Posaconazole is a well-tolerated alternative drug, backed by less clinical experience but with excellent salvage treatment results after failure of other antifungals. Amphotericin B has been useful as alternative therapy in some cases. Combination antifungal therapy is recommended for cerebral abscesses when surgery is not possible and for disseminated infections in immunocompromised patients.

Keywords: Clinical presentation, diagnosis, guideline, mycosis, phaeohyphomycosis, prophylaxis, treatment

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Introduction

A panel of experts of the European Fungal Infection Study Group (EFISG) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) undertook a data review and compiled guidelines for the diagnosis and management of infections caused by melanized (black) fungi. The deep-seated infection caused by these fungi is often referred to as phaeohyphomycosis. Many infections, however, are superficial and mild, or cause cutaneous or pulmonary colonization only. In addition, many species of black fungi have a cosmopolitan presence and are widely distributed in the environment and the possibility that a suspected clinical isolate might be a contaminant must be considered. The course of infection differs with the species, so for clinical management it is paramount to obtain an accurate species identification. Although sizeable numbers of these rare fungal pathogens have been implicated in human infections, we have reviewed only the most common ones.

Methods

The guideline development followed the AGREE II method (Appraisal of guidelines for research and evaluation II; http://www.agreetrust.org/resource-centre/agree-ii/, accessed 13 December 2013). The overall objective of the guidelines has been on the diagnosis and management of deep-seated phaeohyphomycosis, including disseminated infections. In addition, superficial and allergic manifestations caused by these fungi are also briefly discussed. The definition of the strength of recommendation and the quality of the published evidence are defined in Table 1. The health questions covered by the guidelines are specifically described in the Tables 2–4. The population to whom the recommendations are meant to apply is any patient suffering from phaeohyphomycosis. The expert panel (35 members) was set up by ESCMID/EFISG and European Confederation of Medical Mycology (ECMM) including clinical microbiologists, infectious diseases experts, paediatricians, haematologists and intensive care unit experts taking into account the target users of these guidelines. Competing interests of guideline development group members were recorded and addressed. An expert subgroup (AC, MCE, JG, SDH, SK, OAC, JFM) reviewed the available literature. The other experts of the panel acted as external reviewers. The members actively shared their views and documents by email, teleconferences and face-to-face meetings during 2012–2013.

### TABLE 1. System for grading strength of recommendation and quality of evidence about diagnostic procedures and therapy of infections by black fungi

<table>
<thead>
<tr>
<th>Grade of recommendation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of recommendation</td>
<td></td>
</tr>
<tr>
<td>Grade A</td>
<td>ESCMID (EFISG) and ECMM strongly support a recommendation for use</td>
</tr>
<tr>
<td>Grade B</td>
<td>ESCMID (EFISG) and ECMM moderately support a recommendation for use</td>
</tr>
<tr>
<td>Grade C</td>
<td>ESCMID (EFISG) and ECMM marginally support a recommendation for use</td>
</tr>
<tr>
<td>Grade D</td>
<td>ESCMID (EFISG) and ECMM support a recommendation against use</td>
</tr>
<tr>
<td>Level of evidence</td>
<td>Definition</td>
</tr>
<tr>
<td>Level I</td>
<td>Evidence from at least one properly designed randomized, controlled trial</td>
</tr>
<tr>
<td>Level II</td>
<td>Evidence from at least one well-designed clinical trial, without randomization; from cohort or case-control analytical studies (preferably from more than one centre); from multiple time series; or from dramatic results of uncontrolled experiments</td>
</tr>
<tr>
<td>Level III</td>
<td>Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies, or reports of expert committees</td>
</tr>
<tr>
<td>Aetiological agents [references]</td>
<td>Most common described infections</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Alternaria [38,55,167]</td>
<td>Cutaneous and subcutaneous infection, sinustis, keratitis, ABPM, disseminated disease</td>
</tr>
<tr>
<td>Bacillus [211]</td>
<td>Cutaneous and subcutaneous infection, ocular infection, rare deep infection, fungaemia</td>
</tr>
<tr>
<td>Bipolaris [213,242]</td>
<td>Cutaneous and subcutaneous infection, sinustis, keratitis, ABPM, disseminated disease</td>
</tr>
<tr>
<td>C. globosum</td>
<td>C. globosum</td>
</tr>
<tr>
<td>Cladosporium [87,123]</td>
<td>Cutaneous and subcutaneous infection, brain abscess</td>
</tr>
<tr>
<td>C. dematiodos</td>
<td>C. dematiodos</td>
</tr>
<tr>
<td>Curvularia [277,284]</td>
<td>Cutaneous and subcutaneous infection, sinustis, keratitis, ABPM, disseminated disease</td>
</tr>
<tr>
<td>C. lunata</td>
<td>C. lunata</td>
</tr>
<tr>
<td>Exophiala [334–338]</td>
<td>Cutaneous and subcutaneous infection, keratitis, ABPM, disseminated disease</td>
</tr>
<tr>
<td>E. dermatisos</td>
<td>E. dermatisos</td>
</tr>
<tr>
<td>E. spermata</td>
<td>E. spermata</td>
</tr>
<tr>
<td>Exserohilum [340,375]</td>
<td>Cutaneous and subcutaneous infection, keratitis, meningitis and spinal infection, arthritis, disseminated disease</td>
</tr>
<tr>
<td>Fonsecaea [83,378]</td>
<td>Cutaneous and subcutaneous infections brain abscess</td>
</tr>
<tr>
<td>F. pedrosoi</td>
<td>F. pedrosoi</td>
</tr>
<tr>
<td>Fonsecaea spp.</td>
<td>Fonsecaea spp.</td>
</tr>
<tr>
<td>Horlete [396]</td>
<td>Cutaneous infections and encephalomyelitis, very rare deep mycosis</td>
</tr>
<tr>
<td>Neosartorya [103,400,401,409]</td>
<td>Cutaneous infections and encephalomyelitis, very rare deep mycosis</td>
</tr>
<tr>
<td>Oidiodendron [434,437]</td>
<td>Pneumonia, brain abscess, disseminated infections</td>
</tr>
<tr>
<td>Phaeosphaeria [445,446]</td>
<td>Pneumonia, brain abscess, disseminated infections</td>
</tr>
<tr>
<td>Phoma [457,459]</td>
<td>Subcutaneous infection, arthritis, disseminated disease</td>
</tr>
<tr>
<td>Pyrenomycetes [462]</td>
<td>Cutaneous and subcutaneous infection, ulcerative infection, rare deep mycosis</td>
</tr>
<tr>
<td>Rhinocladiella [492]</td>
<td>Cutaneous and subcutaneous infection, keratitis, brain abscess</td>
</tr>
<tr>
<td>R. aquaspera</td>
<td>R. aquaspera</td>
</tr>
<tr>
<td>Veronaea [494]</td>
<td>Cutaneous and subcutaneous infection, disseminated disease</td>
</tr>
</tbody>
</table>

AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; ISA, isavuconazole; FC, flucytosine; FLU, fluconazole; ECHINO, echinocandins; TERB, terbinafine; ABPM, allergic bronchopulmonary mycosis.

*Devises collective MIC ranges from all the references mentioned.
Once the first consensus was reached, the preliminary recommendations were discussed, developed further and finalized as a group consensus. The methods to evaluate the quality of evidence and to reach consensus recommendations were described previously in detail when the first official ESCMID guidelines on the diagnosis and treatment of Candida infections were published [1–6].

The characteristic feature of phaeohyphomycosis is the presence of melanin in the fungal cell walls, which gives a dark colour to the hyphae, and is considered a major virulence factor. The criteria for selecting the evidence were searching the literature using the string ‘melanized’, ‘dark’, ‘phaeoid’ and ‘dematiaceous’ and search results were systematically reviewed. As the clinical syndromes associated with these fungi are common across the different pathogens (Table 2), the first part of this guideline presents recommendations for each clinical entity (localized cutaneous and subcutaneous infection, chromoblastomycosis, mycetoma, keratitis, pulmonary infections, cerebral infection, disseminated disease and allergic manifestations). Subsequently, specific issues for each of the fungal pathogens are presented in alphabetical order. Most recommendations in this guideline are based on dramatic results of uncontrolled experiments, opinions of respected authorities, clinical experience, descriptive case studies, or reports of expert committees. In some cases, in vitro data and animal studies are also included. Unfortunately, much of the older literature could not be included because of the unreliability of the non-molecular strain identification methods used. These guidelines highlight the fact that there is no standard approach for treatment of phaeohyphomycosis. Also, the reference microdilution methodologies for in vitro antifungal susceptibility testing have not been standardized nor are the validated MIC breakpoints that are used for interpretation of the results for antifungal drugs against the phaeoid fungi available. Unlike the other guidelines for fungal infections caused by rare yeasts and the mucorales, which recommend clear-cut therapeutic approaches [7,8], the huge diversity of dematiaceous fungi and their host range make it impossible to advise a uniform approach for phaeohyphomycosis. Length of therapy and choice of intervention (surgery, antifungals or both) for each clinical entity is primarily based on the clinical presentation, the underlying condition of the host and the initial response. The prolonged duration of therapy in the diseases caused by phaeoid fungi generally ranges from several weeks to months or longer. The clinical entities and their therapeutic recommendations are given below and summarized in Tables 1–4. Table 3 includes recommendations for diagnostic procedures and susceptibility testing of these diseases [9–25]. These guidelines will be periodically updated.

### Table 3. Recommendations for microbiological procedures to detect infections by black fungi

<table>
<thead>
<tr>
<th>Disease/Population [references]</th>
<th>Intention</th>
<th>Diagnostic procedure</th>
<th>SoR</th>
<th>QoE</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases with deep infections [8,13,17]</td>
<td>Definitive diagnosis and species identification</td>
<td>To know local species distribution</td>
<td>A</td>
<td>A</td>
<td>A III</td>
</tr>
<tr>
<td>All cases with deep infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect high MIC values and MIC determination</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>All cases with deep infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect local resistance</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>All cases with deep infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in tissues</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>All cases with deep infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in blood, serum or other sterile fluids</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Cerebral abscess and other localized</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in tissues</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Cerebral abscess and other localized</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in blood, serum or other sterile fluids</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Disseminated infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in tissues</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Disseminated infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in blood, serum or other sterile fluids</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Rhinocladiella mackenziei infections [9–13]</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in tissues</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Rhinocladiella mackenziei infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in blood, serum or other sterile fluids</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Cladophialophora bantiana infections [9–13]</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in tissues</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
<tr>
<td>Cladophialophora bantiana infections</td>
<td>Definitive diagnosis and species identification</td>
<td>To detect infection in blood, serum or other sterile fluids</td>
<td>A</td>
<td>X</td>
<td>C III</td>
</tr>
</tbody>
</table>

**Notes:**
- **SoR:** Strength of evidence (A: high, B: moderate, C: low).
- **QoE:** Quality of evidence (A: high, B: moderate, C: low).
- **Comments:** None.

**Abbreviations:**
- BHI: Brain Heart Infusion.
- ELISA: Enzyme-linked immunosorbent assay.
- PCR: Polymerase chain reaction.
- **ESCMID guidelines on the diagnosis and treatment of Candida infections were described previously in detail when the first official ESCMID guidelines on the diagnosis and treatment of Candida infections were published [1–6].**
### TABLE 4. Recommendations for targeted treatment of infection by black fungi. Table includes grade and quality of evidence

<table>
<thead>
<tr>
<th>Disease</th>
<th>Intention</th>
<th>Intervention [references]</th>
<th>SoR</th>
<th>QoE</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized cutaneous infection</td>
<td>Cure</td>
<td>Surgery [12,26–32]</td>
<td>A II</td>
<td></td>
<td>Dramatic results of uncontrolled cases and multiple time series</td>
</tr>
<tr>
<td>or subcutaneous nodule(s)</td>
<td>Cure</td>
<td>Cryotherapy, laser therapy, heat therapy or potassium iodide [33–37]</td>
<td>B III</td>
<td></td>
<td>Reports from areas where antifungal agents are unavailable or failure/contraindication of antifungals</td>
</tr>
<tr>
<td>Subcutaneous nodule</td>
<td>To prevent dissemination</td>
<td>Add itraconazole (400 mg) or voriconazole (400 mg) [12]</td>
<td>B III</td>
<td></td>
<td>Expert opinion (particularly in immunocompromised patients)</td>
</tr>
<tr>
<td>Multiple subcutaneous nodules</td>
<td>Cure</td>
<td>Itraconazole (400 mg) or voriconazole (400 mg) [38–44]</td>
<td>A III</td>
<td></td>
<td>Descriptive case studies; treatment duration 3–12 months</td>
</tr>
<tr>
<td></td>
<td>Cure</td>
<td>Itraconazole (200 mg), posaconazole (800 mg), amphotericin B (1 mg/kg), liposomal amphotericin B (3 mg/kg), caspofungin (70/50 mg), terbinafine (250–500 mg), or combination therapy with itraconazole PLUS terbinafine or voriconazole PLUS amphotericin B [12,41,45–58]</td>
<td>C III</td>
<td></td>
<td>Few descriptive case studies and insufficient data. Some cases including surgery when possible</td>
</tr>
<tr>
<td>Mycetoma</td>
<td>Cure or reduce infections in advanced cases</td>
<td>Itraconazole (400 mg) for at least 3 months (years in some cases) PLUS surgery [59–64]</td>
<td>A II</td>
<td></td>
<td>Dramatic results of uncontrolled cases and some time series</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Vincristine (400 mg), posaconazole (800 mg) or terbinafine (250 mg) PLUS surgery [66–69]</td>
<td>A III</td>
<td></td>
<td>Dramatic results of uncontrolled few cases</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Amphotericin B (1 mg/kg) [12]</td>
<td>D III</td>
<td></td>
<td>Side effects</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Ketocanazole (400 mg) [59,63,64]</td>
<td>D III</td>
<td></td>
<td>Impractical given the therapy duration</td>
</tr>
<tr>
<td></td>
<td>Reduce lesions</td>
<td>Combination antifungal therapy (azoles PLUS terbinafine or fluconazole) [6,67]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Itraconazole (400 mg) for months to years PLUS surgery [72–74,74]</td>
<td>A II</td>
<td></td>
<td>Dramatic results of uncontrolled cases and multiple time series</td>
</tr>
<tr>
<td>Chromoblastomycosis</td>
<td>Cure or reduced infections in advanced cases</td>
<td>Itraconazole (400 mg) [12,41,45–58]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Terbinafine (350 mg) or posaconazole (800 mg) PLUS surgery [72,75,77,78,88]</td>
<td>C III</td>
<td></td>
<td>Expert opinion and descriptive case studies (very few)</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Amphotericin B (1 mg/kg) [12]</td>
<td>D III</td>
<td></td>
<td>Expert opinion</td>
</tr>
<tr>
<td></td>
<td>Reduce lesions</td>
<td>Combination antifungal therapy (itraconazole plus terbinafine) [72,74,75,82–84]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Keratitis</td>
<td>Cure</td>
<td>Natamycin alone or PLUS other topical agents [89,90,92–94]</td>
<td>A II</td>
<td></td>
<td>Multiple time series</td>
</tr>
<tr>
<td>Keratitis</td>
<td>Cure</td>
<td>Topical azoles alone [95–97]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Refractory keratitis</td>
<td>Cure</td>
<td>Oral triazoles (conventional doses) PLUS surgery if needed [89,91–93]</td>
<td>C III</td>
<td></td>
<td>Insufficient data</td>
</tr>
<tr>
<td></td>
<td>Cure</td>
<td>Intravenous voriconazole injection [76,98]</td>
<td>C III</td>
<td></td>
<td>Insufficient data</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td>Cure or control of infection</td>
<td>Systemic liposomal amphotericin B (3 mg/kg), voriconazole (400 mg), or posaconazole (800 mg) [12,102–107]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies in immunocompromised or with underlying pulmonary disease (few cases for posaconazole)</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Amphotericin B (1 mg/kg) [12]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Solitary pulmonary nodule in immunocompetent</td>
<td>Cure</td>
<td>Surgery [12,39,108]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Cerebral abscess</td>
<td>Cure</td>
<td>Complete excision (when possible) [109,112,117,120]</td>
<td>A II</td>
<td></td>
<td>Dramatic results of uncontrolled cases</td>
</tr>
<tr>
<td></td>
<td>Cure when surgery is not possible</td>
<td>Vincristine (400 mg) or posaconazole (800 mg) [12,11–128]</td>
<td>C II</td>
<td></td>
<td>Multiple time series, animal model and in vitro data</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Amphotericin B (several doses) [122–124,129]</td>
<td>D III</td>
<td></td>
<td>Descriptive case studies, failures and results from animal models and in vitro data</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>New combination therapy (voriconazole or posaconazole plus echinocandin plus fluconazole) [12,116,130]</td>
<td>B III</td>
<td></td>
<td>Expert opinion and descriptive case studies (very few)</td>
</tr>
<tr>
<td>Bone and joint infections</td>
<td>Cure</td>
<td>Surgery PLUS itraconazole (400 mg), posaconazole (800 mg) [12,131,132]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Cure (associated with peritoneal dialysis)</td>
<td>Catheter removal PLUS systemic antifungal therapy [133–137]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Disseminated infection</td>
<td>Cure or infection control</td>
<td>Liposomal amphotericin B (3 mg/kg), voriconazole (400 mg), posaconazole (400 mg) [138,141–147]</td>
<td>C III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td></td>
<td>As above</td>
<td>Visceral amphotericin B (400 mg), or posaconazole (800 mg) PLUS terbinafine (250 mg) PLUS colony-stimulating factors/leucocytosis infusion [148–150]</td>
<td>B III</td>
<td></td>
<td>Expert opinion and descriptive case studies (very few and based on experience with Scedosporium infections)</td>
</tr>
<tr>
<td>Allergic sinusitis</td>
<td>Remove the mucin and reduce symptoms</td>
<td>Surgery PLUS systemic steroids [151–154]</td>
<td>A II</td>
<td></td>
<td>Prospective, randomized, placebo-controlled trial (24 patients only) and reviews</td>
</tr>
<tr>
<td></td>
<td>Reduce requirements of steroids</td>
<td>Add itraconazole (several doses) [152,153,155]</td>
<td>C III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Refractory allergic sinusitis</td>
<td>Reduce symptoms</td>
<td>Add itraconazole (several doses) or voriconazole (400 mg) [156–158]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td>Sinus fungus ball</td>
<td>Cure</td>
<td>Surgery [159]</td>
<td>C III</td>
<td></td>
<td>Few descriptive case studies and insufficient data. Some cases including surgery when possible</td>
</tr>
<tr>
<td>Invasive sinusitis</td>
<td>Cure</td>
<td>Liposomal amphotericin B (3 mg/kg) 2 weeks followed by voriconazole (400 mg) 3 months [159]</td>
<td>C III</td>
<td></td>
<td>Insufficient evidence</td>
</tr>
<tr>
<td>Allergic bronchopulmonary mycosis</td>
<td>Reduce symptoms</td>
<td>Steroids [12,151,161,162]</td>
<td>B III</td>
<td></td>
<td>Descriptive case studies</td>
</tr>
<tr>
<td></td>
<td>Reduce symptoms</td>
<td>Add itraconazole (several doses) [160,163]</td>
<td>D III</td>
<td></td>
<td>Expert opinion</td>
</tr>
</tbody>
</table>

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

*The population to whom the recommendations are meant to apply is any patient suffering from phaeohyphomycosis.

*Dosage recommendation for combination antifungal therapy is the conventional dosing.
Recommendations by Clinical Entities

Localized cutaneous infection and subcutaneous nodules
One of the common manifestations of dematiaceous fungi is superficial localized cutaneous and subcutaneous disease. Most superficial infections are secondary to trauma. Lesions typically appear as isolated cystic or popular lesions on exposed areas of the body, such as limbs and hands. *Alternaria* spp. are the most common aetiological agent and others include species of *Exophiala* spp. and *Phialophora*. Clinical presentation is usually indolent, with a gradually enlarging mass. Generally immunocompromised patients are at increased risk of subsequent dissemination. On histopathological examination the phaeohyphomycotic cyst presents as a single dermal lesion with minimal changes in the epidermis and granulomatous inflammation with abundant giant cells. Fungal elements such as yeast-like structures and septate hyphae can be found in the specimen. For subcutaneous nodules in particular, surgery alone has been effective (recommendation All) [12,26–32]. Cryotherapy, laser, heat and photodynamic therapy have also been used successfully in many cases (recommendation BIII) [33–37]. Oral antifungals, mainly azoles, have been widely used as co-adjunctive therapies particularly in immunocompromised patients and to prevent dissemination (recommendation BIII) [12]. Multiple subcutaneous nodules have to be treated with systemic antifungal agents. Itraconazole or voriconazole at 400 mg are recommended (recommendation All) [38–44]. Other antifungal agents have been used in some cases (recommendation CIII, Table 4) [12,41,45–58].

Eumycotic mycetoma
Mycetomas are localized infections that involve cutaneous and subcutaneous tissue, fascia and bone. Lesions consist of abscesses, granuloma and draining sinuses from which granules may be recovered. They may be caused by different fungi, which produce granules of different colours, such as *Acremonium* spp. (white), *Aspergillus nidulans* (white), *Exophiala jeanselmei* (black), *Leptosphaeria senegalensis* (black), *Madurella grisea* (black), *Madurella mycetomatis* (black), *Neotestudina rosatii* (white) and *Pyrenochaeta romeroi* (black). Mycetoma is difficult to cure and therapy includes amputation of the affected limb or large surgical excision of the affected tissue to reduce the disease burden. However, excision alone is rarely sufficient for a complete cure. This condition always requires surgery and prolonged systemic antifungal therapy (recommendation All) [59–64]. Historically, the majority of cases reported used ketoconazole or itraconazole. Itraconazole appears to have consistent clinical activity (recommendation All), and ketoconazole should be avoided because of side effects (recommendation DIII). Also, the newer triazoles (voriconazole and posaconazole; recommendation All) and combination therapy with terbinafine or fluocytosine have been used successfully (recommendation BIII) [65–69].

Chromoblastomycosis
This is a chronic subcutaneous infection by dematiaceous fungi characterized by the presence of muriform cells or sclerotic bodies (medlar bodies) in tissue sections or wet preparations of pus or scrapings. Muriform cells are thick-walled, spherical, dark brown cells, which swell and often develop intersecting septa in various planes. The most commonly involved fungi are *Cladophialophora carrionii*, *Fonsecaea compacta*, *Fonsecaea pedrosoi* and *Phialophora verrucosa*. These causative agents of chromoblastomycosis are rarely recovered from nature but are selectively enriched by the human host [70]. The infection is difficult to cure, and relapses are common, possibly due to resistance development during therapy [71–76]. Overall, several studies suggest that standard of therapy should include itraconazole plus surgery (recommendation All) [72–74,76]. In a few cases of chromoblastomycosis terbinafine monotherapy and surgery have been applied successfully (recommendation BIII) [72,75,77,78]. In addition, laser, heat and potassium iodide therapies have also been used in the past with successful outcome (recommendation BIII) [75,79–81]. Recommendations for refractory cases are combination antifungal therapy including cryotherapy or surgery when possible (recommendation BIII) [72,74,75,82–84]. Based on experimental and in vitro studies the new triazole drug posaconazole is promising and could be useful when other therapy has failed (recommendation BIII) [85–88].

Keratitis
Keratitis due to dematiaceous fungi is mainly reported from India where trauma accounts for up to 20% of cases [89–91]. The majority of patients can be treated with topical agents, the most commonly used are 5% natamycin and topical amphoterin B (0.15–0.3%) with or without topical azoles (1%) for at least 4 weeks to several months (recommendation All) [89,90,92–94]. Topical azoles alone especially itraconazole and voriconazole (1%) can also be used (recommendation BIII) [95–97]. Severe and refractory cases require administration of oral azoles and usually surgery including penetrating and lamellar keratoplasty (recommendation BIII) [89,91–93]. An intracorneal injection of voriconazole (1%) as salvage therapy has been efficient in patients not responding to topical and systemic therapy in some cases (recommendation CIII) [96,98].

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Pulmonary infections
These are potentially life threatening and are mainly seen in immunocompromised patients or those with underlying lung disease although cases in immunocompetent patients have been reported [99–101]. A wide variety of species can be involved and clinical manifestations include pneumonia, pulmonary nodules and endobronchial lesions. Therapy consists of intravenous liposomal amphotericin B or mould-active azoles except ketoconazole for a prolonged period (recommendation BIII). However, mortality rates are high in immunocompromised patients if underlying host defence defects are not resolved [12,102–107]. Solitary pulmonary nodule in immunocompetent patients can be treated with surgery (recommendation BII) [12,99,108].

Cerebral infection
Cerebral abscess due to dematiaceous fungi is rare but frequently fatal and a surprisingly high proportion of these infections occurs in apparently immunocompetent individuals [109–116]. These infections are spread haematogenously, probably from an initial, presumably subclinical pulmonary focus, although spread from the sinus or following surgery may also occur. The neurotropic fungi are often geographically restricted, such as Rhinocladiella mackenzii occurring in the Middle East and Cladosiphialophora bantiana mainly in India. Although most infections with Exophiala dermatitidis are reported from East Asia the fungus is encountered worldwide. Overall, the therapeutic studies suggest that complete excision of brain abscesses has better outcome than only aspiration or partial excision (recommendation All) [109,112,117–120]. Even with antifungal therapy outcome is poor; however, single cases suggest that voriconazole and posaconazole may provide clinical improvement and voriconazole penetrates into brain tissue most effectively (recommendation CII) [121–128]. Amphotericin B therapy generally has a poor outcome (recommendation DIII) [122–124,129]. Combination therapy including a triazole plus an echinocandin plus flucytosine, which also has in vitro activity against many of the black moulds and achieves good brain penetration, could be the first-line therapy when surgery is not possible (recommendation BIII) [12,116,130].

Other localized deep infections
These comprise mainly bone and joint infections and peritonitis. Recommendations can be found in Table 4 [12,131–137].

Disseminated infection
This is uncommon and reported mainly in the immunocompromised population [138]. Occasionally Exophiala asiatica causes dissemination in patients without known immunodeficiency or risk factors and it was recently reported from China [139,140]. There are at present no antifungal regimens associated with improved survival in disseminated infection, including multiple combination therapies (recommendation CIII) [138,141–147]. Combination antifungal therapy with adjunctive treatments has been effective in some cases of infections with hyaline fungi and multi-resistant Scedosporium spp. (recommendation BIII) [148–150].

Allergic fungal sinusitis
This entity is a hypersensitivity reaction, especially in immunocompetent, often atopic patients, and is caused by many species of dematiaceous fungi. The main black fungi involved are Bipolaris, Curvularia, Exserohilum and Alternaria species. Diagnosis depends on a histopathological demonstration of allergic mucin with visible fungal elements. Therapy consists of systemic steroids combined with surgical removal of the mucin (recommendation All) [151–154]. The role of antifungal therapy, mostly azoles, is still under debate but may have a steroid-sparing effect (recommendation CIII) [152,153,155]. Recent reports indicate that oral triazole therapy can reduce symptoms of refractory sinusitis (recommendation BIII) [156–158]. In many instances the dematiaceous fungi can be the aetiological agents of sinus fungus balls. Surgical resection of fungus balls is generally sufficient (recommendation All) unless local tissue invasion of the surrounding mucosa is demonstrated. Additional systemic antifungal drugs are indicated when this occurs (recommendation CIII) [159].

Allergic bronchopulmonary mycosis
This mycosis caused by fungi other than Aspergillus is a rare disease with $<$200 reported cases worldwide [160]. The two most commonly implicated dematiaceous fungi are Bipolaris and Curvularia. Analogous to allergic bronchopulmonary mycosis due to Aspergillus, the treatment of allergic bronchopulmonary mycosis consists of systemic steroids (recommendation BII) [12,151,161,162]. Treatment with azoles is not yet clearly established and therefore, not recommended (recommendation DII) [160,163].

Black Fungal Species with Clinical Relevance

During the last few decades the list of dematiaceous fungi implicated in human infections has continued to evolve and will further expand in line with the increase in the numbers of susceptible patients and the employment of better diagnostic tools. The important black fungi, their clinical manifestations, risk factors for infection, diagnosis and treatment are discussed along with their current taxonomical nomenclature.
Alternaria
The genus Alternaria is a plant pathogen and is commonly isolated from soil, air and plants [164–166]. The majority of cutaneous and subcutaneous infections are by Alternaria alternata followed by Alternaria infectoria, Alternaria tenuissima, Alternaria alternatum and Alternaria tenuis [55,167].

Clinical manifestations. Clinical manifestations of Alternaria infections are usually cutaneous or subcutaneous lesions mainly in immunosuppressed individuals [41,52,55,167]. To a lesser extent immunocompetent subjects can be affected following traumatic inoculation with plant debris and/or soil [168–171]. In cutaneous alternariosis, skin and soft tissue of the dorsal part of the hands and feet, fingers, elbows, knees and pretilial areas are the most commonly affected [55]. Most cases of subcutaneous alternariosis present with erythema, desquamation of skin, crusted ulcers, erythematous macules, yellow papules or violaceous nodules. Rarely sinusitis, keratitis and allergic bronchopulmonary mycosis have been reported, and disseminated infections occur with painless papulo-nodular lesions or cutaneous nodules. Cerebral infections due to Alternaria species are very rare [55,160,172]. The major predisposing factor is organ transplantation, reported in 40% of cases [39,42,55,167,173]. Bone marrow recipients are particularly at risk of sinusitis, whereas lung transplant recipients have a risk of cerebral infection [55,174]. In cutaneous/subcutaneous diseases Cushing syndrome is a major risk factor [175,176]. Other risk factors are long-term corticosteroid therapy, surgery, diabetes, human immunodeficiency virus infection, tuberculosis, neutropenia and haematological malignancies [29,31,43,47,177,178].

Diagnosis. Specific diagnosis is based on the microscopic detection of yellowish-brown hyphae with or without budding cells in tissue biopsies, aspirated pus, surgical drainage or skin scrapings. Culture and microscopic examination are mandatory for the correct identification of Alternaria spp. Amplification of DNA targets can be required for identification of uncommon Alternaria spp. [164,179].

Antifungal susceptibility and treatment. Cutaneous alternariosis usually requires the combination of wide excisional surgery, prolonged antifungal therapy, and reduction of immunosuppression [39,180]. In the case of well-delimited lesions, excision alone can lead to a total resolution of the disease, but antifungal therapy is required to avoid relapse. Itraconazole, voriconazole, posaconazole and amphotericin B constitute the cornerstones of the antifungal management of cutaneous and subcutaneous alternariosis based on clinical data available [38,55,56,167,181]. Also, a solitary case report on the successful use of intravenous caspofungin for the treatment of cutaneous alternariosis has been described [39]. As clinical trials are lacking, the optimal treatment strategy for patients with deep-seated Alternaria infections remains unclear [44,176]. Combination antifungal therapy can be recommended in disseminated cases [159,177,182]. In vitro susceptibility data suggest that the susceptibility of Alternaria species to antifungal agents appears to be species dependent (Table 2) [38]. Most of the species are susceptible to amphotericin B, itraconazole, voriconazole and posaconazole, and with high MIC values of echinocandins, fluconazole and fluycytosine. Terbinafine also has been used successfully in the treatment of cutaneous alternariosis [31,46,173]. The role of echinocandins as part of combination therapy for alternariosis remains to be clarified.

Acrophialophora
The genus Acrophialophora comprises three species but only Acrophialophora fusiispora is of clinical interest. Acrophialophora fusiispora is a thermotolerant fungus with a wide distribution in tropical and temperate regions [164].

Clinical manifestations. Only five cases of phaeohyphomycosis have been reported so far, which include two cases of brain abscess attributed to Acrophialophora fusiispora and three other cases involving the lung in two and cornea in one case [183–185].

Diagnosis. This fungus is similar to Paecilomyces spp. and sometimes misidentified as Scedosporium prolificans [186] but can be differentiated by the presence of pigmented, warted conidiophores, basally inflated verticillate phialides and pigmented fusiform conidia ornamented in spiral bands.

Antifungal susceptibility and treatment. Due to the small number of cases reported, the optimal treatment and management of these infections are unknown. The isolates tested have shown variable susceptibility to itraconazole, voriconazole, posaconazole, amphotericin B and resistance to echinocandins. Response in vivo has been unpredictable [183–185].

Aureobasidium
Aureobasidium is a genus of black yeasts that ubiquitously colonize smooth surfaces of plant leaves, glass and rocks, and may contaminate metal, glassware and tubing systems in the hospital [187]. These fungi are commonly found as contaminants in the clinical laboratory. Clinically significant species are Aureobasidium pullulans, Aureobasidium proteae and Aureobasidium mansoni, all of which are associated with cerebral phaeohyphomycosis [164,188].
**Clinical manifestations.** *Aureobasidium pullulans* has an affinity for synthetic materials and surgically implanted silastic devices, as the fungus has been isolated from indwelling peritoneal dialysis catheters and central venous lines [189–193]. In severely compromised patients deep infections are encountered, and the fungus has been isolated from blood, bronchoalveolar lavage, lymph nodes, splenic abscess or cerebrospinal fluid [187,194–203]. Infections are caused mostly by traumatic inoculation of the skin or eye, and intrathecal administration of cytotoxic drugs [204–210].

**Diagnosis.** Black yeasts are observed by microscopy. Classification of this fungus can be done easily by conventional methods and also by DNA sequencing.

**Susceptibility testing and treatment.** No standard treatment exists for *Aureobasidium* infections but amphotericin B is recommended because it has been successfully used to treat systemic infection, menigitis and peritonitis [190–192]. However, two cases of fungaemia reported to have amphotericin B treatment failure are on record [187,197]. Other alternative treatment options which are reported to be effective in localized infections could be fluconazole and flucytosine [192,199]. *In vitro* studies revealed that this organism showed variable degrees of susceptibility to commonly used antifungals (Table 2) [211]. Apart from amphotericin B in invasive cases, voriconazole could be added concomitantly because it completely cured a chronic meningitis case caused by *Aureobasidium proteae* [188].

**Bipolaris**

*Bipolaris* spp. are ubiquitous in nature and found in soil and decaying matter [212]. The commonest species in human infections are *Bipolaris australiensis*, *Bipolaris hawaiiensis* and *Bipolaris spicifera* [12,213]; however, these three species have recently been transferred to *Curvularia* [214]. *Bipolaris* spp. previously classified as *Drechslera* or *Helminthosporium* are emerging as important aetiological agents of phaeohyphomycosis in humans [164].

**Clinical manifestations.** *Bipolaris* spp. are associated with serious infections in immunocompetent and immunocompromised hosts, such as pansinusitis [215], endophthalmitis and orbital cellulitis [216,217], necrotizing pneumonia and allergic bronchopulmonary mycosis [160,162,218], peritonitis [219], ascending aorta endarteritis [220] and encephalitis [221,222]. Dissemination to the central nervous system via the nasal sinuses has been described [114,223–225]. Dissemination to other deep sites may occur in debilitated or compromised patients such as those having undergone either organ transplantation or other surgical procedures [226–230]. Superficial disease involving cutaneous, subcutaneous and corneal regions afflicts mainly immunocompetent patients [115,231–233].

**Diagnosis.** Diagnostic procedures of cutaneous and invasive infections are summarized in Table 3 and are similar for most black fungi. Molecular identification based on PCR and sequencing of the internal transcribed spacer (ITS) and D1/D2 regions of rDNA is recommended for accurate identification [234]. Direct detection of *Bipolaris* DNA by PCR has been reported [235,236]. As with all fungi in this class, the Fontana–Masson stain is helpful for diagnosis [237].

**Antifungal susceptibility and therapy.** Treatment involves a combination of surgical debridement and antifungal treatment, typically with amphotericin B or an azole [238–241]. With the exception of fluconazole and flucytosine, amphotericin B, itraconazole, posaconazole and voriconazole showed good activity against species of *Bipolaris* [213,242]. Surgical interventions such as removal of foreign objects, catheter tips or sinus debridement are usually necessary as adjunctive therapy, especially in localized infections and those associated with foreign implants [243,244].

**Chaetomium**

The genus *Chaetomium* is a large genus of saprobic ascomycetes including >180 species. *Chaetomium* species are generally found in warm, dry, cellulose-rich media, such as animal dung, straw, seeds, plant debris, bird feathers and many other substrates [245,246]. They are rarely implicated in human disease; the clinically significant species include *Chaetomium globosum*, followed by *Chaetomium strumarium*, *Chaetomium atrorbrunneum*, *Chaetomium funcicola* and *Chaetomium perlucidum* [247–255].

**Clinical manifestations.** The spectrum of mycoses caused by *Chaetomium* species includes onychomycosis, chromoblastomycosis and sinusitis in immunocompetent individuals [249,253], and empyema, pneumonia, and fatal disseminated cerebral disease in immunocompromised hosts and intravenous drug users [247,248,250–252,254,255]. The majority of reports have involved patients with haematological malignancies and/or immunosuppression secondary to bone marrow or solid organ transplantation [102,248,252,254,255].

**Diagnosis.** Diagnostic procedures are similar to those previously described. The main characteristic of *Chaetomium* species is the presence of hairs or setae covering the ascomata. They are differentiated by the size and shape of ascomata, the type
of setae they possess, and the size and shape of their brownish ascospores [143,164,256].

**Susceptibility testing and treatment.** Most patients with reported invasive disease received either conventional or lipid-based amphotericin B empirically during their treatment course [247–255]. Chaetomium perlicum isolates have low MICs of amphotericin B, itraconazole, voriconazole and posaconazole, but high MICs of caspofungin. Amphotericin B had varied susceptibility profiles while itraconazole and voriconazole exhibited good activity against Chaetomium globosum [143,257,258].

**Cladophialophora**

The genus includes neurotropic fungi such as *Cladophialophora bantiana* and *Cladophialophora modesta* causing mainly brain infections [259]. While *Cladophialophora bantiana* is reported worldwide, a general preference for warm climates with high humidity is apparent [260]. *Cladophialophora carrionii* is prevalent in dry countries and desert zones, and other rarely reported species *Cladophialophora devriesii* and *Cladophialophora arxii* cause disseminated disease, while *Cladophialophora boppii*, *Cladophialophora emmonsii* and *Cladophialophora saturnica* cause mild cutaneous infections [164,245,261–263].

**Clinical manifestations.** Human infections, due to *Cladophialophora* range from mild cutaneous lesions to fatal cerebral infection. In a review in 2004, *Cladophialophora bantiana* was the most common species responsible for cerebral disease and accounted for 48 of 101 cases of cerebral phaeohyphomycosis [127]. Single lesions were present in the majority of cases of brain abscess. Also, no evidence of dissemination outside the central nervous system has been observed. Patients with central nervous system phaeohyphomycosis are often immunocompetent and have no known underlying diseases [123,124,264,265]. These species also cause superficial and subcutaneous diseases. Most of the aetiological agents produce only localized disease restricted to skin and subcutaneous tissue. Chromoblastomycosis due to *Cladophialophora* is mainly caused by *Cladophialophora carrionii* [12,266]. Risk factors or underlying diseases associated with infection due to *Cladophialophora* are organ transplantation, diabetes, systemic lupus erythematosus, pulmonary tuberculosis, primary immunodeficiency of unknown origin, recurrent cytomegalovirus viraemia, pneumonitis, neutropenia and nephrectomy [105,126,128,267,268].

**Diagnosis.** *Cladophialophora* is a genus related to black yeast-like fungi but in routine cultures it grows strictly monomorphically as a mould with long, delicate, branching chains of hydrophobic conidia and lacking yeast cells [164]. In cerebral phaeohyphomycosis and other infections a KOH preparation of pus from the lesion may show lightly pigmented yeast-like forms or more often short chains of spores and hyphae. Histopathology is essential for confirmation of subcutaneous infections. Culture is recommended and for species identification, sequencing of ITS regions of rDNA is most appropriate [245]. Although there are no specific clinical or radiological features for the diagnosis of cerebral phaeohyphomycosis, a computed tomography scan of the cranium often reveals unilateral well-circumscribed single or multiple mass lesions localized within the cerebral cortex [117,260,269]. Purulent meningitis, with or without brain abscess, may also be seen [265].

**Susceptibility testing and treatment.** When possible, complete surgical removal of the encapsulated abscess combined with antifungal therapy is recommended, but so far success in treating cerebral phaeohyphomycosis due to *Cladophialophora* is limited regardless of the immune status of the patient (>70% mortality) [12,127]. Adding antifungal monotherapy or combination therapy might improve survival [270,271]. When there are multiple cerebral abscesses and surgery is not practicable, combination therapy with amphotericin B, fluconazole, caspofungin and terbinafine, or an extended spectrum triazole, has been proposed as a regimen [12,128]. Itraconazole and posaconazole had the best activity in vitro, while voriconazole has better central nervous system penetration and better bioavailability [272–275]. Echinocandins and amphotericin B have shown also activity in vitro [87,123]. The newer drug isavuconazole reveals low MICs for *Cladophialophora carrionii*. In murine models of *Cladophialophora bantiana* infections, the combination of the three drugs fluconazole, micafungin and posaconazole was the only therapy that prolonged survival time [276].

**Curvularia**

The genus *Curvularia* comprises nearly 100 species. Most are saprobes in soil, on dead plant material or plant pathogens mainly infecting grasses [212]. The clinically relevant species are *Curvularia aeria*, *Curvularia geniculata/Curvularia senegalensis* and *Curvularia lunata*; less frequently implicated species are *Curvularia brachyspora*, *Curvularia clavata*, *Curvularia inaequalis*, *Curvularia pallescens* and *Curvularia verruculosa* [164,212,277].

**Clinical manifestations.** More commonly, species of *Curvularia* cause allergic sinusitis [278,279], but they can disseminate to the brain even in immunocompetent patients [113]. Other manifestations include subcutaneous infections following traumatic implantation [280,281], onychomykosis [282], keratitis
endophthalmitis [285,286], mycetoma [287], invasive sinusitis [288,289], peritonitis [290,291], invasive cerebral infections [292,293], endocarditis [294] and disseminated infections [295–297].

**Diagnosis.** Colonies of *Curvularia* are blackish, expanding and hairy; the conidiophores are erect and the conidia are ellipsoidal, brown, usually curved and generally with three or four septa. Recent studies have demonstrated that molecular confirmation of species is usually required by sequencing the ITS regions of rDNA and the glyceraldehyde-3-phosphate dehydrogenase gene [164,212,277,298].

**Antifungal susceptibility and treatment.** The in vitro antifungal susceptibility of different clinical isolates of *Curvularia* has been determined in several studies (Table 2) [113,277,284,299]. In general, amphotericin B showed potent in vitro activity and triazoles and echinocandins had less activity. Clinical experience with the treatment of *Curvularia* infections is scarce and mainly based on a few case reports where amphotericin B and azoles have been the most frequently used drugs in monotherapy or combination therapy with variable results (Table 4) [110,279,280,286,293,295,297,300,301].

**Exophiala**

The genus *Exophiala* comprises the most clinically relevant black yeasts, often isolated from environmental substrates, including soil, wood and other plant material [164,298]. The species commonly involved in human infections are *Exophiala dermatitidis*, *Exophiala xenobiotica* and *Exophiala oligosperma*, followed by *Exophiala lecaniicori*, *Exophiala phaeomuriformis*, *Exophiala jeanselmei*, *Exophiala bergeri*, *Exophiala mesophila*, *Exophiala spinfera*, *Exophiala xenobiotica* and *Exophiala oligosperma* [302–304]. Although distributed worldwide, *Exophiala dermatitidis*, a neurotropic agent, is reported mainly from Asia, whereas *Exophiala spinfera* is reported from various parts of the world as the causative agent of phaeohyphomycosis and chromoblastomycosis [259,264,294,305].

**Clinical manifestations.** Most of the infections caused by *Exophiala* are cutaneous and subcutaneous [306–308] whereas fatal systemic infections can occur, including rare cerebral infections [139,140,164,298,309]. *Exophiala* species produce pustules or verrucous plaques in the skin or subcutaneous tissue. These lesions can enlarge and impair mobility but rarely disseminate to the internal organs [35,305,310,311]. Chromoblastomycosis or eumycotic mycetoma is rarely caused by this genus [60,74]. Besides subcutaneous infections, this species can cause pulmonary colonization of the lungs in patients with cystic fibrosis [312] and brain abscess and disseminated, eventually fatal, disease in patients without recognized underlying diseases [304,313,314]. Disseminated disease generally affects elderly and immunosuppressed patients such as individuals with AIDS or those on prolonged use of immuno-suppressive drugs, chemotherapy treatment or systemic corticosteroids [267,315–317]. Additionally, intestinal colonization by the fungus has been reported [318,319].

**Diagnosis.** The histological characteristics of *Exophiala* for a cutaneous deep fungal infection include epidermal hyperkeratosis, hyperplasia, acanthosis, pseudoepitheliomatous and intraepidermal pustule formation. Pigmented fungal elements can be detected most frequently in areas of inflammation, within or adjoining to multinucleate giant cells. Diagnostic techniques are shown in Table 3. Molecular methods of detection and classification have also been reported [303,320].

**Susceptibility testing and treatment.** Apart from surgical resection, which in some cases is curative, treatment requires antifungal agents such as itraconazole or terbinafine alone or in combination [267,321,322]. As an alternative to the prolonged, expensive pharmacological treatments, some authors propose Mohs micrographic surgery as an effective therapeutic option with the important benefit of minimal tissue loss [26]. Other antifungal agents have also been used, and brain and disseminated infections are infections that are difficult to treat [141,323–333]. *In vitro* susceptibility studies demonstrated variable activity of posaconazole, itraconazole, voriconazole and amphotericin B [334–338]. In animal models of disseminated infection by *Exophiala dermatitidis* posaconazole was more effective than amphotericin B and itraconazole [339].

**Exserohilum**

The anamorphic genus *Exserohilum* comprises around 35 species, which are common saprobes fungi on plant debris [164]. Three species *Exserohilum rostratum*, *Exserohilum longirostratum* and *Exserohilum mcginnisii* have been reported in the past as opportunistic pathogens for humans. However, several molecular studies have demonstrated that they belong to a single species, *Exserohilum rostratum* being the accepted one [340].

**Clinical manifestations.** *Exserohilum* is a rare clinically significant pathogen causing invasive infections mainly in immunocompromised patients [222,341–356], keratitis [357–361] or localized infections in immunocompetent individuals usually after accidental inoculation [362–364]. The risk factors for *Exserohilum* infections include aplastic anaemia [345,365] and haematopoietic stem cell transplantation [341,356]. Recently, *Exserohilum rostratum* has been implicated in a fungal meningitis outbreak that was traced back to contami-
nated steroid injections [366–369]. As of 23 October 2013 there were 718 cases of fungal meningitis, stroke due to presumed fungal meningitis, and/or spinal or paraspinal infections; 33 cases of peripheral joint infections and 64 deaths (http://www.cdc.gov/hai/outbreaks/meningitis-map-large.html, accessed 9 December 2013).

**Diagnosis.** Exserohilum species are mainly identified by the conidial morphology when growing in its natural substratum [164]. *In vitro* identification is more difficult, the conidia tending to be smaller and the isolates often losing the ability to sporulate. At the generic level, the most useful microscopic characteristics are the conidial shape with the presence of a protruding scar or hilum. Sequencing of the ITS region of rDNA for molecular identification has been used. In the context of the above mentioned outbreak, species-specific real-time PCR assays were developed for rapid molecular diagnosis [370,371].

**Antifungal susceptibility and treatment.** There are limited data on the treatment of infections due to *Exserohilum*. Experience from the recent meningitis outbreak [368] and case reviews of sinusitis and cutaneous infections by these fungi reveal successful outcomes with amphotericin B [341,346,372] and more recently with itraconazole and voriconazole [347,353]. Based on historical data, amphotericin B might be the first choice in severe infections [340,373,374] but an expert group coordinated by the US Centers for Disease Control advised voriconazole because of its excellent pharmacokinetics/pharmacodynamics in cerebral infections [366,375]. However, clinical failures with voriconazole have been reported [376]. *In vitro* studies showed itraconazole, posaconazole and amphotericin B to be the most potent followed by voriconazole [340,375]. Animal models of Exserohilum central nervous system infection have not yet been developed for therapeutic and prophylactic studies [377].

**Fonsecaea**

Fonsecaea is one of the classical genera of fungi causing human chromoblastomycosis. A small group of three closely related species include *Fonsecaea pedrosoi*, *Fonsecaea monophora* and *Fonsecaea nubica* [378]. *Fonsecaea* particularly occurs in tropical climate zones, especially South America and Japan [379–381]. Most cases outside endemic zones are assumed to have been imported. However, cases that were likely to be autochthonous were reported even in northern Europe [382]. Other *Fonsecaea* species are saprobes in the environment, and occasionally cause infections in animals [383].

**Clinical manifestations.** The classical presentation of chromoblastomycosis caused by *Fonsecaea* is similar to that described previously above [74]. The disease is probably acquired by traumatic inoculation of plant debris and possibly hydrocarbon-rich plant material, such as coconut shells, which are preferentially infested by *Fonsecaea* species [384]. *Fonsecaea* infections other than chromoblastomycosis are rare and mainly concern brain infections by *Fonsecaea monophora* [385–387]. The portal of entry of these infections is unknown but dissemination from a pulmonary focus is likely.

**Diagnosis.** *Fonsecaea* species are recognized by poorly differentiated conidiophores apically producing short, branched chains of conidia [74,298,370]. For species distinction, sequencing of rDNA ITS regions is necessary [378,386]. Genus-specific PCR for detection of *Fonsecaea* species has been applied [71,388]. Detection of 1,3-β-D-glucan was used to diagnose and monitor therapy against cerebral phaeohyphomycosis by *Fonsecaea monophora* in a transplant recipient [125].

**Susceptibility testing and treatment.** Therapy for chromoblastomycosis has already been commented on (Table 4). Surgery plus antifungal therapy is the standard of therapy. In addition, combination therapy with itraconazole plus terbinafine or flucytosine has been successfully used in severe disease [72,83]. *In vitro* susceptibility data of these species revealed lowest MICs for posaconazole followed by itraconazole, voriconazole, terbinafine, amphotericin B and caspofungin [378]. A refractory case of chromoblastomycosis caused by *Fonsecaea monophora* failed treatment with itraconazole and terbinafine. Photodynamic therapy and combination therapy with voriconazole plus terbinafine led to improvement of the lesions [37].

**Hortaea werneckii**

The melanized, polymorphic and yeast-like fungus *Hortaea werneckii*, previously known as *Exophiala werneckii* or *Cladosporum werneckii*, is the black yeast responsible for tinea nigra. *Hortaea werneckii* is best known from tropical climates and lives in saline environments such as seawater and natural or man-made salt pans [389,390]. Most cases of infection originate from rural areas in tropical and humid regions characterized by abundant vegetation and had close contact with plants and grasses with substrata of high salinity.

**Clinical manifestations.** Tinea nigra is a superficial mycosis of one or both hands and sometime affects the sole. The disease has no preference for age or sex, with cases equally occurring...
in adults and children, and males and females [391–393]. Most cases are unilateral but bilateral infections can be observed [394], probably resulting from autoinoculation. The first human case not involving the skin was reported from an immunocompromised patient with endophthalmitis following cataract surgery [395]. Hortaea werneckii has been recovered from blood and splenic abscess of two patients with acute myelomonocytic leukaemia [396].

**Diagnosis.** Conidia of Hortaea werneckii appear as pigmented yeast cells with a dark central septum, the outer wall later becoming thick-walled and heavily pigmented. Conidia finally germinate with hyphae resulting in yeast-like colonies that gradually change into filaments. Molecular identification of Hortaea werneckii has been reported [388,390,397].

**Susceptibility testing and treatment.** The treatment of tinea nigra is simple and effective. Most cases resolve with only keratolytic agents like urea, salicylic acid and Whitfield ointment, applied once or twice a day [391]. In vitro antifungal susceptibility testing showed variable MICs of itraconazole, voriconazole, posaconazole, isavuconazole and amphotericin B (Meis J.F., unpublished data). There are reports available of high MICs of this fungus to amphotericin B, fluconazole, flucytosine and ketoconazole [399]. However, molecular studies have demonstrated that they are two different species, and Nattrassia mangiferae is now accommodated in a different genus with non-pathogenic species [406]. Neoscytalidium dimidiatum is distinguished from dermatophytes by its characteristic sinuous, irregular hyphal appearance and by brown pigmentation on direct microscopy of cutaneous specimens, its fast-growing colonies, and its sensitivity to cycloheximide [401]. On microscopy of cultures, characteristic pigmented hyphae and long chains of barrel-shaped arthroconidia are seen. In deeper tissue the fungus has been described as producing yeast-like cells with short hyphae [404].

**Neoscytalidium dimidiatum**

Neoscytalidium dimidiatum (formerly Scytalidium dimidiatum) is a known plant pathogen in tropical areas that can also be found in soil and wood and can infect humans [398,399]. Scytalidium hyalinum, previously considered a non-pigmented species similar to Neoscytalidium dimidiatum is in fact only a mutant variant [400]. The fungus is endemic in tropical and subtropical areas of South America, the Caribbean, Asia and Africa but has been increasingly reported from other non-endemic regions owing to immigration and travel [401]. It was reported that in Jamaica up to 40% of the population suffer from this infection [402].

**Clinical manifestations.** Neoscytalidium dimidiatum causes mainly onychomycosis and tinea pedis, and in endemic areas may rival dermatophytes as the leading cause of superficial fungal infection. This fungus most often causes chronic superficial infections of the skin and nails, clinically resembling dermatophytosis [399,403]. Rarely mycetoma, subcutaneous lesions, cerebral infections, fungaemia and other deep-seated infections mainly affecting immunocompromised patients [399] have also been reported. Invasive infections have been seen mostly in immunosuppressed patients [399,401,404].

The underlying conditions reported are similar to those of other opportunistic invasive mycoses.

**Diagnosis.** Traditionally, the fungus has been characterized by producing dark arthroconidia when grown in culture whereas in older cultures some isolates developed a picnidial form called Nattrassia mangiferae (formerly Hendersonula toruloidea) [405]. However, molecular studies have demonstrated that they are two different species, and Nattrassia mangiferae is now accommodated in a different genus with non-pathogenic species [406]. Neoscytalidium dimidiatum is distinguished from dermatophytes by its characteristic sinuous, irregular hyphal appearance and by brown pigmentation on direct microscopy of cutaneous specimens, its fast-growing colonies, and its sensitivity to cycloheximide [401]. On microscopy of cultures, characteristic pigmented hyphae and long chains of barrel-shaped arthroconidia are seen. In deeper tissue the fungus has been described as producing yeast-like cells with short hyphae [404].

**Susceptibility testing and treatment.** Antifungal therapy with amphotericin B, voriconazole, posaconazole or ketoconazole has been used with variable results [399–404,407,408]. In vitro studies have shown that amphotericin B was the most active drug followed by terbinafine, whereas voriconazole and posaconazole showed less activity [400,409]. The best treatment of systemic infections by this fungus is unknown; however, in a murine model, amphotericin B, voriconazole and posaconazole had efficacy in the treatment of a disseminated infection [410].

**Ochroconis**

Ochroconis encompasses several species including Ochroconis constricta, Ochroconis gallopava, recently transferred to the new genus Verrucosis, and Ochroconis humicola [136,144,411]. Members of the genus have been isolated worldwide from soil, thermal springs, decaying vegetation, in chicken litter and the effluents of thermal nuclear reactors [101,412–417]. Although the organism has a worldwide distribution, many cases of human infections have been described in the southeastern USA [418,419]. Its exact mode of transmission is unclear, but it is hypothesized that Ochroconis might be acquired from penetrating trauma or via inhalation of conidia [70,420,421]. Although Ochroconis spp. have traditionally been regarded as a cause of deep infections in birds and other animals there have been multiple reports implicating these fungi, particularly Verrucosis gallopava and Ochroconis constricta, as pathogens in humans [104,144,422–427].
Clinical manifestations. The majority of these reports have been in two patient populations: those that have received transplants [415,423,424,426–431], and those with haematological malignancies undergoing chemotherapy [418,419,422,432]. Infections in these two groups presented as a combination of both pulmonary and extra-pulmonary disease, particularly involving the brain, spleen, skin and other organ sites. Although a number of patients with extra-pulmonary disease have survived [101,433], it is more frequently associated with poor clinical outcomes [418,422,424,426]. Other risk factors are HIV and chronic granulomatous disease [144,420,434]. The minority of cases of Ochroconis infections have been in immunocompetent patients [435,436].

Diagnosis. The colonies of Ochroconis species are brown-olive, and have a velvety texture. Microscopically, they are characterized by brown septate hyphae, unbranched conidiophores with apical denticles arranged sympodially, and club-shaped conidia with one to three transverse septa [164,411]. The paucity of Ochroconis infections in humans has two potential consequences. First, clinicians may fail to consider it in their differential diagnosis. Second, the microbiology laboratory may mistakenly dismiss the organism as a contaminant, rather than acknowledging it as a true pathogen [127,413,418]. Similar to other black fungi, sequencing of ITS and D1/D2 regions of rDNA can be used for molecular identification [234].

Susceptibility testing and treatment. Due to the high mortality rate reported in patients (estimated at 50%), proper recognition and treatment of Ochroconis infections are paramount [426,430]. Several studies suggest that posaconazole and itraconazole may be an optimal therapy for Ochroconis infection, with amphotericin B and voriconazole as valid alternatives. Fluconazole and fluconazole are the least effective drugs [423,427,430,434]. Ochroconis gallopava has low MICs for most antifungal drugs with terbinafine, posaconazole and voriconazole showing the best in vitro activity [434,437].

Phaeoacremonium

The genus Phaeoacremonium initially accommodated species with features similar to those seen in both Acremonium and Phialophora [406]. A recent morphological and molecular characterization of the genus using β-tubulin sequences [438] has more clearly defined the genus and provided differential features for clinically significant species. Human pathogens include Phaeoacremonium parasiticum (obsolete Phialophora parasitica), Phaeoacremonium alvesii, Phaeoacremonium amstelodamense, Phaeoacremonium griseorubrum, Phaeoacremonium kraidenii, Phaeoacremonium rubigenum, Phaeoacremonium inflata-

Clinical manifestations. Recently, Phaeoacremonium infections have been increasingly reported in humans including subcutaneous abscesses, cysts, or chronic or acute osteoarthritis and disseminated infection mostly in immunocompromised patients (solid organ transplantation and haematological diseases) [28–30,441–443]. Colonization of cracked skin on the extremities has also been described [438]. In the majority of cases, a preceding trauma leading to inoculation from the environment was reported [30,442,443]. In immunocompromised patients with disseminated infections, endocarditis, brain abscess and fungaemia have been reported [12,438,440].

Diagnosis. Infections by Phaeoacremonium are diagnosed by biopsy of the cysts. Direct examination reveals medium brown hyphae, which become pale brown to hyaline and verrucous in the aspirated pus, biopsy material or skin scrapings [30,164]. The phialides have a funnel-shaped collarette and show a wide variety of conidia with diverse forms, including ellipsoidal, obovate, cylindrical or allantoid (sausage-like) [438,439]. PCR amplifying ITS regions of rDNA followed by sequencing was shown to be able to detect and identify species of Phaeoacremonium [444].

Susceptibility testing and treatment. The most active drugs in vitro against Phaeoacremonium parasiticum isolates were voriconazole, posaconazole and itraconazole whereas reduced susceptibility to amphotericin B was reported [445,446]. When possible, complete surgical removal of the encapsulated abscess combined with antifungal therapy such as posaconazole and itraconazole is the recommended treatment [28–30]. However, antifungal therapy for infections caused by some of the species of Phaeoacremonium in immunocompromised hosts is at present unsatisfactory [438,440,441].

Phoma

Phoma species are ubiquitous saprobes on plant material found worldwide [164,447]. Of the more than 200 species of Phoma currently accepted, fewer than 10 species have occasionally been found in human infections [164,448].

Clinical manifestations. Phaeohyphomycosis caused by Phoma has been sporadically described in the literature. Most reported cases are subcutaneous [449–456] and ocular infections [457,458]. Systemic infection with Phoma spp. is generally seen in severely immunocompromised patients and generally has a poor outcome [459–461]. Often the aetiological agent is not identified to the species level. The risk factors
or underlying diseases associated with Phoma infections may include diabetes mellitus, corticosteroid therapy and cancer chemotherapy [450,456,459–461].

Diagnosis. Phoma species produce slow-growing, dark-grey-olive, or dark-brown colonies. The fungus produces ostiolated fruiting bodies known as pycnidia and numerous, small, asexual conidia. Pycnidia are black, globose, subglobose, or pyriform and either submerged or on the surface of agar. Conidia (pycnidiospores) are produced from the phialides that line the inner wall of pycnidia and are hyaline, one-celled, elliptical, rod shaped or curved [164,460]. A PCR assay for detecting Phoma exigua DNA in deparaffinized lung biopsy material has been developed [459].

Susceptibility testing and treatment. Excision of phaeomycotic cysts without antifungal treatment is usually curative. For the treatment of cutaneous lesions triazoles (itraconazole and voriconazole) [451,457] and amphotericin B [450] are recommended. In vitro susceptibility data on Phoma species is based on sporadic case reports with itraconazole and voriconazole MICs ranging from 0.25 to 8 mg/L and amphotericin B MICs from 0.5 to 1 mg/L [457,459].

Pyrenochaeta

Pyrenochaeta is a genus that comprises pycnidial coelomycetes that are widely distributed in the environment, being found in soil, on wood and on plant debris and also as plant pathogens [462]. The species implicated in human infections include Pyrenochaeta keratinophila, Pyrenochaeta unguis-hominis, Pyrenochaeta romeroi and Pyrenochaeta mackinnonii [463–468]. In a recent phylogenetic study based on the analysis of large subunit, ITS, small subunit, β-tubulin and chitin synthase 1 sequences, Pyrenochaeta romeroi and Pyrenochaeta mackinnonii were accommodated in the new genera Medicopsis and Nigrograna as Medicopsis romeroi and Nigrograna mackinnonii, respectively [469].

Clinical manifestations. Pyrenochaeta keratinophila and Pyrenochaeta unguis-hominis are rarely reported as agents of keratitis and onychomycosis, respectively [464,465]. Pyrenochaeta romeroi and Pyrenochaeta mackinnonii have a higher clinical relevance as agents of mycetoma and subcutaneous infections in tropical areas [462,466–472].

Diagnosis. Colonies grow fairly rapidly, and are flat, velvety or floccose and produce dark olive-grey aerial hyphae with an olivaceous-black reverse. Pycnidia are produced after 2–3 weeks and are submerged, ostiolate, olivaceous to black, spherical to pyriform, with thick walls, and often covered with erect, stiff, dark hyphae. Conidia are produced from ampulliform phialides lining the innermost pycnidial wall and oozing out of the ostiolum in slimy drops, and are hyaline, one-celled and ellipsoidal to bacilliform [164,473]. Sequencing of ITS and D1/D2 regions of rDNA was successfully used for molecular identification [234].

Susceptibility testing and treatment. No standard therapy is available for infection with Pyrenochaeta and little is known about the relation between MIC and clinical outcome in this disease. Itraconazole has so far been used in the treatment of cases with mycetoma due to Pyrenochaeta romeroi [182]. Ketoconazole, itraconazole and terbinafine appear active in vitro against Pyrenochaeta romeroi, although systemic ketoconazole would not be the first choice due to unfavourable side effects [473].

Rhinocladiella

The genus Rhinocladiella is a small, polyphyletic genus comprising a few clinically significant species, Rhinocladiella aquaspersa, Rhinocladiella similis, Rhinocladiella basitona, Rhinocladiella mackenziei and Rhinocladiella obovoidum [164,474]. Rhinocladiella mackenziei and Rhinocladiella obovoidum are the neurotropic fungi affecting only the central nervous system [122,474–476]. Rhinocladiella mackenziei has never been isolated from the environment so the natural niche of this organism remains unknown [477]. Most of cases are restricted to the Middle East, Persian Gulf, Somalia and Pakistan [118,120,130,478,479]. Rhinocladiella aquaspersa is an agent of chromoblastomycosis reported from South America, and Rhinocladiella similis and Rhinocladiella basitona are occasional opportunists [480–482].

Clinical manifestations. Most patients (60%) with Rhinocladiella mackenziei brain abscess presented with solitary brain abscesses and the remainder had multiple brain lesions [109]. Among all reported cases of Rhinocladiella mackenziei infections, 25% of patients had no reported underlying conditions [130,477,479]. Diabetes mellitus was the predominant risk factor seen in some patients followed by solid organ failure and/or transplant [120,130,477–484]. Rhinocladiella mackenziei infections are associated with poor outcome and nearly 100% mortality in both immunocompetent and immunocompromised individuals despite surgical intervention and antifungal therapy [12,122,130]. Nine cerebral cases due to Rhinocladiella obovoidum, of which five were fatal, despite administration of amphotericin B in three of them, have been reported so far [482].

Diagnosis. General diagnostic recommendations for cerebral infections are stated in previous sections. These species appear
in culture as olive dark colonies that on microscopic examination show erect, thick-walled and darkly pigmented conidiophores that give rise to conidia only at their distal portions [298,474]. Definitive identification of the species requires sequencing of ITS and or D1/D2 regions of the rDNA gene [118].

Susceptibility testing and treatment. There is no standard therapy for cerebral infections and surgical drainage as opposed to aspiration alone did not improve survival. Medical treatment mostly involved high-dose lipid amphotericin B, itraconazole and flucytosine, or a combination of these drugs [118,120,122,130,477–484]. In vitro antifungal susceptibility studies of the most common pathogenic species showed that this organism has high MICs to amphotericin B and echinocandins, and low MICs to itraconazole, posaconazole and voriconazole [130,478,485]. There are many reported fatal cases of cerebral abscesses where patients failed to respond to antifungal therapy with amphotericin B [109,130,480]. A single case of successful treatment of Rhinocladiella mackenziei brain abscess was reported in which the patient showed improvement after switching from itraconazole to posaconazole [122]. The in vitro data are also consistent with animal studies of a murine model of Rhinocladiella mackenziei cerebral phaeohyphomycosis, where posaconazole was found to be superior to amphotericin B and itraconazole and reduced the brain fungal burden [121].

Veronaea

The genus Veronaea, defined by its type species Veronaea botryosa, is a small group containing several opportunistic species infecting vertebrates [164]. Veronaea botryosa is an environmental fungus but with a currently undiscovered ecological niche. The phylogenetically nearest neighbours of Veronaea botryosa are found in Exophiala species inhabiting water and causing opportunistic infections in waterborne animals [245].

Clinical manifestations. The clinical presentation of the infection is a cutaneous lesion or nodular subcutaneous infection, resembling that of chromoblastomycosis, with muriform cells in tissue but with a strong tendency to disseminate. The infection has been described in both immunocompetent patients [85,486–492], and those with debilitated immunity such as liver [40] and heart transplant recipients [493].

Diagnosis. Veronaea botryosa is readily recognizable by its microscopical morphology. Its large, erect conidiophores with sympodial, uni-septate conidia on flat scars give easy clues for identification in culture [164,298]. Molecular identification using sequencing of the ITS rDNA region is applicable [494].

Antifungal susceptibility and treatment. Published cases of cutaneous and subcutaneous infections show much variation in therapeutic regimens with effective treatment mostly involving itraconazole [40,489]. There were cases that failed to respond to treatment with terbinafine, itraconazole and amphotericin B, but some showed significant improvement with posaconazole [85,487,494]. Very few studies on the in vitro susceptibility of this pathogen have been reported; it demonstrates high MICs for most antifungal drugs (Table 2) with the exception of posaconazole and itraconazole [487,494].

Conclusion

Although previously reported as rare agents of infections the melanized fungi are now emerging as an important fungal disease in humans and animals. These infections have not been studied in clinical trials and so far the available therapeutic data are primarily based on sporadic case reports. Furthermore, the diagnosis depends on a high index of clinical suspicion along with accurate mycological findings. There are no standardized therapies for infections caused by dematiaceous fungi but voriconazole, posaconazole, itraconazole and in some cases amphotericin B demonstrate the most consistent in vitro activity against this group of fungi. Oral itraconazole had been considered the drug of choice for most situations, given the extensive clinical experience with this agent. However, voriconazole may have advantages for central nervous system infections because of its ability to achieve good cerebrospinal fluid levels, unlike itraconazole. Posaconazole is a broad-spectrum alternative that is well-tolerated, though backed by less clinical experience but with excellent salvage treatment results after failure of other antifungal agents. Amphotericin B has been useful in some cases. As a result of the large variability in the spectrum of dematiaceous fungi, it is important to obtain in vitro susceptibilities of the individual patient’s fungal isolate although it has not been firmly established that results obtained from susceptibility testing translate into better clinical outcomes.

Transparency Declaration

AnC has no conflicts of interest to declare. JaM has received research grants from Astellas, Merck and MSD, is a consultant to Astellas, Basilea, Merck and MSD, received travel support from Astellas, and received lecture honoraria from Merck. JoG has no conflicts of interest to declare. SdH has no conflicts of interest to declare. SK has no conflicts of interest to declare.
MCA has received research grants from Astellas, Gilead, Merck/Schering and Pfizer, is a consultant to Merck, Gilead, Pfizer, received travel support from Astellas, Merck/Schering and Pfizer and received lecture honoraria from Astellas, Gilead, Merck/Schering, and Pfizer. SAA has received research grants from Pfizer, is a consultant to Pfizer, and received lecture honoraria from Merck, and Pfizer. MA has received research grants from Gilead, Merck and Pfizer, is a consultant to Gilead, Merck and Pfizer, has received travel support from Merck, Gilead, and Pfizer, and received lecture honoraria from Gilead, Merck, and Pfizer. TB has received royalties from Elsevier. MoC has no conflicts of interest to declare. JeG has received research grants from Basilea, BioMerieux, Astellas, Pfizer, Fundacion Mutua Madrileña, Fondo de Investigacion Sanitaria (FIS), and received lecture honoraria from Astellas, Pfizer, Gilead, MSD, and Hickma Pharma. ArC has no conflict of interest to declare. ED has received research grants from BioRad, Gilead and Pfizer, is a consultant to Astellas and Innothera, received travel support from Merck/Schering, Astellas and Gilead, and received lecture honoraria from Gilead and Merck/Schering. AvD has no conflict of interest to declare. TF is a consultant to Hutman AG. AHG has received research grants from Gilead and Merck Sharp & Dohme, is a consultant to Astellas, Gilead, Merck Sharp & Dohme and Schering-Plough, and received lecture honoraria from Astellas, Gilead, Merck Sharp & Dohme, Schering-Plough, and Zeneus/Cephalon. WH has received research grants from Pfizer, Astellas, Gilead and F2G, is a consultant to Pfizer, Astellas, Gilead and F2G, and received lecture honoraria from Astellas, Gilead, Merck/Schering, and Pfizer. Ej is a consultant to Astellas, Gilead, Merck/Schering and Pfizer, received travel support from Astellas, Merck/Schering and Pfizer, received payment for development of educational presentations from Astellas, Merck/Schering and Pfizer, and received lecture honoraria from Astellas, Gilead, Merck/Schering, and Pfizer. ML has no conflicts of interest to declare. KL has received research grants from Gilead, MSD and Pfizer, has given expert testimony for Merck/Schering and Pfizer, is a consultant to Gilead, Merck/Schering and Pfizer, received travel support from MSD, Pfizer and Gilead and received lecture honoraria from Gilead, Merck/Schering, and Pfizer. FL has received research grants from Gilead, received travel support from Gilead, MSD and Schering, and received lecture honoraria from Gilead. 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LP is a board member of Gilead and Merck is a consultant to Gilead, Merck and Pfizer, and received lecture honoraria from Astellas, Gilead, Merck, and Pfizer. GP has received research grants from Pfizer, Gilead, AstraZeneca, Novartis, Astellas, GSK, is a consultant to MSD, received travel support from Gilead, Astellas and Pfizer and received lecture honoraria from MSD, and Astellas. MR has received payment for development of educational presentations from Pfizer, received royalties from Blackwell Publishing, received travel support from Astellas, is a consultant to Gilead and MSD, and received lecture honoraria from Astellas, and Pfizer. ER has received research grants from Enzon, Gilead, Pfizer and Schering, is a consultant to Astellas, Gilead, Merck, Pfizer and Schering, and received lecture honoraria from Astellas, and Pfizer. AT has received research grants from Astellas and MSD, and received lecture honoraria from Astellas, Gilead, and MSD. AJU has received research grants from Astellas, Gilead, Merck/Schering and Pfizer, is a consultant to Astellas, Basilea, Gilead, Merck/Schering and Pfizer, received payment for development of educational presentations from Gilead, and received lecture honoraria from Astellas, Gilead, Merck/Schering and Pfizer. PV has received research grants from Astellas, Gilead, Merck/Schering, and Pfizer, is a consultant to Astellas, Basilea, Gilead, Merck/Schering and Pfizer, received payment for development of educational presentations from Merck and Pfizer, and received lecture honoraria from Astellas, Gilead, Merck/Schering, and Pfizer. 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Case Report

Exophiala (Wangiella) dermatitidis Prosthetic Aortic Valve Endocarditis and Prosthetic Graft Infection in an Immune Competent Patient

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Exophiala (Wangiella) dermatitidis is an emerging dematiaceous fungus associated with high mortality rates and is a rare cause of endocarditis. We describe the first case of E. dermatitidis endocarditis of a prosthetic aortic valve and aortic graft in an immune competent patient with no clear risk factors of hematological acquisition.

1. Introduction

Exophiala (Wangiella) dermatitidis is a dematiaceous fungus that has been isolated from soil, decaying organic matter, plant debris, and human feces. Recent reports have also described isolation in indoor environments such as kitchen sinks and dishwashers and in steam baths and tubs [1–5]. Infections caused by E. dermatitidis are called phaeohyphomycosis due to the presence of dark pigmented hyphal elements that are seen on histopathology [3]. Phaeohyphomycosis may involve any organ of the body and infections involving the skin, brain, lung, eye, joints, and endocardium have been previously reported [3, 6]. However, the route of human systemic infection remains elusive. Infections with this fungus are rare but tend to be associated with a high mortality rate [7]. To our knowledge, there have been only two previous cases reported of aortic valve endocarditis secondary to E. dermatitidis infection. One occurred in a patient who was immune compromised and the second case was in a patient with a clear risk of acquisition in the setting of active intravenous drug use [8, 9]. The case we discuss below is the first report of E. dermatitidis infection of a prosthetic aortic valve and aortic graft in an immune competent patient.

2. Case

A 53-year-old African American male presented to the Weiler Campus of Montefiore Medical Center on January 12, 2016, with a two-week history of intermittent fevers and chest pain. His past medical history was significant for hypertension and an emergency repair of a ruptured aortic root aneurysm with a valve-sparing aortic root replacement in August 2014. His postoperative course at this time was unremarkable. Four days prior to his current admission, he was seen by his primary care physician with complaints of fever and cough. He received a course of oral antibiotics for “walking pneumonia.” His symptoms did not improve and he presented to the hospital after a syncopal episode at home.

He was born in New York with no relevant travel history. He was a former interior decorator and routinely did woodwork but stopped working after his surgery in 2014. He lived
On hospital day 16, Micafungin 100mg daily was added to stain and acid-fast bacilli (AFB) stain were also negative. In addition, a bacterial gram was negative for fungal organisms. In addition, a bacterial gram form do not the histological specimen was available and a amplicon to be 100% identical to coding.org/BioloMICSSequences.aspx), identified the PCR Bank (https://www.ncbi.nlm.nih.gov/genbank/) and Cen-Sequencing and BLAST search using two databases, GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Centralbureau voor Schimmelcultures (http://www.fungalbar-coding.org/BioloMICSSequences.aspx), identified the PCR amplicon to be 100% identical to E. dermattidis.

Gomori methenamine silver (GMS) staining was performed on the histological specimens available and was negative for fungal organisms. In addition, a bacterial gram stain and acid-fast bacilli (AFB) stain were also negative. On hospital day 16, Micafungin 100 mg daily was added to his antimicrobial regimen once yeast was isolated from his operative cultures. After the identification of the yeast as E. dermatitidis, antifungal therapy was switched to intravenous Voriconazole (loading dose of 6 mg/kg twice daily; maintenance dose 4 mg/kg twice daily) and later converted to oral. The patient's postoperative course was marked by three separate reoperations for bleeding and tamponade (hospital days 10, 20, and 32), each time in the setting of an INR between 2.0 and 2.5. His postoperative course was further complicated by acute kidney injury, which later improved. A six-week course of empiric antibiotics was completed for possible bacterial endocarditis. He remained on oral Voriconazole until discharge from the hospital on day 63 with plans to continue as long as tolerated. He was kept on low dose Warfarin with an INR goal of 1.5–2.0. Extensive workup to evaluate an underlying immunodeficiency was unremarkable.

The in vitro antifungal susceptibility of our isolate of E. dermatitidis was determined using the broth microdilution method according to the guidelines of Clinical Laboratory Standards Institute (CLSI) at the NYSDOH. The minimum inhibitory concentrations (MIC) of Amphotericin B, Posaconazole, Voriconazole, Itraconazole, Fluconazole, Caspofungin, and Micafungin against the culture isolate were 0.5 mcg/mL, 0.06 mcg/mL, 0.25 mcg/mL, 4.0 mcg/mL, 4.0 mcg/mL, and 2.0 mcg/mL, respectively. The interpretations for the drugs were based on the CLSI M38-A2 document [12].

3. Discussion

E. dermatitidis rarely causes clinical infection in immune competent individuals. Clinical manifestations of disease can range from localized subcutaneous nodules to highly invasive infections such as brain abscesses, meningitis, and endocarditis [1, 6, 8]. Fungi are a less common cause of infective endocarditis and account for less than 10 percent of cases [13]. There are only two previous reports of E. dermatitidis endocarditis in the literature [8, 9]. As described earlier, Vartian et al. reported the first known case of endocarditis secondary to E. dermatitidis with a clear mode of acquisition in the setting

at home with his wife and eight-year-old daughter. They have one cat and no other pets. He occasionally smokes marijuana.

On presentation to the Emergency Department he was hypotensive and hypoxic, but afebrile. His initial white blood cell (WBC) count was 19 and his hemoglobin was 8.2 mg/dl. Computed tomography angiography (CTA) of his chest showed fluid around the aortic root without extravasation. A tranesophageal echocardiogram revealed multiple small mobile densities (3-4 mm) on the aortic valve and inner surface of the aortic graft with moderate aortic valve regurgitation. He also had circumferential thickening outside the aortic graft suggestive of a paragraft abscess. He was started on broad spectrum antibiotics (vancomycin 1 g, 12 hourly and piperacillin-tazobactam 4.5 g every 6 hours) and multiple sets of blood cultures were sent which remained negative.

On hospital day 7, he underwent a reoperative mechanical aortic valve and partial aortic arch replacement with reimplantation of the innominate artery. Intraoperative findings were significant for a peri-aortic abscess. A gram stain of the graft showed many polymorphonuclear leukocytes. Cytopathology revealed marked acute and chronic inflammation of the aortic valve consistent with acute endocarditis as well as focal necrosis and exudate of the aortic graft consistent with an abscess.

Culture of the aortic valve initially on thioglycolate broth showed budding yeast. Subculture of the isolate on Sabouraud Dextrose agar grown at 37 degrees Celsius yielded black colonies of yeast (Figure 1). On observing the colonies with Lactophenol cotton blue by doing tape preparation, dark, septate, and cylindrical to flask shaped conidiogenous cells were seen. Round to oval single celled pale brown conidia accumulate at the apex of the conidiogenous cell and down the sides of the conidiophore were observed. The isolate from the aortic valve culture was identified as E. dermattidis by the microbiology laboratory at our institution using the BD Phoenix automated microbiology system (Becton Dickinson Diagnostic Systems Phoenix Instrument, Maryland, United States) [10].

Further molecular characterization and confirmation of the pathogenic fungal organism as Exophiala dermattidis was performed by the Mycology Laboratory at the New York State Department of Health (NYSDOH). In addition to phenotypic characterization, internal transcribed spacer (ITS) sequencing was completed. The genomic DNA from the mold culture was isolated and the ITS region (ITS1-5.8S-ITS2) of the ribosomal gene was amplified using primer set V1827 (ITS5) 5'-GGAAATCAAAGCTGATAACGG-3' and V50 (ITS4) 5'-TTCCTCCTTATTGATATGC-3'. Polymerase chain reaction (PCR) was performed as described previously [11]. Sequencing and BLAST search using two databases, GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Centralbureau voor Schimmelcultures (http://www.fungalbar-coding.org/BioloMICSSequences.aspx), identified the PCR amplicon to be 100% identical to E. dermattidis.

Gomori methenamine silver (GMS) staining was performed on the histological specimens available and was negative for fungal organisms. In addition, a bacterial gram stain and acid-fast bacilli (AFB) stain were also negative. On hospital day 16, Micafungin 100 mg daily was added to
of intravenous drug use. This patient’s endocarditis affected the native aortic valve and was complicated by relapsing infection of subsequent aortic prosthesis and dissemination to the spine [9]. Patel et al. reported a case of native aortic valve endocarditis in a posttransplant patient on immune suppression with prednisolone, tacrolimus, and mycophenolate, who responded well to medical and surgical management [8]. Our case is the first report of endocarditis and prosthetic graft infection in a patient with no known underlying immunodeficiency and no clear risk factors of hematogenous acquisition such as intravenous drug use.

Diagnosis of fungal endocarditis can be challenging as the most common presenting clinical features such as fever, new heart murmur, and peripheral embolization are nonspecific for fungal etiologies [13, 14]. Even when there is a high index of suspicion, microbiological identification of fungi remains cumbersome. Identification of phaeohyphomycosis is particularly difficult as cultures for E. dermatitidis may remain negative even when infection is present [14]. This organism grows slowly on primary isolation media and recovery may be missed if blood cultures are incubated for short periods of time.

In this case, budding yeasts were seen from the patient’s operative cultures after nine days of incubation. However, multiple sets of blood cultures remained negative despite an extended period of incubation of four weeks to ensure recovery of E. dermatitidis. Given his CTA, echocardiogram, and cytology findings, we considered the positive intraoperative prosthetic graft culture as evidence of infection with E. dermatitidis even though it was not identified from the patient’s blood.

Extensive workup was completed to evaluate underlying immunodeficiency in our patient which was all unremarkable. Screening tests for human immunodeficiency virus (HIV) infection, immunoglobulin levels, lymphocyte subsets, and peripheral flow cytometry were all within normal limits. In addition, serological markers of connective tissue disease were negative as well. No underlying immunologic predisposition was identified and we presumed that our patient acquired this pathogen via inhalational exposure leading to hematogenous spread. However, our patient did have two of the most common risk factors for fungal endocarditis: previous cardiac surgery for an aortic root aneurysm and recent antibiotic treatment for pneumonia [13, 14].

Perhaps even more challenging than accurate diagnosis is the appropriate clinical management of this infection. There are no previous randomized controlled trials addressing treatment guidelines for E. dermatitidis and all present data in current literature is based on case reports and series. Treatment is dependent on the site of infection and can range from surgical excision alone for localized subcutaneous infection to a multipronged approach with surgical debridement combination antifungal therapy as well as immune enhancement for cerebral phaeohyphomycosis [3, 7, 8, 13, 15].

With involvement of the prosthetic aortic valve and graft, our patient underwent aortic valve replacement with a bioprosthetic valve [3, 16]. Voriconazole was chosen because of favorable treatment responses in previous reports of E. dermatitidis endocarditis, tolerability, and the isolate’s susceptibility profile [8]. Furthermore, due to postoperative complications of acute kidney injury we decided to avoid Amphotericin B because of its known risk of nephrotoxicity.

Another important issue with E. dermatitidis invasive infections is to define an ideal duration of treatment. We plan to continue an extended course of oral Voriconazole with close clinical monitoring given the severity of his infection and the involvement of prosthetic materials. At this juncture it is hard to define a finite duration of treatment weighing the risks of re-infection of the new valve and graft with the side effects of treatment. With the lack of clinical trials data and no established treatment guidelines, further investigation is needed to define the long-term management of E. dermatitidis infections.

**Conflicts of Interest**

The authors declare there are no conflicts of interest.

**References**


Antifungal susceptibility testing of *Exophiala* spp.: a head-to-head comparison of amphotericin B, itraconazole, posaconazole and voriconazole

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Frequently, diseases caused by black yeasts are chronic in nature with a high morbidity. In addition, these infections are often fatal and relapse is common, even after prolonged treatment. Although the CLSI Document M38-A outlines methods for antifungal susceptibility testing of moulds, *Exophiala* spp. are not directly discussed. In an effort to determine the antifungal susceptibility patterns of *Exophiala* spp. we tested 160 clinical isolates against amphotericin B, itraconazole, posaconazole, and voriconazole in a head-to-head comparison. Posaconazole and itraconazole were the most active *in vitro* with MICs falling well below the achievable serum levels typically observed with standard dosing regimens.

**Keywords** *Exophiala* species, antifungal susceptibility testing, CLSI methods

**Introduction**

Recent reports of infections caused by *Exophiala* species suggest they are being diagnosed with increasing frequency [1–5]. They have been reported from numerous clinical presentations varying from subcutaneous disease to deep seated infection in immunocompromised patients. Despite this increase, there is little information regarding the antifungal susceptibility patterns of this genus against currently available antifungal agents.

**Methods**

A total of 160 *Exophiala* spp. were tested. All strains were clinical isolates that were submitted to the Fungus Testing Laboratory for antifungal susceptibility testing and/or identification from the period 2001 to 2006. Species distribution confirmed by molecular characterization [6] included *Exophiala attenuata* (n = 3, subcutaneous infection), *E. bergeri* (n = 6, cutaneous and subcutaneous infections), *E. dermatitidis* (n = 27, cutaneous, subcutaneous, and deep infection), *Exophiala* sp. (n = 3, mucosal and subcutaneous infection), *E. jeansielsmei* (n = 8, subcutaneous and deep infection), *E. lecanii-corni* (n = 9, subcutaneous and deep infection), *E. mesophila* (n = 6, subcutaneous and deep infections), *E. oligosperma* (n = 40, cutaneous, subcutaneous, and deep infection), *E. phaeomuriformis* (n = 11, cutaneous, subcutaneous, and deep infection), *E. spinifera* (n = 8, cutaneous and subcutaneous infection), *E. xenobiotica* (n = 39, cutaneous, subcutaneous, and deep infection).

All isolates were tested according to methods outlined in M38-A modified to accommodate macrobroth dilution testing. This included testing in RPMI-1640 for itraconazole (ITRA – Janssen Pharmaceutica), posaconazole (POSA – Schering Plough), and voriconazole (VORI – Pfizer) and antibiotic medium 3 (M3) for amphotericin B (AMB – Bristol Meyers Squibb), an inoculum of 1–4 × 10^4, incubation at 35°C for 48–96 h, and an endpoint determination at the first drug concentrations exhibiting an 80% reduction in turbidity as compared to the drug-free control tube.

**Results**

Minimum inhibitory concentration ranges, MIC<sub>50</sub> and MIC<sub>90</sub> data are presented in Table 1 for species with
Table 1 Susceptibility trends for *Exophiala* spp.

<table>
<thead>
<tr>
<th>Isolate (N)</th>
<th>Range (µg/ml)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
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<tbody>
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<td><em>Exophiala attenuata</em> (3)</td>
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<tr>
<td>Amphotericin B</td>
<td>4–16</td>
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<td>≤0.015</td>
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<tr>
<td>Posaconazole</td>
<td>≤0.015</td>
<td>ND</td>
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<tr>
<td>Voriconazole</td>
<td>0.03–0.06</td>
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<td>Itraconazole</td>
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<td>Posaconazole</td>
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<tr>
<td>Voriconazole</td>
<td>0.03–0.25</td>
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<tr>
<td><em>E. jeanselmei</em> (8)</td>
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<td><em>E. spinifera</em> (8)</td>
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<td><em>E. xenobiotica</em> (39)</td>
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ND, not determined due to insufficient numbers of isolates tested.

sufficient numbers tested to calculate these data. In general, all species appear susceptible to AMB with the exception of *E. attenuata* where MICs ranged from 4–16 µg/ml for all isolates tested. Although breakpoints have not been established for AMB against moulds, a general guideline for comparison purposes is anything greater than 1.0 µg/ml being described as most likely resistant. Among the azoles, POSA appeared the most active as all MICs were ≤0.25 µg/ml. ITRA and VORI gave similar results suggesting that *Exophiala* species, in general, are susceptible to theazole class of agents.

**Discussion**

Although standard methods exist for many moulds in CLSI document M38-A [1], *Exophiala* species are not specifically discussed. Numerous papers exist detailing susceptibility testing of *Exophiala* spp. [8–12]. Despite the lack of a described method, it is not typically a problem to determine testing parameters that will permit testing of this group of fungi. By assessing conidial size, a starting point for preparing the inoculum can be determined. Species with conidia roughly the size of *Aspergillus* sp. should begin by adjusting the inoculum to 80–82%T while those with larger conidia should be adjusted to 68–70%T. Colony counts performed on these inocula will provide information allowing adjustments to be made that will result in the final desired concentration of CFU/ml.

*Exophiala* species pose unique problems when preparing an inoculum. In most cultures, despite which *Exophiala* species is being tested, both the yeast and mould phase are present within the same colony. Yeast cells are larger than the conidia produced by the mould resulting in a mixed population of conidial sizes. Despite this, adjusting the inoculum to 80–82%T gave the desired final CFU/ml concentration for our study. In addition to the inoculum difficulties, *Exophiala* spp. tend to grow very slowly in susceptibility testing methods. Although *E. dermatitidis* typically has sufficient growth to determine 48 h MICs, most of the other species require extended incubation to obtain MIC results. Because of this, some strains must be held up to 72 h or more before the first MIC reading can be determined. The slow growth rate often results in dehydration of media from the test wells in a microtiter method. This being the case, we opted to test the *Exophiala* spp. in a macrobroth method. The macrobroth method is discussed in CLSI document M27-A2 [7] which describes testing for yeast fungi. Numerous studies have shown the microtiter and macrobroth methods to be equivalent [8].

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Conclusions

The methods outlined in by the CLSI may be used to assess the susceptibility of *Exophiala* spp. to antifungals. Testing is made possible by making only minor adjustments to the existing method. *Exophiala* species appear susceptible to the newer azoles *in vitro* although correlation studies are desirable to determine if the low MICs noted in the test tube correspond to favorable patient response.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


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Review
Fungal endocarditis: current challenges

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β-1,3-1-Glucans

A B S T R A C T

Whilst it used to affect mostly intravenous drug users and patients who underwent valvular surgery with suboptimal infection control procedures, fungal endocarditis is now mostly observed in patients with severe immunodeficiency (onco-haematology), in association with chronic central venous access and broad-spectrum antibiotic use. The incidence of fungal endocarditis has probably decreased in most developed countries with access to harm-reduction policies (i.e. needle exchange programmes) and with improved infection control procedures during cardiac surgery. Use of specific blood culture bottles for diagnosis of fungal endocarditis has decreased due to optimisation of media and automated culture systems. Meanwhile, the advent of rapid techniques, including fungal antigen detection (galactomannan, mannan/anti-mannan antibodies and β-1,3-1-glucans) and PCR (e.g. universal fungal PCR targeting 18S rRNA genes), shall improve sensitivity and reduce diagnostics delays, although limited data are available on their use for the diagnosis of fungal endocarditis. New antifungal agents available since the early 2000s may represent dramatic improvement for fungal endocarditis: (i) a new class, the echinocandins, has the potential to improve the management of Candida endocarditis owing to its fungicidal effect on yeasts as well as tolerability of increased dosages; and (ii) improved survival in patients with invasive aspergillosis with voriconazole compared with amphotericin B, and this may apply to Aspergillus sp. endocarditis as well, although its prognosis remains dismal. These achievements may allow selected patients to be cured with prolonged medical treatment alone when surgery is considered too risky.

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1. Introduction

Fungal endocarditis is a rare disease with a dismal prognosis related to the population affected (e.g. immunocompromised patients), suboptimal diagnostic tools responsible for long diagnostic delays in most cases, and poorly defined activity of most antifungal agents in endocarditis. The characteristics of patients affected by fungal endocarditis have dramatically changed over the last decades, with an overall decrease in the number of cases managed each year in most institutions. Hence, the number of physicians who maintained expertise in this field has probably declined. We aimed to provide an update on the current challenges in the management of fungal endocarditis based on a literature review and on our own experience.

2. Epidemiology

2.1. Changing profile of fungal endocarditis

The characteristics of patients affected by infective endocarditis have dramatically changed since the 1990s, as illustrated by prospective cohort and population-based studies [1–7]. Characteristics of the 270 cases reported during 1965–1995 are as follows: male:female ratio, 2.2; mean age, 44.3 ± 14.3 years; and main risk factors being previous valve surgery/prosthetic valve endocarditis (54%), prolonged use of antibiotics (48%), rheumatic heart disease (24%), surgery other than cardiac (23%), vascular lines (18%), immunosuppressive treatment (17%), non-iatrogenic...
immunodeficiency (17%) and intravenous drug use (IVDU) (13%). A subsequent review of 152 cases reported between 1995 and 2000 found that prosthetic valves (45%), central venous catheters (30%) and broad-spectrum antibiotic use (20%) were the main risk factors, whilst intravenous drug users were much less affected (4%). Two major observations emerged from these large literature reviews: (i) over time, dramatic changes have occurred in the major risk factors for fungal endocarditis; once a disease affecting mostly patients with recent valvular surgery, rheumatic heart disease or IVDU, fungal endocarditis is currently more common in immunocompromised patients, with long-term vascular lines, and who underwent complex non-cardiac surgery; and (ii) the prognosis gradually improved, with a mortality rate that decreased from 86% during 1966–1971 to 56% during 1995–2000 [1,3–5]. Although these figures, mostly based on case reports, are subject to publication bias, an improved outcome over the years would be expected owing to significant success achieved in the field of diagnosis as well as new antifungal agents. For example, diagnosis of fungal endocarditis was obtained before surgery in 43% of cases reported before 1988 compared with 72% of cases reported during 1988–1995 (P = 0.0001), which may be related to the advent of echocardiography (since 1975) as well as progress in automated blood culture systems, with a sensitivity estimated at 54% during 1965–1995 [1] and 81% during 1995–2000 [5]. Likewise, development of lipid formulations of amphotericin B (AmB), new azoles with extended spectrum, and echinocandins, over the last 20 years significantly enhanced the therapeutic armamentarium against fungal endocarditis.

2.2. Characteristics of fungal endocarditis in the 21st century

In most contemporary studies, fungal endocarditis represents <2% of all cases of endocarditis. This was the case in the International Collaboration on Endocarditis – Prospective Cohort Studies (ICE-PCS) [8] and in nationwide studies from Italy [9], the USA [10] and France [11]. However, higher proportions of fungal endocarditis have been reported in various settings, including hospitals with a high incidence of fungal endocarditis following cardiac surgery owing to infection control issues, or in areas with a high prevalence of IVDU and no access to harm-reduction policies such as needle exchange programmes.

The spectrum of fungal species causing endocarditis can be summarised as follow: (i) Candida endocarditis is the most common, representing 50–80% of all cases of fungal endocarditis; (ii) among these, although Candida albicans remains the most common species (30–40% of all fungal endocarditis), non-albicans Candida endocarditis is emerging, especially in patients previously exposed to azoles. Candida parapsilosis, Candida glabrata and Candida tropicalis are the main non-albicans Candida spp. causing endocarditis [4]; and (iii) Aspergillus endocarditis represents 20–25% of all fungal endocarditis and is due to Aspergillus fumigatus in two-thirds of cases. Aspergillus terreus, Aspergillus niger and Aspergillus flavus may rarely be encountered. Aspergillus endocarditis mostly occurs on prosthetic valves in patients recently operated and/or in immunocompromised patients, especially with haematological malignancies [7,12,13]. These features are summarised in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Host</th>
<th>Characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio</td>
<td>Male:female ratio ca. 2</td>
<td>Dramatic changes in risk factors from 1965 to 2014:</td>
</tr>
<tr>
<td>Age</td>
<td>Mean, 44 ± 14 years</td>
<td>- haematological malignancies, immunosuppressive drugs, CVCs and broad-spectrum antibiotics are emerging;</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Main current risk factors:</td>
<td>- intravenous drug users rarely affected in countries with needle exchange programmes</td>
</tr>
<tr>
<td></td>
<td>- prosthetic valve, 45%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- CVCs, 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- broad-spectrum antibiotic use, 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- malignancy, 9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- intravenous drug use, 4%</td>
<td></td>
</tr>
</tbody>
</table>

### Pathogens

<table>
<thead>
<tr>
<th>Candida endocarditis, 50–80%</th>
<th>Candida albicans, 30–40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-albicans Candida endocarditis:</td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Candida tropicalis</td>
</tr>
<tr>
<td>Mostly Aspergillus fumigatus</td>
<td>Others:</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison of Candida endocarditis and non-fungal endocarditis within the International Collaboration on Endocarditis–Prospective Cohort Studies (ICE-PCS).

<table>
<thead>
<tr>
<th>Candida endocarditis (n = 33) [n (%)]</th>
<th>Non-fungal endocarditis (n = 2716) [n (%)]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosthetic valve</td>
<td>16 (48.5)</td>
<td>533 (19.6)</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>7 (21.2)</td>
<td>115 (4.4)</td>
</tr>
<tr>
<td>Healthcare-associated endocarditis</td>
<td>17 (51.5)</td>
<td>702 (25.8)</td>
</tr>
<tr>
<td>Previous infective endocarditis</td>
<td>7 (21.2)</td>
<td>213 (7.8)</td>
</tr>
<tr>
<td>Reasons for surgery*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial abscess</td>
<td>7/15 (46.7)</td>
<td>289/1302 (22.2)</td>
</tr>
<tr>
<td>Embolic risk</td>
<td>6/15 (40.0)</td>
<td>257/1302 (19.7)</td>
</tr>
<tr>
<td>Persistent positive blood cultures</td>
<td>5/15 (33.3)</td>
<td>129/1302 (9.9)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>2/15 (13.3)</td>
<td>554/1302 (42.5)</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>10 (30.3)</td>
<td>464 (17.1)</td>
</tr>
</tbody>
</table>

* The denominator is the number of patients who underwent cardiac surgery for endocarditis. Patients may have multiple reasons for surgery.

3. Diagnosis

3.1. Clinical diagnosis

Fungal endocarditis shares many features with bacterial endocarditis, and modified Duke criteria are commonly used to classify Candida endocarditis based on the same microbiological and echocardiographic major criteria [8,9]. However, clinical diagnosis of fungal endocarditis is more difficult, as documented by the significant proportion of cases not diagnosed before valvular surgery. For cases reported during 1965–1995, the mean duration of symptoms before hospitalisation was 32 ± 39 days, and fungal endocarditis was considered in the initial differential diagnosis in only 18% of patients [7].

Classically, fungal endocarditis is characterised by: (i) its propensity to develop large (‘bulky’) vegetations, with an increased risk of dramatic embolic events (massive stroke, limb ischaemia); (ii) a high frequency of ophthalmological complications, with specific fundoscopic findings; and (iii) typical cutaneous lesions unique to particular fungal organisms (i.e. macronodules or maculopapules in candidaemia, black haemorrhagic lesions in Aspergillus endocarditis) [4]. However, when Baddley et al., compared 33 consecutive cases of Candida endocarditis with 2716 cases of non-fungal endocarditis enrolled in the ICE-PCS database during 2000–2005 [8], the only significant differences were risk factors, reasons for surgery and mortality (Table 2).
Table 3
New diagnostic tests for fungal antigen detection in serum.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diagnostic performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannan and anti-mannan antibodies</td>
<td>Only for the diagnosis of Candida bloodstream infections [17]</td>
</tr>
<tr>
<td></td>
<td>For candidaemia: sensitivity, 80%; specificity, 85%</td>
</tr>
<tr>
<td></td>
<td>For Candida endocarditis: sensitivity, 83% [19]</td>
</tr>
<tr>
<td>β-1,3-D-Glucan</td>
<td>Pan-fungal diagnostic method as β-1,3-D-glucan is present in most pathogenic fungal species</td>
</tr>
<tr>
<td></td>
<td>For candidaemia, with a cut-off value of 80 pg/mL: sensitivity, &gt;65%; specificity, &gt;80%</td>
</tr>
<tr>
<td></td>
<td>For Candida endocarditis: sensitivity, 100% [19]</td>
</tr>
<tr>
<td>Galactomannan</td>
<td>Major constituent of Aspergillus cell wall. Cross-reactivity with histoplasmosis, blastomycosis, cryptococcosis, penicilliosis and with concomitant antibiotics (piperacillin/tazobactam, amoxicillin/clavulanic acid)</td>
</tr>
<tr>
<td></td>
<td>Validated for the diagnosis of invasive aspergillosis in neutropenic patients.</td>
</tr>
<tr>
<td></td>
<td>Not evaluated for the diagnosis of Aspergillus endocarditis</td>
</tr>
</tbody>
</table>

3.2. Blood cultures

Blood cultures remain the main evidence for diagnosis of Candida endocarditis. Most cases are diagnosed in the setting of prolonged candidaemia, which is a standalone indication for transoesophageal echocardiography and fundoscopic examination. Diagnostic procedures for Candida diseases have been reviewed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in 2012 [14] and by the Infectious Diseases Society of America (IDSA) in 2013 [15]. Briefly, these guidelines recommend to inoculate 60 mL of blood obtained by venipuncture and divided into six 10-ml aliquots among three aerobic and three anaerobic bottles to be incubated for ≥5 days. Although the sensitivity of automated blood culture systems is lower for fungal than for bacterial bloodstream infections, there is no evidence that use of specific blood culture bottles for fungal detection increases the diagnostic yield. Hence, guidelines for blood culture sampling in patients suspected of infective endocarditis applies for fungal as for bacterial endocarditis [16]. The sensitivity of blood culture for the diagnosis of C. albicans endocarditis has been estimated at 50–75% [14], and somewhat lower for non-albicans Candida endocarditis. Unfortunately, the yield of blood cultures is almost zero in Aspergillus endocarditis, estimated at 4% (2/53) in a recent review [12]. Most cases of Aspergillus endocarditis are diagnosed by tissue culture (e.g. valves in patients who underwent valve replacement), galactomannan antigen assay or post-mortem examination.

3.3. Innovative diagnostic tests (Table 3)

Given the shortcomings of blood cultures for the diagnosis of fungal endocarditis, innovative biological diagnostic tests are being developed and were recently reviewed [14]. The combined detection of mannann and anti-mannann antibodies in serum has been developed for the diagnosis of Candida bloodstream infections [17], and may also apply to Candida endocarditis. Its diagnostic performance for the diagnosis of candidaemia has been estimated at ca. 80% for sensitivity and 85% for specificity, which translates into an estimated accuracy of 50–70%.

The most promising test for the diagnosis of fungal endocarditis may be β-1,3-D-glucan (BDG) detection in serum, considered to be a pan-fungal diagnostic method, as BDG is present in most pathogenic fungal species. Its performance for the diagnosis of candidaemia has been evaluated in a meta-analysis [18]: with a cut-off value of 80 pg/mL, sensitivity would be >65%, specificity >80% and the negative predictive value >85%. In a prospective study of 18 patients with definite cases of Candida endocarditis, Lefort et al. found that the sensitivity of BDG and mannann/anti-mannann antibody detection in serum were 100% and 83%, respectively, for the diagnosis of Candida endocarditis [19].

For the diagnosis of Aspergillus endocarditis, potential diagnostic tests include the detection of BDG and the detection of galactomannan antigen in serum. Galactomannan is a major constituent of the Aspergillus cell wall and has been validated for the diagnosis of invasive aspergillosis in neutropenic patients. Caveats include cross-reactivity with other fungal infections (histoplasmosis, blastomycosis, cryptococcosis, penicilliosis) and with concomitant antibiotics (piperacillin/tazobactam, amoxicillin/clavulanic acid), and a suboptimal sensitivity for A. fumigatus [12]. Recent data suggest that galactomannan may be of interest for the diagnosis of Aspergillus endocarditis [13]. However, owing to the very low incidence of Aspergillus endocarditis since galactomannan antigen and BDG assays have been available, the diagnostic performance of these tests for the diagnosis of Aspergillus endocarditis remains poorly documented.

4. Treatment

4.1. Antifungal agents

Most cases of fungal endocarditis reported in the literature have been treated with amphotericin B deoxycholate (AmB): 93% of patients reported during 1965–1995 (of whom 22% also received flucytosine) [7], and 78% of those reported during 1995–2000 (of whom 28% also received flucytosine or azoles) [5]. AmB is a polycye, fungicidal for most yeast and moulds potentially involved in fungal endocarditis, but tolerability of conventional AmB (i.e. AmB) is poor, especially when a prolonged duration of treatment is mandatory for cure, as is the case for fungal endocarditis. Hence, owing to its improved tolerability and the ability to administer higher doses, lipid formulations of AmB are favoured over the deoxycholate preparation in all guidelines recently published, based on experimental studies and expert opinion rather than on clinical studies [16,20–22]. In addition, lipid formulations of AmB may also exert enhanced fungicidal activity on biofilms, which may be of interest in patients with prosthetic valve fungal endocarditis, especially when surgery is not feasible. Of note, Candida lusitaniae is naturally resistant to polyenes.

For the treatment of Candida endocarditis, the echinocandins are promising alternatives to polyenes (i.e. AmB and derivatives) for the following reasons: (i) they are rapidly fungicidal against most Candida spp., including azole-resistant Candida spp.; (ii) they remain active against Candida biofilms [23]; and (iii) their tolerability is much better than that of polyenes, allowing prolonged treatment, at high doses, when necessary. Most recent reports illustrate the trend towards increased use of echinocandins (e.g. 77% of patients with Candida endocarditis in the recent French observational prospective study [19]), either instead of, or in association with, lipid formulations of AmB.

Azoles are only fungistatic in yeasts; hence, they cannot be considered as primary treatment of Candida endocarditis. However, these compounds retain some potential indications for the treatment of fungal endocarditis in at least three situations: (i) as a primary choice for the medical treatment of Aspergillus endocarditis, on the grounds that voriconazole is fungicidal against Aspergillus spp. and has proven its superiority over AmB in invasive
aspergillosis in a landmark randomised controlled trial [24]; (ii) in combination with an echinocandin (preferably) or a polylene in difficult-to-treat fungal endocarditis; and (iii) as a long-term suppressive treatment to prevent late relapses of Candida endocarditis. Indeed, relapses have been reported in as many as 30–40% of patients who survive after the acute phase of fungal endocarditis and these relapses may occur as late as 9 years after patients have been considered as ‘cured’ [25]. Hence, most experts would recommend lifelong treatment with fluconazole in any situations when Candida endocarditis could not be adequately managed: this includes patients who were not treated with valvular replacement, patients who had positive valve cultures when valvular replacement was performed, patients with prolonged candidaemia, or patients with intracardiac devices that were not extracted.

Flucytosine demonstrates broad antifungal activity against most Candida spp., with the exception of Candida krusei. It is sometimes used at a dosage of 100 mg/kg/day in combination with a lipid formulation of AmB or an echinocandin during initial treatment of difficult-to-treat Candida endocarditis cases. Owing to bone marrow toxicity, therapeutic drug monitoring is recommended in prolonged treatment, as well as dose adjustment in patients with renal dysfunction [21].

4.2. Cardiac surgery

Fungal endocarditis has long been considered as a ‘standalone’ indication for surgical valvular replacement for the following reasons: (i) prognosis in patients who received only medical treatment has consistently been very poor, with mortality rates estimated at 96% for Aspergillus endocarditis [12] and 50–70% for Candida endocarditis [5,19]; (ii) most antifungal agents have limited activity within biofilm, vegetations and prosthetic devices; (iii) late relapses are common in patients who did not undergo valve replacement; and (iv) large vegetations commonly encountered in fungal endocarditis may lead to severe embolic events, even under optimised antifungal treatment.

However, with the advent of new antifungal agents, including the echinocandins, and better use of older antifungal agents, a significant proportion of patients with native valve Candida endocarditis may be controlled without cardiac surgery [26]. A recent meta-analysis of medical versus surgical therapy for Candida endocarditis found a non-significant impact of surgical valve replacement, with an odds ratio of 0.56 (95% confidence interval 0.16–1.99) for death [27]. Another meta-analysis of 64 cases of Candida endocarditis who did not undergo surgical valvular replacement found that failure was more common in patients who received only fluconazole compared with patients who received fluconazole combined with another antifungal agent (42% vs. 16%; P = 0.02) [28].

4.3. Current guidelines for the treatment of fungal endocarditis (Table 4)

Although polyenes are proposed as the potential first choice for most cases of fungal endocarditis, a switch towards preferred use of echinocandins for Candida endocarditis has been observed in recent cohorts [8,19] as well as in updated guidelines [16,20–22]. Accordingly, the recommended first-line treatment of Candida endocarditis includes high doses of echinocandins as an alternative to lipid formulations of AmB for primary treatment of Candida endocarditis in the USA (caspofungin 50–150 mg/day, anidulafungin 100–200 mg/day or micafungin 100–150 mg/day) [21], caspofungin ± fluconazole as an alternative to lipid formulations of AmB ± fluconazole in Europe [20], and micafungin (200 mg/day), caspofungin (70 mg loading dose, then 50–100 mg/day) or anidulafungin (licensed doses) as primary choices (preferably to AmB or derivatives) in the UK [22].

Regarding surgical indications, although case reports of Candida endocarditis controlled by medical treatment alone have accumulated over recent years, fungal endocarditis is still considered as a standalone indication for valvular surgery by most experts. Interestingly, guidelines differ in the way they express these requirements: 2009 European guidelines for endocarditis stated that ‘treatment of fungal endocarditis necessitates dual therapy with antifungal agent and valve replacement’ [16]; 2009 US guidelines for Candida diseases stated that ‘valve replacement is strongly recommended. For those who are unable to undergo surgical replacement of the valve, lifelong therapy with fluconazole, 400–800 mg/day is recommended’ [21]; 2012 European guidelines for Candida diseases stated that ‘in patients with native valve Candida endocarditis, surgery within a week is recommended, and in prosthetic valve Candida endocarditis, earlier surgery may even be beneficial’ [20]; and lastly, 2012 guidelines for endocarditis in the UK separated Aspergillus endocarditis where ‘surgical valve replacement is mandatory for survival’ and Candida endocarditis where

### Table 4

<table>
<thead>
<tr>
<th>European guidelines on infective endocarditis, 2009 [16]</th>
<th>Antifungal agents</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Amphotericin B or derivatives with or without azoles</td>
<td>• Valve replacement for all patients</td>
<td></td>
</tr>
<tr>
<td>• or caspofungin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Prolonged or lifelong fluconazole suppressive treatment</td>
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<tbody>
<tr>
<td>• Liposomal amphotericin B or other lipid formulations with or without fluconazole</td>
<td>• Valve replacement strongly recommended</td>
<td></td>
</tr>
<tr>
<td>• or an echinocandin at high doses (caspofungin 50–150 mg/day, micafungin 100–150 mg/day or anidulafungin 100–200 mg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Prolonged fluconazole suppressive treatment (400–800 mg/day)</td>
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</thead>
<tbody>
<tr>
<td>• Liposomal amphotericin B with or without fluconazole</td>
<td>• Surgical valve replacement within a week if native valve</td>
<td></td>
</tr>
<tr>
<td>• or caspofungin 70 mg/day or 50 mg/day with or without fluconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Prolonged, fluconazole suppressive treatment (400–800 mg/day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>British guidelines on infective endocarditis, 2012 [22]</th>
<th>Antifungal agents</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>• First-line: an echinocandin at high doses (caspofungin 70 mg loading dose, then 50–100 mg/day, micafungin 200 mg/day or anidulafungin, licensed doses)</td>
<td>• Valve replacement highly desirable if technically feasible for Candida endocarditis</td>
<td></td>
</tr>
<tr>
<td>• Second-line: liposomal amphotericin B or other lipid formulations with or without fluconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Prolonged, fluconazole suppressive treatment (400–800 mg/day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Guidelines apply both for Candida and Aspergillus endocarditis.  
** Guidelines apply only for Candida endocarditis.
surgical valve replacement is highly desirable if technically feasible [22].

5. Perspectives and challenges

Significant progress in the prevention, diagnosis and management of fungal endocarditis has been achieved over the last decades. These include: (i) dramatic reduction in the proportion of infective endocarditis due to fungus in most settings, thanks to improvement in infection control procedures during cardiac surgery as well as harm-reduction policies with access to needle exchange programmes; (ii) development of antifungal agents with rapid fungicidal activity against most Candida spp. (echinocandins) or improved survival in patients with invasive aspergillosis (voriconazole); and (iii) although less dramatic, significant improvement in diagnostic tests (transosophageal echocardiography, automated blood culture systems).

Challenges for the future include: (i) expanded access to policies that have proven effective in the reduction of fungal endocarditis (e.g. needle exchange programmes in the USA and Eastern Europe countries); (ii) development of innovative diagnostic tests based on antigen or specific nucleic acid detection in serum (e.g. universal fungal PCR targeting 18S rRNA genes, galactomannan, mannan/anti-mannan antibodies and BGDGs); (iii) identification of clinical or biological criteria to select patients who may be cured without surgical valvular replacement; and (iv) optimised treatment of difficult-to-treat fungal endocarditis (e.g. C. parapsilosis endocarditis with diminished susceptibility to echinocandins).

6. Conclusions

In conclusion, fungal endocarditis remains one of the most severe infectious diseases, with mortality rates ranging from 96% in Aspergillus endocarditis patients who could not undergo surgical valvular replacement to 32% in patients with Candida endocarditis treated with medical and surgical treatment. However, significant progress has been achieved over the last decades, illustrated by the dramatic decrease in the proportion of infective endocarditis caused by fungus and the development of new antifungal agents that increase the rate of success in patients who cannot undergo surgical valve replacement.

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Ethical approval: Not required.

References

Clinical Practice Guidelines for the Management of Candidiasis: 2009 Update by the Infectious Diseases Society of America

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Guidelines for the management of patients with invasive candidiasis and mucosal candidiasis were prepared by an Expert Panel of the Infectious Diseases Society of America. These updated guidelines replace the previous guidelines published in the 15 January 2004 issue of Clinical Infectious Diseases and are intended for use by health care providers who care for patients who either have or are at risk of these infections. Since 2004, several new antifungal agents have become available, and several new studies have been published relating to the treatment of candidemia, other forms of invasive candidiasis, and mucosal disease, including oropharyngeal and esophageal candidiasis. There are also recent prospective data on the prevention of invasive candidiasis in high-risk neonates and adults and on the empiric treatment of suspected invasive candidiasis in adults. This new information is incorporated into this revised document.

EXECUTIVE SUMMARY

There have been several significant changes in the management of candidiasis since the last publication of these guidelines in January 2004. Most of these changes relate to the appropriate use of echinocandins and expanded spectrum azoles in the management of candidemia, other forms of invasive candidiasis, and mucosal candidiasis. For some of the less common forms of invasive candidiasis (e.g., chronic disseminated candidiasis, osteomyelitis, and CNS disease), there are few new treatment data since 2004, with only anecdotal experience, case reports, or small series providing some evidence to support new approaches to therapy. Each section of the Guideline begins with a specific clinical question and is followed by numbered recommendations and a summary of the most-relevant evidence in support of the recommendations. The most significant changes and/or additions to existing recommendations are described below in the Executive Summary. The remaining topics are discussed in greater detail in the main body of the guidelines.

Candidemia in Nonneutropenic Patients

- Fluconazole (loading dose of 800 mg [12 mg/kg], then 400 mg [6 mg/kg] daily) or an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose of 200 mg, then 100 mg daily) is recommended as initial therapy for most adult patients (A-I). The Expert Panel favors an echinocandin for patients with moderately severe to severe illness or...
• For patients who have had recent azole exposure (A-III). Fluconazole is recommended for patients who are less critically ill and who have had no recent azole exposure (A-III). The same therapeutic approach is advised for children, with attention to differences in dosing regimens.

• Transition from an echinocandin to fluconazole is recommended for patients who have isolates that are likely to be susceptible to fluconazole (e.g., Candida albicans) and who are clinically stable (A-II).

• For infection due to Candida glabrata, an echinocandin is preferred (B-III). Transition to fluconazole or voriconazole therapy is not recommended without confirmation of isolate susceptibility (B-III). For patients who have initially received fluconazole or voriconazole, are clinically improved, and whose follow-up culture results are negative, continuing use of an azole to completion of therapy is reasonable (B-III).

• For infection due to Candida parapsilosis, treatment with fluconazole is recommended (B-III). For patients who have initially received an echinocandin, are clinically improved, and whose follow-up culture results are negative, continuing use of an echinocandin is reasonable (B-III).

• Amphotericin B deoxycholate (AmB-d) administered at a dosage of 0.5–1.0 mg/kg daily or a lipid formulation of AmB (LFAmB) administered at a dosage of 3–5 mg/kg daily are alternatives if there is intolerance to or limited availability of other antifungals (A-I). Transition from AmB-d or LFAmB to fluconazole is recommended for patients who have isolates that are likely to be susceptible to fluconazole (e.g., C. albicans) and who are clinically stable (A-I).

• Voriconazole administered at a dosage of 400 mg (6 mg/kg) twice daily for 2 doses and then 200 mg (3 mg/kg) twice daily thereafter is effective for candidemia (A-I), but it offers little advantage over fluconazole and is recommended as step-down therapy for selected cases of candidiasis due to Candida krusei or voriconazole-susceptible C. glabrata (B-III).

• The recommended duration of therapy for candidemia without obvious metastatic complications is for 2 weeks after documented clearance of Candida from the bloodstream and resolution of symptoms attributable to candidemia (A-III).

• Intravenous catheter removal is strongly recommended for nonneutropenic patients with candidemia (A-II).

Candidemia in Neutropenic Patients

• An echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micaflufungin: 100 mg daily [A-II]; anidulafungin: loading dose of 200 mg, then 100 mg daily [A-III]) or LFAmB (3–5 mg/kg daily [A-II]) is recommended for most patients.

• For patients who are less critically ill and who have no recent azole exposure, fluconazole (loading dose of 800 mg [12 mg/kg], then 400 mg [6 mg/kg] daily) is a reasonable alternative (B-III). Voriconazole can be used in situations in which additional mold coverage is desired (B-III).

• For infections due to C. glabrata, an echinocandin is preferred (B-III). LFAmB is an effective but less attractive alternative (B-III). For patients who were already receiving voriconazole or fluconazole, are clinically improved, and whose follow-up culture results are negative, continuing use of the azole to completion of therapy is reasonable (B-III).

• For infections due to C. parapsilosis, fluconazole or LFAmB is preferred as initial therapy (B-III). If the patient is receiving an echinocandin, is clinically stable, and follow-up culture results are negative, continuing the echinocandin until completion of therapy is reasonable. For infections due to C. krusei, an echinocandin, LFAmB, or voriconazole is recommended (B-III).

• Recommended duration of therapy for candidemia without persistent fungemia or metastatic complications is for 2 weeks after documented clearance of Candida from the bloodstream, resolution of symptoms attributable to candidemia, and resolution of neutropenia (A-III).

• Intravenous catheter removal should be considered (B-III).

Empirical Treatment for Suspected Invasive Candidiasis in Nonneutropenic Patients

• Empirical therapy for suspected candidiasis in nonneutropenic patients is similar to that for proven candidiasis. Fluconazole (loading dose of 800 mg [12 mg/kg], then 400 mg [6 mg/kg] daily), caspofungin (loading dose of 70 mg, then 50 mg daily), anidulafungin (loading dose of 200 mg, then 100 mg daily), or micaflufungin (100 mg daily) is recommended as initial therapy (B-III). An echinocandin is preferred for patients who have had recent azole exposure, whose illness is moderately severe or severe, or who are at high risk of infection due to C. glabrata or C. krusei (B-III).

• AmB-d (0.5–1.0 mg/kg daily) or LFAmB (3–5 mg/kg daily) are alternatives if there is intolerance to other antifungals or limited availability of other antifungals (B-III).

• Empirical antifungal therapy should be considered for critically ill patients with risk factors for invasive candidiasis and no other known cause of fever, and it should be based on clinical assessment of risk factors, serologic markers for invasive candidiasis, and/or culture data from nonsterile sites (B-III).

Empirical Treatment for Suspected Invasive Candidiasis in Neutropenic Patients

• LFAmB (3–5 mg/kg daily), caspofungin (loading dose of 70 mg, then 50 mg daily) (A-I), or voriconazole (6 mg/kg administered intravenously twice daily for 2 doses, then 3 mg/kg twice daily) are recommended (B-I).

• Fluconazole (loading dose of 800 mg [12 mg/kg], then 400 mg [6 mg/kg] daily) and itraconazole (200 mg [3 mg/kg] twice daily) are alternative agents (B-I).

• AmB-d is an effective alternative, but there is a higher risk
of toxicity with this formulation than with LFAmB (A-I).

- Aminopyrine should not be used for empirical therapy in patients who have received an azole for prophylaxis (B-II).

**Treatment for Neonatal Candidiasis**

- AmB-d (1 mg/kg daily) is recommended for neonates with disseminated candidiasis (A-II). If urinary tract involvement is excluded, LFAmB (3–5 mg/kg daily) can be used (B-II). Fluconazole (12 mg/kg daily) is a reasonable alternative (B-II). The recommended length of therapy is 3 weeks (B-II).
- A lumbar puncture and a dilated retinal examination, preferably by an ophthalmologist, are recommended in neonates with sterile body fluid and/or urine cultures positive for *Candida* species (B-III). Imaging of the genitourinary tract, liver, and spleen should be performed if the results of sterile body fluid cultures are persistently positive (B-III).
- Echinocandins should be used with caution and are generally limited to situations in which resistance or toxicity precludes the use of fluconazole or AmB-d (B-III).
- Intravascular catheter removal is strongly recommended (A-II).
- In nurseries with high rates of invasive candidiasis, fluconazole prophylaxis may be considered in neonates whose birth weight is <1000 g (A-I). Antifungal drug resistance, drug-related toxicity, and neurodevelopmental outcomes should be observed (A-III).

**Antifungal Prophylaxis for Solid-Organ Transplant Recipients, Patients Hospitalized in Intensive Care Units (ICUs), Neutropenic Patients receiving Chemotherapy, and Stem Cell Transplant Recipients at Risk of Candidiasis**

- For solid-organ transplant recipients, fluconazole (200–400 mg [3–6 mg/kg] daily) or liposomal AmB (L-Amb) (1–2 mg/kg daily for 7–14 days) is recommended as postoperative antifungal prophylaxis for liver (A-I), pancreas (B-II), and small bowel (B-III) transplant recipients at high risk of candidiasis.
- For patients hospitalized in the ICU, fluconazole (400 mg [6 mg/kg] daily) is recommended for high-risk patients in adult units that have a high incidence of invasive candidiasis (B-I).
- For patients with chemotherapy-induced neutropenia, fluconazole (400 mg [6 mg/kg] daily) (A-I), posaconazole (200 mg 3 times daily) (A-I), or caspofungin (50 mg daily) (B-II) is recommended during induction chemotherapy for the duration of neutropenia. Oral itraconazole (200 mg twice daily) is an effective alternative (A-1), but it offers little advantage over other agents and is less well tolerated.
- For stem cell transplant recipients with neutropenia, fluconazole (400 mg [6 mg/kg] daily), posaconazole (200 mg 3 times daily), or miconazole (50 mg daily) is recommended during the period of risk of neutropenia (A-I).

**INTRODUCTION**

*Candida* species are the most common cause of invasive fungal infections in humans, producing infections that range from non–life-threatening mucocutaneous disorders to invasive disease that can involve any organ. Invasive candidiasis is largely a disease of medical progress, reflecting the tremendous advances in health care technology over the past several decades [1–5]. The most frequently implicated risk factors include the use of broad-spectrum antibacterial agents, use of central venous catheters, receipt of parenteral nutrition, receipt of renal replacement therapy by patients in ICUs, neutropenia, use of implantable prosthetic devices, and receipt of immunosuppressive agents (including glucocorticosteroids, chemotherapeutic agents, and immunomodulators) [2–7]. *Candidiasis* is the fourth most common cause of nosocomial bloodstream infections in the United States and in much of the developed world [5, 8–10]. Invasive candidiasis has a significant impact on patient outcomes, and it has been estimated that the attributable mortality of invasive candidiasis is as high as 47% [11], although many authorities estimate the attributable mortality to be 15%–25% for adults and 10%–15% for neonates and children [12, 13]. The estimated additional cost of each episode of invasive candidiasis in hospitalized adults is ~$40,000 [1, 13].

The Expert Panel addressed the following clinical questions:

I. What is the treatment of candidemia in nonneutropenic patients?
II. What is the treatment of candidemia in neutropenic patients?
III. What is the empirical treatment for suspected invasive candidiasis in nonneutropenic patients?
IV. What is the empirical treatment for suspected invasive candidiasis in neutropenic patients?
V. What is the treatment for urinary tract infections due to *Candida* species?
VI. What is the treatment for vulvovaginal candidiasis?
VII. What is the treatment for chronic disseminated candidiasis?
VIII. What is the treatment for osteoarticular infections due to *Candida* species?
IX. What is the treatment for CNS candidiasis in adults?
X. What is the treatment for *Candida* endophthalmitis?
XI. What is the treatment for infections of the cardiovascular system due to *Candida* species?
XII. What is the treatment for neonatal candidiasis?
XIII. What is the significance of *Candida* species isolated from respiratory secretions?
XIV. What is the treatment for nongenital mucocutaneous candidiasis?
Table 1. Infectious Diseases Society of America–US Public Health Service Grading System for ranking recommendations in clinical guidelines.

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

**NOTE.** Adapted from Canadian Task Force on the Periodic Health Examination [15].

XV. Should antifungal prophylaxis be used for solid-organ transplant recipients, ICU patients, neutropenic patients receiving chemotherapy, and stem cell transplant recipients at risk of candidiasis?

**PRACTICE GUIDELINES**

Practice guidelines are systematically developed statements to assist practitioners and patients in making decisions about appropriate health care for specific clinical circumstances [14]. Attributes of good guidelines include validity, reliability, reproducibility, clinical applicability, clinical flexibility, clarity, multidisciplinary process, review of evidence, and documentation [14].

**UPDATE METHODOLOGY**

**Panel Composition**

The Infectious Diseases Society of America (IDSA) Standards and Practice Guidelines Committee (SPGC) convened experts in the management of patients with candidiasis. The specialties of the members of the Expert Panel are listed at the end of the text.

**Literature Review and Analysis**

For the 2009 update, the Expert Panel completed the review and analysis of data published since 2004. Computerized literature searches of the English-language literature using PubMed were performed.

**Process Overview**

In evaluating the evidence regarding the management of candidiasis, the Expert Panel followed a process used in the development of other IDSA guidelines. The process included a systematic weighting of the quality of the evidence and the grade of recommendation (table 1) [15].

**Consensus Development on the Basis of Evidence**

The Expert Panel met in person on 1 occasion and via teleconference 11 times to discuss the questions to be addressed, to make writing assignments, and to deliberate on the recommendations. All members of the Expert Panel participated in the preparation and review of the draft guidelines. Feedback from external peer reviews was obtained. The guidelines were reviewed and approved by the IDSA SPGC and the IDSA Board of Directors prior to dissemination. A summary of the recommendations is included in table 2.

**Guidelines and Conflict of Interest**

All members of the Expert Panel complied with the IDSA policy on conflicts of interest, which requires disclosure of any financial or other interest that might be construed as constituting an actual, potential, or apparent conflict. Members of the Expert Panel were provided with the IDSA’s conflict of interest disclosure statement and were asked to identify ties to companies developing products that might be affected by promulgation of the guidelines. Information was requested regarding employment, consultancies, stock ownership, honoraria, research funding, expert testimony, and membership on company advisory committees. The Expert Panel made decisions on a case-by-case basis as to whether an individual’s role should be limited as a result of a conflict. Potential conflicts of interest are listed in the Acknowledgments section.

**Revision Dates**

At annual intervals, the Expert Panel Chair, the SPGC liaison advisor, and the Chair of the SPGC will determine the need for revisions to the guidelines on the basis of an examination.
of current literature. If necessary, the entire Expert Panel will be reconvened to discuss potential changes. When appropriate, the Expert Panel will recommend revision of the guidelines to the SPGC and the IDSA Board for review and approval.

**LITERATURE REVIEW**

**Pharmacologic Considerations of Therapy for Candidiasis**

Systemic antifungal agents shown to be effective for the treatment of candidiasis comprise 4 major categories: the polyenes (AmB-d, L-AmB, AmB lipid complex [ABLc], and AmB colloidal dispersion [ABCD]), the triazoles (fluconazole, itraconazole, voriconazole, and posaconazole), the echinocandins (caspofungin, anidulafungin, and micafungin), and flucytosine. Clinicians should become familiar with strategies to optimize efficacy through an understanding of relevant pharmacokinetic properties.

**Amphotericin B (AmB)**

Most experience with AmB is with the deoxycholate preparation (AmB-d). Three LFAmBs have been developed and approved for use in humans: ABLc, ABCD, and L-AmB. These agents possess the same spectrum of activity as AmB-d. The 3 LFAmBs have different pharmacological properties and rates of treatment-related adverse events and should not be interchanged without careful consideration. In this document, a reference to AmB, without a specific dose or other discussion of form, should be taken to be a reference to the general use of any of the AmB preparations. For most forms of invasive candidiasis, the typical intravenous dosage for AmB-d is 0.5–0.7 mg/kg daily, but dosages as high as 1 mg/kg daily should be considered for invasive Candida infections caused by less susceptible species, such as *C. glabrata* and *C. krusei*. The typical dosage for LFAmB is 3–5 mg/kg daily when used for invasive candidiasis [16, 17]. Nephrotoxicity is the most common serious adverse effect associated with AmB-d therapy, resulting in acute renal failure in up to 50% of recipients [18]. LFAmBs are considerably more expensive than AmB-d, but all have considerably less nephrotoxicity [19–21]. These agents retain the infusion-related toxicities associated with AmB-d. Among these agents, a comparative study suggests that L-AmB may afford the greatest renal protection [21]. The impact of the pharmacokinetics and differences in toxicity of LFAmB has not been formally examined in clinical trials. We are not aware of any forms of candidiasis for which LFAmB is superior to AmB-d, nor are we aware of any situations in which these agents would be contraindicated, with the exception of urinary tract candidiasis, in which the protection of the kidney afforded by the pharmacological properties of these formulations has the theoretical potential to reduce delivery of AmB [22]. Animal model studies suggest a pharmacokinetic and therapeutic advantage for L-AmB in the CNS [23]. Data demonstrating that AmB-d–induced nephrotoxicity is associated with a 6.6-fold increase in mortality have led many clinicians to use LFAmB as initial therapy for individuals who are at high risk of nephrotoxicity [24].

**Triazoles**

Fluconazole, itraconazole, voriconazole, and posaconazole demonstrate similar activity against most Candida species [25, 26]. Each of the azoles has less activity against *C. glabrata* and *C. krusei*. All of the azole antifungals inhibit cytochrome P450 enzymes to some degree. Thus, clinicians must carefully consider the influence on a patient’s drug regimen when adding or removing an azole. In large clinical trials, fluconazole demonstrated efficacy comparable to that of AmB-d for the treatment of candidemia [27, 28] and is also considered to be standard therapy for oropharyngeal, esophageal, and vaginal candidiasis [29, 30]. Fluconazole is readily absorbed, with oral bioavailability resulting in concentrations equal to ∼90% of those achieved by intravenous administration. Absorption is not affected by food consumption, gastric pH, or disease state. Among the triazoles, fluconazole has the greatest penetration into the CSF and vitreous body, achieving concentrations of at least 50% of those in serum [31]; for this reason, it is used in the treatment of CNS and intraocular Candida infections. Fluconazole achieves urine concentrations that are 10–20 times the concentrations in serum. For patients with invasive candidiasis, fluconazole should be administered with a loading dose of 800 mg (12 mg/kg), followed by a daily dose of 400 mg (6 mg/kg); a lower dosage is required in patients with creatinine clearance <50 mL/min.

Itraconazole is generally reserved for patients with mucosal candidiasis, especially those who have experienced treatment failure with fluconazole [32]. There are few data that examine the use of itraconazole in the treatment of invasive candidiasis. Gastrointestinal absorption differs for the capsule and the oral solution formulations. Histamine receptor antagonists and proton pump inhibitors result in decreased absorption of the capsule formulation, whereas acidic beverages, such as carbonated drinks and cranberry juice, enhance absorption [33]. Administration of the capsule formulation with food increases absorption, but the oral solution is better absorbed on an empty stomach [34]. Oral formulations are dosed in adults at 200 mg 3 times daily for 3 days, then 200 mg once or twice daily thereafter.

Voriconazole is effective for both mucosal and invasive candidiasis. Its clinical use has been primarily for step-down oral therapy for patients with infection due to *C. krusei* and fluconazole-resistant, voriconazole-susceptible *C. glabrata*. CSF and vitreous penetration is excellent [35, 36]. Voriconazole is available in both oral and parenteral preparations. The oral bioavailability of voriconazole is >90% and is not affected by
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<th>Condition or treatment group</th>
<th>Therapy</th>
<th>Alternative</th>
<th>Comments</th>
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<tbody>
<tr>
<td><strong>Candidemia</strong></td>
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<tr>
<td>Nonneutropenic adults</td>
<td>Fluconazole 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily or an echinocandin^{a} (A-I). For species-specific recommendations, see text.</td>
<td>LFAmB 3–5 mg/kg daily; or AmB-d 0.5–1 mg/kg daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses, then 200 mg (3 mg/kg) bid (A-I)</td>
<td>Choose an echinocandin for moderately severe to severe illness and for patients with recent azole exposure. Transition to fluconazole after initial echinocandin is appropriate in many cases. Remove all intravascular catheters, if possible. Treat 14 days after first negative blood culture result and resolution of signs and symptoms associated with candidemia. Ophthalmological examination recommended for all patients.</td>
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<tr>
<td>Neutropenic patients</td>
<td>An echinocandin^{a} or LFAmB 3–5 mg/kg daily (A-II). For species-specific recommendations, see text.</td>
<td>Fluconazole 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses then 200 mg (3 mg/kg) bid (B-I)</td>
<td>An echinocandin or LFAmB is preferred for most patients. Fluconazole is recommended for patients without recent azole exposure and who are not critically ill. Voriconazole is recommended when additional coverage for molds is desired. Intravascular catheter removal is advised but is controversial.</td>
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<td><strong>Suspected candidiasis</strong></td>
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<td>treated with empiric anti-</td>
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<td>fungal therapy</td>
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<td>Nonneutropenic patients</td>
<td>Treat as above for candidemia. An echinocandin or fluconazole is preferred (B-III).</td>
<td>LFAmB 3–5 mg/kg daily or AmB-d 0.5–1 mg/kg daily (B-III)</td>
<td>For patients with moderately severe to severe illness and/or recent azole exposure, an echinocandin is preferred. The selection of appropriate patients should be based on clinical risk factors, serologic tests, and culture data. Duration of therapy is uncertain, but should be discontinued if cultures and/or serodiagnostic tests have negative results.</td>
</tr>
<tr>
<td>Neutropenic patients</td>
<td>LFAmB 3–5 mg/kg daily, caspofungin 70-mg loading dose, then 50 mg daily (A-I), or voriconazole 400 mg (6 mg/kg) bid for 2 doses then 200 mg (3 mg/kg) bid (B-I).</td>
<td>Fluconazole 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily; or itraconazole 200 mg (3 mg/kg) bid (B-I)</td>
<td>In most neutropenic patients, it is appropriate to initiate empiric antifungal therapy after 4 days of persistent fever despite antibiotics. Serodiagnostic tests and CT imaging may be helpful. Do not use an azole in patients with prior azole prophylaxis.</td>
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<tr>
<td><strong>Urinary tract infection</strong></td>
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<tr>
<td>Asymptomatic cystitis</td>
<td>Therapy not usually indicated, unless patients are at high risk (e.g., neonates and neutropenic adults) or undergoing urologic procedures (A-III)</td>
<td>…</td>
<td>Elimination of predisposing factors recommended. For high-risk patients, treat as for disseminated candidiasis. For patients undergoing urologic procedures, fluconazole, 200–400 mg (3–6 mg/kg) daily or AmB-d 0.3–0.6 mg/kg daily for several days before and after the procedure.</td>
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<tr>
<td>Symptomatic cystitis</td>
<td>Fluconazole 200 mg (3 mg/kg) daily for 2 weeks (A-III)</td>
<td>AmB-d 0.3–0.6 mg/kg for 1–7 days; or fluconazole 25 mg/kg qid for 7–10 days (B-III)</td>
<td>Alternative therapy as listed is recommended for patients with fluconazole-resistant organisms. AmB-d bladder irrigation is recommended only for patients with refractory fluconazole-resistant organisms (e.g., Candida krusei and Candida glabrata).</td>
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<tr>
<td>Pyelonephritis</td>
<td>Fluconazole 200–400 mg (3–6 mg/kg) daily for 2 weeks (B-III)</td>
<td>AmB-d 0.5–0.7 mg/kg daily with or without 5-FC 25 mg/kg qid; or 5-FC alone for 2 weeks (B-III)</td>
<td>For patients with pyelonephritis and suspected disseminated candidiasis, treat as for candidemia.</td>
</tr>
<tr>
<td>Condition or treatment group</td>
<td>Therapy</td>
<td>Alternative</td>
<td>Comments</td>
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<tr>
<td>Urinary fungus balls</td>
<td>Surgical removal strongly recommended (B-III); fluconazole 200–400 mg (3–6 mg/kg) daily; or AmB-d 0.5–0.7 mg/kg daily with or without 5-FC 25 mg/kg qid (B-III)</td>
<td>...</td>
<td>Local irrigation with AmB-d may be a useful adjunct to systemic antifungal therapy.</td>
</tr>
<tr>
<td>Vulvovaginal candidiasis</td>
<td>Topical agents or fluconazole 150 mg single dose for uncomplicated vaginitis (A-I)</td>
<td>...</td>
<td>Recurrent vulvovaginal candidiasis is managed with fluconazole 150 mg weekly for 6 months after initial control of the recurrent episode. For complicated vulvovaginal candidiasis, see section VI.</td>
</tr>
<tr>
<td>Chronic disseminated candidiasis</td>
<td>Fluconazole 400 mg (6 mg/kg) daily for stable patients (A-III); LFAmB 3–5 mg/kg daily or AmB-d 0.5–0.7 mg/kg daily for severely ill patients (A-III); after patient is stable, change to fluconazole (B-III)</td>
<td>An echinocandin for several weeks followed by fluconazole (B-III)</td>
<td>Transition from LFAmB or AmB-d to fluconazole is favored after several weeks in stable patients. Duration of therapy is until lesions have resolved (usually monthly) and should continue through periods of immunosuppression (e.g., chemotherapy and transplantation).</td>
</tr>
<tr>
<td>Candida osteoarticular infection</td>
<td>Fluconazole 400 mg (6 mg/kg) daily for 6–12 months or LFAmB 3–5 mg/kg daily for several weeks, then fluconazole for 6–12 months (B-III)</td>
<td>An echinocandin or AmB-d 0.5–1 mg/kg daily for several weeks then fluconazole for 6–12 months (B-III)</td>
<td>Duration of therapy usually is prolonged (6–12 months). Surgical debridement is frequently necessary.</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>Fluconazole 400 mg (6 mg/kg) daily for at least 6 weeks or LFAmB 3–5 mg/kg daily for several weeks, then fluconazole to completion (B-III)</td>
<td>An echinocandin or AmB-d 0.5–1 mg/kg daily for several weeks then fluconazole to completion (B-III)</td>
<td>Duration of therapy usually is for at least 6 weeks, but few data are available. Surgical debridement is recommended for all cases. For infected prosthetic joints, removal is recommended for most cases.</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid for several weeks, followed by fluconazole 400–800 mg (6–12 mg/kg) daily (B-III)</td>
<td>Fluconazole 400–800 mg (6–12 mg/kg) daily for patients unable to tolerate LFAmB</td>
<td>Treat until all signs and symptoms, CSF abnormalities, and radiologic abnormalities have resolved. Removal of intraventricular devices is recommended.</td>
</tr>
<tr>
<td>CNS candidiasis</td>
<td>LFAmB 3–5 mg/kg daily; or fluconazole 400–800 mg (6–12 mg/kg) daily (B-III)</td>
<td>LFAmB 3–5 mg/kg daily; voriconazole 6 mg/kg q12h for 2 doses, then 3–4 mg/kg q12h; or an echinocandin (B-III)</td>
<td>Alternative therapy is recommended for patients intolerant of or experiencing failure of AmB and 5-FC therapy. Duration of therapy is at least 4–6 weeks as determined by repeated examinations to verify resolution. Diagnostic vitreal aspiration should be done if etiology unknown.</td>
</tr>
<tr>
<td>Candida endophthalmitis</td>
<td>AmB-d 0.7–1 mg/kg with 5-FC 25 mg/kg q12h for 2 doses, then 3–4 mg/kg q12h; or an echinocandin (B-III)</td>
<td>LFAmB 3–5 mg/kg daily; voriconazole 6 mg/kg q12h for 2 doses, then 3–4 mg/kg q12h; or an echinocandin (B-III)</td>
<td>Alternative therapy is recommended for patients intolerant of or experiencing failure of AmB and 5-FC therapy. Duration of therapy is at least 4–6 weeks as determined by repeated examinations to verify resolution. Diagnostic vitreal aspiration should be done if etiology unknown.</td>
</tr>
<tr>
<td>Candida infection of the cardiovascular system</td>
<td>LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid; or AmB-d 0.6–1 mg/kg daily with or without 5-FC 25 mg/kg qid; or an echinocandin (B-III)</td>
<td>Step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily for susceptible organism in stable patient with negative blood culture results (B-III)</td>
<td>Valve replacement is strongly recommended. For those who are unable to undergo surgical removal of the valve, chronic suppressive with fluconazole 400–800 mg (6–12 mg/kg) daily is recommended. Lifelong suppressive therapy for prosthetic valve endocarditis if valve cannot be replaced is recommended.</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>LFAmB 3–5 mg/kg daily; or fluconazole 400–800 mg (6–12 mg/kg) daily; or an echinocandin (B-III)</td>
<td>After stable, step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily (B-III)</td>
<td>Therapy is often for several months, but few data are available. A pericardial window or pericardiectomy is recommended.</td>
</tr>
<tr>
<td>Pericarditis or myocarditis</td>
<td>LFAmB 3–5 mg/kg daily; or fluconazole 400–800 mg (6–12 mg/kg) daily; or an echinocandin (B-III)</td>
<td>After stable, step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily (B-III)</td>
<td>Surgical incision and drainage or resection of the vein is recommended if feasible. Treat for at least 2 weeks after candidemia has cleared.</td>
</tr>
</tbody>
</table>
Table 2.  (Continued.)

<table>
<thead>
<tr>
<th>Condition or treatment group</th>
<th>Primary</th>
<th>Alternative</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected pacemaker, ICD, or VAD</td>
<td>LFAmB 3–5 mg/kg with or without 5-FC 25 mg/kg qid; or AmB-d 0.6–1 mg/kg daily with or without 5-FC 25 mg/kg qid; or an echinocandin&lt;sup&gt;b&lt;/sup&gt; (B-III)</td>
<td>Step-down therapy to fluconazole 400–800 mg (6–12 mg/kg) daily for susceptible organism in stable patient with negative blood culture results (B-III)</td>
<td>Removal of pacemakers and ICDs strongly recommended. Treat for 4–6 weeks after the device removed. For VAD that cannot be removed, chronic suppressive therapy with fluconazole is recommended.</td>
</tr>
<tr>
<td>Neonatal candidiasis</td>
<td>AmB-d 1 mg/kg daily (A-II); or fluconazole 12 mg/kg daily (B-II) for 3 weeks</td>
<td>LFAmB 3–5 mg/kg daily (B-III)</td>
<td>A lumbar puncture and dilated retinal examination should be performed on all neonates with suspected invasive candidiasis. Intravascular catheter removal is strongly recommended. Duration of therapy is at least 3 weeks. LFAmB used only if there is no renal involvement. Echinocandins should be used with caution when other agents cannot be used.</td>
</tr>
<tr>
<td>Candida isolated from respiratory secretions</td>
<td>Therapy not recommended (A-III)</td>
<td>...</td>
<td>Candida lower respiratory tract infection is rare and requires histopathologic evidence to confirm a diagnosis.</td>
</tr>
<tr>
<td>Nongenital mucocutaneous candidiasis</td>
<td>Clotrimazole troches 10 mg 5 times daily; nystatin suspension or pastilles qid (B-II); or fluconazole 100–200 mg daily (A-II)</td>
<td>Itraconazole solution 200 mg daily; or posaconazole 400 mg qd (A-II); or voriconazole 200 mg bid; or AmB oral suspension (B-II); IV echinocandin&lt;sup&gt;a&lt;/sup&gt; or AmB-d 0.3 mg/kg daily (B-II)</td>
<td>Fluconazole is recommended for moderate-to-severe disease, and topical therapy with clotrimazole or nystatin is recommended for mild disease. Treat uncomplicated disease for 7–14 days. For refractory disease, itraconazole, voriconazole, posaconazole, or AmB suspension is recommended.</td>
</tr>
<tr>
<td>Oropharyngeal</td>
<td>Fluconazole 200–400 mg (3–6 mg/kg) daily (A-I); an echinocandin&lt;sup&gt;b&lt;/sup&gt;; or AmB-d 0.3–0.7 mg/kg daily (B-II)</td>
<td>Itraconazole oral solution 200 mg daily; or posaconazole 400 mg bid; or voriconazole 200 mg bid (A-III)</td>
<td>Oral fluconazole is preferred. For patients unable to tolerate an oral agent, IV fluconazole, an echinocandin, or AmB-d is appropriate. Treat for 14–21 days. For patients with refractory disease, the alternative therapy as listed or AmB-d or an echinocandin is recommended.</td>
</tr>
<tr>
<td>Esophageal</td>
<td>Fluconazole 200–400 mg (3–6 mg/kg) daily (A-I); an echinocandin&lt;sup&gt;b&lt;/sup&gt;; or AmB-d 0.3–0.7 mg/kg daily (B-II)</td>
<td>Itraconazole oral solution 200 mg daily; or posaconazole 400 mg bid; or voriconazole 200 mg bid (A-III)</td>
<td>Oral fluconazole is preferred. For patients unable to tolerate an oral agent, IV fluconazole, an echinocandin, or AmB-d is appropriate. Treat for 14–21 days. For patients with refractory disease, the alternative therapy as listed or AmB-d or an echinocandin is recommended.</td>
</tr>
</tbody>
</table>

NOTE.  AmB, amphotericin B; AmB-d, amphotericin B deoxycholate; bid, twice daily; ICD, implantable cardiac defibrillator; IV, intravenous; LFAmB, lipid formulation of amphotericin B; qid, 4 times daily; VAD, ventricular assist device; 5-FC, flucytosine.

<sup>a</sup> Echinocandin dosing in adults is as follows: anidulafungin, 200-mg loading dose, then 100 mg/day; caspofungin, 70-mg loading dose, then 50 mg/day; and micafungin, 100 mg/day.

<sup>b</sup> For patients with endocarditis and other cardiovascular infections, higher daily doses of an echinocandin may be appropriate (e.g., caspofungin 50–150 mg/day, micafungin 100–150 mg/day, or anidulafungin 100–200 mg/day).

gastric pH, but it decreases when the drug is administered with food [37]. In adults, the recommended oral dosing regimen includes a loading dose of 400 mg twice daily, followed by 200 mg twice daily. Intravenous voriconazole is complexed to a cyclodextrin molecule; after 2 loading doses of 6 mg/kg every 12 h, a maintenance dosage of 3–4 mg/kg every 12 h is recommended. Because of the potential for cyclodextrin accumulation among patients with significant renal dysfunction, intravenous voriconazole is not recommended for patients with a creatinine clearance <50 mL/min [38]. Oral voriconazole does not require dosage adjustment for renal insufficiency, but it is the only triazole that requires dosage reduction for patients with mild-to-moderate hepatic impairment. Common polymorphisms in the gene encoding the primary metabolic enzyme for voriconazole result in wide variability of serum levels [39, 40]. Drug-drug interactions are common with voriconazole and should be considered when initiating and discontinuing treatment with this compound.

Posaconazole does not have an indication for primary candidiasis therapy. It demonstrates in vitro activity against Candida species that is similar to that of voriconazole, but clinical data are inadequate to make an evidence-based recommendation for treatment of candidiasis other than oropharyngeal candidiasis. Posaconazole is currently available only as an oral suspension with high oral bioavailability, especially when given with fatty foods [41], but absorption is saturated at relatively
modest dosage levels. Thus, despite a prolonged elimination half-life (>24 h), the drug must be administered multiple times daily (e.g., 200 mg 4 times daily or 400 mg twice daily). Similar to itraconazole capsules, posaconazole absorption is optimal in an acidic gastric environment.

**Echinocandins**

Caspofungin, anidulafungin, and micafungin are available only as parenteral preparations [42–44]. The MICs of the echinocandins are low for a broad spectrum of *Candida* species, including *C. glabrata* and *C. krusei*. *C. parapsilosis* demonstrates less in vitro susceptibility to the echinocandins than do most other *Candida* species, which raises the concern that *C. parapsilosis* may be less responsive to the echinocandins. However, in several clinical trials, this has not been demonstrated [45, 46]. Each of these agents has been studied for the treatment of esophageal candidiasis [47–50] and invasive candidiasis [51–54] in noncomparative and comparative clinical trials, and each has been shown to be effective in these clinical situations. All echinocandins have few adverse effects. The pharmacologic properties in adults are also very similar and are each administered once daily intravenously [42–44]; the major route of elimination is nonenzymatic degradation. None of the echinocandins require dosage adjustment for renal insufficiency or dialysis. Both caspofungin and micafungin undergo minimal hepatic metabolism, but neither drug is a major substrate for cytochrome P450. Caspofungin is the only echinocandin for which dosage reduction is recommended for patients with moderate to severe hepatic dysfunction.

Based on existing data, intravenous dosing regimens for invasive candidiasis with the 3 compounds are as follows: caspofungin, loading dose of 70 mg and 50 mg daily thereafter; anidulafungin, loading dose of 200 mg and 100 mg daily thereafter; and micafungin, 100 mg daily.

**Flucytosine**

Flucytosine demonstrates broad antifungal activity against most *Candida* species, with the exception of *C. krusei*. The compound is available only as an oral formulation. The drug has a short half-life (2.4–4.8 h) and is ordinarily administered at a dosage of 25 mg/kg 4 times daily for patients with normal renal function. Flucytosine demonstrates excellent absorption after oral administration (80%–90%), and most of the drug (>90%) is excreted unchanged in the urine [55]. Thus, dose adjustment is necessary for patients with renal dysfunction [56].

Flucytosine is rarely administered as a single agent but is usually given in combination with AmB for patients with invasive diseases, such as *Candida* endocarditis or meningitis. Occasionally, it is used for the treatment of urinary tract candidiasis due to susceptible organisms.

**Pediatric Dosing**

The pharmacokinetics of antifungal agents vary between adult and pediatric patients, but the data on dosing for antifungal agents in pediatric patients are limited. The pharmacological properties of antifungal agents in children and infants have been reviewed in detail [57, 58]. AmB-d kinetics are similar in neonates and adults [59]. There are few data describing the use of LFAmB in neonates and children. A phase I/II study of ABLC (2–5 mg/kg per day) in the treatment of hepatosplenic candidiasis in children found that the area under the curve and the maximal concentration of drug were similar to those in adults [17]. There are anecdotal data reporting successful use of L-AmB in neonates [60].

Flucytosine clearance is directly proportional to glomerular filtration rate, and infants with a very low birth weight may accumulate high plasma concentrations because of poor renal function due to immaturity [61]. Thus, the use of flucytosine without careful monitoring of serum drug levels is discouraged in this group of patients.

The pharmacokinetics of fluconazole vary significantly with age [62–64]. Fluconazole is rapidly cleared in children (plasma half-life, ∼14 h). To achieve comparable drug exposure, the daily fluconazole dose needs to be doubled, from 6 to 12 mg/kg daily, for children of all ages and neonates [62]. In comparison with the volume of distribution (0.7 L/kg) and half-life (30 h) seen in adults, neonates may have a higher volume of distribution and longer half-life [63, 64]; it has been recently reported that the volume of distribution in young infants and neonates is 1 L/kg and that the half-life is 30–50 h [65]. These data indicate that once-daily dosing of 12 mg/kg in premature and term neonates will provide exposure similar to that in adults who receive 400 mg daily. If the young infant’s creatinine level is >1.2 mg/dL for >3 consecutive doses, the dosing interval for 12 mg/kg may be increased to once every 48 h until the serum creatinine level is <1.2 mg/dL.

When administered to infants and children, the oral solution of itraconazole (5 mg/kg per day) provides potentially therapeutic concentrations in plasma [66]. Levels in children 6 months to 2 years of age are substantially lower than those attained in adult patients; thus, children usually need twice-daily dosing. A recent study of itraconazole solution in HIV-infected children documented its efficacy for treating oropharyngeal candidiasis in pediatric patients [67]. Voriconazole kinetics vary significantly between children and adults [68], demonstrating linear elimination in children after doses of 3 mg/kg and 4 mg/kg every 12 h. Thus, children up to ∼12 years of age require higher doses of voriconazole than do adults to attain similar serum concentrations. A dosage of 7 mg/kg every 12 h is currently recommended to achieve plasma exposures comparable to those in an adult receiving 4 mg/kg given every 12 h.
suggest safety and efficacy in the pediatric population. Data for each of the echinocandins have been studied in children 2–17 years of age and should be dosed to achieve therapeutic concentrations [73]. Anidulafungin has been studied in children and neonates; children should be treated with 2–4 mg/kg daily, but neonates may require as much as 10–12 mg/kg daily. A single 500-mg dose of caspofungin. Micafungin has been studied in children and neonates during pregnancy (category C). There are fewer data concerning the echinocandins, but these should be used with caution during pregnancy (category C). Flucytosine and voriconazole are contraindicated during pregnancy because of fetal abnormalities observed in animals (category D) [74].

Considerations during Pregnancy
Systemic AmB is the treatment of choice for invasive candidiasis in pregnant women [74]. Most azoles, including fluconazole, itraconazole, and posaconazole, should generally be avoided in pregnant women because of the possibility of birth defects associated with their use (category C). There are fewer data concerning the echinocandins, but these should be used with caution during pregnancy (category C). Flucytosine and voriconazole are contraindicated during pregnancy because of fetal abnormalities observed in animals (category D) [74].

Antifungal Susceptibility Testing
Intensive efforts to develop standardized, reproducible, and clinically relevant susceptibility testing methods for fungi have resulted in the development of the Clinical and Laboratory Standards Institute M27-A3 methodology for susceptibility testing of yeasts [76]. Data-driven interpretive breakpoints determined with use of this method are available for testing the susceptibility of Candida species to fluconazole, itraconazole, voriconazole, flucytosine, and the echinocandins [25, 76]. Although the susceptibility of Candida to the currently available antifungal agents is generally predictable if the species of the infecting isolate is known, individual isolates do not necessarily follow this general pattern (table 3) [25, 76]. For this reason, susceptibility testing is increasingly used to guide the management of candidiasis, especially in situations in which there is a failure to respond to initial antifungal therapy. Expert opinion suggests that laboratories perform routine antifungal susceptibility testing against fluconazole for C. glabrata isolates from blood and sterile sites and for other Candida species that have failed to respond to antifungal therapy or in which azole resistance is strongly suspected. Currently, antifungal resistance in C. albicans is uncommon, and routine testing for antifungal susceptibility against this species is not generally recommended.

### Table 3. General patterns of susceptibility of Candida species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
<th>Flucytosine</th>
<th>Amphotericin B</th>
<th>Candidins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S</td>
<td>S to I</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S</td>
<td>I to R</td>
<td>S to I</td>
<td>S</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
<td>S</td>
</tr>
</tbody>
</table>

**NOTE.** I, intermediately susceptible; R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

* Echinocandin resistance among C. parapsilosis isolates is uncommon.
Non-Culture-Based Diagnostic Techniques
Several new diagnostic techniques offer promise for the early diagnosis of invasive candidiasis. Several of these assays are approved as adjuncts to the diagnosis of invasive candidiasis, but their role in clinical practice is poorly defined. Several other assays are under development but are not yet approved (see below).

RECOMMENDATIONS FOR THE MANAGEMENT OF CANDIDIASIS

I. WHAT IS THE TREATMENT OF CANDIDEMIA IN NONNEUTROPENIC PATIENTS?

Recommendations
1. Fluconazole (loading dose of 800 mg [12 mg/kg], then 400 mg [6 mg/kg] daily) or an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose of 200 mg, then 100 mg daily) is recommended as initial therapy for most adult patients with candidemia (A-I). The Expert Panel favors an echinocandin for patients with moderately severe to severe illness or patients who have had recent azole exposure (A-III). Fluconazole is recommended for patients who are less critically ill and who have no recent azole exposure (A-III). The same therapeutic approach is advised for children, with attention to differences in dosing regimens (B-III).

2. Transition from an echinocandin to fluconazole is recommended for patients who have isolates that are likely to be susceptible to fluconazole (e.g., C. albicans) and who are clinically stable (A-II).

3. For infection due to C. glabrata, an echinocandin is preferred (B-III). Transition to fluconazole or voriconazole is not recommended without confirmation of isolate susceptibility (B-III). For patients who initially received fluconazole or voriconazole, are clinically improved, and whose follow-up culture results are negative, continuing anazole to completion of therapy is reasonable (B-III).

4. For infection due to C. parapsilosis, fluconazole is recommended (B-III). For patients who have initially received an echinocandin, are clinically improved, and whose follow-up culture results are negative, continuing use of an echinocandin is reasonable (B-III).

5. AmB-d (0.5–1.0 mg/kg daily) or LFAmB (3–5 mg/kg daily) are alternatives if there is intolerance to or limited availability of other antifungal agents (A-I). Transition from AmB-d or LFAmB to fluconazole therapy is recommended for patients who have isolates that are likely to be susceptible to fluconazole (e.g., C. albicans) and who are clinically stable (A-I).

6. Voriconazole (400 mg [6 mg/kg] twice daily for 2 doses, then 200 mg [3 mg/kg] twice daily) is effective for candidemia (A-I), but it offers little advantage over fluconazole and is recommended as step-down oral therapy for selected cases of candidiasis due to C. krusei or voriconazole-susceptible C. glabrata (B-III).

7. Recommended duration of therapy for candidemia without obvious metastatic complications is for 2 weeks after documented clearance of Candida species from the bloodstream and resolution of symptoms attributable to candidemia (A-III).

8. Intravenous catheter removal is strongly recommended for nonneutropenic patients with candidemia (A-II).

Evidence Summary
The selection of any particular agent for the treatment of candidemia should optimally take into account any history of recent azole exposure, a history of intolerance to an antifungal agent, the dominant Candida species and current susceptibility data in a particular clinical unit or location, severity of illness, relevant comorbidities, and evidence of involvement of the CNS, cardiac valves, and/or visceral organs. Early initiation of effective antifungal therapy is critical in the successful treatment of candidemia, as demonstrated by recent data suggesting higher mortality rates among patients with candidemia whose therapy was delayed [77, 78].

Flucanazole remains standard therapy for selected patients with candidemia, on the basis of abundant data from well-designed clinical trials [27, 28, 53, 79]. There is little role for itraconazole in this setting, given similar antifungal spectrum, ease of administration, superior pharmacokinetics, and better tolerability of fluconazole. Fluconazole should be considered first-line therapy for patients who have mild to moderate illness (i.e., are hemodynamically stable), who have no previous exposure to azoles, and who do not belong in a group at high risk of C. glabrata (infection e.g., elderly patients, patients with cancer, and patients with diabetes). Patients with candidemia and suspected concomitant endocardial or CNS involvement should probably not receive fluconazole as initial therapy; rather, they should receive an agent that is fungicidal, such as AmB (for endocardial or CNS candidiasis) or an echinocandin (for endocardial candidiasis). On the basis of data from recent clinical trials [28, 51, 52, 54, 79], step-down therapy to fluconazole is reasonable for patients who have improved clinically after initial therapy with an echinocandin or AmB and who are infected with an organism that is likely to be susceptible to fluconazole (e.g., C. albicans, C. parapsilosis, and Candida tropicalis).

The echinocandins demonstrate significant fungicidal activity against all Candida species, and each has demonstrated success in ~75% of patients in randomized clinical trials. Because of their efficacy, favorable safety profile, and very few drug interactions, the echinocandins are favored for initial therapy for patients who have a recent history of exposure to an azole,
moderately severe to severe illness (i.e., are hemodynamically unstable), allergy or intolerance to azoles or AmB, or high risk of infection with C. krusei or C. glabrata. A short course of intravenous echinocandin therapy (3–5 days) followed by transition to oral fluconazole or voriconazole (for C. krusei infection) is a reasonable approach to the treatment of candidemia in the stable patient, but there are few clinical data to support this management strategy. The Expert Panel favors fluconazole over the 3 available echinocandins for treatment of candidemia due to C. parapsilosis on the basis of the decreased in vitro activity of echinocandins against C. parapsilosis [45, 46] and reports of echinocandin resistance among selected isolates [80]. Most experts agree that the echinocandins are sufficiently similar to be considered interchangeable.

Data from a recent randomized study suggest that an echinocandin may be superior to fluconazole as primary therapy for candidemia [53]. Although many experts agree that an echinocandin is favored as initial therapy for patients with moderately severe to severe disease due to invasive candidiasis, few agree that an echinocandin is favored for all episodes, and it is reasonable to consider the history of recent azole exposure, severity of illness, and the likelihood of fluconazole resistance in making a choice for initial antifungal therapy.

Voriconazole was shown to be as effective as AmB induction therapy for 4–7 days, followed by fluconazole for candidemia and invasive candidiasis [79]. Voriconazole possesses activity against most Candida species, including C. krusei [26, 81], but the need for more-frequent administration, less predictable pharmacokinetics, more drug interactions, and poor tolerance to the drug, compared with other systemic antifungals, make it a less attractive choice for initial therapy. Voriconazole does not provide predictable activity against fluconazole-resistant C. glabrata [26, 81]. It does, however, fill an important niche for patients who have fluconazole-resistant isolates of C. krusei, C. guilliermondii, or C. glabrata that have documented voriconazole susceptibility and who are ready for transition from an echinocandin or AmB to oral therapy.

Posaconazole has excellent in vitro activity against most Candida species, but there are few clinical data to support its use among patients with candidemia. On the basis of available data and the lack of an intravenous formulation, it is difficult to envision a significant role for posaconazole in the treatment of candidemia, other than in select patients for whom transition to an expanded-spectrum azole is warranted.

AmB-d is recommended as initial therapy when alternative therapy is unavailable or unaffordable, when there is a history of intolerance to echinocandins or azoles, when the infection is refractory to other therapy, when the organism is resistant to other agents, or when there is a suspicion of infection due to non-Candida yeast, such as Cryptococcus neoformans. L-AmB at doses of 3 mg/kg daily has been shown to be effective for treatment of candidemia, based on a recent prospective clinical trial [52]. Similarly, ABLC administered at 3 mg/kg/day has been successfully used for the treatment of candidemia (E. J. Anaissie, unpublished data). Infections due to Candida luisitianae are uncommon; for this organism, the Expert Panel favors the use of fluconazole or an echinocandin over AmB because of the observation of in vitro polyene resistance.

For all patients with candidemia, a dilated funduscopic examination sometime within the first week after initiation of therapy and routine blood cultures to document clearance of Candida from the bloodstream is strongly advised (see Performance Measures). If there are no metastatic complications, the duration of antifungal therapy is 14 days after resolution of signs and symptoms attributable to infection and clearance of Candida species from the bloodstream. This recommendation is based on the results of several prospective, randomized trials in which this rule has been successfully applied, and it is generally associated with few complications and relapses [27, 28, 51–54, 79]. The recommended length of therapy pertains to all systemic antifungal therapy and includes sequential therapy with AmB or an echinocandin followed by an azole.

Central venous catheters should be removed when candidemia is documented, if at all possible [82–84]. The data supporting this are strongest among nonneutropenic patients and show that catheter removal is associated with shorter duration of candidemia [82, 83] and reduced mortality in adults [82, 84] and neonates [85]. Recently completed trials in adults suggest better outcomes and shorter duration of candidemia among patients in whom central venous catheters were removed or replaced [28, 54]. Among neutropenic patients, the role of the gastrointestinal tract as a source for disseminated candidiasis is evident from autopsy studies, but in an individual patient, it is difficult to determine the relative contributions of the gastrointestinal tract versus catheter as primary sources of candidemia [82, 86]. An exception is made for candidemia due to C. parapsilosis, which is very frequently associated with catheters [87]. There are no randomized studies on this topic, but the Expert Panel strongly favors catheter removal when feasible. The role for antifungal lock solutions is not well defined.

II. WHAT IS THE TREATMENT OF CANDIDEMIA IN NEUTROPENIC PATIENTS?

Recommendations

9. An echinocandin (caspofungin, loading dose of 70 mg, then 50 mg daily; micafungin, 100 mg daily (A-II); anidulafungin, loading dose of 200 mg, then 100 mg daily (A-III)) or LAmB (3–5 mg/kg daily) (A-II) is recommended for most patients.

10. For patients who are less critically ill and who have no recent azole exposure, fluconazole (800 mg [12 mg/kg] loading...
dose, then 400 mg [6 mg/kg] daily) is a reasonable alternative (B-III). Voriconazole (400 mg [6 mg/kg] twice daily for 2 doses, then 200 mg [3 mg/kg] [twice daily] can be used in situations in which additional mold coverage is desired (B-III).

11. For infections due to _C. glabrata_, an echinocandin is preferred (B-III); LFAmB is an effective but less attractive alternative because of cost and the potential for toxicity (B-III). For patients who were already receiving voriconazole or fluconazole, are clinically improved, and whose follow-up culture results are negative, continuing use of the azole to completion of therapy is reasonable (B-III).

12. For infections due to _C. parapsilosis_, fluconazole or LFAmB is preferred as initial therapy (B-III). If the patient is receiving an echinocandin and is clinically stable and if follow-up culture results are negative, continuing use of the echinocandin until completion of therapy is reasonable. For infections due to _C. krusei_, an echinocandin, LFAmB, or voriconazole is recommended (B-III).

13. Recommended duration of therapy for candidemia without persistent fungemia or metastatic complications is 2 weeks after documented clearance of _Candida_ from the bloodstream and resolution of symptoms attributable to candidemia and resolution of neutropenia (A-III).

14. Intravenous catheter removal should be considered (B-III).

**Evidence Summary**

Candidemia in neutropenic patients is a life-threatening infection that is associated with acute disseminated candidiasis, a sepsis-like syndrome, multiorgan failure, and death. Candidemia associated with _C. tropicalis_ is particularly virulent in neutropenic hosts. Chronic disseminated candidiasis can ensue as a complication of candidemia in neutropenic patients despite antifungal therapy.

There are no adequately powered randomized controlled trials of treatment of candidemia in neutropenic patients. The data are largely derived from single-arm studies or from small subsets of randomized controlled studies that have enrolled mostly nonneutropenic patients. Historically, candidemia in the neutropenic patient has been treated with an AmB formulation. The availability of voriconazole and the echinocandins have led to greater use of these agents in this clinical scenario but without compelling clinical data. The extensive use of fluconazole for prophylaxis to prevent invasive candidiasis in neutropenic patients and the lack of significant prospective data has led to a diminished therapeutic role for this agent among these patients.

The numbers of neutropenic patients included in recent candidemia treatment studies are small, but response rates are encouraging. In these trials, 50% of caspofungin recipients versus 40% of AmB-d recipients [51], 68% of micafungin recipients versus 61% of L-AmB recipients [52], and 69% of micafungin recipients versus 64% of caspofungin recipients [54] with neutropenia at onset of therapy were successfully treated. Data from the recent randomized controlled trial of anidulafungin versus fluconazole enrolled too few neutropenic patients with candidemia to generate meaningful data regarding efficacy [53]. In 2 retrospective studies, successful outcomes for primary treatment of neutropenic patients were reported in 64% of those receiving AmB-d, 64% of those receiving fluconazole, and 68% of those receiving caspofungin [88, 89].

An extremely important factor influencing the outcome of candidemia in neutropenic patients is the recovery of neutrophils during therapy. In a large retrospective cohort of 476 patients with cancer who had candidemia, persistent neutropenia was associated with a greater chance of treatment failure [87].

Additional insights can be gleaned from data derived from studies of empirical antifungal therapy involving febrile patients with neutropenia who had candidemia at baseline. In these studies, baseline candidemia was cleared in 73% of those treated with AmB-d versus 82% of those treated with L-AmB [90] and in 67% of those treated with caspofungin versus 50% of those treated with L-AmB [91]. Data from a large randomized trial also suggest that voriconazole is a reasonable choice for febrile patients with neutropenia and suspected invasive candidiasis for whom additional mold coverage is desired [92].

On the basis of these limited data, the success rates of antifungal therapy for candidemia in patients with neutropenia do not appear to be substantially different from those reported in the large randomized trials of nonneutropenic patients. Moreover, these data do not suggest less favorable outcomes associated with fluconazole and voriconazole, but many physicians prefer LFAmB or an echinocandin, which may be more fungicidal, as first-line agents. Similar to the approach in nonneutropenic patients, the recommended duration of therapy for candidemia in neutropenic patients is for 14 days after resolution of attributable signs and symptoms and clearance of the bloodstream of _Candida_ species, provided that there has been recovery from neutropenia. This recommendation is based on the limited data from prospective randomized trials and has been associated with few complications and relapses [51, 52, 54].

The management of intravascular catheters in neutropenic patients with candidemia is less straightforward than in their nonneutropenic counterparts. Distinguishing gut-associated from vascular catheter–associated candidemia can be difficult in these patients [86], the data for catheter removal is less compelling, and the consequences of catheter removal often create significant intravenous access problems. Nonetheless, the Expert Panel suggests consideration of venous catheter removal (including removal of tunneled catheters) for neutropenic patients who have persistent candidemia and in whom it is logically feasible.
III. WHAT IS THE EMPIRICAL TREATMENT FOR SUSPECTED INVASIVE CANDIDIASIS IN NONNEUTROPENIC PATIENTS?

Recommendations

15. Empirical therapy for suspected candidiasis in non-neutropenic patients is similar to that for proven candidiasis. Fluconazole (800-mg [12-mg/kg] loading dose, then 400 mg [6 mg/kg] daily), caspofungin (70-mg loading dose, then 50 mg daily), anidulafungin (200-mg loading dose, then 100 mg daily), or micafungin (100 mg daily) is recommended as initial therapy (B-III). An echinocandin is preferred for patients with recent azole exposure, patients with moderately severe to severe illness, or patients who are at high risk of infection due to C. glabrata or C. krusei (B-III).

16. AmB-d (0.5–1.0 mg/kg daily) or LFAmB (3–5 mg/kg daily) are alternatives if there is intolerance to other antifungals or limited availability of other antifungals (B-III).

17. Empirical antifungal therapy should be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever and should be based on clinical assessment of risk factors, serologic markers for invasive candidiasis, and/or culture data from nonsterile sites (B-III).

Evidence Summary

Candida species are an increasing cause of sepsis among non-neutropenic patients receiving intensive care; one-half to two-thirds of all episodes of candidemia occur in an ICU or surgical ward [5, 8]. Identification of patients at risk of Candida infection and prompt initiation of antifungal therapy are critical [77, 78]. Candida colonization, severity of illness, number of broad-spectrum antibiotic agents used and duration of use, previous surgery (especially bowel surgery), receipt of dialysis, use of central venous catheters, receipt of parenteral nutrition, and length of ICU stay are important risk factors for invasive candidiasis [93–98]. The level of Candida colonization has a low positive predictive value, and routine assessment of colonization is labor intensive and expensive [93, 99]. Signs and symptoms of candidiasis are nonspecific, and microbiology and imaging techniques lack sensitivity and specificity.

Early diagnosis of invasive candidiasis remains a challenge; thus, clinical prediction rules have been developed to identify patients in the ICU who are at high risk of candidiasis [100–102]. Characterized by high specificity but a low sensitivity, these rules allow the identification of only a small proportion of ICU patients who will develop candidiasis. Newer serological diagnostic tests have become available to assist in the assessment of patients with suspected candidiasis. Combined measurement of mannan and anti-mannan antibodies has yielded encouraging results and is worthy of additional evaluation [103]. Detection of β-D-glucan has shown good overall performance characteristics, with a sensitivity of 80%–90% in patients with candidemia [104, 105], confirming previous results obtained in patients with hematological malignancies [106]. Real-time PCR is a nonvalidated but intriguing methodology that holds promise as an early diagnostic aid for candidemia [107]. These encouraging data offer new perspectives for early diagnosis of Candida infections, but continued evolution of these assays will be required before they can be used routinely.

Few clinical studies have carefully examined the impact of empirical or preemptive treatment strategies. In one study, preemptive therapy with fluconazole in selected colonized patients in a surgical ICU was associated with reduced incidence of proven candidiasis [108], and in another study, early preemptive fluconazole therapy in patients who had had gastrointestinal surgery for bowel obstruction or perforation had some impact on the resolution of fever, the incidence of candidemia, the length of ICU stay, and mortality [109]. In a more recent study of ICU patients at risk of invasive candidiasis and with unexplained fever, empiric fluconazole (800 mg daily for 14 days) was not associated with better outcomes, compared with placebo [110].

Criteria for starting empirical antifungal therapy in non-neutropenic patients remain poorly defined. Early initiation of antifungal therapy may reduce morbidity, mortality, and length of stay in critically ill patients, but the widespread use of these agents must be balanced against the risk of toxicity, costs, and the emergence of resistance. Empirical antifungal therapy should be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever. Preference should be given to an echinocandin in hemodynamically unstable patients, in patients previously exposed to an azole, and in those known to be colonized with azole-resistant Candida species. LFAmB and AmB-d are alternatives to an echinocandin, but the risk of toxicity is a concern. Empirical therapy with fluconazole may be considered in non–critically ill patients who are known to be colonized with azole-susceptible Candida species or who have no prior exposure to azoles.

IV. WHAT IS THE EMPIRICAL TREATMENT FOR SUSPECTED INVASIVE CANDIDIASIS IN NEUTROPENIC PATIENTS?

Refer to the 2002 IDSA guidelines for the use of antimicrobial agents in neutropenic patients with cancer [111].

Recommendations

18. LFAmB (3–5 mg/kg daily), caspofungin (70-mg loading dose, then 50 mg daily) (A-I), or voriconazole (6 mg/kg twice daily for 2 doses, then 3 mg/kg twice daily) are recommended (B-I).

19. Fluconazole (800-mg [12-mg/kg] loading dose, then 400 mg [6 mg/kg] daily) and itraconazole (200 mg [3mg/kg] twice daily) are alternative agents (B-I).

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Evidence Summary

Empirical antifungal therapy in persistently febrile and neutropenic patients with hematological malignancies, allogeneic hematopoietic stem cell transplantation, and other underlying conditions became a standard of care in the late 1980s when it became clear that the lack of sensitivity of microbiological and clinical findings resulted in delayed diagnosis. AmB-d in neutropenic patients with persistent, unexplained fever despite 4–7 days of broad-spectrum antibiotics was shown to reduce the occurrence of invasive fungal infections and related mortality in 2 randomized, prospective clinical trials [112, 113]. Although these studies provided the scientific basis for empirical antifungal therapy, they were statistically underpowered. Since that time, several clinical trials have compared the efficacy and safety of various antifungal agents for this indication. In a majority of studies, the overall response was assessed by a composite end point consisting of a combination of resolution of fever during neutropenia, successful treatment of baseline fungal infections, absence of breakthrough fungal infection, discontinuation of therapy because of drug-related lack of efficacy or toxicity, and survival.

In recent years, imaging techniques, such as chest CT, and serial measurements of fungal antigens have become integral to the evaluation of the neutropenic patient with persistent, unexplained fever despite broad-spectrum antibacterial therapy [114, 115]. Serial measurements of Aspergillus galactomannan, mannan and anti-mannan antibodies, or β-D-glucan have been shown to be useful additions to culture methods [105, 116–118]. Combined serum galactomannan screening, chest CT, and bronchoalveolar lavage can enhance the diagnosis of invasive fungal infections and reduce the use of empirical therapy in neutropenic patients [119].

Empirical therapy in persistently febrile and neutropenic patients should cover infections caused by yeasts and molds. Given its toxicity, AmB-d is no longer a treatment of first choice unless other safer agents are unavailable. L-AmB is as efficacious as AmB-d and is associated with fewer breakthrough fungal infections and less infusion-related toxicity and nephrotoxicity [90]. ABCD and ABLC are efficacious but associated with a higher incidence of infusion-related toxicity than L-AmB [120, 121].

Fluconazole is less toxic, but its usefulness is limited by its relatively narrow spectrum [122–124]. Itraconazole has been shown to be as efficacious as AmB-d and less toxic [125]; it is available only as an oral formulation and has variable oral bioavailability and frequent gastrointestinal adverse effects. Voriconazole has been shown to prevent breakthrough fungal infections and is effective for aspergillosis and candidemia [79, 92]. Posaconazole has been shown to be effective prophylaxis against invasive fungal infections in high-risk neutropenic patients and allogeneic hematopoietic stem cell transplant recipients [126, 127], but its role as empirical therapy has not been determined. Azoles are unsuitable for empirical therapy if they have been used for prior prophylaxis.

Among the echinocandins, caspofungin has been shown to be as effective as and better tolerated than L-AmB for empirical therapy [91]. Micafungin has been shown to prevent fungal infections in hematopoietic stem cell transplant recipients [42, 128], but micafungin and anidulafungin have not been studied as empirical therapy for neutropenic patients.

V. WHAT IS THE TREATMENT FOR URINARY TRACT INFECTIONS DUE TO CANDIDA SPECIES?

Recommendations: asymptomatic candiduria

20. AmB-d is an effective alternative, but there is a higher risk of toxicity than there is with LFAmB (A-I).

21. Azoles should not be used for empirical therapy in patients who have received an azole for prophylaxis (B-II).

22. Treatment is not recommended unless the patient belongs to a group at high risk of dissemination (A-III). Elimination of predisposing factors often results in resolution of candiduria (A-III).

23. High-risk patients include neutropenic patients, infants with low birth weight, and patients who will undergo urologic manipulations. Neutropenic patients and neonates should be managed as described for invasive candidiasis. For those patients undergoing urologic procedures, fluconazole administered at a dosage of 200–400 mg (3–6 mg/kg) daily or AmB-d administered at a dosage of 0.3–0.6 mg/kg daily for several days before and after the procedure is recommended (B-III).

24. Imaging of the kidneys and collecting system to exclude abscess, fungus ball, or urologic abnormality is prudent when treating asymptomatic patients with predisposing factors (B-III).

Recommendations: symptomatic candiduria

25. For candiduria with suspected disseminated candidiasis, treatment as described for candidemia is recommended (A-III).

26. For cystitis due to a fluconazole-susceptible Candida species, oral fluconazole at a dosage of 200 mg (3 mg/kg) daily for 2 weeks is recommended (A-III). For fluconazole-resistant organisms, AmB-d at a dosage of 0.3–0.6 mg/kg daily for 1–7 days or oral flucytosine at a dosage of 25 mg/kg 4 times daily for 7–10 days are alternatives (B-III). AmB-d bladder irrigation is generally not recommended but may be useful for treatment of patients with fluconazole-resistant Candida species, especially C. glabrata (B-III).

27. For pyelonephritis due to fluconazole-susceptible organisms, oral fluconazole at a dosage of 200–400 mg (3–6 mg/
yield be until symptoms have resolved and urine cultures no longer
of 50 mg/L of sterile water (B-III). Treatment duration should
to systemic therapy is irrigation with AmB-d at a concentration
at a dosage of 0.5–0.7 mg/kg daily with or without flucytosine
at a dosage of 25 mg/kg 4 times daily (B-III). If access to the renal collecting system is available, an adjunct
to systemic therapy is irrigation with AmB-d at a dosage of 25 mg/kg 4 times daily is an alternative (B-III).
If the presence of yeast in the urine, whether microscopically
visualized or grown in culture, must be evaluated in the context
of the particular clinical setting to determine its relevance and
the need for antifungal therapy. If no predisposing condition
is uncovered in an asymptomatic patient, only observation is
warranted [129, 130]. Among patients with predisposing condi-
tions, management of that condition alone, such as removal
of an indwelling catheter, may be sufficient to eliminate candi-
duria without specific antifungal therapy. Several conditions
require an aggressive approach to persistent candiduria, even
among asymptomatic patients. These include neonates with low
birth weight and severely immunocompromised patients with
fever and candiduria, in whom disseminated candidiasis should
be considered. For Candida cystitis, fluconazole is the drug of
choice. However, C. glabrata accounts for ~20% of urine isolates obtained from adults [132, 133], and such
infections frequently require treatment with AmB-d. LFAmB
should not be considered as a first choice because of presumed
low concentrations of the drug in renal tissue. Failure of LFAmB
therapy has been described in the treatment of Candida pyelo-
nephritis in experimental animals and patients [22, 134].

There are several animal studies and a report describing a
small number of patients in which echinocandins were used
successfully for the treatment of renal parenchymal infections
[135, 136]. Although there are clinical circumstances, such as
renal insufficiency and/or the isolation of fluconazole-resistant
organisms, in which an echinocandin or voriconazole may be
considered for the treatment of Candida pyelonephritis, the
Expert Panel does not currently recommend these agents be-
cause of very limited clinical data and poor urinary
concentrations.

Candida prostatitis and epididymo-orchitis are rare [137–
139]. Most patients will require surgical drainage of abscesses
or other surgical debridement, as well as antifungal therapy.
Fluconazole is the agent of choice, but treatment recommenda-
tions are based on anecdotal data.

Fungus balls can occur anywhere in the urinary collecting
system. Aggressive surgical debridement is central to successful
treatment in most nonneonatal cases. Systemic treatment with
AmB-d (with or without flucytosine) or fluconazole has been
eliminated most often [140, 141]. If a percutaneous device provides
direct access to the renal pelvis, ureters, or bladder, local irri-
gation with AmB-d at a dosage of 50 mg/L of sterile water
should be considered as an adjunct to systemic antifungal ther-
apy, but the optimal dose and duration of AmB-d irrigation
have not been defined [141]. Other methods to facilitate the
breakdown and passage of fungus balls include intermittent
saline irrigation, debulking of the fungal mass through a per-
cutaneous device, and irrigation with streptokinase [142–144].

VI. WHAT IS THE TREATMENT FOR
VULVOVAGINAL CANDIDIASIS (VVC)?

Recommendations
29. Several topical antifungal agents are effective therapy
for VVC, and no agent is clearly superior (table 4) (A-I).
30. A single 150-mg dose of fluconazole is recommended
for the treatment of uncomplicated Candida VVC (A-I).
31. For recurring Candida VVC, 10–14 days of induction
therapy with a topical or oral azole, followed by fluconazole at
a dosage of 150 mg once per week for 6 months, is recom-
manded (A-I).

Evidence Summary
VVC is usually caused by C. albicans but can be caused by
other Candida species. A diagnosis of Candida VVC can usually
be made clinically when a woman complains of pruritus, irritation, vaginal soreness, external dysuria, and dyspareunia often accompanied by a change in vaginal discharge. Signs include vulvar edema, erythema, excoriation, fissures, and a white, thick, curd-like vaginal discharge. Unfortunately, these symptoms and signs are nonspecific and can be the result of a variety of infectious and noninfectious etiologies. Before proceeding to empirical antifungal therapy, diagnosis should be confirmed by a wet mount preparation with use of saline and 10% potassium hydroxide to demonstrate the presence of yeast or hyphae. In addition, VVC is associated with normal pH (<4.5). For those with negative wet mount findings, vaginal cultures should be obtained.

VVC can be classified as either uncomplicated (as in ~90% of cases) or complicated (~10% of cases) on the basis of clinical presentation, microbiological findings, host factors, and response to therapy [145]. Complicated VVC is defined as severe or recurrent disease, infection due to Candida species other than C. albicans, and/or VVC in an abnormal host [145]. A variety of topical and systemic or oral agents are available. No evidence exists to show the superiority of any topical agent formulation or regimen [146, 147]. Similarly, oral and topical antifungics achieve entirely equivalent results [148]. Uncomplicated VVC can be effectively treated with either single-dose or short-course therapy, both of which achieve >90% response. Complicated VVC requires topical therapy administered intravaginally daily for ~7 days or multiple doses of fluconazole (150 mg every 72 h for 3 doses) [147]. Therapy with an azole, including voriconazole, is frequently unsuccessful for C. glabrata VVC. Topical boric acid, administered in a gelatin capsule at a dosage of 600 mg daily for 14 days, may be successful [149]. Other alternatives include topical 17% flucytosine cream alone or in combination with 3% AmB cream administered daily for 14 days; these agents must be compounded by a pharmacy. Azole-resistant C. albicans infections are extremely rare [150]. Recurrent VVC is defined as ≥4 episodes of symptomatic VVC within 1 year and is usually caused by azole-susceptible C. albicans [151]. After control of contributing factors, such as diabetes, induction therapy with 10–14 days of a topical or oral azole should be followed by a suppressive regimen for at least 6 months. The most convenient and well-tolerated regimen is once weekly oral fluconazole at a dose of 150 mg, which achieves control of symptoms in >90% of patients [151]. After cessation of maintenance therapy, a 40%–50% recurrence rate can be anticipated. If fluconazole therapy is not feasible, topical clotrimazole (200 mg twice weekly) or clotrimazole (500-mg vaginal suppository once weekly) or other intermittent topical azole treatments are advised. Treatment of VVC should not differ on the basis of HIV infection status; identical response rates are anticipated for HIV-positive and HIV-negative women.

VII. WHAT IS THE TREATMENT FOR CHRONIC DISSEMINATED CANDIDIASIS?

Recommendations

32. Fluconazole at a dosage of 400 mg (6 mg/kg) daily is recommended for clinically stable patients (A-III). LFAmB at a dosage of 3–5 mg/kg daily or AmB-d at a dosage of 0.5–0.7 mg/kg daily can be used to treat acutely ill patients or patients with refractory disease (A-III). Induction therapy with AmB for 1–2 weeks, followed by oral fluconazole at a dosage of 400 mg (6 mg/kg) daily, is also recommended (B-III).

33. Anidulafungin (loading dose of 200 mg, then 100 mg daily), micafungin (100 mg daily), or caspofungin (loading dose of 70 mg, then 50 mg daily for 1–2 weeks) are alternatives for initial therapy, followed by oral fluconazole when clinically appropriate (B-III).

34. Therapy should be continued for weeks to months, until calcification occurs or lesions resolve (A-III). Premature discontinuation of antifungal therapy can lead to recurrent infection.

35. Patients with chronic disseminated candidiasis who require ongoing chemotherapy or undergo stem cell transplantation should continue to receive antifungal therapy throughout the period of high risk to prevent relapse (A-III).

Evidence Summary

Approaches to this syndrome, also termed hepatosplenic candidiasis, are based on anecdotal case reports and open-label series. The bulk of the data and clinical experience are with AmB-d [152, 153], LFAmB [154], and fluconazole [155, 156].

<table>
<thead>
<tr>
<th>Table 4. Intravaginal agents.</th>
</tr>
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<tbody>
<tr>
<td>Butoconazole 2% cream 5 g intravaginally for 3 days OR</td>
</tr>
<tr>
<td>Butoconazole 2% cream 5 g (butoconazole1-sustained release), single intravaginal application OR</td>
</tr>
<tr>
<td>Clotrimazole 1% cream 5 g intravaginally for 7–14 days OR</td>
</tr>
<tr>
<td>Clotrimazole 100-mg vaginal tablet for 7 days OR</td>
</tr>
<tr>
<td>Clotrimazole 100-mg vaginal tablet, 2 tablets for 3 days OR</td>
</tr>
<tr>
<td>Miconazole 2% cream 5 g intravaginally for 7 days OR</td>
</tr>
<tr>
<td>Miconazole 100-mg vaginal suppository, 1 suppository for 7 days OR</td>
</tr>
<tr>
<td>Miconazole 200-mg vaginal suppository, 1 suppository for 3 days OR</td>
</tr>
<tr>
<td>Miconazole 1200-mg vaginal suppository, 1 suppository for 1 day OR</td>
</tr>
<tr>
<td>Nystatin 100,000-unit vaginal tablet, 1 tablet for 14 days OR</td>
</tr>
<tr>
<td>Tioconazole 6.5% ointment 5 g intravaginally in a single application OR</td>
</tr>
<tr>
<td>Terconazole 0.4% cream 5 g intravaginally for 7 days OR</td>
</tr>
<tr>
<td>Terconazole 0.4% cream 5 g intravaginally for 3 days OR</td>
</tr>
<tr>
<td>Terconazole 80-mg vaginal suppository, 1 suppository for 3 days OR</td>
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Some experts feel it helpful to begin treatment with AmB for 1–2 weeks, followed by fluconazole therapy for as long as several months. Caspofungin [157], micafungin [54], and voriconazole [158] have also been used successfully in small numbers of cases. Receipt of therapy for several months and until lesions have either calcified or cleared radiographically is essential to prevent relapse. Additional chemotherapy and stem cell transplantation can proceed when clinically appropriate, provided that antifungal therapy is continued. A novel approach recently put forward is to consider this syndrome, which almost always appears during recovery from neutropenia, to be a form of immune reconstitution inflammatory syndrome and to use corticosteroids in conjunction with antifungal agents for treatment [159]. Additional studies will be required in order to establish the benefit of this approach.

VIII. WHAT IS THE TREATMENT FOR OSTEOARTICULAR CANDIDA INFECTIONS?

Recommendations

36. For osteomyelitis, the Expert Panel recommends fluconazole at a dosage of 400 mg (6 mg/kg) daily for 6–12 months or LFAmB at a dosage of 3–5 mg/kg daily for at least 2 weeks, followed by fluconazole at a dosage of 400 mg daily for 6–12 months (B-III). Alternatives include an echinocandin or AmB-d at a dosage of 0.5–1 mg/kg daily for up to 2 weeks, followed by fluconazole at a dosage of 400 mg daily for at least 6 months (B-III). Surgical debridement in selected cases is advised (B-III).

37. For septic arthritis, the Expert Panel recommends treatment for at least 6 weeks with fluconazole at a dosage of 400 mg (6 mg/kg) daily or LFAmB at a dosage of 3–5 mg/kg daily for up to 2 weeks, followed by fluconazole at a dosage of 400 mg daily (B-III). Alternatively include an echinocandin or AmB-d at a dosage of 0.5–1 mg/kg daily for at least 2 weeks, followed by fluconazole at a dosage of 400 mg daily for the remainder of therapy (B-III). Surgical debridement is indicated in all cases (A-III).

38. For infection involving a prosthetic device, device removal is recommended for most cases (A-III). Therapy for at least 6 weeks with the above dosages of fluconazole, LFAmB, an echinocandin, or AmB-d is recommended (B-III). If the device cannot be removed, chronic suppression with fluconazole is recommended (B-III).

Evidence Summary

Approaches to osteoarticular infections are based on anecdotal case reports and open-label series. The published experience is heavily dominated by reports of use of AmB-d, fluconazole, and more recently, caspofungin. Use of LFAmB, other azoles, and other echinocandins would appear to be reasonable, but experience is limited.

Candida osteomyelitis appears to be best treated with surgical debridement of the affected area in conjunction with antifungal therapy. Some authors have shown that surgical therapy is important for vertebral osteomyelitis [160, 161], but this is not a commonly held view. AmB-d at a dosage of 0.5–1 mg/kg daily for 6–10 weeks has been used successfully [161]. Fluconazole has been used successfully as initial therapy for patients who have susceptible isolates, although treatment failures have also been reported [162–165]. There are reports of the use of itraconazole [166] and caspofungin [167]. The addition of AmB-d to bone cement appears to be safe and may be of value in complicated cases [168]. The data suggest that surgical debridement and an initial course of AmB for 2–3 weeks, followed by treatment with fluconazole for a total duration of therapy of 6–12 months, would also be rational.

IX. WHAT IS THE TREATMENT FOR CNS CANDIDIASIS IN ADULTS?

Recommendations

39. LFAmB at a dosage of 3–5 mg/kg daily, with or without flucytosine at a dosage of 25 mg/kg 4 times daily, is recommended for the initial several weeks of treatment (B-III).

40. Fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily is recommended as step-down therapy after the patient responds to initial treatment with LFAmB and flucytosine. Therapy should continue until all signs and symptoms, CSF
abnormalities, and radiologic abnormalities have resolved (B-III).

41. Removal of infected ventricular devices is recommended (A-III).

Evidence Summary

CNS Candida infections in adults can occur as a manifestation of disseminated candidiasis, as a complication of a neurosurgical procedure (especially after CSF shunt placement), or as an isolated chronic infection [175–181]. Meningitis is the most common presentation, but multiple small abscesses throughout the brain parenchyma, large solitary brain abscesses, and epidural abscesses have been reported [182–184]. Most cases are due to C. albicans, with very few reports of C. glabrata and other species causing infection [176, 178, 179, 181, 183].

No randomized controlled trials have been performed to evaluate the most appropriate treatment. Single cases and small series are reported, and most experience has accrued with the use of AmB-d, with or without flucytosine [175, 176, 178–181]. The Expert Panel favors LFAmB because of the decreased risk of nephrotoxicity. L-AmB attained higher levels in the brain than did ABLC and AmB-d in a rabbit model of Candida meningoencephalitis [23], and there is some clinical experience with use of this formulation for Candida meningitis in neonates [185]. The combination of AmB and flucytosine is appealing because of the in vitro synergism noted with the combination and the excellent CSF concentrations achieved by flucytosine [181]. The length of therapy with AmB alone or in combination with flucytosine has not been defined, but the Expert Panel favors several weeks of therapy before transition to treatment with an azole (after the patient has shown clinical and CSF improvement).

Fluconazole achieves excellent levels in CSF and brain tissue and has proved useful for treatment of Candida CNS infections as step-down therapy after AmB and flucytosine [175–177]. Fluconazole has been used successfully as sole therapy [175, 176, 179, 186], but treatment failures also have been noted, and it is not favored by the Expert Panel as primary therapy [175, 176, 179, 187]. On the basis of these limited data, the Expert Panel recommends that fluconazole as initial therapy be reserved for those patients for whom LFAmB is contraindicated. Fluconazole combined with flucytosine has been reported to cure Candida meningitis in a few patients [178, 188].

There are no reports of the use of voriconazole or posaconazole for CNS candidiasis. Voriconazole achieves excellent levels in CSF [36], but posaconazole CSF levels are low [189]. For the rare case of C. glabrata or C. krusei meningitis, voriconazole seems to be appropriate therapy after initial treatment with AmB and flucytosine.

Echinocandins have been used infrequently for CNS candidiasis. There are case reports of both treatment failure and success [184, 190], and there are reports of CNS breakthrough infections after therapy for candidemia. These agents cannot be recommended for CNS candidiasis.

Removal of an infected ventricular device without the administration of an antifungal agent has proved curative in some patients [176, 177, 179]. However, most physicians combine device removal with systemic antifungal therapy or use both systemic and intraventricular AmB-d injected into the device before its removal [175, 179].

X. WHAT IS THE TREATMENT FOR CANDIDA ENDOPHTHALMITIS?

Recommendations

42. AmB-d at a dosage of 0.7–1 mg/kg daily, combined with flucytosine at a dosage of 25 mg/kg administered 4 times daily, is recommended for advancing lesions or lesions threatening the macula (A-III). Fluconazole at a dosage of 400–800 mg daily (loading dose of 12 mg/kg then 6–12 mg/kg daily) is an acceptable alternative for less severe endophthalmitis (B-III). LFAmB at a dosage of 3–5 mg/kg daily, voriconazole at a dosage of 6 mg/kg twice daily for 2 doses and 3–4 mg/kg twice daily thereafter, or an echinocandin can be used to treat patients who are intolerant of or experiencing treatment failure with AmB-d in combination with flucytosine or fluconazole (B-III).

43. The recommended duration of therapy is at least 4–6 weeks and is determined by the stabilization or resolution of lesions as documented by repeated ophthalmological examinations (A-III).

44. All patients with candidemia should have at least 1 dilated retinal examination early in the course of therapy, preferably performed by an ophthalmologist (A-II). It is especially important to examine patients who cannot communicate regarding visual disturbances.

45. A diagnostic vitreal aspirate is recommended for patients with endophthalmitis of unknown origin (A-III). The Expert Panel strongly recommends ophthalmologic consultation for consideration of partial vitrectomy and intravitreal antifungal therapy with AmB-d for all patients with severe endophthalmitis and vitreitis (B-III).

Evidence Summary

There are no prospective studies for the treatment of Candida endophthalmitis. The majority of published cases report the use of intravenous and/or intravitreal AmB-d with or without oral flucytosine as initial therapy [191–195]. Oral or intravenous fluconazole has also been used successfully as initial, salvage, and transition therapy [195, 196]. Although the data are very limited, LFAmB, the echinocandins, and voriconazole are reasonable options for treatment of patients who are not responding to conventional therapy with AmB-d or fluconazole [197–200]. However, caution is advised with use of the echinocandins because of their poor ocular penetration. Voriconazole
at a dosage of 3–4 mg/kg twice daily appears to be safe and achieves excellent intravitreal levels [35]; it can also be given topically [201]. Among the newer antifungal agents, the published experience is greatest with voriconazole [35, 197, 200, 201].

Early surgical intervention with a partial vitrectomy is an important adjunct to antifungal therapy in more-advanced cases and can be a sight-saving procedure [195]. The value of intraocular instillation of antifungals at the time of vitrectomy, in addition to standard systemic and topical therapy, has not been well studied, but it is commonly practiced. The optimal duration of antifungal therapy has not been determined, but most experts advise at least 4–6 weeks of systemic treatment and continuation of treatment until all clinical evidence of intraocular infection has resolved.

The definitive diagnosis of Candida endophthalmitis still rests on the isolation of the organism from the vitreous body by culture methods or histopathological identification of the organism. There are few data on the value of non–culture-based methodology, such as PCR, in this condition [202].

**XI. WHAT IS THE TREATMENT FOR CANDIDA INFECTIONS OF THE CARDIOVASCULAR SYSTEM?**

**Recommendations**

46. For native valve endocarditis, LFAmb at a dosage of 3–5 mg/kg daily with or without flucytosine at a dosage of 25 mg/kg 4 times daily is recommended (B-III). Alternatives include AmB-d at a dosage of 0.6–1 mg/kg daily with or without flucytosine at a dosage of 25 mg/kg 4 times daily or an echinocandin (higher dosages may be necessary than for treatment of candidemia; e.g., caspofungin at a dosage of 50–150 mg daily, micafungin at a dosage of 100–150 mg daily, or anidulafungin at a dosage of 100–200 mg daily) (B-III). Step-down therapy to fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily should be considered among patients with susceptible Candida isolates who have demonstrated clinical stability and clearance of Candida from the bloodstream (B-III). Valve replacement is recommended, and treatment should continue for at least 6 weeks after valve replacement and should continue for a longer duration in patients with perivalvular abscesses and other complications (B-III).

47. For patients who cannot undergo valve replacement, long-term suppression with fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily is recommended (B-III).

48. For prosthetic valve endocarditis (PVE), the recommendations above apply, and suppressive therapy should be lifelong if valve replacement is not possible (B-III).

49. For pericarditis, LFAmb at a dosage of 3–5 mg/kg daily, AmB-d at a dosage of 0.6–1 mg/kg daily, an echinocandin administered at the dosages noted in recommendation 46, or fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily for as long as several months, in combination with either a pericardial window or pericardectomy, is recommended (B-III).

Step-down therapy to fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily should be considered for patients who have initially responded to AmB or an echinocandin and who are clinically stable (B-III).

50. For myocarditis, treatment as for endocarditis (as outlined in recommendation 46) is recommended (B-III).

51. For suppurrative thrombophlebitis, catheter removal and incision and drainage or resection of the vein, if feasible, is recommended (B-III). LFAmb at a dosage of 3–5 mg/kg daily, AmB-d at a dosage of 0.6–1 mg/kg daily, fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily, or an echinocandin at the dosages noted in recommendation 46 for at least 2 weeks after candidemia has cleared is recommended (B-III). Step-down therapy to fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily should be considered for patients who have initially responded to AmB or an echinocandin and who are clinically stable (B-III). Resolution of the thrombus can be used as evidence to discontinue antifungal therapy if clinical and culture data are supportive (B-III).

52. For pacemaker and implantable cardiac defibrillator wire infections, removal of the entire device and systemic antifungal therapy with LFAmb at a dosage of 3–5 mg/kg daily with or without flucytosine at a dosage of 25 mg/kg 4 times daily, AmB-d at a dosage of 0.6–1 mg/kg daily with or without flucytosine at a dosage of 25 mg/kg 4 times daily, or an echinocandin at the dosages noted in recommendation 46 is recommended (B-III). Step-down therapy to fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily should be considered for patients with susceptible Candida isolates who have demonstrated clinical stability and clearance of Candida from the bloodstream (B-III). For infections limited to generators and/or pockets, 4 weeks of antifungal therapy after removal of the device is recommended (B-III). For pacemaker and implantable cardiac defibrillator wire infections, at least 6 weeks of antifungal therapy after wire removal is recommended (B-III).

53. For ventricular assist devices that cannot be removed, treatment with LFAmb, AmB-d, or an echinocandin at the dosages noted in recommendation 46 is recommended (B-III). After candidemia has cleared and the patient has responded clinically, fluconazole at a dosage of 400–800 mg (6–12 mg/kg) daily is recommended as step-down therapy (B-III). Chronic suppressive therapy with fluconazole is warranted until the device is removed (B-III).

**Evidence Summary**

Medical therapy of endocarditis has occasionally been curative [203–211], but the optimum therapy for both native and prosthetic valve endocarditis in adults is a combination of valv...
replacement and a long course of antifungal therapy [212, 213]. This recommendation is based on anecdotal case reports, case series, and clinical experience. Valve repair and vegetectomy are alternatives to valve replacement. Most of the cases reported in the literature have been treated with AmB-d with or without fluconazole [205, 212–217]. Azoles, usually fluconazole, have been used for completion of therapy. Because of less toxicity and the ability to administer higher dosages, LFAmB are currently favored over AmB-d. A prospective, open-label clinical trial and several case reports show a role for the echinocandins in the treatment of endocarditis [209, 211, 218–224]. Caspofungin at a dosage of 50–150 mg daily has been used successfully; data are limited for the other echinocandins. Higher-than-usual dosages of the echinocandins may be necessary to treat Candida endocarditis. Other cases have been reported of successful treatment using caspofungin in combination with LFAmB, fluconazole, or voriconazole [209, 221, 222].

In neonates, medical therapy alone, usually with AmB-d, has been most frequently used [216, 217]. Success rates for the treatment of neonatal Candida endocarditis are comparable for those treated medically and those treated with combined medical and surgical therapy [216]. Mural endocarditis, an entity associated with a high failure rate, has been successfully treated with caspofungin combined with voriconazole [222].

Lifelong suppressive therapy with fluconazole at a dosage of 400–800 mg daily has been successfully used after a course of primary therapy in patients for whom cardiac surgery is judged to be unacceptably risky and also has been advocated to prevent late recurrence of Candida PVE [221, 225]. Because Candida endocarditis has a propensity to relapse months to years later, follow-up should be maintained for several years [214, 215].

Most experience in the treatment of pericarditis is with AmB-d or fluconazole, as noted in case reports and small series [226, 227]. A few patients have been cured with only pericardiocentesis and antifungal therapy [226], but the preferred procedures are creation of a pericardial window or pericardiectomy. There are few other data to guide therapy. Antifungal treatment should continue for several months until resolution of signs and symptoms of pericardial inflammation.

AmB-d or LFAmB, with or without fluconazole, and voriconazole have all been used successfully to treat myocarditis. Treatment duration may need to be for as long as several months if myocardial abscesses are present [228].

Although most experience treating suppurative thrombophlebitis has been with AmB-d, the Expert Panel supports the use of LFAmB because of decreased nephrotoxicity and the need to treat for prolonged periods. Fluconazole therapy has also been successful in some cases [229, 230]. Any of the agents used for primary treatment of candidemia, including echinocandins and voriconazole, should be effective [231]. Surgical excision of the vein plays an important role in the treatment of peripheral Candida thrombophlebitis. When a central vein is involved, surgery is not usually an option. In some cases, systemic anticoagulation or thrombolytic therapy has been used as adjunctive therapy, but there are insufficient data to routinely recommend their use.

There are a few case reports of Candida infections of venous pacemakers [232, 233] and implantable cardiac defibrillators [234, 235]. Combined surgical and medical therapy is advocated [232, 234, 235]. Medical therapy alone has failed [233]. There is a paucity of data on Candida infections of ventricular assist devices, but the Expert Panel feels that suppressive azole therapy after initial AmB or echinocandin therapy is warranted until the device is removed.

XII. WHAT IS THE TREATMENT FOR NEONATAL CANDIDIASIS?

Recommendations

54. AmB-d at a dosage of 1 mg/kg daily is recommended for neonates with disseminated candidiasis (A-II). If urinary tract involvement is excluded, LFAmB at a dosage of 3–5 mg/kg daily can be used (B-II). Fluconazole at a dosage of 12 mg/kg daily is a reasonable alternative (B-II). The recommended length of therapy is 3 weeks (B-II).

55. A lumbar puncture and a dilated retinal examination are recommended in neonates with sterile body fluid and/or urine cultures positive for Candida species (B-III). Imaging of the genitourinary tract, liver, and spleen should be performed if sterile body fluid cultures have persistently positive results (B-III).

56. Echinocandins should be used with caution and generally limited to situations in which resistance or toxicity preclude the use of fluconazole or AmB-d (B-III).

57. Intravascular catheter removal is strongly recommended (A-II).

58. In nurseries with high rates of invasive candidiasis, fluconazole prophylaxis may be considered in neonates with birth weights <1000 g (A-I). Antifungal drug resistance, drug-related toxicity, and neurodevelopmental outcomes should be observed (A-III).

Evidence Summary

Neonatal candidiasis differs from invasive disease in older patients. Neonates present with subtle symptoms. The primary risk factors are prematurity and day of life; younger and premature infants are more often infected. Dosing of antifungal agents is substantially different for neonates than it is for older children. Outcomes for neonates differ markedly from those for older patients. Although mortality is lower (~20%) in neonates, these infants frequently have CNS disease [236]. CNS infection in the neonate usually manifests as meningoencephalitis and should be assumed to be present in the neonate.
with candidemia because of the high incidence of this complication. Neurologic impairment is common in survivors; therefore, careful follow-up of neurodevelopmental parameters is important.

Failure to promptly remove or replace central venous catheters for infants with candidemia places the infant at increased risk of prolonged infection, mortality, and long-term irreversible neurodevelopmental impairment [85, 236]. Removal or replacement of the catheter at an anatomically distinct site should be performed unless contraindicated.

Treatment of neonatal candidiasis with fluconazole and AmB-d has been evaluated in small, single-center trials [237–239] and in a multi-center cohort study [236]. Fluconazole and AmB-d both appear to be acceptable choices for therapy. The role of fluconazole in neonates with Candida meningitis is questionable and is not routinely recommended [181, 236].

Fluconazole prophylaxis at a dosage of 3 mg/kg or 6 mg/kg twice weekly significantly reduces rates of invasive candidiasis in premature neonates in nurseries that have a very high incidence of Candida infections. In 2 recent studies, the incidence of candidiasis in the placebo arms were 20% in neonates weighing <1000 g and 13% in neonates weighing 1000–1500 g [240, 241]. In contrast, most neonatal intensive care units have an incidence of <5% in neonates who weigh <1000 g and ~1% in neonates who weigh 1000–1500 g. Almost 40% of neonatal nurseries have an incidence that is 10-fold less (<2% in neonates who weigh <1000 g) than that reported in the randomized trials [236, 242, 243]. Pharmacokinetic and prospective safety data are very limited for fluconazole in premature infants, and systematic long-term neurologic follow-up data after routine prophylaxis have not been reported. Because there are unknown risks for neurologic and cognitive disorders after fluconazole exposure in neonates, neurodevelopmental parameters should be followed in neonates who receive this agent. The Expert Panel recommends routine fluconazole prophylaxis for premature infants and infants with extremely low birth weights in nurseries that have a high incidence of invasive candidiasis.

XIII. WHAT IS THE SIGNIFICANCE OF CANDIDA ISOLATED FROM RESPIRATORY SECRETIONS?

Recommendation

59. Growth of Candida from respiratory secretions rarely indicates invasive candidiasis and should not be treated with antifungal therapy (A-III)

Evidence Summary

Candida pneumonia and lung abscess are very uncommon [244, 245]. Candida colonization of the bronchial tree in critically ill patients who receive mechanical ventilation is common, but the lungs have innate defense mechanisms that render them relatively resistant to tissue invasion by Candida species. Only rarely after aspiration of oropharyngeal material does a primary Candida pneumonia or abscess develop. More commonly, hematogenously disseminated candidiasis produces lesions in the lung, as well as in other organs. Diagnosis of bona fide Candida pneumonia requires histopathological confirmation.

In contrast to pneumonia, colonization of the airway with Candida species and/or contamination of the respiratory secretions with oropharyngeal material are extremely common. Unfortunately, a positive culture from respiratory secretions is frequently used as an indication to initiate antifungal therapy in febrile patients who have no other evidence of invasive disease. Multiple prospective and retrospective studies, including autopsy studies, consistently demonstrate the poor predictive value of the growth of Candida from respiratory secretions, including bronchoalveolar lavage fluid. Because of the rarity of Candida pneumonia, the extremely common finding of Candida in respiratory secretions, and the lack of specificity of this finding [246–248], a decision to initiate antifungal therapy should not be made on the basis of respiratory tract culture results alone.

XIV. WHAT IS THE TREATMENT FOR NONGENITAL MUCOCUTANEOUS CANDIDIASIS?

Recommendations: oropharyngeal candidiasis

60. For mild disease, clotrimazole troches at a dosage of 10 mg 5 times daily, nystatin suspension at a concentration of 100,000 U/mL and a dosage of 4–6 mL 4 times daily, or 1–2 nystatin pastilles (200,000 U each) administered 4 times daily for 7–14 days is recommended (B-II).

61. For moderate to severe disease, oral fluconazole at a dosage of 100–200 mg (3 mg/kg) daily for 7–14 days is recommended (A-I).

62. For fluconazole-refractory disease, either itraconazole solution at a dosage of 200 mg daily or posaconazole suspension at a dosage of 400 mg twice daily for 3 days, then 400 mg daily for up to 28 days, are recommended (A-II). Voriconazole at a dosage of 200 mg twice daily or a 1-mL oral suspension of AmB-d, administered at a dosage of 100 mg/mL 4 times daily, are recommended when treatment with other agents has failed (B-II). Intravenous echinocandin or AmB-d at a dosage of 0.3 mg/kg daily can be used in treating patients with refractory disease (B-II).

63. Chronic suppressive therapy is usually unnecessary for patients with HIV infection (A-I). If suppressive therapy is required, fluconazole at a dosage of 100 mg 3 times weekly is recommended (A-I). Treatment with HAART is recommended to reduce recurrent infections (A-I).
64. For denture-related candidiasis, disinfection of the denture, in addition to antifungal therapy, is recommended (B-II).

**Recommendations: esophageal candidiasis**

65. Systemic antifungal therapy is always required (A-II). Oral fluconazole at a dosage of 200–400 mg (3–6 mg/kg) daily for 14–21 days is recommended (A-I). Intravenous fluconazole at a dosage of 400 mg (6 mg/kg) daily, AmB-d at a dosage of 0.3–0.7 mg/kg daily, or an echinocandin should be used for patients who cannot tolerate oral therapy (B-II). A diagnostic trial of antifungal therapy is appropriate before performing an endoscopic examination (B-II).

66. For fluconazole-refractory disease, itraconazole solution at a dosage of 200 mg daily, posaconazole suspension at a dosage of 400 mg twice daily, or voriconazole at a dosage of 200 mg twice daily administered intravenously or orally for 14–21 days is recommended (A-III). Micafungin at a dosage of 150 mg daily, caspofungin at a dosage of 50 mg daily, anidulafungin at a dosage of 200 mg daily, or AmB-d at a dosage of 0.3–0.7 mg/kg daily are acceptable alternatives (B-II).

67. Suppressive therapy with fluconazole at a dosage of 100–200 mg 3 times weekly is recommended for recurrent infections (A-I).

68. In patients with AIDS, treatment with HAART is recommended to reduce recurrent infections (A-I).

**Evidence Summary**

Most cases of oropharyngeal and esophageal candidiasis are caused by *C. albicans*, either alone or in mixed infection [249, 250]. Symptomatic infections caused by *C. glabrata* and *C. krusei* alone have been described [251]. Multiple randomized prospective studies of oropharyngeal candidiasis have been performed involving patients with AIDS and patients with cancer. Most patients respond initially to topical therapy [249, 252, 253]. In HIV-infected patients, symptomatic relapses may occur sooner with topical therapy than with fluconazole [252], and resistance may develop with either regimen. Fluconazole and itraconazole solution are superior to ketoconazole and itraconazole capsules [254–256]. A dosage of itraconazole solution of 2.5 mg/kg twice daily has been recommended for pediatric patients ≥ 5 years of age [67]. Local effects of oral solutions may be as important as systemic effects. Posaconazole suspension is also as efficacious as fluconazole in patients with AIDS [257].

Recent infections typically occur in patients with ongoing immunosuppression, especially those who have AIDS. Long-term suppressive therapy with fluconazole is effective in the prevention of oropharyngeal candidiasis [30, 258, 259]. Long-term suppressive therapy with fluconazole was compared with the episodic use of fluconazole in response to symptomatic disease. Continuous suppressive therapy reduced the relapse rate more effectively than did intermittent therapy, but it was associated with increased microbiological resistance. The frequency of refractory disease was the same for the 2 groups [30]. Oral AmB-d, nystatin, and itraconazole capsules are less effective than fluconazole in preventing oropharyngeal candidiasis [260, 261].

Fluconazole-refractory infections should be treated initially with itraconazole solution. Between 64% and 80% of patients will respond to this therapy [251, 262]. Posaconazole suspension is efficacious in ~ 74% of patients with refractory oropharyngeal or esophageal candidiasis [263], and voriconazole may be efficacious for fluconazole-refractory infections [264]. Intravenous caspofungin, micafungin, or anidulafungin are reasonable alternatives to the triazoles [47–50]. Oral or intravenous AmB-d is also effective in some patients [265]. Immunosuppression with adjunctive granulocyte-macrophage colony-stimulating factor [266] and IFN-γ [267] have been used for refractory oral candidiasis.

The presence of oropharyngeal candidiasis and dysphagia or odynophagia is predictive of esophageal candidiasis. A therapeutic trial with fluconazole for patients with presumed esophageal candidiasis is a cost-effective alternative to endoscopic examination; most patients with esophageal candidiasis will have resolution of their symptoms within 7 days after the start of therapy [268]. Fluconazole is superior to ketoconazole, itraconazole capsules, and flucytosine; itraconazole solution is comparable to fluconazole for the treatment of esophageal candidiasis [269, 270]. Up to 80% of patients with fluconazole-refractory infections will respond to itraconazole solution [262]. Voriconazole is as efficacious as fluconazole and has shown success in the treatment of cases of fluconazole-refractory disease, but it is associated with a higher rate of adverse events [264, 271].

The echinocandins are associated with relapse rates that are higher than those noted with fluconazole [47–50]. Fluconazole-refractory disease responds to caspofungin, and it is likely that micafungin and anidulafungin are similarly effective. In patients with advanced AIDS, recurrent infections are common, and long-term suppressive therapy with fluconazole is effective in preventing recurrences [30].

In HIV-infected patients, the use of HAART has been associated with decreasing rates of oral carriage of *C. albicans* and reduced frequency of symptomatic oropharyngeal candidiasis [272]. Thus, HAART should be used as adjunctive therapy whenever possible for all HIV-infected patients with oropharyngeal or esophageal candidiasis.

Chronic mucocutaneous candidiasis is a rare condition that is characterized by chronic, persistent onychomycosis and mucocutaneous lesions due to *Candida* species. Some patients have a thymoma or autoimmune polyendocrinopathy syndrome type 1 [273]. Fluconazole should be used as initial therapy for candidiasis in these patients. Response to antifungal therapy
may be delayed when there is extensive skin or nail involvement, and relapses almost invariably occur. Thus, most patients require chronic suppressive antifungal therapy. Development of fluconazole-refractory infections is common [274]. Patients with fluconazole-refractory Candida infections should be treated similar to patients with AIDS who have fluconazole-refractory infections.

XV. SHOULD ANTIFUNGAL PROPHYLAXIS BE USED FOR SOLID-ORGAN TRANSPLANT RECIPIENTS, ICU PATIENTS, NEUTROPENIC PATIENTS RECEIVING CHEMOTHERAPY, AND STEM CELL TRANSPLANT RECIPIENTS AT RISK OF CANDIDIASIS?

Recommendations

69. For solid-organ transplant recipients, fluconazole at a dosage of 200–400 mg (3–6 mg/kg) daily or L-AmB at a dosage of 1–2 mg/kg daily, each for at least 7–14 days, is recommended as postoperative prophylaxis for high-risk liver (A-I), pancreas (B-II), and small bowel (B-III) transplant recipients.

70. For ICU patients, fluconazole at a dosage of 400 mg (6 mg/kg) daily is recommended for high-risk patients in adult units with a high incidence of invasive candidiasis (B-I).

71. For patients with chemotherapy-induced neutropenia, fluconazole at a dosage of 400 mg (6 mg/kg) daily (A-I), posaconazole at a dosage of 200 mg 3 times per day (A-I), or caspofungin at a dosage of 50 mg daily (B-II) is recommended during induction chemotherapy for the duration of neutropenia. Oral itraconazole at a dosage of 200 mg daily is an effective alternative (A-I) but offers little advantage and is less well tolerated than these agents.

72. For stem cell transplant recipients with neutropenia, fluconazole at a dosage of 400 mg (6 mg/kg) daily, posaconazole at a dosage of 200 mg 3 times daily, or micafungin at a dosage of 50 mg daily is recommended during the period of risk of neutropenia (A-I).

Evidence Summary

Patients who undergo liver transplantation who have at least 2 key risk factors, including retransplantation, creatinine level >2.0 mg/dL, choledochojejunostomy, intraoperative use of >40 U of blood products, prolonged intraoperative time (>1 h), and fungal colonization detected at least 2 days before and 3 days after transplantation have been identified as being at high risk of invasive candidiasis [275, 276]. One retrospective trial using fluconazole [277] and several prospective trials using L-AmB [278] or fluconazole [279, 280] showed reduced rates of invasive fungal infection. The largest study compared fluconazole with placebo, both given for 70 days after surgery, and showed fungal infections in 6% of fluconazole recipients, compared with 23% of placebo recipients [280]. The most recent study of antifungal prophylaxis in high-risk liver transplant recipients compared L-AmB administered at a dosage of 2 mg/kg/day with placebo for 14 days after transplantation and demonstrated a numerical benefit for L-AmB [281].

The risk of candidiasis among pancreas transplant recipients is probably less than that among liver transplant recipients. However, a retrospective review of 445 consecutive pancreas transplant recipients revealed a 6% frequency of intra-abdominal fungal infection in those who received fluconazole prophylaxis at a dosage of 400 mg/day for 7 days after transplantation, compared with a 10% frequency (P = not significant) for those who did not receive prophylaxis [282]. There were also significant improvements in 1-year graft survival rate and overall survival among patients without infection. Small bowel transplant recipients are a group at high risk of invasive fungal infection [283]. There are no randomized trials of antifungal prophylaxis among this small group of patients, but most experts agree that fluconazole at a dosage of 400 mg daily (6 mg/kg daily in children) for at least 2 weeks after transplantation is reasonable. The risk of invasive candidiasis after transplantation of other solid organs, such as kidneys and hearts, appears to be too low to warrant routine prophylaxis [284].

For ICUs that show very high rates of invasive candidiasis, compared with the normal rates of 1%–2%, antifungal prophylaxis may be warranted [285], and selected ICU patients who are at highest risk (>10%) of invasive candidiasis may benefit from antifungal prophylaxis [102]. There are 3 randomized, placebo-controlled trials that have shown a reduction in the incidence of invasive candidiasis in single units or single hospitals selecting patients at high risk of infection [286–288]. Recent meta-analyses have confirmed this finding; however, it is important to stress that the primary studies and subsequent analysis have all failed to show a survival benefit associated with this strategy [289, 290]. None of the above studies of antifungal prophylaxis in the ICU have demonstrated increased resistance to fluconazole or major ecological shifts in Candida species.

In neutropenic chemotherapy recipients, a meta-analysis of randomized, placebo-controlled trials has shown that systematically active antifungal agents can reduce the number of superficial and invasive Candida infections [291]. A randomized controlled trial showed that receipt of posaconazole decreased invasive fungal infections, compared with receipt of fluconazole or itraconazole in patients with chemotherapy-induced neutropenia who had acute leukemia and myelodysplastic syndrome [127], and an open-label study of prophylaxis with caspofungin versus itraconazole in patients with hematologic malignancies undergoing induction chemotherapy found the 2 drugs to be equivalent [292]. A meta-analysis of 13 randomized controlled trials demonstrated the efficacy of itraconazole (administered orally and intravenously) as antifungal prophylaxis in neutropenic patients with hematologic malignancies, but itra-
conazole offers little advantage over many other antifungal agents and is less well tolerated [293].

In stem cell transplant recipients, micafungin administered at a dosage of 50 mg daily before engraftment significantly reduced episodes of candidiasis, compared with fluconazole administered at a dosage of 400 mg daily, and was associated with a trend toward lower rates of aspergillosis [128]. After transplantation, posaconazole was shown to be more effective than fluconazole in preventing invasive fungal infections in stem cell transplant recipients who had severe graft-versus-host disease [126]. A recently completed randomized, double-blind study that compared fluconazole (400 mg daily) with voriconazole (200 mg twice daily) for 100 days after transplantation as primary antifungal prophylaxis in allogeneic stem cell transplant recipients demonstrated no significant differences in the incidence of invasive fungal infection or fungus-free survival [294]. The usefulness of other potentially active agents, such as itraconazole and AmB, in stem cell transplant recipients is limited by toxicity, drug-drug interactions, logistical issues, or bioavailability [295]. The optimal duration of prophylaxis is not known but should, at a minimum, include the period of risk of neutropenia.

**PERFORMANCE MEASURES**

1. All patients with candidemia should undergo a dilated ophthalmological evaluation to exclude *Candida* endophthalmitis. This procedure has direct therapeutic implications, because patients with endophthalmitis may require surgery and local therapy, and patients with disseminated disease require longer courses of systemic therapy. We suggest that this be performed at a time when the candidemia appears to be controlled and when new spread to the eye is unlikely. Neutropenic patients may not manifest visible endophthalmitis until recovery from neutropenia; therefore, ophthalmological examination in neutropenic patients should be performed after recovery of the neutrophil count.

2. Antifungal therapy should be started on all candidemic patients within 24 h after a blood culture positive for yeast. Recent studies stress the importance of addressing a positive blood culture result with prompt initiation of systemic antifungal therapy, because delays are associated with increased mortality.

Follow-up blood cultures should be obtained for all patients with candidemia to ensure clearance of *Candida* from the bloodstream. The Expert Panel recommends that blood cultures be performed daily or every other day until they no longer yield yeast.

**EXPERT PANEL SPECIALTIES**


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Isavuconazole: A New Broad-Spectrum Triazole Antifungal Agent

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Isavuconazole is a new extended-spectrum triazole with activity against yeasts, molds, and dimorphic fungi. It is approved for the treatment of invasive aspergillosis and mucormycosis. Advantages of this triazole include the availability of a water-soluble intravenous formulation, excellent bioavailability of the oral formulation, and predictable pharmacokinetics in adults. A randomized, double-blind comparison clinical trial for treatment of invasive aspergillosis found that the efficacy of isavuconazole was noninferior to that of voriconazole. An open-label trial that studied primary as well as salvage therapy of invasive mucormycosis showed efficacy with isavuconazole that was similar to that reported for amphotericin B and posaconazole. In patients in these studies, as well as in normal volunteers, isavuconazole was well tolerated, appeared to have few serious adverse effects, and had fewer drug–drug interactions than those noted with voriconazole. As clinical experience increases, the role of this new triazole in the treatment of invasive fungal infections will be better defined.

Keywords. isavuconazole; isavuconazonium sulfate; aspergillosis; mucormycosis; triazoles.

Currently available antifungal agents for the treatment of invasive fungal infections in humans include polyenes, azoles, echinocandins, and pyrimidine analogues. All of these drug classes have disadvantages that can limit their use in clinical practice. Major advantages of the second-generation triazoles, posaconazole and voriconazole, include their extended antifungal spectrum and their availability in both oral and intravenous formulations. However, the use of these agents is often limited by their variable bioavailability, severe adverse events, significant drug–drug interactions, and the emergence of resistance. Isavuconazole is a new extended-spectrum triazole with activity against yeasts, molds, and dimorphic fungi. The safety profile of this agent and its excellent pharmacokinetic characteristics make isavuconazole an attractive option for treatment of invasive fungal infections. The aim of this review is to summarize the in vitro activity, pharmacological attributes, and clinical efficacy that led to the recent US Food and Drug Administration (FDA) approval of isavuconazole for the treatment of invasive aspergillosis and mucormycosis.

IN VITRO ACTIVITY

Mechanism of Action

Similar to other azoles, isavuconazole inhibits cytochrome P450 (CYP)–dependent 14α-lanosterol demethylation, which is essential for fungal cell membrane ergosterol synthesis. This blockade produces methylated sterols in the fungal membrane, altering its function and allowing the accumulation of ergosterol toxic precursors in the cytoplasm, which leads to cell death [1]. The side arm of the active isavuconazole molecule allows greater avidity of isavuconazole for the binding pocket in the fungal CYP51 protein, conferring broader antifungal spectrum even to pathogens resistant to other azoles [1]. In vitro, isavuconazole has broad antifungal activity against yeasts, molds, and dimorphic fungi [2]. The data that follow give a general idea of the activity of isavuconazole with the caveat that to date, no breakpoints have been established.
Yeasts
Isavuconazole appears to be active against all Candida species (Table 1). In general, isavuconazole activity against most Candida species is comparable to that of voriconazole and posaconazole [2–4]. The minimum inhibitory concentration (MIC) values for 90% (MIC90) of almost 3000 unique Candida isolates collected from 2011 and 2012 by the global SENTRY Antimicrobial Surveillance Program were ≤1 µg/mL for all Candida species, with the exception of C. glabrata [3, 4]. For most Candida species, the MIC90 values were ≤0.12 µg/mL; the MIC90 for C. krusei and C. guilliermondii was 1 µg/mL, and for C. glabrata, 2 µg/mL. Isavuconazole MIC values for those isolates of C. glabrata that were resistant to fluconazole and/or voriconazole ranged from 1 µg/mL to ≥8 µg/mL [4]. It appears that for C. glabrata especially, isavuconazole is similar to other azoles in regard to the development of resistance.

Isavuconazole shows in vitro activity against Cryptococcus neoformans and Cryptococcus gattii; most isolates are susceptible to <0.25 µg/mL [5, 7]. Low MIC values against less common yeasts, including Trichosporon species, Geotrichum capitatum, Saccharomyces cerevisiae, Rhodotorula species, and Pichia species have been reported [3, 6, 8] (Table 1).

Molds
Isavuconazole appears to have potent in vitro activity against the most common Aspergillus species, A. fumigatus and A. flavus [2, 3, 9–12] (Table 2). The MIC90 for these Aspergillus species was 1 µg/mL. Higher MIC values have been observed with A. niger (MIC90 2 µg/mL) [3, 10–12]. In one study, isavuconazole retained activity against preselected A. fumigatus isolates that were resistant to other azoles [9], but cross-resistance was shown in another study of azole-resistant isolates [12]. These resistant isolates were shown to have mutations of the CYP51 gene, leading to azole cross-resistance, an emerging problem mostly in Europe, but also described in India [12, 15, 16].

The spectrum of activity of isavuconazole includes some other hyaline molds, such as Paecilomyces lilacinus and Scedosporium apiospermum, but the drug has limited in vitro activity against Fusarium species [2, 11]. Scedosporium prolificans appears to be resistant to isavuconazole, as it is to most antifungal agents [11]. Dematiaceous molds, including Bioplaris spicifera, Alternaria species, Curvularia lunata, and Exophiala species, have MICs that vary from 0.25 µg/mL to 16 µg/mL to isavuconazole [13, 17, 18] (Table 3). MIC90 values for most pigmented molds range from 1 µg/mL to 4 µg/mL. Additionally, isavuconazole has activity against many dermatophytes [19].

Genera in the family Mucoraceae (order Mucorales) are resistant to most azoles, with the exception of posaconazole. In vitro studies with isavuconazole show that this drug is active against many genera of Mucoraceae [10, 11, 13, 14] (Table 2). However, the MICs vary widely within a given genera. For example, some isolates in the genus Rhizopus have MIC

<table>
<thead>
<tr>
<th>Table 1. In Vitro Susceptibilities of Isavuconazole Against Candida Species, Cryptococcus Species, and Less Common Yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism and Species</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td><strong>Candida spp</strong></td>
</tr>
<tr>
<td>C. albicans</td>
</tr>
<tr>
<td>C. glabrata</td>
</tr>
<tr>
<td>C. parapsilosis</td>
</tr>
<tr>
<td>C. tropicalis</td>
</tr>
<tr>
<td>C. krusei</td>
</tr>
<tr>
<td>C. lusitaniae</td>
</tr>
<tr>
<td>C. dubliniensis</td>
</tr>
<tr>
<td>C. kefyr</td>
</tr>
<tr>
<td>C. guilliermondii</td>
</tr>
<tr>
<td><strong>Cryptococcus spp</strong></td>
</tr>
<tr>
<td>C. neoformans</td>
</tr>
<tr>
<td>C. gattii</td>
</tr>
<tr>
<td><strong>Uncommon yeasts</strong></td>
</tr>
<tr>
<td>Trichosporon asahii</td>
</tr>
<tr>
<td>Trichosporon mucoides</td>
</tr>
<tr>
<td>Trichosporon inkin</td>
</tr>
<tr>
<td>Geotrichum capitatum</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>Rhodotorula spp</td>
</tr>
<tr>
<td>Pichia spp</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, minimum inhibitory concentration.
values as low as 0.12 µg/mL, but others have MIC values as high as 32 µg/mL.

**Dimorphic Fungi**

The MICs of isavuconazole for *Histoplasma capsulatum* and *Coccidioides* species range from 0.12 µg/mL to 2 µg/mL [13] (Table 3). Only 6 isolates of *Blastomyces dermatitidis* have been studied, and MIC values ranged from 0.5 µg/mL to 4 µg/mL. MICs against a small number of isolates of *Sporothrix* species were reported to be high for isavuconazole and similar to those noted with voriconazole [2].

**PHARMACOLOGY**

Isavuconazonium sulfate, the prodrug of isavuconazole, is available in both intravenous and oral formulations. The dosage strength of 372 mg isavuconazonium sulfate corresponds to 200 mg of isavuconazole, the active component, in both formulations. The capsules each contain 186 mg isavuconazonium sulfate (100 mg isavuconazole). The recommended dosing regimen for both oral and intravenous formulations is a loading dose of 600 mg, given as 200 mg every 8 hours, for 2 days followed by 200 mg daily thereafter [20].

Unlike voriconazole and posaconazole, the prodrug, isavuconazonium sulfate, is highly water soluble, and therefore the intravenous formulation does not require solubilization by a cyclodextrin vehicle. This is an advantage as this eliminates the concerns of nephrotoxicity from the cyclodextrin vehicle, which is used in the intravenous formulations of voriconazole and posaconazole [21].

After intravenous infusion, the prodrug is broken down quickly to the active component, isavuconazole, and an inactive cleavage product. In healthy adults, plasma concentrations of the prodrug and the inactive cleavage product are detectable only during the intravenous infusion and are not detectable 30 minutes later. Following oral administration, plasma concentrations of the active compound reach maximum concentrations (C\text{max}) by 2–3 hours; the prodrug and cleavage product are not measurable in plasma after oral administration.

In healthy adult volunteers, isavuconazole exhibits linear and dose-proportional pharmacokinetics. The oral bioavailability of isavuconazole is 98% [22, 23]. Absorption of isavuconazole is not affected by food intake. In addition to excellent bioavailability, isavuconazole serum concentrations show low intersubject variability. In healthy volunteers, the C\text{max} at steady state was 2.5 ± 1.0 µg/mL [23]. In a small number of patients with acute myeloid leukemia and neutropenia, similar pharmacokinetic profiles for intravenous isavuconazole were found [24].

Isavuconazole has a large volume of distribution, is >99% protein bound, and has a long terminal half-life of 100–130 hours [22, 23]. Consistent with this long terminal elimination half-life, tissue levels persist long after plasma levels become undetectable [25]. The route of elimination in humans has not been established, but animal studies have shown that excretion occurs primarily via feces. Urine elimination of isavuconazole is negligible, and thus this agent is unlikely to be useful for the treatment of urinary tract infections.

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**Table 2. In Vitro Susceptibilities of Isavuconazole Against Hyaline Molds and Mucorales**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No.</th>
<th>MIC\text{50}, µg/mL</th>
<th>MIC\text{90}, µg/mL</th>
<th>MIC Range, µg/mL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> spp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>855</td>
<td>0.5</td>
<td>1</td>
<td>0.06–2</td>
<td>[12]</td>
</tr>
<tr>
<td>A. flavus</td>
<td>444</td>
<td>0.5</td>
<td>1</td>
<td>0.12–4</td>
<td>[12]</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>106</td>
<td>0.125</td>
<td>1</td>
<td>0.06–1</td>
<td>[12]</td>
</tr>
<tr>
<td>A. niger</td>
<td>207</td>
<td>1</td>
<td>2</td>
<td>0.06–2</td>
<td>[12]</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>75</td>
<td>0.25</td>
<td>0.5</td>
<td>0.03–2</td>
<td>[12]</td>
</tr>
<tr>
<td>A. terreus</td>
<td>384</td>
<td>0.25</td>
<td>0.5</td>
<td>0.06–2</td>
<td>[12]</td>
</tr>
<tr>
<td>Fusarium spp</td>
<td>50</td>
<td>16</td>
<td>&gt;16</td>
<td>1–16</td>
<td>[11, 13]</td>
</tr>
<tr>
<td>Paecilomyces lilacinus</td>
<td>22</td>
<td>1</td>
<td>2</td>
<td>0.2–2</td>
<td>[13]</td>
</tr>
<tr>
<td><em>Scedosporium apiospermum</em></td>
<td>44</td>
<td>2</td>
<td>4</td>
<td>0.5–8</td>
<td>[11, 13]</td>
</tr>
<tr>
<td><em>Scedosporium prolificans</em></td>
<td>6</td>
<td>...</td>
<td>...</td>
<td>&gt;16</td>
<td>[11]</td>
</tr>
<tr>
<td><em>Mucorales</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absidia spp</td>
<td>80</td>
<td>1</td>
<td>8</td>
<td>0.03–16</td>
<td>[14]</td>
</tr>
<tr>
<td>Cunninghamella spp</td>
<td>18</td>
<td>2</td>
<td>16</td>
<td>0.12–16</td>
<td>[14]</td>
</tr>
<tr>
<td>Mucor spp</td>
<td>79</td>
<td>4</td>
<td>16</td>
<td>&lt;0.015–128</td>
<td>[14, 15]</td>
</tr>
<tr>
<td>Rhizomucor spp</td>
<td>29</td>
<td>2</td>
<td>16</td>
<td>0.015–64</td>
<td>[14]</td>
</tr>
<tr>
<td>Rhizopus spp</td>
<td>199</td>
<td>1</td>
<td>4</td>
<td>0.12–32</td>
<td>[14, 15]</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, minimum inhibitory concentration.
Metabolism of isavuconazole takes place in the liver via the CYP enzyme family, specifically CYP3A4 and CYP3A5 isoenzymes. The metabolites do not have antifungal activity. Data suggest that patients with liver disease have higher exposure to isavuconazole. The clearance of isavuconazole in patients with mild to moderate hepatic impairment (Child-Pugh class A and B) is impaired [26], but no dosage adjustment is recommended for these patients. There are no data for patients who have Child-Pugh class C liver disease.

Area under the curve (AUC) values and Cmax are not significantly affected in patients with renal impairment; no dose adjustment is necessary in this patient group [20]. Isavuconazole is likely not dialyzable by either hemodialysis or continuous hemofiltration; however, no pharmacokinetic studies have been reported in patients undergoing renal replacement therapy.

Similar to observations with voriconazole, the metabolism of isavuconazole may be affected by race. Compared with healthy white subjects, Chinese subjects were found to have a lower clearance of isavuconazole (2.6 L/h vs 1.6 L/h, respectively) and a 50% higher AUC [21]. However, at this point, no dosage adjustment has been recommended based on race. Age and sex minimally affect the pharmacokinetics of isavuconazole. No pharmacokinetic studies have been reported in children.

Isavuconazole is considered a pregnancy class C drug and should not be given to pregnant women. Because the drug was found in breast milk of lactating rats, it should not be used in women who are breastfeeding.

**DRUG–DRUG INTERACTIONS**

Isavuconazole is a substrate for CYP3A4, so inhibitors of this enzyme lead to increased levels of isavuconazole and should be used with caution (Table 4).

Potent inducers of CYP3A4, such as rifampin, carbamazepine, and long-acting barbiturates, significantly decrease isavuconazole serum levels and should not be used with this antifungal [21].

Isavuconazole is a moderate inhibitor of CYP3A4 and inhibits to a variable extent the metabolism of sirolimus, tacrolimus, and cyclosporine, which can lead to higher levels of these drugs. Serum levels need to be monitored if these drugs are given with isavuconazole. The effects on other CYP3A4 substrates, such as midazolam and atorvastatin, appear to be mild and dosage

### Table 3. In Vitro Activity of Isavuconazole Against Dematiaceous Molds and Dimorphic Fungi

<table>
<thead>
<tr>
<th>Organism</th>
<th>No.</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;, µg/mL</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;, µg/mL</th>
<th>MIC Range, µg/mL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dematiaceous molds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophiala spp</td>
<td>106</td>
<td>2</td>
<td>4</td>
<td>0.25–16</td>
<td>[18]</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>0.5–2</td>
<td>[13]</td>
</tr>
<tr>
<td>Alternaria infectoria</td>
<td>50</td>
<td>4</td>
<td>4</td>
<td>2–4</td>
<td>[17]</td>
</tr>
<tr>
<td>Bipolaris spicifera</td>
<td>30</td>
<td>2</td>
<td>4</td>
<td>0.5–4</td>
<td>[13]</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>24</td>
<td>2</td>
<td>4</td>
<td>1–4</td>
<td>[13]</td>
</tr>
<tr>
<td><strong>Dimorphic fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
<td>6</td>
<td>. . .</td>
<td>. . .</td>
<td>0.5–4</td>
<td>[13]</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>28</td>
<td>0.5</td>
<td>2</td>
<td>0.12–2</td>
<td>[13]</td>
</tr>
<tr>
<td>Coccidioides posadasi</td>
<td>30</td>
<td>0.25</td>
<td>0.5</td>
<td>0.12–1</td>
<td>[13]</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, minimum inhibitory concentration.

### Table 4. Drug–Drug Interactions With Isavuconazole

<table>
<thead>
<tr>
<th>Type of Interaction, Drug</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increases isavuconazole level</td>
<td>Use with caution</td>
</tr>
<tr>
<td>Lopinavir/ritonavir</td>
<td>Use with caution</td>
</tr>
<tr>
<td><strong>Decreases isavuconazole level</strong></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>Contraindicated</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td></td>
</tr>
<tr>
<td>Long-acting barbiturates</td>
<td></td>
</tr>
<tr>
<td>St John’s wort</td>
<td></td>
</tr>
<tr>
<td><strong>Levels increased by isavuconazole</strong></td>
<td></td>
</tr>
<tr>
<td>Sirolimus</td>
<td>Use with caution. Monitor serum levels of these drugs and adjust dose when given with isavuconazole</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td></td>
</tr>
<tr>
<td>Colchicine</td>
<td>Use with caution. May require dose adjustment</td>
</tr>
<tr>
<td>Dagibatran</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>No dose adjustment recommended when given with isavuconazole; monitor patient</td>
</tr>
<tr>
<td>Midazolam</td>
<td></td>
</tr>
<tr>
<td><strong>Levels decreased by isavuconazole</strong></td>
<td></td>
</tr>
<tr>
<td>Bupropion</td>
<td>Use with caution. Dose increase of bupropion may be necessary</td>
</tr>
<tr>
<td>Lopinavir/ritonavir</td>
<td>Use with caution</td>
</tr>
</tbody>
</table>

Source: Adapted from [21].
adjustment is not recommended [27]. However, patients should be monitored for adverse effects if these drugs are used concomitantly with isavuconazole.

Isavuconazole has a minimal effect on CYP2C9 and CYP2C19, and thus can be used with warfarin and omeprazole without dosage adjustment of these drugs [28]. Isavuconazole is a weak inhibitor of P-glycoprotein, and if given with digoxin may lead to higher serum digoxin levels [21]. Overall, when compared with voriconazole and posaconazole, isavuconazole seems to have fewer drug–drug interactions.

SIDE EFFECTS

Isavuconazole appears to be relatively safe and well tolerated. The most common side effects reported are gastrointestinal, including nausea, vomiting, and diarrhea, but these rarely have led to discontinuation of the drug [23, 29–31] (Table 5). Other common side effects reported to be associated with isavuconazole in <15% of volunteers and patients include headache, rash, and peripheral edema [23, 29–31]. Commonly noted side effects with voriconazole, such as visual disturbances, hallucinations, and photosensitivity, have not been described with isavuconazole.

Elevations in hepatic enzyme levels can occur with isavuconazole therapy, as with other azoles. The usual pattern is elevation of alanine aminotransferase and aspartate aminotransferase, but elevations in alkaline phosphatase levels also have been noted. There are reports of a few patients who had severe hepatotoxicity, but whether this was caused solely by isavuconazole or was related to other underlying illness is not clear [20]. Liver enzymes should be monitored in patients taking isavuconazole, as is recommended for all other azole drugs.

Differing from other triazoles that cause QTc segment prolongation, isavuconazole has been noted to cause dose-dependent QTc shortening by as much as 13 msec with 200 mg daily and 24.6 msec with 600 mg daily in healthy volunteers [20]. Among 257 patients treated with isavuconazole in a clinical trial of invasive mold infections, 17 (7.5%) had QTc shortening >60 msec from baseline [21]. At this point, the clinical significance of QTc shortening is unclear, except for patients who have the rare disease familial short QT syndrome, who should not receive isavuconazole.

Infusion reactions that include acute respiratory distress, chills, dyspnea, and hypotension have been noted in a few patients [21]. Whether these reactions are related to particulates in the intravenous formulation, which are composed of isavuconazole that has been cleaved from the prodrug while being infused, is not clear [21]. It is recommended that an in-line filter (0.2–1.2 µm) be used for the infusion of isavuconazole [20].

CLINICAL USES

Aspergillosis

Isavuconazole has been approved for the treatment of invasive aspergillosis based on the results of a randomized, double-blind, noninferiority clinical trial that compared isavuconazole with voriconazole for primary treatment of invasive aspergillosis and other invasive mold infections [21, 32–34]. A total of 516 adult patients with proven, probable, or possible invasive fungal disease, as defined by European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [35], were randomized 1:1 to receive isavuconazole or voriconazole. The primary endpoint of the study was all-cause mortality at day 42 for this entire population.

The analysis of efficacy for treating invasive aspergillosis focused on the subset of 272 patients who had proven (65 patients) or probable (207 patients) invasive fungal infection [21]. Probable infection was based on a positive galactomannan assay in half of the patients in both treatment arms. Efficacy was defined as success at the end of treatment by EORTC/MSG criteria [36] and was adjudicated by a blinded data review committee.

It should be noted that the actual number of patients who had proven or probable aspergillosis was 231; the other 41 patients included in the analysis of efficacy at the end of treatment had other invasive mold infections. Of the 231 patients who had invasive aspergillosis, 123 received isavuconazole and 108 received voriconazole, and 16% had proven infection and 84% had probable infection.

Hematological malignancies were the most common underlying condition (84%); 65% were neutropenic and 20% had received an allogeneic hematopoietic cell transplant. The median

<table>
<thead>
<tr>
<th>Table 5. Adverse Effects Associated With Isavuconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Most frequent side effects or other abnormalities</strong></td>
</tr>
<tr>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Rash</td>
</tr>
<tr>
<td>Elevation of liver enzymes</td>
</tr>
<tr>
<td>QTc shortening</td>
</tr>
<tr>
<td><strong>Less common side effects or other abnormalities</strong></td>
</tr>
<tr>
<td>Peripheral edema</td>
</tr>
<tr>
<td>Hypokalemia</td>
</tr>
<tr>
<td>Infusion reactions</td>
</tr>
<tr>
<td>Acute respiratory distress</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Dyspnea</td>
</tr>
<tr>
<td>Hypotension</td>
</tr>
</tbody>
</table>
duration of intravenous therapy was 5 days in both arms, and the total duration of antifungal treatment was 45 and 46.5 days for isavuconazole and voriconazole, respectively.

Day 42 all-cause mortality for all patients who received study drug did not differ significantly (18.6% for the isavuconazole arm and 20.2% for the voriconazole arm). Similarly, the overall success (complete and partial response) at the end of treatment was not different (35% and 36.4% for the isavuconazole and voriconazole arms, respectively). For the 231 patients who had proven or probable invasive aspergillosis, both the 42-day mortality (18.7% and 22.2%) and success at the end of treatment (35% vs 38.9%) were not different between the isavuconazole and voriconazole arms, respectively.

Mucomycosis
The data on which the approval for the treatment of invasive mucomycosis is based are not as strong as those for aspergillosis. In large measure this is because of the rarity of mucomycosis and resultant inability to enroll enough cases into a randomized controlled treatment trial. The study that was used for licensing was an open-label, noncomparative trial that evaluated isavuconazole for treatment of invasive aspergillosis in patients with renal impairment and for treatment of invasive fungal infections caused by rare molds, including members of the order Mucorales [21, 30]. This study allowed both primary treatment of invasive mold infections and salvage treatment of patients who were intolerant of or failing prior antifungal therapy. Of 149 patients enrolled in this study, 37 had proven (86%) or probable (14%) invasive mucomycosis by EORCT/MSG criteria [35]. Twenty-one of the 37 had not received prior antifungal therapy, 11 had refractory disease, and 5 were intolerant of prior therapy. A hematologic malignancy was present in 59%; 35% had received a hematopoietic cell transplant, and 27% were neutropenic. Diabetes was a risk factor in only 4 patients. Pulmonary involvement was present in 22 patients (59%), and half of those patients also had another site of infection, mainly sinus, ocular, and central nervous system. The median duration of treatment was 84 days (range, 2–882 days).

The endpoints of this study were all-cause mortality at day 42 and overall response at day 42 as assessed by an independent data review committee. The mortality at day 42 was 38%; mortality was higher among patients who had refractory disease or were intolerant of prior therapy (43.7%) compared with those who were treated for primary mucomycosis (33.3%). The overall response rate at day 42, as judged by the data review committee, was 31.4%.

A matched case-control analysis was performed comparing the 21 patients who had primary treatment with isavuconazole with the cases of 33 patients who were treated with amphotericin B and whose data had been entered into the global Fungiscope Registry, an ongoing observational database. Matching was done by severity of infection, hematologic malignancy, and whether surgical debridement was performed. In this analysis, isavuconazole-treated and amphotericin B–treated patients had similar mortality (33.3% vs 41.3%, respectively). These mortality rates are consistent with historical data showing mortality rates of 35%–45% in patients who were treated with amphotericin B [37, 38] and in those who were entered into several posaconazole salvage studies [39, 40].

Other Fungal Infections
The data on the role of isavuconazole for the treatment of patients with invasive mold infection caused by Fusarium and Scedosporium species are limited to 9 and 3 patients, respectively [41]. Only 3 patients with fusariosis and 1 with scedosporiosis had a complete or partial response at the end of therapy [41].

Twenty-nine patients with endemic mycoses—paracoccidioidomycosis (10), coccidioidomycosis (9), histoplasmosis (7), and blastomycosis (3)—were entered into the rare molds treatment trial [31]. Disseminated infection was present in 70% and 57% of patients with paracoccidioidomycoses and histoplasmosis, respectively. Successful response at the end of therapy was 64%, but 3 patients died from progression of disease (2 with paracoccidioidomycosis and 1 with blastomycosis). These data suggest that isavuconazole could be an alternative for the treatment of dimorphic fungi infections, but the number of patients treated is too small to recommend the use of this agent at this time.

A phase 2, randomized, double-blind, multicenter trial that evaluated 3 dosing regimens of isavuconazole compared with fluconazole in 160 immunocompromised patients with esophageal candidiasis noted an overall 96% clinical and microbiological success rate [29]. However, given the efficacy of fluconazole, it seems unlikely that isavuconazole will assume a major role in the treatment of mucosal candidiasis.

SUMMARY
Isavuconazole has been approved by the FDA for the treatment of invasive aspergillosis and mucomycosis. Advantages of this triazole are the availability of a water-soluble intravenous formulation, excellent bioavailability of the oral formulation, predictable pharmacokinetics, and, to date, few adverse effects. However, clinical experience is limited for the treatment of invasive fungal infections when compared with voriconazole and posaconazole. Voriconazole remains the drug of choice for the treatment of invasive aspergillosis, but isavuconazole could be considered an attractive alternative for patients who cannot tolerate voriconazole. Lipid formulations of amphotericin B remain the treatment of choice for mucomycosis. Posaconazole is typically used for step-down therapy and for those patients who cannot tolerate amphotericin B. Isavuconazole is an
acceptable alternative to posaconazole in those patients. The role of isavuconazole in treating endemic mycoses and other fungal infections should be clarified by further studies.

**Note**

**Potential conflict of interest.** Both authors: No reported conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


A meta-analysis of medical versus surgical therapy for Candida endocarditis

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Abstract Objectives. The optimal management of Candida infective endocarditis (IE) is unknown.

Methods. We reviewed all 879 cases of Candida IE reported from 1966-2002 in the peer-reviewed literature to better understand the role of medical and surgical therapies. This review included 163 patients from 105 reports that met our inclusion criteria: 31 cases treated with antifungal monotherapy, 25 cases treated with medical antifungal combination therapy, and 107 cases treated with adjunctive surgical plus medical antifungal therapy. We also used meta-analytic techniques to evaluate 22 observational case-series (72 patients) of the 105 reports with two or more patients with definite Candida IE.

Results. We found that in patients who underwent adjunctive surgery there was a lower reported proportion of deaths [prevalence odds ratio (POR) = 0.56; 95% confidence interval (CI) = 0.16, 1.99)]. Higher mortality was noted in patients treated prior to 1980 (POR = 2.03; 95% CI = 0.55, 7.61), treated with antifungal monotherapy (POR = 1.49; 95% CI = 0.39, 5.81), infected with Candida parapsilosis (POR = 1.51; 95% CI = 0.41, 5.52), or with left-sided endocarditis (POR = 2.36; 95% CI = 0.55, 10.07).

Conclusions. Medical antifungal therapy of Candida IE is poorly characterized, and recent antifungal developments lend promise for those patients who cannot undergo surgery.

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Introduction

Fungal infective endocarditis (IE) is increasing in incidence; fungi now comprise between 1 and 10% of organisms isolated in IE, including approximately 10% of prosthetic valve endocarditis cases. In recent reviews of fungal endocarditis, 53–68% were Candida species and Candida albicans was the most common. Surgical intervention over the last several decades has decreased overall mortality of infective endocarditis, and surgery is generally regarded as a standard treatment for fungal IE because of the risk of septic embolization and the difficulty in sterilizing a fungal vegetation. The American College of Cardiology (ACC) and the American Heart Association (AHA) published guidelines list ‘fungal endocarditis’ as an indication for surgery as defined by a Class I recommendation—‘conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective’. Unfortunately, some patients are poor surgical candidates. Furthermore, the requirement for surgery for Candida IE has never been formally tested, which is particularly relevant as new fungicidal antifungal agents now become available.

The most recent 2004 Infectious Diseases Society of America (IDSA) guidelines for management of Candida IE state that ‘both native-valve and prosthetic-valve infection should be managed with surgical replacement of the infected valve’. The key antifungal recommendations from the IDSA are ‘medical therapy with amphotericin B with or without flucytosine at maximal tolerated doses has most often been used. If valve replacement is not possible, long-term (possibly life-long) suppressive therapy with fluconazole may be used’.

Most patients with Candida IE treated without surgery have traditionally received extended courses of amphotericin B deoxycholate. Such medical management of Candida IE has been associated with high rates of death and amphotericin B-induced nephrotoxicity, making it an unappealing treatment option. However, these observations were reported prior to the recent availability of safe, potent antifungal agents, including the echinocandins and second generation azoles. As a result, the optimal medical management of patients with Candida IE in whom cardiac surgery is not an option is unknown. In the current report, we systematically review the published literature of medical and surgical management of Candida IE and provide data to question the untested yet accepted practice of required surgical intervention for Candida IE in this new era of safer, potent antifungals.

Patients and methods

Review of literature

We conducted a MEDLINE search using the keywords ‘Candida’ and ‘endocarditis’ and text word searching. All English language clinical reports of medical or medical with adjunctive surgical therapy for patients with Candida IE from January 1, 1966 to December 31, 2002 were reviewed. Publications cited in these reports and abstracts from recent (2000-2003) scientific meetings (Interscience Conference on Antimicrobial Agents and Chemotherapy, Infectious Diseases Society of America) were also considered for inclusion in the current analysis. In order to increase reliability, a single reviewer (WJS) abstracted data from all included publications using a data collection form developed specifically for the current analysis.

Inclusion and exclusion criteria

Cases were included in the current analysis if they (a) met criteria for definite Candida IE according to the modified Duke criteria, and (b) contained specific information regarding therapy and outcome. Cases were excluded if they (a) received <7 days of systemic antifungal therapy, (b) involved a permanent pacemaker or defibrillator, or (c) presented with a cardiac mass without apparent valvular involvement.

Study definitions

Three treatment categories were included in the current analysis: medical antifungal monotherapy, medical antifungal combination therapy, and adjunctive surgical plus medical antifungal therapy. Medical antifungal monotherapy was defined as patients who were treated with a single antifungal agent at one time, and did not undergo surgery. Medical antifungal combination therapy was defined as patients who were treated with two or more antifungal agents concurrently, and did not undergo surgery. Adjunctive surgical plus medical therapy was defined as patients who underwent...
surgery for IE as well as received either antifungal monotherapy or antifungal combination therapy.

Analysis plan

Data from included cases were presented in two stages. The first stage of data presentation consisted of a detailed summary of all Candida IE cases (1966-2002) identified in the literature which met study inclusion criteria. Mortality was deemed to be due to fungal infection in the majority of reviewed cases, but due to the reporting style of many of the individual retrospective cases this could not be verified. In the next stage, a meta-analysis of all reports meeting study inclusion criteria and describing ≥2 cases of definite Candida IE was performed. The following variables were considered within the meta-analysis as dichotomous variables: year of publication (prior versus subsequent to 1980), left- versus right-sided valvular involvement, use of adjunctive surgery, use of antifungal monotherapy, and infection with C. parapsilosis. The year 1980 was selected due to the widespread clinical use of transthoracic echocardiography for the diagnosis of endocarditis beginning in this decade. We used as reference categories the group with less than 100% of patients in the series with adjunctive surgery, antifungal monotherapy, infection with C. parapsilosis, and left-sided endocarditis.

We transformed risk-factor specific mortality rates into their respective logits (natural log of the probability divided by 1-probability) to take advantage of the properties of the logit (normal distribution and stable variance) transformation. We then evaluated the data with the Egger test. In order to address heterogeneity, we compared average prevalence estimates between groups of studies using random-effects meta-regression. The dependent variable was the logit of the prevalence of mortality and the independent variables were year of study publication, use of surgery, medical antifungal monotherapy, affected heart side of IE, and isolation of C. parapsilosis.

The regressions were fit with random-effects weights (reciprocal sum of the estimated within-study and among-study variances) using restricted maximum likelihood estimation of the between-study variance to account for heterogeneity. For each independent variable, we compared studies and present the 95% confidence intervals (CI) of the prevalence odds ratios (POR). The meta-regression techniques presented are analogous to logistic regression. However, because most reviewed studies are primarily observational or explanatory, p-values are not presented, and formal inferences are not drawn from the presented 95% confidence intervals (CI).

Results

A total of 879 cases of Candida endocarditis in 418 reports were reviewed. Of these, 163 cases of definite Candida endocarditis from 105 reports met inclusion criteria (Appendices A–C). Those cases were divided as treatment with medical antifungal monotherapy alone (n=31), treatment with medical antifungal combination therapy (n=25), and treatment with adjunctive surgical plus medical antifungal therapy (n=107).

Summary of reported cases

Because we observed a greater association with reported mortality in patients treated prior to 1980 (POR 2.03) in our meta-analysis, and considering the advances in echocardiographic diagnostic techniques as well as the frequency of adjunctive surgical intervention after that time period, in order to better reflect current medical practice only results from the 92 patients reported after 1980 are described in greater detail below.

These 92 cases were grouped as follows: medical antifungal monotherapy (n=15), medical antifungal combination therapy (n=19), and adjunctive surgical plus medical antifungal therapy (n=58). After 1980, C albicans and C. parapsilosis were the most common Candida species isolated. In the medical antifungal monotherapy cohort, 9 (60%) patients were infected with C. albicans, 3 (20%) were infected with C. parapsilosis, and 3 (20%) were infected with other Candida species. In the medical antifungal combination therapy cohort, 9 (47.4%) patients were infected with C. albicans, 8 (42.1%) with C. parapsilosis, and 2 (10.5%) with other Candida species. Finally, in the adjunctive surgical plus medical antifungal cohort, 25 (43.1%) patients were infected with C. albicans, 21 (36.2%) with C. parapsilosis, and 12 (20.7%) with other Candida species.

The majority of reported cases of Candida IE were left-sided disease. After 1980, in the medical antifungal monotherapy cohort there were 80% (12/15) reported with left-sided infection, 63.1% (12/19) with left-sided infection in the medical antifungal combination cohort, and 82.7% (48/58) with left-sided IE in the adjunctive surgical plus
Candida endocarditis therapy

antifungal therapy group. The majority of cases were also native valve IE. After 1980, in the medical antifungal monotherapy cohort there were 73.3% (11/15) reported with native valve infection, 63.1% (12/19) with native valve infection in the medical antifungal combination cohort, 62.1% (36/58) with native valve IE in the adjunctive surgical plus antifungal therapy group.

The most common antifungal therapy reported after 1980 remained amphotericin B, generally reported at doses of 0.5–1.0 mg/kg/day. Amphotericin B was reported as treatment in 53.3% (8/15) of patients with medical antifungal monotherapy, and 48.3% (28/58) of patients with adjunctive surgical plus antifungal therapy. Combination antifungal therapy with amphotericin B + flucytosine was used in 73.7% (14/19) of patients with medical antifungal combination therapy, and 36.2% (21/58) of patients with adjunctive surgical plus antifungal therapy.

Patient outcome in the cases reported after 1980 was dichotomized based on reported overall patient mortality. In the medical antifungal monotherapy cohort there were 6/11 reported survivors with native valve endocarditis and 2/4 reported survivors with prosthetic valve endocarditis. In the combination antifungal therapy cohort there were 8/12 reported survivors with native valve endocarditis and 4/7 reported survivors with prosthetic valve endocarditis. In the adjunctive surgical plus antifungal therapy cohort there were 24/36 reported survivors with native valve endocarditis and 19/22 reported survivors with prosthetic valve endocarditis.

We evaluated 22 observational case-series from all the reviewed series of Candida endocarditis using meta-analytic techniques (Table 3). A total of 97 patients were reported in those 22 observational series, and 72 patients met the definition of definite Candida endocarditis by modified Duke criteria for analysis. Mortality ranged from 0 to 100%, and there was evidence of heterogeneity (Egger p-value 0.09). We found that in the reports in which a higher proportion of patients received medical antifungal monotherapy (POR = 1.49; 95% CI = 0.39, 5.81) and in those reports with a higher frequency of left-sided endocarditis (POR = 2.36; 95% CI = 0.55, 10.07) patients trended toward a higher reported mortality. Also associated with higher reported mortality was the proportion of patients infected with C. parapsilosis (POR = 1.51, 95% CI = 0.41, 5.52), and cases reported before 1980 (POR = 2.03, 95% CI = 0.55, 7.61). Adjunctive surgery (POR = 0.56, 95% CI = 0.16–1.99) was associated with a lower reported mortality.

Discussion

Candida IE has been reviewed and in several studies the overall mortality is approximately 80%. Some studies noted no difference in mortality with antifungal treatment versus surgical intervention, but mortality did decrease in those patients who underwent both surgical replacement and antifungal therapy. This analysis is the largest literature review of Candida IE treatment and outcome, presenting the cases as both a descriptive literature review (Tables 1 and 2, Appendices A–C) and a meta-analysis of evaluable cases (Table 3).

The findings in this investigation suggest that antifungal monotherapy (primarily with amphotericin B) without adjunctive surgery was associated with the poorest patient outcome. Interestingly, the clinical outcomes were similar for those patients receiving combination antifungal agents without surgery and patients receiving adjunctive surgical intervention. This observation suggests that while surgical therapy remains the cornerstone of therapy for most patients with Candida IE, combination antifungal therapy may provide an incremental advantage to monotherapy for those patients in whom surgery is not an option.

Several patient characteristics were associated with mortality in the meta-analysis. Left-sided involvement, infection prior to 1980, and antifungal monotherapy were associated with patient mortality. Patients reported prior to 1980 had a higher reported mortality (POR = 2.03), which could be related to delays in diagnosis due to a lack of echocardiography or the reported use of generally lower doses of amphotericin B (<1 mg/kg/day) in cases during that time period. Interestingly, surgery appeared to confer a protective effect to patients with Candida IE, as series in which all patients underwent surgery had lower mortality rates (POR = 0.56).

Although historically amphotericin B has been considered the ‘gold standard’ for antifungal therapy for decades, a growing body of evidence suggests that it may not be an optimal therapy for Candida IE. Amphotericin B fails to penetrate well into fibrin clots and vegetations, leading to lower in vivo activity than predicted by in vitro testing. For example, in vitro testing of amphotericin B on C. albicans enmeshed in human fibrin clots showed amphotericin B was unable to sterilize even with constant exposure for 96 h at concentrations higher than safely attainable in the blood. Additionally, acute and chronic toxicities of amphotericin B are common and often severe.
Both fluconazole and flucytosine also have important limitations. Fluconazole is fungistatic and has been shown to be inferior compared to amphotericin B in IE animal models.26,27 Flucytosine monotherapy is also ineffective27,28 and rapidly results in drug resistance. The echinocandins are new fungicidal antifungals with excellent activity against Candida biofilms.29–31 While there are little clinical data on echinocandin activity against Candida endocarditis, success in a biofilm model may possibly mimic the vegetation environment of endocarditis.31

The findings of the current review suggest that combination antifungal therapies, primarily amphotericin B + flucytosine, might be associated with better clinical outcomes compared with antifungal monotherapy. The findings also suggest that in select patients in whom surgical therapy is not an alternative, combination therapy can optimize the chance for treatment success. Whether the availability of new rapidly fungicidal agents such as echinocandins can make it possible to treat this lethal infection in select patients with medical therapy alone is unknown.

This literature review and meta-analysis have the limitations of any retrospective analyses of a large number of heterogeneous, uncontrolled observational studies and case reports. We attempted to standardize the diagnosis by using only definitive IE cases according to the modified Duke criteria,12 and a case was disregarded if we could not reliably conclude it was truly definite IE.

### Table 1

Details of 92 reported cases of *Candida* infective endocarditis (1980–2002)

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. cases</th>
<th>Reported successful outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of cardiac valve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right-sided</td>
<td>20</td>
<td>70.0% (14/20)</td>
</tr>
<tr>
<td>Medical antifungal monotherapy</td>
<td>3</td>
<td>33.3% (1/3)</td>
</tr>
<tr>
<td>Medical antifungal combination</td>
<td>7</td>
<td>71.4% (5/7)</td>
</tr>
<tr>
<td>Medical antifungal with surgery</td>
<td>10</td>
<td>80.0% (8/10)</td>
</tr>
<tr>
<td>Left-sided</td>
<td>72</td>
<td>68.1% (49/72)</td>
</tr>
<tr>
<td>Medical antifungal monotherapy</td>
<td>12</td>
<td>58.3% (7/12)</td>
</tr>
<tr>
<td>Medical antifungal combination</td>
<td>12</td>
<td>58.3% (7/12)</td>
</tr>
<tr>
<td>Medical antifungal with surgery</td>
<td>48</td>
<td>72.9% (35/48)</td>
</tr>
<tr>
<td>Type of infected valve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native valve</td>
<td>59</td>
<td>64.4% (38/59)</td>
</tr>
<tr>
<td>Medical antifungal monotherapy</td>
<td>11</td>
<td>54.5% (6/11)</td>
</tr>
<tr>
<td>Medical antifungal combination</td>
<td>12</td>
<td>66.7% (8/12)</td>
</tr>
<tr>
<td>Medical antifungal with surgery</td>
<td>36</td>
<td>66.7% (24/36)</td>
</tr>
<tr>
<td>Prosthetic valve</td>
<td>33</td>
<td>75.8% (25/33)</td>
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<tr>
<td>Medical antifungal monotherapy</td>
<td>4</td>
<td>50.0% (2/4)</td>
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<td>Medical antifungal combination</td>
<td>7</td>
<td>57.1% (4/7)</td>
</tr>
<tr>
<td>Medical antifungal with surgery</td>
<td>22</td>
<td>86.4% (19/22)</td>
</tr>
<tr>
<td>Specific treatment details</td>
<td></td>
<td></td>
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<tr>
<td>Medical antifungal monotherapy</td>
<td>15</td>
<td>53.3% (8/15)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>8</td>
<td>75.0% (6/8)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>6</td>
<td>16.7% (1/6)</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>1</td>
<td>100% (1/1)</td>
</tr>
<tr>
<td>Medical antifungal combination</td>
<td>19</td>
<td>63.2% (12/19)</td>
</tr>
<tr>
<td>Amphotericin B + flucytosine</td>
<td>14</td>
<td>64.3% (9/14)</td>
</tr>
<tr>
<td>Amphotericin B + fluconazole</td>
<td>3</td>
<td>66.7% (2/3)</td>
</tr>
<tr>
<td>Amphotericin B + rifampin + flucytosine</td>
<td>1</td>
<td>100% (1/1)</td>
</tr>
<tr>
<td>Amphotericin B + fluconazole + flucytosine</td>
<td>1</td>
<td>0% (0/1)</td>
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<td>Medical antifungal with surgery</td>
<td>58</td>
<td>63% (67/107)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>28</td>
<td>67.9% (19/28)</td>
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<tr>
<td>Amphotericin B + flucytosine</td>
<td>21</td>
<td>76.2% (16/21)</td>
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<tr>
<td>Miconazole</td>
<td>3</td>
<td>66.7% (2/3)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>3</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Amphotericin B + fluconazole</td>
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<td>100% (1/1)</td>
</tr>
<tr>
<td>Fluconazole + flucytosine</td>
<td>1</td>
<td>100% (1/1)</td>
</tr>
<tr>
<td>Amphotericin B + fluconazole + flucytosine</td>
<td>1</td>
<td>100% (1/1)</td>
</tr>
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</table>
However, we are dependent on the individual investigator’s reports for other variables of patient care including the inherent differences in the hosts, antifungal dosing, supportive care management, and investigator-assessed outcome. Importantly, the reported outcomes are subjective and based on the individual investigator’s own biases, thereby making it impossible to draw firm recommendations from the descriptive results. There is also potential for significant selection bias for better outcome in the choice of patients who are reported in the literature. Additionally, there is possible bias in those patients who might be considered surgical candidates, with the sickest patients often excluded from surgical options.

While the decision to exclude cases not receiving at least 7 days of antifungal therapy biases the review by excluding early episodes of failed medical therapy, we felt it would create worse bias by including the myriad of cases where a dying patient was given 1-2 days of an antifungal and that patient’s death deemed due to antifungal therapy failure. On the other hand, there was little mention in the individual reports of long-term suppressive antifungal therapy, so it is impossible to analyse how that may have impacted treatment outcome. Additionally, issues of valvular dysfunction have not been considered, and particularly how it might relate to medical versus surgical therapy. Finally, for most cases the outcome was reported after

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Patient age and pathogen for 92 reported cases of Candida infective endocarditis reviewed (1980-2002)</th>
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<tr>
<td>Variable</td>
<td>No. cases</td>
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<td>Infant (&lt;4 months)</td>
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<td>Medical antifungal monotherapy</td>
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<td>7</td>
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<td>Medical antifungal with surgery</td>
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<tr>
<td>Child (4 months-18 years)</td>
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<td>Medical antifungal monotherapy</td>
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<td>Medical antifungal combination</td>
<td>3</td>
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<tr>
<td>Medical antifungal with surgery</td>
<td>5</td>
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<td>Adult (&gt;18 years)</td>
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<td>Medical antifungal with surgery</td>
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<td>Candida species</td>
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<td>Candida albicans</td>
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<tr>
<td>Medical antifungal with surgery</td>
<td>25</td>
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<td>Candida parapsilosis</td>
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<tr>
<td>Medical antifungal with surgery</td>
<td>21</td>
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<tr>
<td>Other Candida species</td>
<td>17</td>
</tr>
<tr>
<td>Medical antifungal monotherapy</td>
<td>3</td>
</tr>
<tr>
<td>Medical antifungal combination</td>
<td>2</td>
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<tr>
<td>Medical antifungal with surgery</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Meta-regression analysis of Candida infective endocarditis (1966-2002) using mortality as outcome (n=22 studies; 72 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable</td>
<td>Prevalence odds ratio</td>
</tr>
<tr>
<td>Left-sided Candida endocarditis</td>
<td>2.36</td>
</tr>
<tr>
<td>Studies prior to 1980</td>
<td>2.03</td>
</tr>
<tr>
<td>Infection with C. parapsilosis</td>
<td>1.51</td>
</tr>
<tr>
<td>Antifungal monotherapy</td>
<td>1.49</td>
</tr>
<tr>
<td>Adjunctive surgery</td>
<td>0.56</td>
</tr>
</tbody>
</table>
some post-therapy follow-up but for some the exact duration was not specified, making it impossible to incorporate that aspect into any meaningful analysis.

In conclusion, Candida IE is a difficult disease to treat effectively and the present guidelines have acknowledged a weak evidence-based foundation. Clinical care has changed dramatically over the review period, but the general perception of Candida IE therapy seems to be unchanged based on the lack of prospective studies and controlled clinical trials, as demonstrated by unchanging published recommendations.\textsuperscript{10,11} Antifungal monotherapy has led to relatively poor outcomes, while combination antifungal therapy alone appears to possibly approach the success of adjunctive surgery. With the recent availability of less-toxic agents such as the echinocandins or the extended spectrum triazoles, the medical options for Candida IE have increased. The optimal antifungal regimen for Candida IE is not clear, and no specific regimen can be firmly recommended. However, medical antifungal therapy of Candida IE deserves study. With newer fungicidal agents now available and the development of a multicentre collaborative effort such as the 23-site International Collaboration on Endocarditis,\textsuperscript{32} we are poised for a prospective pilot study to determine optimal antifungal therapy and question the primary requirement for surgery.

\textbf{Acknowledgements}

WJS is supported by NIAID 1 K08 A1061149-01, and DKB received support from NICHD 1 R03 HD42940-02.

\textbf{Appendix A: Previously reported cases of medical antifungal monotherapy for definite cases of Candida endocarditis (1966-2002; \textit{n} = 31 cases)}

\begin{center}
\begin{tabular}{llllllll}
Age (yrs) & Underlying risk factor & Affected valve (native or prosthetic) & Organism & Medical treatment & Duration & Outcome & Year & Reference \\
--- & --- & --- & --- & --- & --- & --- & --- & --- \\
26 & Rheumatic heart disease & (N) Aortic & \textit{C. albicans} & AmB & 50 days & Alive & 1962 & 33 \\
34 & Mitral valve commissurotomy & (N) Mitral & \textit{C. albicans} & AmB & 11 weeks & Expired & 1966 & 34 \\
73 & None & (N) Aortic & \textit{C. albicans} & AmB, then flucytosine & 1 week, then 40 days & Alive & 1971 & 35 \\
53 & Aortic valve debridement & (N) Aortic & \textit{C. albicans} & AmB, then flucytosine, then clotrimazole Nystatin & 9 months & Expired & 1972 & 37 \\
53 & Tricuspid valve trauma & (N) Tricuspid & \textit{C. albicans} & Flucytosine & 12 days & Expired & 1973 & 38 \\
60 & Aortic, tricuspid, mitral valve replacement & (P) Aortic, tricuspid, mitral & \textit{C. albicans} & Flucytosine & 46 days & Expired & 1973 & 39 \\
29 & Aortic valve replacement & (P) Aortic & \textit{C. albicans} & AmB & 8 weeks & Alive & 1968 & 40 \\
26 & Aortic valve replacement & (P) Aortic & \textit{C. albicans} & AmB & 4 months & Expired & 1971 & 41 \\
58 & Aortic valve replacement & (P) Aortic & \textit{C. albicans} & AmB & NR & Expired & 1971 & 41 \\
57 & Aortic valve replacement & (P) Aortic & \textit{C. parapsilosis} & AmB, then flucytosine & 2 weeks, then 11 weeks & Expired & 1971 & 35 \\
\end{tabular}
\end{center}

(continued on next page)
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Medical treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Aortic, tricuspid, mitral valve replacement</td>
<td>Aortic, tricuspid, (P) mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>46 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>62</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>14 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>60</td>
<td>Aortic, tricuspid, mitral valve replacement, radiation therapy</td>
<td>Aortic, tricuspid, (P) mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>69 days</td>
<td>Expired</td>
<td>1975</td>
<td>42</td>
</tr>
<tr>
<td>48</td>
<td>Mitral, aortic valve replacement</td>
<td>Mitral, (P) aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>37 days</td>
<td>Expired</td>
<td>1975</td>
<td>42</td>
</tr>
<tr>
<td>83</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>58 days</td>
<td>Expired</td>
<td>1989</td>
<td>43</td>
</tr>
<tr>
<td>64</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>Fluconazole</td>
<td>50 days</td>
<td>Expired</td>
<td>1991</td>
<td>44</td>
</tr>
<tr>
<td>64</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>Fluconazole</td>
<td>50 days</td>
<td>Expired</td>
<td>1991</td>
<td>44</td>
</tr>
<tr>
<td>43 days</td>
<td>29 week gestation</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>75 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
</tr>
<tr>
<td>47</td>
<td>Broad spectrum antibiotics</td>
<td>(N) Mitral, tricuspid</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>17 months</td>
<td>Alive</td>
<td>1995</td>
<td>46</td>
</tr>
<tr>
<td>44</td>
<td>HIV</td>
<td>(N) Mitral</td>
<td><em>C. zeylanoides</em></td>
<td>AmB</td>
<td>2 g</td>
<td>Alive</td>
<td>1996</td>
<td>47</td>
</tr>
<tr>
<td>51</td>
<td>Mitral valve commissurotomy</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>8</td>
</tr>
<tr>
<td>12 days</td>
<td>32 weeks gestation</td>
<td>(N) Tricuspid, Pulmonary</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>7 days</td>
<td>Expired</td>
<td>1996</td>
<td>48</td>
</tr>
<tr>
<td>14</td>
<td>Liver transplantation</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>25 days</td>
<td>Abdominal surgery, hyperalimentation</td>
<td>(N) Pulmonary</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>20 days, then NR</td>
<td>Expired</td>
<td>2000</td>
<td>50</td>
</tr>
<tr>
<td>72</td>
<td>Abdominal surgery, hyperalimentation</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>Fluconazole</td>
<td>16 weeks, then NR</td>
<td>Alive</td>
<td>2001</td>
<td>51</td>
</tr>
<tr>
<td>74</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1997</td>
<td>52</td>
</tr>
<tr>
<td>56</td>
<td>Rheumatic heart disease, mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB, then fluconazole</td>
<td>8 weeks, then 1 year</td>
<td>Alive</td>
<td>2001</td>
<td>53</td>
</tr>
<tr>
<td>58</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB, then fluconazole</td>
<td>26 months</td>
<td>Alive</td>
<td>1993</td>
<td>54</td>
</tr>
<tr>
<td>45</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>Fluconazole, then AmB, then ABLC</td>
<td>12 months</td>
<td>Expired</td>
<td>1995</td>
<td>55</td>
</tr>
</tbody>
</table>

AmB, amphotericin B; ABLC, amphotericin B lipid complex; IVDA, intravenous drug abuser; HIV, human immunodeficiency virus; NR, not recorded.
Appendix B: Previously reported cases of medical combination antifungal therapies for definite cases of *Candida* endocarditis (1966–2002; *n* = 25 cases)

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Heroin abuse, aortic patch</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ clotrimazole, AmB+ fluconazole</td>
<td>7 days</td>
<td>Expired</td>
<td>1974</td>
<td>56</td>
</tr>
<tr>
<td>57</td>
<td>Alcoholism, malnutrition</td>
<td>(N) Aortic</td>
<td><em>C. glabrata</em></td>
<td>AmB+ flucytosine</td>
<td>3 months</td>
<td>Alive</td>
<td>1975</td>
<td>19</td>
</tr>
<tr>
<td>42</td>
<td>Heroin abuse</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine</td>
<td>4 months</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
</tr>
<tr>
<td>37 days</td>
<td>34 week gestation, TEF, PDA, VSD</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then AmB for 7 weeks</td>
<td>7 days</td>
<td>Alive</td>
<td>1977</td>
<td>57</td>
</tr>
<tr>
<td>43</td>
<td>Heroin abuse, mitral valve regurgitation</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine</td>
<td>7 weeks</td>
<td>Alive</td>
<td>1978</td>
<td>58</td>
</tr>
<tr>
<td>17</td>
<td>Rheumatic heart disease, heroin addict, mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine</td>
<td>7 days</td>
<td>Expired</td>
<td>1976</td>
<td>18</td>
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<tr>
<td>36</td>
<td>Heroin abuse</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine, then flucytosine for life</td>
<td>3 weeks</td>
<td>Alive</td>
<td>1980</td>
<td>59</td>
</tr>
<tr>
<td>6 days</td>
<td>29 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine</td>
<td>8 days</td>
<td>Expired</td>
<td>1983</td>
<td>60</td>
</tr>
<tr>
<td>12 days</td>
<td>27 week gestation</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine</td>
<td>102 days</td>
<td>Alive</td>
<td>1990</td>
<td>61</td>
</tr>
<tr>
<td>73 days</td>
<td>24 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine</td>
<td>98 days</td>
<td>Expired</td>
<td>1991</td>
<td>62</td>
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<td>91 days</td>
<td>Trisomy 21, ASD, PDA, pulmonic, mitral</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine, then AmB for 4 weeks</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1991</td>
<td>62</td>
</tr>
<tr>
<td>73 days</td>
<td>24 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine</td>
<td>98 days</td>
<td>Alive</td>
<td>1991</td>
<td>62</td>
</tr>
<tr>
<td>26 days</td>
<td>Apneic episode, central venous catheter</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB+ Rifampin+ flucytosine</td>
<td>30 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
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<tr>
<td>29 days</td>
<td>26 week gestation</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then AmB for 4 weeks</td>
<td>4 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
</tr>
<tr>
<td>73</td>
<td>Candidemia three months earlier, not treated</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>AmB+ flucytosine</td>
<td>NR</td>
<td>Expired</td>
<td>1998</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>Acute lymphoblastic leukemia</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB+ fluconazole, then fluconazole for 2 weeks</td>
<td>3 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>64</td>
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</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Tetralogy of fallot</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then fluconazole for life</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>16</td>
<td>Renal transplantation</td>
<td>(N) Mitral, aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine + fluconazole</td>
<td>5 months</td>
<td>Expired</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>37</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine, then fluconazole</td>
<td>NR</td>
<td>Alive</td>
<td>1988</td>
<td>65</td>
</tr>
<tr>
<td>58</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ fluconazole, then fluconazole</td>
<td>2 weeks</td>
<td>Expired</td>
<td>1993</td>
<td>66</td>
</tr>
<tr>
<td>57</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB+ fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1994</td>
<td>67</td>
</tr>
<tr>
<td>61</td>
<td>Aortic valve prosthesis</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>68</td>
</tr>
<tr>
<td>61</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine</td>
<td>5 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>8</td>
</tr>
<tr>
<td>41</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. lusitaniae</em></td>
<td>AmB+ flucytosine, fluconazole + flucytosine, then fluconazole 4 months, then AmB+ flucytosine for relapse for 3 months</td>
<td>5 weeks</td>
<td>Expired</td>
<td>1998</td>
<td>69</td>
</tr>
<tr>
<td>51</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine (7 days) then caspofungin + fluconazole (6 weeks), then caspofungin then ABLC</td>
<td>7 weeks, 6 weeks, 20 days, 2 g</td>
<td>Expired</td>
<td>2002</td>
<td>70</td>
</tr>
</tbody>
</table>

NR, not recorded; AmB, amphotericin B; TEF, tracheal-esophageal fistula; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus.
Appendix C: Previously reported cases of adjunctive surgical and medical antifungal therapies for definite cases of *Candida* endocarditis (1966–2002; \( n = 107 \) cases)

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Medical treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Bacterial endocarditis</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>9 months</td>
<td>Expired</td>
<td>1968</td>
<td>71</td>
</tr>
<tr>
<td>34</td>
<td>None</td>
<td>(N) Aortic</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>12 weeks</td>
<td>Alive</td>
<td>1968</td>
<td>71</td>
</tr>
<tr>
<td>29</td>
<td>Rheumatic heart disease</td>
<td>(N) Aortic</td>
<td><em>C. parakrusei</em></td>
<td>AmB</td>
<td>2 months</td>
<td>Alive</td>
<td>1968</td>
<td>71</td>
</tr>
<tr>
<td>30</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. guilliermondii</em></td>
<td>AmB, Clotrimazole</td>
<td>NR</td>
<td>Expired</td>
<td>1972</td>
<td>72</td>
</tr>
<tr>
<td>39</td>
<td>IVDA</td>
<td>(N) Aortic, mitral, tricuspid</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
</tr>
<tr>
<td>28</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. krusei</em></td>
<td>AmB + flucytosine</td>
<td>33 days</td>
<td>Expired</td>
<td>1972</td>
<td>73</td>
</tr>
<tr>
<td>23</td>
<td>IVDA</td>
<td>(N) Tricuspid</td>
<td><em>C. tropicalis</em></td>
<td>AmB + flucytosine (10d)</td>
<td>1 month</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
</tr>
<tr>
<td>30</td>
<td>IVDA Corticosteroid</td>
<td>(N) Pulmonary tricuspid</td>
<td><em>C. stellatoidea</em></td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1972</td>
</tr>
<tr>
<td>49</td>
<td>None</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>Flucytosine</td>
<td>3 months</td>
<td>Alive</td>
<td>1974</td>
<td>75</td>
</tr>
<tr>
<td>23</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>15 days</td>
<td>Expired</td>
<td>1974</td>
<td>76</td>
</tr>
<tr>
<td>22</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>AmB</td>
<td>15 days</td>
<td>Alive</td>
<td>1974</td>
<td>76</td>
</tr>
<tr>
<td>56</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1974</td>
<td>77</td>
</tr>
<tr>
<td>44</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. parakrusei</em></td>
<td>AmB</td>
<td>1 week</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
</tr>
<tr>
<td>38</td>
<td>IVDA</td>
<td>(N) Mitral</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1975</td>
<td>78</td>
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<td>26</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. glabrata</em></td>
<td>AmB</td>
<td>10 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>35</td>
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<td>(N) Aortic</td>
<td><em>C. guilliermondii</em></td>
<td>AmB</td>
<td>1 week</td>
<td>Alive</td>
<td>1975</td>
<td>78</td>
</tr>
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<td>35</td>
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<td><em>C. parakrusei</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td><em>C. guilliermondii</em></td>
<td>AmB</td>
<td>5 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>10 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
</tr>
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<td>63</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td>Candida spp.</td>
<td>AmB, then flucytosine</td>
<td>19 days</td>
<td>Expired</td>
<td>1975</td>
<td>72</td>
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<td>42</td>
<td>Peritonitis Rheumatic heart disease</td>
<td>(N) Mitral</td>
<td><em>C. glabrata</em></td>
<td>AmB</td>
<td>1.5 g</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
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<td>Peritonitis Rheumatic heart disease</td>
<td>(N) Mitral and mitral</td>
<td><em>C. stellatoidea</em></td>
<td>AmB</td>
<td>2 weeks</td>
<td>Expired</td>
<td>1975</td>
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<td>None</td>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>1.7 g</td>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>1 g</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
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<td>Rheumatic heart disease</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>3 g</td>
<td>Alive</td>
<td>1975</td>
<td>19</td>
</tr>
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<td>27</td>
<td>Bacterial endocarditis</td>
<td>(N) Mitral</td>
<td><em>C. tropicalis</em></td>
<td>AmB</td>
<td>1 g</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
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<td>55</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>NR</td>
<td>Alive</td>
<td>1976</td>
<td>79</td>
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<td>54</td>
<td>Rheumatic heart disease</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>14 days</td>
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<td>Abdominal surgery</td>
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<td>AmB</td>
<td>30 days</td>
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<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1976</td>
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<td>(N) Mitral</td>
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<td>AmB</td>
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<td>1977</td>
<td>82</td>
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<td>Age (yrs)</td>
<td>Underlying risk factor</td>
<td>Affected valve (native or prosthetic)</td>
<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
<td>Reference</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine AmB</td>
<td>2 months</td>
<td>Alive</td>
<td>1979</td>
<td>83</td>
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<td>40</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>5 weeks</td>
<td>Alive</td>
<td>1968</td>
<td>84</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parakrusei</td>
<td>AmB</td>
<td>5 months</td>
<td>Expired</td>
<td>1970</td>
<td>85</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parakrusei</td>
<td>AmB</td>
<td>NR</td>
<td>Alive</td>
<td>1970</td>
<td>86</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>Candida spp.</td>
<td>AmB + flucytosine AmB</td>
<td>3 months</td>
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<td>1971</td>
<td>87</td>
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<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td>C. albicans</td>
<td>AmB + flucytosine AmB</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1971</td>
<td>35</td>
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<td>51</td>
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<td>Aortic valve replacement</td>
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<td>C. parapsilosis</td>
<td>AmB</td>
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<td>Expired</td>
<td>1973</td>
<td>39</td>
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<td>(P) Aortic</td>
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<td>AmB + flucytosine AmB</td>
<td>2 months</td>
<td>Alive</td>
<td>1975</td>
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<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td>C. albicans</td>
<td>AmB</td>
<td>2.2 g</td>
<td>Alive</td>
<td>1975</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. glabrata</td>
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<td>8 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>90</td>
</tr>
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<td>(P) Aortic</td>
<td>C. albicans</td>
<td>AmB</td>
<td>25 days</td>
<td>Alive</td>
<td>1976</td>
<td>91</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>Candida spp.</td>
<td>Flucytosine</td>
<td>1 year</td>
<td>Alive</td>
<td>1977</td>
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<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB, then flucytosine, then AmB + flucytosine AmB</td>
<td>3 months</td>
<td>Expired</td>
<td>1977</td>
<td>93</td>
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<td>36</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB, then flucytosine, then miconazole AmB, then flucytosine, then miconazole Miconazole</td>
<td>4 months</td>
<td>Expired</td>
<td>1977</td>
<td>93</td>
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<td>(P) Aortic</td>
<td>C. albicans</td>
<td>AmB + flucytosine</td>
<td>10 weeks</td>
<td>Alive</td>
<td>1977</td>
<td>94</td>
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<td>63</td>
<td>IDDM, abdominal surgery</td>
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<td>C. glabrata</td>
<td>AmB + flucytosine</td>
<td>41 days</td>
<td>Alive</td>
<td>1980</td>
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<td>IVDA</td>
<td>(N) Mitral</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>4 g</td>
<td>Alive</td>
<td>1984</td>
<td>96</td>
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<td>52</td>
<td>IHSS</td>
<td>(N) Mitral</td>
<td>C. tropicalis</td>
<td>AmB</td>
<td>3.0 g</td>
<td>Expired</td>
<td>1984</td>
<td>97</td>
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<td>31</td>
<td>Renal and pancreatic tail transplantation</td>
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<td>C. albicans</td>
<td>AmB, then ketoconazole</td>
<td>4 weeks</td>
<td>Alive</td>
<td>1986</td>
<td>98</td>
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<td>36</td>
<td>Corticosteroid therapy for SLE</td>
<td>(N) Mitral</td>
<td>C. albicans</td>
<td>AmB</td>
<td>15 days</td>
<td>Expired</td>
<td>1986</td>
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<td>8 weeks</td>
<td>32 week gestation</td>
<td>(N) Tricuspid</td>
<td>C. albicans</td>
<td>Miconazole, then AmB + flucytosine Miconazole</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1987</td>
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<td>16</td>
<td>Ventricular septal defect</td>
<td>(N) Aortic</td>
<td>C. albicans</td>
<td>AmB</td>
<td>2 weeks</td>
<td>Expired</td>
<td>1987</td>
<td>101</td>
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<td>30</td>
<td>Pancreaticoduodenectomy</td>
<td>(N) Aortic</td>
<td>C. tropicalis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1987</td>
<td>101</td>
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<td>25</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td>C. albicans</td>
<td>AmB</td>
<td>8 days</td>
<td>Expired</td>
<td>1987</td>
<td>102</td>
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<td>Age (yrs)</td>
<td>Underlying risk factor</td>
<td>Affected valve (native or prosthetic)</td>
<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
<td>Reference</td>
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<td>62</td>
<td>Candidemia with central catheter</td>
<td>Tricuspid</td>
<td>C. albicans</td>
<td>AmB + flucytosine</td>
<td>NR</td>
<td>Expired</td>
<td>1988</td>
<td>103</td>
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<td>Ruptured esophagus</td>
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<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>4 weeks</td>
<td>Alive</td>
<td>1989</td>
<td>104</td>
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<td>43</td>
<td>Mitral commissurotomy Colectomy</td>
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<td>C. albicans</td>
<td>AmB, then flucytosine</td>
<td>2 months</td>
<td>Alive</td>
<td>1989</td>
<td>105</td>
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<td>22</td>
<td></td>
<td>(N) Tricuspid</td>
<td>C. albicans</td>
<td>AmB, Miconazole, flucytosine</td>
<td>NR</td>
<td>Alive</td>
<td>1989</td>
<td>106</td>
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<td>Testicular carcinoma</td>
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<td>C. albicans</td>
<td>AmB + flucytosine, then AmB</td>
<td>NR</td>
<td>Expired</td>
<td>1991</td>
<td>107</td>
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<td>IVDA</td>
<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>12 weeks</td>
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<td>C. parapsilosis</td>
<td>AmB + flucytosine, then ketoconazole</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>41</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>78</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>5 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>Allogeneic BMT</td>
<td>(N) Mitral</td>
<td>C. parapsilosis</td>
<td>AmB, then fluconazole</td>
<td>4 months</td>
<td>Alive</td>
<td>1994</td>
<td>110</td>
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<td>26</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1994</td>
<td>111</td>
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<td>66</td>
<td>None</td>
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<td>C. glabrata</td>
<td>Miconazole, fluconazole</td>
<td>16 days</td>
<td>Alive</td>
<td>1994</td>
<td>112</td>
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<td>67</td>
<td>Abdominal surgery</td>
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<td>C. albicans</td>
<td>L-Amb + flucytosine, then fluconazole</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>8</td>
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<td>38</td>
<td>Aortic regurgitation</td>
<td>(N) Aortic</td>
<td>C. albicans</td>
<td>AmB, then ketoconazole</td>
<td>30 days</td>
<td>Alive</td>
<td>1996</td>
<td>68</td>
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<td>Hyper-immunoglobulinemia E syndrome</td>
<td>Tricuspid</td>
<td>C. albicans</td>
<td>AmB + flucytosine, then fluconazole</td>
<td>4 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>113</td>
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<td>57</td>
<td>Alcoholic cirrhosis, candidemia untreated</td>
<td>Tricuspid</td>
<td>C. glabrata</td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>114</td>
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<td>Sickle cell disease</td>
<td>(N) Tricuspid</td>
<td>C. tropicalis</td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>115</td>
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<td>42</td>
<td>Percutaneous transluminal coronary angioplasty</td>
<td>(N) Aortic</td>
<td>C. parapsilosis and C. albicans</td>
<td>AmB, then fluconazole</td>
<td>1 g</td>
<td>Alive</td>
<td>1998</td>
<td>116</td>
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<tr>
<td>57</td>
<td>Prolonged hyper-alimentation</td>
<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>Fluconazole</td>
<td>18 months</td>
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<td>1998</td>
<td>117</td>
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<td>Age (yrs)</td>
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<td>Affected valve (native or prosthetic)</td>
<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
<td>Reference</td>
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<td>42</td>
<td>Aortic abscess</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>Fluconazole + flucytosine + AmB, then fluconazole</td>
<td>1 g</td>
<td>Alive</td>
<td>1998</td>
<td>118</td>
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<td>2</td>
<td>VSD, PDA 25 week</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB, then AmB</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1999</td>
<td>49</td>
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<td>1 week</td>
<td>25 week gestation</td>
<td>(N) Pulmonary</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>4 weeks</td>
<td>Alive</td>
<td>2000</td>
<td>119</td>
</tr>
<tr>
<td>28</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>2000</td>
<td>120</td>
</tr>
<tr>
<td>29</td>
<td>HIV, IVDA</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>2000</td>
<td>121</td>
</tr>
<tr>
<td>42</td>
<td>IVDA</td>
<td>(N) Mitral</td>
<td>Pichia ohmeri (telemorph of C. guilliermondii)</td>
<td>AmB + flucytosine</td>
<td>8 weeks</td>
<td>Alive</td>
<td>2002</td>
<td>122</td>
</tr>
<tr>
<td>24</td>
<td>IVDA</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>2 g</td>
<td>Alive</td>
<td>1980</td>
<td>123</td>
</tr>
<tr>
<td>5</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>4 months</td>
<td>Alive</td>
<td>1980</td>
<td>124</td>
</tr>
<tr>
<td>34</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>2 months</td>
<td>Alive</td>
<td>1980</td>
<td>124</td>
</tr>
<tr>
<td>53</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>20 weeks</td>
<td>Alive</td>
<td>1980</td>
<td>125</td>
</tr>
<tr>
<td>26</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. glabrata</em></td>
<td>AmB + flucytosine</td>
<td>4 weeks</td>
<td>Alive</td>
<td>1980</td>
<td>125</td>
</tr>
<tr>
<td>67</td>
<td>Mitral valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1984</td>
<td>126</td>
</tr>
<tr>
<td>44</td>
<td>Aortic and mitral valve replacement</td>
<td>(P) Aortic</td>
<td>Candidaspp.</td>
<td>Flucytosine</td>
<td>NR</td>
<td>Alive</td>
<td>1988</td>
<td>127</td>
</tr>
<tr>
<td>27</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>16 months</td>
<td>Alive</td>
<td>1990</td>
<td>128</td>
</tr>
<tr>
<td>60</td>
<td>Aortic and mitral valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB, then ketoconazole</td>
<td>3 g</td>
<td>Alive</td>
<td>1997</td>
<td>129</td>
</tr>
<tr>
<td>47</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB, then ketoconazole, then AmB + flucytosine</td>
<td>6 months</td>
<td>Alive</td>
<td>1997</td>
<td>129</td>
</tr>
<tr>
<td>NR</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>57 days</td>
<td>Alive</td>
<td>1997</td>
<td>130</td>
</tr>
<tr>
<td>61</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1998</td>
<td>131</td>
</tr>
<tr>
<td>50</td>
<td>Rheumatic heart disease, mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. tropicalis</em></td>
<td>AmB, then relapse and AmB again</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1996</td>
<td>132</td>
</tr>
<tr>
<td>71</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine, then fluconazole</td>
<td>4 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>129</td>
</tr>
<tr>
<td>38</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB, then ketoconazole</td>
<td>1 g</td>
<td>Alive</td>
<td>1997</td>
<td>129</td>
</tr>
<tr>
<td>34</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>16 days</td>
<td>Expired</td>
<td>1997</td>
<td>129</td>
</tr>
<tr>
<td>67</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>L-AmB + flucytosine</td>
<td>5 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>133</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Underlying risk factor</td>
<td>Affected valve (native or prosthetic)</td>
<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------</td>
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<td>------</td>
<td>-----------</td>
</tr>
<tr>
<td>45</td>
<td>Rheumatic heart disease, mitral valvotomy</td>
<td>(P) Mitral</td>
<td>C. albicans</td>
<td>Fluconazole</td>
<td>3 weeks</td>
<td>Alive</td>
<td>2001</td>
<td>134</td>
</tr>
<tr>
<td>77</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>Candida spp.</td>
<td>AmB + flucytosine, then L-AmB + flucytosine, then fluconazole</td>
<td>2 weeks</td>
<td>Alive</td>
<td>2001</td>
<td>135</td>
</tr>
<tr>
<td>46</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. tropicalis</td>
<td>Fluconazole + flucytosine, then fluconazole</td>
<td>45 days</td>
<td>Alive</td>
<td>2001</td>
<td>136</td>
</tr>
<tr>
<td>82</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB</td>
<td>7.6 g</td>
<td>Expired</td>
<td>2002</td>
<td>137</td>
</tr>
<tr>
<td>59</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB, then L-AmB + flucytosine, then fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>2002</td>
<td>137</td>
</tr>
</tbody>
</table>

AmB, amphotericin B; L-AmB, liposomal amphotericin B; flucytosine, flucytosine; IVDA, intravenous drug abuser; IDDM, insulin dependent diabetes mellitus; SLE, systemic lupus erythematosus; PDA, patent ductus arteriosus; VSD, ventricular septal defect; HIV, human immunodeficiency virus; NR, not recorded.

References

Candida endocarditis therapy


Candida endocarditis therapy


Infective endocarditis (IE) refers to an inflammation of the endocardium caused by infectious agents. IE was first described as a disease condition as early as in the 16th century. Since the first descriptions of IE, significant advances have been made in our understanding of the disease, its prevention, diagnosis and treatment. A number of people have contributed to these advances including Sir William Osler, who in 1885 drew a distinction between “simple” and “malignant” forms of endocarditis, which we now refer to as subacute and acute IE respectively.

The incidence of IE has essentially remained unchanged over the last two decades, ranging between 3 and 10 episodes/100,000 person-years. However, the epidemiological profile of IE has changed dramatically in recent years with a large increase in the proportion of cases with Staphylococcal IE due to intravenous drug abuse, infection of prosthetic heart valves and the use of invasive vascular devices. Nosocomial IE (NIE), which is defined as infective endocarditis in patients admitted to a hospital at least 72 h prior to the onset of symptoms of IE or IE in patients with a history of an invasive procedure carried out in a recent hospital admission less than 8 weeks prior to onset of symptoms, has similarly increased in incidence and is a growing problem.

The diagnosis and management of infective endocarditis in the ICU setting can be quite challenging. ICU teams encounter patients with IE who are hemodynamically unstable due to severe sepsis, heart failure or cardiogenic shock as well as patients who have severe valvular pathology due to IE and require cardiac surgery. Patients are also admitted to the ICU after major cardiac surgery for post-operative care and are very susceptible to NIE and other nosocomial infections. Patients on ICU are in general more susceptible to developing IE due to the widespread use of invasive monitoring and therapeutic devices such as central venous lines, mechanical ventilation, in-dwelling urinary catheters, hemofiltration devices and so on.

Despite advances in diagnosis and treatment along with improved antimicrobial treatments and potentially curative surgery, infective endocarditis continues to cause significant morbidity and mortality. Mortality from IE is particularly high in ICU patients ranging from 29 to 54%.

This review article will
provide a concise overview of the pathophysiology, diagnosis and management of IE and provides a specific update on recent developments in all aspects of IE in the ICU setting.

2. Causative organisms

There is limited data on the causative organisms for IE in ICU patients. A few data series available in literature on patients with IE in the ICU setting have found Staphylococci to be the most commonly isolated organisms\(^9\)–\(^11\) with Streptococci being the second most common. In a study of 90 ICU patients with IE, Mouly et al. reported that majority (44%) of the cases were caused by Staphylococci, followed by Streptococci which were responsible for 25% cases.\(^9\) Meticillin Resistant Staphylococcus aureus (MRSA) were seen in 4% cases. Streplococcal species isolated were Streptococcus bovis and Enterococci. Staphylococci were also the predominant organisms responsible for NIE in these patients accounting for 74% of all nosocomial endocarditis. A small number of patients (3.3% of all cases) had fungal endocarditis with 2 cases of Candida and 2 cases of Aspergillus endocarditis. There was 1 case each of endocarditis with gram negative bacilli and HACEK (Haemophilus, Actinobacillus, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae) group of organisms. In another series from France involving 228 patients with endocarditis in the intensive care setting, Staphylococci were again the most commonly isolated organisms\(^10\) and were responsible for 50% of all cases and the majority of nosocomial IE cases. MRSA were seen in 11% of total cases. 4% of cases had fungal endocarditis and in 5% of the cases gram negative bacilli were isolated. Staphylococci were also the predominant organisms in patients with endocarditis secondary to intravenous drug use admitted to the ITU in a series reported from USA.\(^11\) Fungal endocarditis and in particular Candida endocarditis, although still uncommon, is a rising problem in the ICU setting. Candida endocarditis is ten times more common in the ICU as compared to general hospital patients.\(^13,14\) Candida nosocomial endocarditis has also been reported in neonatal intensive care setting, where morbidity and mortality in patients with Candida endocarditis is very high.\(^15,16\) Hence in patients with endocarditis in the ICU setting, there should be a high index of suspicion for fungal endocarditis especially in patients not responding to empirical antibiotics.

3. Pathophysiology

The endothelium represents the internal lining of heart valves and cavities. In normal conditions it forms a continuous smooth surface which, if subjected to injury, can become susceptible to fibrin and platelet deposits leading to the formation of sterile micro-vegetations.\(^1,12\) This endothelial injury can occur as a result of high-velocity turbulent blood flow associated with certain valvular pathologies like aortic incompetence, mitral incompetence and ventricular septal defects\(^15\) or due to intra-cardiac device insertion. These sterile micro-vegetations constitute an ideal environment for colonisation and infection by microorganisms. Endocardial inflammation itself, which can occur as a result of endothelial injury, promotes bacterial colonisation which may lead to infective endocarditis.\(^18\) Prosthetic heart valves also provide a suitable environment for microbial colonisation in the presence of bacteremia. Trauma to skin and mucosal linings as seen with dental procedures involving manipulation of the gingival surface,\(^7\) intravenous drug abuse and insertion of invasive vascular devices or their manipulation can provide an opportunity for microbes to enter the circulation and cause IE.\(^1,12,18\) Once established, IE can lead to perforation, obstruction or incompetence of heart valves, abscess formation and valve dehiscence (in cases of prosthetic heart valves). Vegetations on valve leaflets can also cause the formation of micro-thrombi which can then embolise to the brain, kidney, spleen, peripheral vasculature and so forth,\(^1\) or induce septic emboli with subsequent metastatic infection, as in the case of pulmonary abscesses seen in patients with tricuspid valve endocarditis.

4. Clinical features

IE may present with a variety of clinical manifestations including atypical symptoms, particularly in patients on ICU. Common presentations on the ICU are pyrexia of unknown origin; peripheral thromboembolism; neurological complications such as stroke or intracranial hemorrhage; hypotension; new or changing cardiac murmur; tachycardia; heart failure; unexplained rise in inflammatory markers; acute kidney injury and anemia\(^8,19,20\) (please see Table 1).

The classical manifestations of IE are not usually seen in critically ill patients, making it difficult to diagnose in the ICU environment. Central nervous system signs of IE can often be blunted by sedation given to intensive care patients. Fever and bacteremia can frequently be attributed to other possible co-existing hospital-acquired infections and acute kidney injury is common due to co-existing pathologies. Hence, the timely diagnosis of IE requires a high index of suspicion particularly in patients more predisposed to IE such as in patients with prosthetic valves, post-cardiac surgery and with long-term in-dwelling invasive monitoring or therapeutic devices.

5. Predictors of outcome

Various clinical factors have been associated with higher overall mortality in patients with IE and the majority of these factors are common predictors of outcome in ICU patients as well as in patients in general. These factors include older age,\(^21\) presence of heart failure, presence of severe sepsis, immunocompromised status, presence of acute kidney injury,\(^5,8,9,12,22,23\) and antimicrobial treatment failure.\(^24\) Other factors linked with increased mortality are prosthetic valve infection,\(^9,25\) and neurological complications.\(^10\) In patients undergoing heart valve surgery for infective endocarditis predictors for mortality were higher age, severity of sepsis, acute kidney injury and hemodynamic instability,\(^6,26\) (please see Table 2). In patients undergoing aortic root replacement surgery for IE, patients with prosthetic valves have higher mortality as compared with those having native valves.\(^27\) Staphylococcal,\(^21\) fungal and gram negative IE are also associated with poorer outcome.\(^23\) Certain echocardiographic features are associated with higher mortality. These include large vegetation size, severe prosthetic valve dysfunction, severe left sided regurgitant lesions, periannular complications such as abscesses and left ventricular

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Common presentations of infective endocarditis in the ICU.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrexia of unknown cause</td>
<td></td>
</tr>
<tr>
<td>Peripheral thromboembolism</td>
<td></td>
</tr>
<tr>
<td>Neurological complications such as stroke or intracranial hemorrhage</td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td></td>
</tr>
<tr>
<td>New or changing cardiac murmur</td>
<td></td>
</tr>
<tr>
<td>Tachycardia</td>
<td></td>
</tr>
<tr>
<td>Heart failure</td>
<td></td>
</tr>
<tr>
<td>Unexplained rise in inflammatory markers</td>
<td></td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
</tr>
</tbody>
</table>
dysfunction. Closer monitoring and attention may reduce morbidity and mortality in these high-risk patient groups.

6. Diagnosis

The diagnosis of IE is derived from a combination of clinical findings, laboratory investigations and imaging data. The Modified Duke’s Criteria can be used for the diagnosis of IE, although these have not been specifically validated for the diagnosis of IE in the ICU setting (please see Table 3).

Essentially, diagnosis is based on strength of clinical suspicion in conjunction with evidence from blood cultures and imaging modalities such as echocardiography, CT and MRI. At least 3 sets of blood cultures should be taken in patients with suspected infective endocarditis. The ACC/AHA guidelines also recommend blood cultures to be taken in patients with any unexplained fever lasting more than 48 h as well as prior to institution of antibiotics in patients with known valve disease or prosthetic valves who have unexplained pyrexia.

In patients with suspected IE, echocardiography is essential for the detection of the presence, location and size of vegetations; the evaluation of valvular dysfunction and the detection of other intracardiac complications (e.g. abscess formation). Echocardiography is also extremely crucial for the assessment of heart function, which is not only a determinant of clinical outcome but also helps to decide the need for valve surgery (see Fig. 1). Although transthoracic echocardiography (TEE) can be used as a screening tool for these purposes, transesophageal echocardiography (TEE) is the recommended investigation for patients with suspected IE in the ICU setting because of its significantly higher sensitivity, specificity and accuracy in detecting IE, identifying complications such as abscess formation, and assessing the severity of valve lesions as a result of IE.28,31 TEE is recommended in patients with prosthetic heart valves30,32 as TEE can be less reliable in these cases. TEE should also be done in all cases of IE being considered for surgery.32 TEE can be a technically challenging procedure in critically ill patients due to problems with accessing the esophagus in the presence of nasogastric tubes and invasive ventilation. This is further confounded by the presence of pre-existing respiratory and cardiovascular compromise. However, complication rates with TEE in critically ill patients remain extremely low.31,33,34 TEE in ICU should be carried out by a sufficiently skilled person and if possible with naso-endoscopy guidance to prevent injury to the esophagus or oropharynx. Patients should be fasting for at least 4–6 h prior to procedure to prevent risk of aspiration.

New diagnostic modalities such as MRI, PET/CT scanning can also be employed in the diagnosis of peripheral embolism or metastatic infection in patients with infective endocarditis.35–40 In particular, MRI of the brain has been shown to detect cerebral emboli, even in asymptomatic patients. Hence MRI should be considered in patients with confirmed IE as the presence of neurological sequelae can be an important indicator of disease severity, treatment response and prognosis.35–38 of IE.

**Table 2**

Predictors of outcome in patients with infective endocarditis.

<table>
<thead>
<tr>
<th>Age</th>
<th>Pre-existing heart pathologies: valve lesions, prosthetic heart valves, congenital heart disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure</td>
<td>Acute kidney injury</td>
</tr>
<tr>
<td>Neurological complications</td>
<td>Valve surgery for endocarditis</td>
</tr>
<tr>
<td>Antimicrobial treatment failure</td>
<td>Staphylococcal, fungal or gram negative endocarditis</td>
</tr>
<tr>
<td><strong>ECHO features such as large vegetations, severe valve dysfunction, prosthetic valve dehiscence</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3**

Modified Duke’s criteria for infective endocarditis.

**Major criteria**

Microbiological evidence of infective endocarditis

- Positive blood cultures (typical organisms consistent with infective endocarditis isolated from 2 separate blood cultures; microorganisms consistent with IE isolated from at least 2 blood culture done more than 12 h apart or all of 3 or majority of Í.4 separate blood cultures spread over more than 1 h; single positive blood culture for Coxiella burnetii or antiphase IgG titer .1:800)

Evidence of endocardial involvement

- Echocardiogram positive for infective endocarditis (oscillating mass on valves or supporting structures, in path of regurgitant jets or unexplained mass on implanted material; abscess; new prosthetic valve dehiscence)
- New valve regurgitation

**Minor criteria**

- Predisposing heart condition or history of intravenous drug abuse
- Fever > 38 degree Celsius
- Vascular phenomena (such as major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, Janeway’s lesions)
- Immunologic phenomena (such as glomerulonephritis, Osler’s nodes, Roth’s spots, positive rheumatoid factor)
- Microbiological evidence: blood cultures not meeting major criteria or serological evidence of active infection with organisms consistent with IE but excluding single positive cultures with coagulase negative staphylococci and organisms not known to cause IE

**Definite IE**

2 Major criteria; 1 major and 3 minor criteria or 5 minor criteria

**Possible IE**

1 Major and 1 minor criteria or 3 minor criteria

**No IE**

Definite alternative diagnosis explaining findings, resolution of findings with ≤4 days of antibiotics, absence of pathological evidence of IE at surgery or biopsy with antibiotic treatment ≤4 days or not meeting criteria for possible IE.

![Fig. 1. Transthoracic echocardiography view of a tricuspid valve vegetation.](image-url)
Fig. 2. Transesophageal image of a vegetation on the aortic valve — TEE has a much higher sensitivity and specificity for detecting infective endocarditis, for the assessment of vegetation size and the severity of valve lesions as well as for the detection of abscesses and fistulae. TEE is much more reliable in the investigation of infective endocarditis in patients with prosthetic heart valves as well as in the presence of complications such as perivalvular abscess formation and should be the imaging modality of choice in these cases.

7. Treatment

Treatment of IE includes antimicrobial therapy alone or in combination with surgery when indicated. The former should be instituted early, after at least three sets of blood culture samples have been taken from peripheral venous sites. Empirical antibiotics can be started based on local hospital policy and standard national or international guidelines such as those issued by the ACC or ESC. The microbiology team should be consulted in all cases to guide treatment as per local protocols. The selection of antimicrobial agents can then be adjusted based on antibiotic susceptibility testing after isolation of the causative microbe.

Surgery is required in about 50% of all cases of infective endocarditis. Careful consideration has to be given to the need for surgery and its appropriate timing. This decision has to be individualized in each case and requires a multidisciplinary approach involving the cardiac surgical team. Heart failure is the most common indication for surgery in patients with infective endocarditis. Heart failure in the majority of patients with IE is a result of severe valvular regurgitation. Other main indications for surgery include uncontrolled infection, presence of perivalvular extension such as abscess or fistula formation, large vegetation size and the prevention of systemic embolism (please see Table 4).

Surgery has to be performed on an urgent basis in patients with persistent cardiogenic shock or pulmonary edema despite optimal medical therapy. In some cases of IE, surgery can be delayed if the valvular regurgitant lesions are well tolerated in order to allow for complete treatment of IE (provided there are no other indications for surgery). Surgery for the prevention of thromboembolism can be considered in patients with isolated vegetations > 15 mm in size as well as in patients with aortic or mitral vegetations > 10 mm in size if one or more embolic episodes have occurred despite antibiotic therapy. Surgery is also indicated in cases of IE due to microorganisms which are difficult to treat or eradicate with antibiotics. These include IE caused by Pseudomonas aeruginosa, Coxiella burnetii, Brucella, Staphylococcus lugdunensis, Candida or Aspergillus. Finally, surgery is also indicated in the majority of early prosthetic valve infections as well as late prosthetic valve infections caused by Staphylococci which can be difficult to treat with antibiotics alone.

In all of the above cases prompt surgical opinion should be sought. In some cases surgery has to be delayed despite indications, such as in patients with intracranial hemorrhage where surgery should be postponed for at least 1 month. The ACC/AHA guidelines recommend surgery in patients with congestive cardiac failure or cardiogenic shock caused by surgically treatable valve disease with or without infective endocarditis, provided there is a reasonable prospect of recovery with satisfactory quality of life.

Thus surgery may not be suitable in some patients with severe embolic brain injury or patients with significant comorbidities where there is no prospect of recovery to a satisfactory quality of life, even if the valve pathology is correctable by surgery.

Decisions such as appropriate antibiotics therapy, duration of antibiotic therapy, need for surgery as well as its timing and appropriateness are complicated and hence need a multidisciplinary approach involving the microbiology, cardiology and cardiothoracic surgery teams in addition to the ICU team.

8. Prevention

As previously highlighted nosocomial IE is a growing problem particularly in the ICU. There is a clear need to have robust ICU protocols to prevent infections such as IE occurring as a result of invasive devices. Central venous catheter associated blood stream infections are common in the ICU setting and are known to cause IE in susceptible patients such as those with prosthetic valves, known valve disease or patients post-cardiac surgery. Adequate precautions and care should be taken in these patients to prevent nosocomial IE as a result of their ICU stay. In addition, local practice should be regularly monitored and reviewed to address the issue of nosocomial infections in the ICU.

Both the American Heart Association and the European Society of Cardiology have recently provided revised guidelines for IE prophylaxis for the general population which are also applicable for the ITU patients. Prophylaxis is now only recommended for those at the highest risk of IE. These include patients with prosthetic heart valves; patients with prosthetic materials used for valve repair; patients with previous IE and patients with cyanotic congenital heart diseases without repair or with a persistent defect or palliative shunt/conduit. Prophylaxis is also indicated in patients with congenital heart diseases either fully repaired with a prosthetic device (during first 6 months following implantation of prosthetic device) or with a residual defect after repair. Prophylaxis is only indicated in patients undergoing dental procedures involving manipulation of the gingival or peri-apical region of teeth and perforation of oral mucosa. There is currently no recommendation for prophylactic antibiotics in patients undergoing respiratory (unless involving incision of respiratory mucosa), gastrointestinal, genito-urinary, skin or musculoskeletal procedures.

For patients undergoing implantation of a prosthetic valve or prosthetic material for valve repair, prophylaxis for Coagulase Negative Staphylococci and S. aureus should be given just before the

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Indications for surgery in patients with infective endocarditis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestive heart failure</td>
<td>Peri-annular complications (such as abscess or fistula formation)</td>
</tr>
<tr>
<td>Severe valvular dysfunction (severe aortic or mitral regurgitation)</td>
<td>Large vegetation size</td>
</tr>
<tr>
<td>Neurological complications</td>
<td>Persistent sepsis</td>
</tr>
<tr>
<td>Isolation of difficult to eradicate organisms (such as Pseudomonas, Coxiella, Brucella, Candida, Aspergillus, S. lugdunensis)</td>
<td>Prosthetic valve endocarditis (early prosthetic valve endocarditis, S. aureus prosthetic valve endocarditis, heart failure in prosthetic valve endocarditis caused my mechanical failure such as dehiscence or obstruction)</td>
</tr>
</tbody>
</table>
procedure and continued up to 48 h after surgery. Any potential source of dental sepsis should be resolved at least 2 weeks prior to implantation of any prosthetic material.

9. Conclusions

IE is a condition which is increasingly encountered in the ICU. Mortality can be very high in this setting due to the higher complexity of cases admitted to ICU and the co-existing pathologies. The ICU stay itself represents a risk factor for nosocomial IE in susceptible patients groups. Diagnosis involves the identification of the causative organism by serial blood cultures (which allows for effective antibiotic therapy to be administered based on sensitivities) along with imaging studies. Transesophageal echo is the investigation of choice for determining the presence of endocarditis as well as to ascertain the severity of the disease. Newer imaging modalities such as MRI and PET/CT can help to identify embolic complications arising from IE particularly in cases with neurological complications due to IE, which may not be clinically apparent in the ventilated ICU patient. Treatment involves a multidisciplinary approach comprising microbiologists, cardiologists and cardiothoracic surgeons working in conjunction with the ICU team. IE patients with evidence of heart failure, severe IE-related valve lesions, severe prosthetic valve dysfunction or dehiscence and patients with large vegetations should be promptly referred for surgery. Emphasis on preventing nosocomial infections for example by protocols aimed at reducing central venous catheter induced blood stream infections along with endocarditis prophylaxis where appropriate will help prevent IE in ICU patients, a challenging condition often associated with poor clinical outcomes.

Conflict of interest

The authors have no conflicts of interest to declare.

References


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The BSAC guidelines on treatment of infectious endocarditis (IE) were last published in 2004. The guidelines presented here have been updated and extended to reflect developments in diagnostics, new trial data and the availability of new antibiotics. The aim of these guidelines, which cover both native valve and prosthetic valve endocarditis, is to standardize the initial investigation and treatment of IE. An extensive review of the literature using a number of different search criteria has been carried out and cited publications used to support any changes we have made to the existing guidelines. Publications referring to in vitro or animal models have only been cited if appropriate clinical data are not available. Randomized, controlled trials suitable for the development of evidenced-based guidelines in this area are still lacking and therefore a consensus approach has again been adopted for most recommendations; however, we have attempted to grade the evidence, where possible. The guidelines have also been extended by the inclusion of sections on clinical diagnosis, echocardiography and surgery.

Keywords: antimicrobial therapy, staphylococci, enterococci, Streptococcus spp., fungal infections

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1. Introduction

In 2004 the Endocarditis Working Party of the British Society for Antimicrobial Chemotherapy (BSAC) published updated guidelines for the treatment of streptococcal, enterococcal and staphylococcal endocarditis, as well as HACEK (Haemophilus
In the light of the introduction of new antibiotic agents, developments in diagnostics and new trial data, the existing guidelines have been revised. In addition to considering the microbiological and therapeutic aspects of infective endocarditis (IE), we have now included sections on clinical diagnosis, echocardiography and surgery. The guidelines include native valve endocarditis (NVE) and prosthetic valve endocarditis (PVE). For the purposes of these guidelines, PVE includes prosthetic valves of all types, annuloplasty rings, intracardiac patches and shunts. We have excluded IE where it is related to pacemakers, defibrillators or ventricular-assist devices, which are the subject of a separate BSAC Working Party review. The aim of these guidelines is to standardize the initial investigation and treatment of IE; however, it is well recognized that patients can develop adverse drug reactions to the recommended regimens and/or fail to respond to initial antimicrobial therapy and may require a change in therapy. Several treatment options are therefore provided for most scenarios.

Guidelines such as these have, in the past, received criticism for not being evidence based. We appreciate that clinical guidelines should ideally be based on high-quality, prospective, randomized controlled trials; however, few such trials have been performed to assess the benefit of antibiotic regimens in the treatment of endocarditis. Since the last guidelines were published, there has been at least one randomized controlled trial that included patients with endocarditis. Therefore, for the first time we have graded the evidence for our recommendations, although the majority remain based on consensus.

For clarity, recommendations are presented in bold text, and throughout this document we have inserted identifying letters after recommendations to identify their provenance. These letters are: A, high-quality randomized controlled trials and meta-analysis of randomized controlled trials; B, observational data and non-randomized trials; and C, expert opinion or Working Party consensus.

An extensive review of the literature using a number of different search methods incorporating a range of criteria (e.g. endocarditis, staphylococci) has been carried out and cited publications used to support any changes we have made to the existing guidelines. Publications referring to in vitro or animal models have only been cited if appropriate clinical data are not available. The text has been largely confined to justification for changes to previous recommendations and differences from European Society for Cardiology (ESC) recommendations.

2. Clinical assessment and diagnosis

2.1 Clinical features

Recommendation 2.1: IE should be considered and actively investigated in patients with any of the criteria shown in Figure 1. [B/C]

The diverse nature and evolving epidemiological profile of IE ensure it remains a diagnostic challenge and delayed or missed diagnoses continue to be a problem. For this reason we have attempted to highlight key clinical scenarios where IE should be considered. Initial investigation in this context may involve appropriate blood culture or echocardiography or both, depending on the index of suspicion or the situation.

The clinical presentation is highly variable, according to the causative microorganism, the presence or absence of pre-existing cardiac disease, and the presence of co-morbidities and risk factors for the development of IE. It may present as an acute, rapidly progressive infection, but also as a subacute or chronic disease, with low-grade fever and non-specific symptoms that may thwart or confuse initial assessment. Patients present to a variety of specialists who may consider a range of alternative diagnoses, including chronic infection, rheumatological and autoimmune disease or malignancy. The early and ongoing involvement of a cardiologist and an infection specialist to guide investigation and management is highly recommended.

The majority (~90%) of patients present with fever, often associated with systemic symptoms of chills, poor appetite and weight loss. Heart murmurs are found in up to 85% and new murmurs have been recently reported in 48%. A pre-existing heart murmur is frequently indicative of a pre-existing ‘at risk’ valvular pathology and should heighten awareness of the possibility of IE, while new valvar regurgitation is more specific for a diagnosis of IE in an appropriate clinical setting. Classic textbook signs may still be seen in the developing world, but peripheral stigmata of IE are increasingly uncommon elsewhere, because patients generally present at an early stage of the disease. Immunological phenomena, such as splinter haemorrhages, Roth spots and glomerulonephritis, are now less common, but emboli to brain, lung or spleen occur in 30% of patients and are often the presenting feature. A high index of suspicion and low threshold for investigation to exclude IE are therefore essential in at-risk groups (see Figure 2). Laboratory signs of infection, such as elevated C-reactive protein or erythrocyte sedimentation rate, leucocytosis, anaemia and microscopic haematuria, may be present in patients with IE but are non-specific findings. Atypical presentation (e.g. absence of fever) is more common in the elderly, after antibiotic pre-treatment, in the immunocompromised patient and in IE involving less virulent or atypical organisms. The diagnosis of IE should also be considered in patients who present with a stroke or transient ischaemic attack and a fever.

2.2 Echocardiography

Recommendation 2.2: Echocardiography must be performed as soon as possible (ideally within 24 h) in all patients with suspected IE. [C]

Recommendation 2.3: Transthoracic echocardiography (TTE) is the initial investigation of choice (Figure 3). [C]

Recommendation 2.4: In cases with an initially negative TTE/transoesophageal echocardiography (TOE) examination, repeat TTE/TOE should be performed 7–10 days later if the clinical suspicion of IE remains high. [C]

Recommendation 2.5: All patients with Staphylococcus aureus bacteraemia or candidaemia require echocardiography (ideally within the first week of treatment or within 24 h if there is other evidence to suggest IE). [B]

Recommendation 2.6: TTE is recommended at completion of antibiotic therapy for evaluation of cardiac and valve morphology and function. [C]

Recommendation 2.7: Follow-up echocardiography should be performed if there is evidence of cardiac complications or...
a suboptimal response to treatment—the timing and mode of assessment (TTE or TOE) is a clinical decision. [B]

Recommendation 2.8: Routine repeat echocardiography while in therapy is not required. [C]

TTE/TOE are now ubiquitous, and their fundamental importance in the diagnosis, management and follow-up of IE is clearly recognized (Figure 3). The recommendations are summarized in Figure 4 and an algorithm for scanning is shown in Figure 2, which highlights the prominent role that TOE plays in the contemporary management of patients in whom there is a high suspicion of IE. The utility of both modes of investigation is diminished when applied indiscriminately, however, and appropriate application in the context of simple clinical criteria improves diagnostic yield. Two exceptions are patients with S. aureus bacteraemia or candidaemia, where routine echocardiography is justified in view of the frequency of IE in this setting, the virulence of these organisms, the devastating effects once intracardiac infection is established and/or the need for surgery. Sometimes multiple scans are needed to demonstrate vegetations.

Echocardiographic findings are major criteria in the diagnosis of IE, and may include the presence of a vegetation, abscess, new dehiscence of a prosthetic valve and newly noted valvular regurgitation. The sensitivity of TTE ranges from 70% to 80% and that of TOE from 90% to 100%.

2.3 Diagnostic criteria and their limitations

Recommendation 2.9: Duke criteria can be used to assist in the diagnosis of IE but are not a substitute for clinical judgement. [C]

The Duke criteria (Table 1), based upon clinical, echocardiographic and microbiological findings, were developed as a research tool, and therefore provide high specificity and moderate sensitivity for the diagnosis of IE. These criteria can help by providing an objective tool for evaluating the strength of evidence to support a diagnosis of IE, particularly in difficult cases. Clinical judgement remains essential, especially in settings where the sensitivity of the modified Duke criteria is diminished, e.g. when blood cultures are negative, when too few blood

Figure 1. Criteria for consideration and investigation of possible infective endocarditis.

1. A febrile illness and a murmur of new valvular regurgitation;
2. A febrile illness, a pre-existing at-risk cardiac lesion (see Figure 2) and no clinically obvious site of infection;
3. A febrile illness associated with any of:
   - Predisposition and recent intervention with associated bacteraemia,
   - Evidence of congestive heart failure,
   - New conduction disturbance,
   - Vascular or immunological phenomena: embolic event, Roth spots, splinter haemorrhages, Janeway lesions, Osler’s nodes,
   - A new stroke,
   - Peripheral abscesses (renal, splenic, cerebral, vertebral) of unknown cause;
4. A protracted history of sweats, weight loss, anorexia or malaise and an at-risk cardiac lesion (Figure 2);
5. Any new unexplained embolic event (e.g. cerebral or limb ischaemia);
6. Unexplained, persistently positive blood cultures;
7. Intravascular catheter-related bloodstream infection with persistently positive blood cultures 72 h after catheter removal.

The Duke criteria (Table 1), based upon clinical, echocardiographic and microbiological findings, were developed as a research tool, and therefore provide high specificity and moderate sensitivity for the diagnosis of IE. These criteria can help by providing an objective tool for evaluating the strength of evidence to support a diagnosis of IE, particularly in difficult cases. Clinical judgement remains essential, especially in settings where the sensitivity of the modified Duke criteria is diminished, e.g. when blood cultures are negative, when too few blood
• Valvular heart disease with stenosis or regurgitation
• Valve replacement
• Structural congenital heart disease, including surgically corrected or palliated structural conditions, but excluding isolated atrial septal defect, fully repaired ventricular septal defect or fully repaired patent ductus arteriosus, and closure devices that are judged to be endothelialized
• Previous infective endocarditis
• Hypertrophic cardiomyopathy

Figure 2. Cardiac conditions considered to increase a patient’s risk of developing infective endocarditis, i.e. ‘at risk’ heart valve lesions.5

2.4 The multidisciplinary team

Recommendation 2.10: A cardiologist and infection specialist should be closely involved in the diagnosis, treatment and follow-up of patients with IE. [C]

Recommendation 2.11: Specialist teams managing patients with IE should have rapid access to cardiac surgical services. [C]

There is no evidence to support these recommendations other than a widely held view that this represents good clinical care.

3. Microbiological diagnosis

3.1 Blood cultures

Recommendation 3.1: Blood cultures remain a cornerstone of the diagnosis of IE cases and should be taken prior to starting treatment in all cases. [B]

Recommendation 3.2: Meticulous aseptic technique is required when taking blood cultures, to reduce the risk of contamination with skin commensals, which can lead to misdiagnosis. Guidelines for best practice should be consulted.13 [B]

Recommendation 3.3: In patients with a chronic or subacute presentation, three sets of optimally filled blood cultures should be taken from peripheral sites with ≥6 h between them prior to commencing antimicrobial therapy. [C]

Figure 3. Indications for echocardiography in suspected infective endocarditis. IE, infective endocarditis; TTE, transthoracic echocardiography; TOE, transoesophageal echocardiography. TOE is not mandatory in isolated right-sided native valve IE with good quality TTE examination and unequivocal echocardiographic findings.
Diagnosis

- TTE is the first-line imaging modality.
- Use TOE in patients with high clinical suspicion of IE and a non-diagnostic TTE.
- Consider TOE in all adults with a positive TTE.
- TOE is not indicated in patients with a good-quality negative TTE and low clinical suspicion of IE.
- Repeat TTE/TOE 7–10 days after a negative scan when clinical suspicion of IE remains high.

Follow-up during medical therapy

- Repeat TTE or TOE are recommended as soon as a new complication is suspected.

Intra-operative echocardiography

- All cases of IE requiring surgery.

Following completion of therapy

- TTE is recommended for baseline evaluation.

Table 1. Modified Duke criteria for diagnosis of infective endocarditis

| Criterion                                      | Diagnostic | Type                                      | Tick if met
<table>
<thead>
<tr>
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<tr>
<td><strong>Major criteria</strong></td>
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<tr>
<td>Positive blood culture for infective endocarditis</td>
<td>typical microorganism consistent with IE from two separate blood cultures, as noted below microorganisms consistent with IE from persistently positive blood cultures, defined as:</td>
<td>viridans streptococci, Streptococcus bovis or HACEK group, OR community-acquired S. aureus or enterococci, in the absence of a primary focus</td>
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<tr>
<td>Evidence of endocardial involvement</td>
<td>positive echocardiogram</td>
<td>oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation, OR abscess, OR new partial dehiscence of prosthetic valve</td>
<td></td>
</tr>
<tr>
<td>Minor criteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predisposition</td>
<td>predisposing heart condition or intravenous drug use</td>
<td>temperature &gt;38.0°C (100.4°F)</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhages and Janeway lesions</td>
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<tr>
<td>Vascular phenomena</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunological phenomena</td>
<td>glomerulonephritis, Osler’s nodes, Roth spots and rheumatoid factor</td>
<td>positive blood culture but does not meet a major criterion as noted above or serological evidence of active infection with organism consistent with IE</td>
<td></td>
</tr>
<tr>
<td>Microbiological phenomena</td>
<td>broad-range PCR of 16S</td>
<td>consistent with IE but do not meet a major criterion as noted above</td>
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IE, infective endocarditis.

Clinical criteria for definite infective endocarditis requires: two major criteria; or one major and three minor criteria; or five minor criteria.
There is no evidence to support the commonly perpetuated view that blood cultures should be taken from different sites. All skin surfaces are colonized by bacteria and adequate skin disinfection is key to reducing contamination. Taking blood cultures at different times is critical to identifying a constant bacteraemia, a hallmark of endocarditis.

Recommendation 3.4: In patients with suspected IE and severe sepsis or septic shock at the time of presentation, two sets of optimally filled blood cultures should be taken at different times within 1 h prior to commencement of empirical therapy, to avoid undue delay in commencing empirical antimicrobial therapy. [C]

This recommendation reflects recent evidence of improved outcomes in severe infection with rapid instigation of appropriate therapy. It is not always appropriate to withhold antimicrobial therapy while three sets of blood cultures are taken over a 12 h period. This recommendation is intended to be pragmatic, allowing time to take at least two sets of blood cultures (the minimum for a secure microbiological diagnosis) prior to commencing antimicrobial therapy. Taking three sets of blood cultures within 1 h does not add anything to the diagnostic pathway (which ideally attempts to confirm sustained/persistent bacteraemia). Although modified Duke criteria specify 1 h between blood cultures, the Working Party did not feel that the evidence to support this criterion was sufficient to justify the inevitable delay in administering antibiotics.

Recommendation 3.5: Bacteraemia is continuous in IE rather than intermittent, so positive results from only one set out of several blood cultures should be regarded with caution. [B]

Recommendation 3.6: Sampling of intravascular lines should be avoided, unless part of paired through-line and peripheral sampling to diagnose concurrent intravascular catheter-related bloodstream infection. [B] [C]

Recommendation 3.7: In groin-injecting intravenous drug users, a groin sinus should not be used to sample blood for culture. [C]

Recommendation 3.8: If a stable patient has suspected IE but is already on antibiotic treatment, consideration should be given to stopping treatment and performing three sets of blood cultures off antibiotics. Antibiotic therapy may need to be stopped for 7–10 days before blood cultures become positive. [C]

Previous ESC guidelines and the experience of Working Party members indicate that blood cultures may only become positive in partially treated IE after 7–10 days off antibiotic therapy. Routine incubation for >7 days is not necessary. [B]

In the previous BSAC guideline, the traditional recommendation for extended incubation and terminal subculture was maintained to increase the yield of fastidious and slow-growing bacteria, although the evidence for this was tenuous in the era of automated continuous-monitoring blood culture systems. In the light of further data and the proven utility of complementary non-culture-based technologies, we feel that the case for extended incubation and blind subculture is not justified and therefore it is not recommended.

Recommendation 3.10: Once a microbiological diagnosis has been made, routine repeat blood cultures are not recommended. [C]

Recommendation 3.11: Blood cultures should be repeated if a patient is still febrile after 7 days of treatment. [C]

3.2 Susceptibility testing

Recommendation 3.12: When the causative microorganism has been isolated, the MIC of the chosen antimicrobial should be established by a standardized laboratory method to ensure susceptibility. [C]

Recommendation 3.13: Gradient tests (such as Etest) may be useful for establishing the susceptibility of fastidious or slow-growing bacteria, such as the HACEK group. [B]

Recommendation 3.14: Routine measurement of the MBC or serum bactericidal titres is not required. [C]

As documented in previous guidelines, these measurements are affected by a range of technical factors that result in poor intralaboratory reproducibility and there remains a lack of evidence regarding their clinical value.

3.3 Serology

Failure to culture a causative microorganism in IE is often due to the administration of antimicrobials prior to blood culture, but may also be due to infection caused by fastidious or slow-growing microorganisms. Diagnostic methods should include serological investigations where they are available and a systematic approach is advised, based on the clinical history of the patient and their exposure to possible risk factors.

Recommendation 3.15: In patients with blood culture-negative IE, serological testing for Coxiella and Bartonella should be performed. [B]

Microorganisms that should be considered first include Coxiella burnetii (Q fever) and Bartonella spp. In a large study of 348 cases of blood culture-negative IE in France, the documented aetiological agent was C. burnetii and Bartonella spp. in 48% and 28% of cases, respectively.

Recommendation 3.16: In patients with blood culture-negative IE, routine serological testing for Chlamydia, Legionella and Mycoplasma should not be performed, but considered if serology in Recommendation 3.15 is negative. [C]

The combined total of infections attributed to Mycoplasma species, Legionella species and Chlamyphera whipplei in a recent study amounted to <1% of all culture-negative cases, and there were no cases in which Chlamydia species were implicated during an 18 year study period. IE due to Chlamydia is rarer than previously thought, owing to false-positive Chlamydia serology caused by antibodies to Bartonella. Endocarditis caused by these microorganisms is extremely rare and serology has not been shown to be of value. Given their rarity, there is also a significant risk of false-positive serology leading to erroneous therapy.

Recommendation 3.17: Consider Brucella in patients with negative blood cultures and a risk of exposure (dietary, occupational or travel). [C]

The serology of Q fever is considered positive when anti-phase 1 IgG antibody titres are ≥1:800 and for Bartonella when anti-Bartonella quintana or anti-Bartonella henselae IgG antibody titres are ≥1:800. Serology may be useful for the diagnosis of IE caused by Brucella species in areas where the clinical history suggests exposure to this agent.
3.4 Investigation of excised heart valves

Recommendation 3.19: Tissues from excised heart valves or vegetations following surgical intervention in patients with suspected IE should be investigated for the presence of infection, including culture and histological examination. [B]

At least 25% of patients with IE will have valve tissue removed.29 Culture of the homogenized tissue is recommended, but results should be regarded with caution due to the relatively poor predictive value. This is due to the high percentage of false-negative results attributable to antimicrobial treatment and the possibility that tissue may have been contaminated during manipulation, leading to frequent false positives.30

Recommendation 3.20: Samples of excised heart valve (or tissue from embolotomy) from cases of culture-negative IE should be referred for broad-range bacterial PCR and sequencing. [B]

Recommendation 3.21: A positive broad-range bacterial PCR result can be reliably used to identify the cause of endocarditis, but cannot be used to infer ongoing presence of infection and should not therefore be used alone to judge the duration of post-operative antimicrobial therapy. [B]

An increasing number of studies have demonstrated the diagnostic utility of broad-range PCR plus sequencing for detecting microbial pathogens in heart valve tissue.22,29,31-37 DNA is extracted from homogenized tissue and subjected to PCR using broad-range primers targeting the bacterial DNA that codes for the 16S ribosomal subunit (16S rDNA). Universal primers may also be used to target the 28S ribosomal subunit of fungi. Any amplicons generated are then sequenced to identify the species present. These PCR assays are particularly useful in assisting the diagnosis of IE in patients who have had prior antimicrobial therapy, as detectable microbial DNA has been shown to persist for many months or even years in vivo after successful therapy.38,39 Such procedures can also identify the presence of rare causes of IE that may not be detected using routine procedures, such as Mycoplasma species30 or fungi.31 Broad-range PCR can be attempted from histopathological specimens, but sensitivity may be reduced.

PCR assays are not without their drawbacks, and these include the presence of PCR inhibitors in clinical samples or the risk of contamination in clinical samples and PCR reagents. The risk of false-positive results can be reduced by the use of real-time PCR, the use of specially designed PCR laboratories, carry-over prevention techniques and limiting the sensitivity of the PCR assay by reducing the number of PCR cycles.35,42 The clinical history of the patient must also be considered given that DNA may persist in valve tissue from past infections and may therefore not be indicative of current active infection. In conclusion, there is accumulating evidence that such techniques, if rigorously controlled, can provide a useful adjunct to blood culture and serology for the diagnosis of IE. DNA sequencing is not available in most laboratories, but many reference laboratories will provide a service for the investigation of tissue samples. Laboratories with ready access to such techniques are likely to use them more widely to support an existing diagnosis, even when blood cultures are positive.

Real-time PCR has been applied to whole blood and serum for the detection of fastidious bacteria and fungi causing IE, but there are insufficient data, at present, to recommend the routine use of such techniques for the diagnosis of culture-negative IE.53-45

The above recommendations have concentrated on the investigations available to the microbiology laboratory, but a comprehensive diagnosis will involve integration of clinical, microbiological, biochemical, haematological, histopathological and echocardiographic data.46-50

4. The role of surgery

Recommendation 4.1: A surgical opinion should be sought at the earliest opportunity for every patient with endocarditis affecting intracardiac prosthetic material. [C]

Recommendation 4.2: A surgical opinion should be sought for every patient with endocarditis and any of the indications for surgery listed in Figure 5. [C]

Recommendation 4.3: The timing of surgery should be judged on a case-by-case basis, but the relative urgency of different indications is given in Figure 5. [C]

Recommendation 4.4: Samples of valve or other infected tissue should be sent for microbiological and histopathological investigation. [B]

5. Antibiotic dosing, delivery and monitoring

5.1 Aminoglycosides

Recommendation 5.1: Gentamicin should be dosed according to actual body weight unless patients are obese, in which case dosing should be discussed with a pharmacist. [C]

Recommendation 5.2: When used for treatment of Gram-positive endocarditis, serum gentamicin levels should be measured regularly to ensure pre-dose (trough) levels remain <1 mg/L and post-dose levels 3–5 mg/L. [C]

Recommendation 5.3: In patients with impaired renal function, dose should be adjusted according to measured or estimated creatinine clearance and serum levels should be monitored daily. [C]

Recommendation 5.4: If ‘once-daily’ gentamicin dosing regimens (e.g. Hartford regimen) are used as part of treatment regimens for IE caused by Enterobacteriaceae or Pseudomonas aeruginosa, use local protocols to monitor and adjust dosing regimens. [C]

The use of aminoglycosides is regularly questioned and is discussed in more detail in the individual sections. Gentamicin dose regimens in IE are usually based on the administration of 1 mg/kg body weight, intravenously (iv)/intramuscularly every 12 h. Gentamicin is poorly lipid soluble and there is a risk of accidental overdose in obese patients dosed according to actual body weight. Evidence to support the recommended therapeutic levels is limited. Once-daily regimens are now widely used for other infections, but data regarding their efficacy in endocarditis...
Heart failure

<table>
<thead>
<tr>
<th>Heart failure</th>
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<tbody>
<tr>
<td>Aortic or mitral IE with:</td>
</tr>
<tr>
<td>1. Severe acute regurgitation or valve obstruction causing refractory pulmonary oedema/shock (emergency).</td>
</tr>
<tr>
<td>2. Fistula into a cardiac chamber or pericardium causing refractory pulmonary oedema/shock (emergency).</td>
</tr>
<tr>
<td>3. Severe acute regurgitation or valve obstruction and persisting heart failure or echocardiographic signs of poor haemodynamic tolerance (urgent).</td>
</tr>
<tr>
<td>4. Severe regurgitation and no heart failure (elective).</td>
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Uncontrolled infection

<table>
<thead>
<tr>
<th>Uncontrolled infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Locally uncontrolled infection including abscess, false aneurysm, enlarging vegetation (urgent).</td>
</tr>
<tr>
<td>2. Persisting fever and positive blood culture for ≥10 days after commencing appropriate antimicrobial therapy (urgent).</td>
</tr>
<tr>
<td>3. Infection caused by fungi or multiresistant micro organisms (urgent/elective).</td>
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Prevention of embolism

<table>
<thead>
<tr>
<th>Prevention of embolism</th>
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<tbody>
<tr>
<td>1. Aortic or mitral IE with large vegetations (&gt;10 mm) resulting in one or more embolic episodes despite appropriate antibiotic therapy (urgent).</td>
</tr>
<tr>
<td>2. Aortic or mitral IE with large vegetations (&gt;10 mm) and other predictors of complicated course like heart failure, persistent infection or abscess (urgent).</td>
</tr>
<tr>
<td>3. Isolated very large vegetations &gt;15 mm (urgent).</td>
</tr>
</tbody>
</table>

Figure 5. Indications for cardiac surgery in the management of infective endocarditis (IE) adapted from the European Society for Cardiology guidelines and the American Heart Association.

5.2 Glycopeptides

5.2.1 Vancomycin

Recommendation 5.5: Vancomycin should be dosed and levels monitored according to local protocols. [C]

Recommendation 5.6: Vancomycin levels should be monitored and dose adjusted to maintain a serum pre-dose level between 15 and 20 mg/L. [C]

Since the previous version of these guidelines, vancomycin breakpoints have been revised and higher pre-dose vancomycin levels have been recommended. Vancomycin dosing is in a state of flux as hospitals attempt to consistently achieve the higher pre-dose levels recommended for serious infections. Until new protocols have been evaluated, the optimum dosing regimen is not known and more detailed guidelines cannot be provided.

Recommendation 5.7: There is insufficient evidence to support the use of continuous infusions of vancomycin in IE patients.

5.2.2 Teicoplanin

Recommendation 5.8: Teicoplanin should be administered initially at a high dose (10 mg/kg body weight every 12 h then 10 mg/kg daily) with dosing interval adjusted according to renal function. [B]

Recommendation 5.9: Teicoplanin serum trough levels must be measured to ensure levels of ≥20 mg/L (and <60 mg/L) and repeated at least weekly. [C]

There is no new evidence to justify a change to these previous recommendations.

Recommendation 5.10: Teicoplanin is less nephrotoxic than vancomycin and should be considered for susceptible isolates (excluding staphylococci) when combination therapy with gentamicin is required.
5.3 β-Lactams

Amoxicillin and ampicillin are considered microbiologically equivalent and either can be used. Amoxicillin may be used instead of benzylpenicillin for susceptible isolates, but is broader spectrum and has a greater risk of Clostridium difficile infection. The time-dependent killing of streptococci by penicillin means that it should be given six times a day, because of its short serum half-life. There are no prospective comparisons of continuous with intermittent penicillin administration for streptococcal endocarditis. Dose modifications for β-lactams may be necessary in patients with impaired renal function and according to the patient’s body weight.

5.4 Alternative antibiotics for patients with penicillin allergy

Where β-lactams are recommended as first-line agents, alternative regimens are listed in the Tables for patients with a β-lactam allergy. It is important to establish the nature of a reported ‘allergy’ to penicillin, as there is less experience with alternative antibiotics, a higher rate of side effects and concerns about the efficacy of alternatives. For example, a history of a rash with ampicillin or amoxicillin may not indicate true allergy. Unless signs of immediate-type hypersensitivity reaction (anaphylaxis, angio-oedema, bronchospasm and urticaria) were reported, a trial with penicillin may be warranted, but access to resuscitation facilities should be available immediately. Penicillin antibody testing and skin prick testing can be useful.

If a rash occurs after 72 h it is likely to be a delayed-type hypersensitivity reaction rather than an immediate IgE-mediated reaction (type I hypersensitivity). In a recent study, 72% of patients with a delayed-type hypersensitivity reaction to amoxicillin had no cross-reactivity with penicillin. There may be a role for skin testing in the ‘penicillin allergic’ patient who does not have a history of anaphylaxis or angio-oedema, rather than avoidance of all β-lactam agents for the treatment of endocarditis. The American Heart Association (AHA) advises ceftriaxone for the penicillin-allergic patient—but this should only be used for allergy other than immediate-type hypersensitivity, because of the risk of cross-sensitivity with penicillin.

5.5 Other antibiotics

Since the previous guidelines were published, other antibiotics such as linezolid and daptomycin have been introduced. Their use, where relevant, is described in the text of the individual sections.

5.6 Home therapy

Recommendation 5.10: Home/community/outpatient intravenous therapy is an appropriate method for managing selected patients with IE. [B]

Recommendation 5.11: IE patients need to satisfy general suitability criteria for home/community/outpatient therapy in addition to the condition-specific requirements in Recommendation 5.12.

Recommendation 5.12: IE patients who might be considered for home/community/outpatient therapy would include those: who are stable and responding well to therapy; without signs of heart failure; without any of the indications for surgery listed in Figure 5; or without uncontrolled extracardiac foci of infection. [C]

Recommendation 5.13: IE caused by any microorganism may be appropriate for home/community/outpatient therapy provided the conditions in Recommendation 5.12 are satisfied. However, S. aureus is the microorganism associated with highest mortality and complications, and caution is therefore advised where this is the cause. [C]

Recommendation 5.14: Patients who have valve replacement surgery for IE and are in hospital solely to complete a planned treatment course and satisfy the conditions in Recommendation 5.12 may be suitable for home/community/outpatient therapy. [C]

Recommendation 5.15: When patients are managed using home/community/outpatient intravenous therapy, systems should be in place to monitor the patient’s clinical condition on a daily basis. [C]

Recommendation 5.16: Ceftriaxone, teicoplanin, daptomycin and vancomycin are suitable agents for home/community/outpatient therapy for endocarditis, depending whether once- or twice-daily administration is available locally. [B]

Recommendation 5.17: The dosing regimens for treating patients on home/community/outpatient therapy are the same as those recommended for specific pathogens. [C]

Home/community/outpatient therapy for endocarditis has been described. Suitability for home therapy will depend on the patient, the availability of the infrastructure to support such therapy and the susceptibility of the infecting microorganism to antibiotics, which lend themselves to home therapy. Home/community/outpatient therapy for endocarditis treatment is often considered for streptococcal endocarditis, as these microorganisms can be less destructive with fewer complications than IE caused by other microorganisms. Trials of home therapy have been reviewed. Antibiotics such as ceftriaxone, daptomycin or teicoplanin that can be given once daily iv are suitable agents, but others can be used depending on who is administering the antimicrobials. Patients may not need a central venous catheter (such as a peripherally inserted central catheter), if antimicrobial therapy can be administered via peripheral cannulae. This approach may be preferable, as these devices have the lowest infection and complication rates of all vascular access devices. Agents such as teicoplanin or daptomycin, which can be given as a bolus, can be administered via a butterfly needle; thus, avoiding the need for any indwelling vascular access and minimizing the risk of infection.

Any of the recommended antimicrobial agents have potential side effects. For example, neutropenia is a well-described side effect of ceftriaxone, occurring in 2 of 55 patients in one study and can predispose to C. difficile infection; teicoplanin also has side effects, including drug fever (25% of cases in one IE series) and daptomycin may cause a myositis and resistance may develop on therapy. Patients being managed in this way need to be carefully monitored for side effects as well as their response to therapy.

5.7 Oral therapy

Oral therapy for endocarditis has been described but is rarely advocated in guidelines, owing to the paucity of data and concerns about efficacy. In general, intravenous therapy is...
6. Empirical treatment regimens

The recommended regimens are summarized in Table 2.

Recommendation 6.1: Empirical antimicrobial regimens for patients with suspected endocarditis should be based on severity of infection, type of valve affected and risk factors for unusual or resistant pathogens. [C]

Recommendation 6.2: Empirical therapy should be directed towards the most common causes of endocarditis. [C]

Recommendation 6.3: If a patient with suspected IE is clinically stable, we recommend waiting for the results of blood cultures before starting any antimicrobials. [C]

Recommendation 6.4: If the diagnosis of IE is in doubt, the patient is clinically stable and has already received antibiotics, we recommend stopping any antibiotics and reculturing. [C]

The most common causes of NVE in non-intravenous drug users are currently *S. aureus* (28%), coagulase-negative staphylococci (CoNS; 9%), streptococci (35%) and enterococci (11%); 9% are culture-negative.³ Meticillin resistance is common among staphylococci. *S. aureus* infection and severity of illness at presentation (APACHE II score) are independent predictors of mortality in IE patients.⁵⁸ IE occasionally presents acutely with severe sepsis when caused by less-virulent microorganisms, such as enterococci, oral streptococci and CoNS. It is likely, though unproven, that early administration of effective antimicrobial therapy in the most severely ill patients will improve outcomes, as is the case for other critically ill patients with infection.¹⁶ Empirical regimens for the critically ill patient therefore need to provide broad-spectrum coverage. Patient risk factors for multiresistant pathogens need to be taken into consideration, e.g. colonization with meticillin-resistant *S. aureus* or extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, or intravenous drug use. If the patient is critically ill and has risk factors for ESBL-producing Enterobacteriaceae or *P. aeruginosa*, we recommend vancomycin plus meropenem [C].

Conversely, to avoid the risks and toxicity of broad-spectrum regimens, it is entirely reasonable to wait for the results of blood cultures in patients who are stable. If empirical therapy is indicated, for NVE with indolent presentation we recommend 2 g of amoxicillin every 4 h. The addition of empirical gentamicin in this situation is controversial. When intracardiac prosthetic material is present, the previous recommendation for vancomycin, gentamicin and rifampicin is unchanged. This applies to both

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### Table 2. Empirical treatment regimens for endocarditis (pending blood culture results)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Dose/route</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NVE—indolent presentation</td>
<td>Amoxicillin⁹ AND (optional) 2 g q4h iv</td>
<td>If patient is stable, ideally await blood cultures. Better activity against enterococci and many HACEK microorganisms compared with benzylpenicillin. Use Regimen 2 if genuine penicillin allergy.</td>
</tr>
<tr>
<td></td>
<td>Gentamicina⁹ 1 mg/kg ABW</td>
<td>If patient is clinically stable and has already received antibiotics, cultures before starting any antimicrobials.</td>
</tr>
<tr>
<td></td>
<td>Meropenema⁹ 2 g q8h iv</td>
<td>In severe sepsis, staphylococci (including meticillin-resistant staphylococci) need to be covered. If allergic to vancomycin, replace with daptomycin 6 mg/kg q12h iv.</td>
</tr>
<tr>
<td>2. NVE, severe sepsis (no risk factors for Enterobacteriaceae, Pseudomonas)</td>
<td>Vancomycin⁹ AND dosed according to local guidelines</td>
<td>Will provide cover against staphylococci (including meticillin-resistant staphylococci), streptococci, enterococci, HACEK, Enterobacteriaceae and <em>P. aeruginosa</em>.</td>
</tr>
<tr>
<td></td>
<td>Gentamicina⁹ 1 mg/kg IBW q12h iv</td>
<td>If there are concerns about nephrotoxicity/acute kidney injury, use ciprofloxacin in place of gentamicin⁹</td>
</tr>
<tr>
<td>3. NVE, severe sepsis AND risk factors for multiresistant Enterobacteriaceae, Pseudomonas</td>
<td>Vancomycina⁹ AND meropenema⁹ 2 g q4h iv</td>
<td>Will provide cover against staphylococci (including meticillin-resistant staphylococci), streptococci, enterococci, HACEK, Enterobacteriaceae and <em>P. aeruginosa</em>.</td>
</tr>
<tr>
<td></td>
<td>Rifampicin⁹ 300–600 mg q12h po/iv</td>
<td>Use lower dose of rifampicin in severe renal impairment.</td>
</tr>
</tbody>
</table>

NVE, native valve endocarditis; PVE, prosthetic valve endocarditis; ABW, actual body weight; IBW, ideal body weight; iv, intravenous; po, orally; q4h, every 4 h; q8h, every 8 h; q12h, every 12 h.

⁹Doses require adjustment according to renal function.
Table 3. Summary of treatment recommendations for staphylococcal endocarditis

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/route</th>
<th>Duration (weeks)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NVE, methicillin-susceptible Staphylococcus spp.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>2 g every 4–6 h iv</td>
<td>4</td>
<td>Use q4h regimen if weight &gt;85 kg.</td>
</tr>
<tr>
<td><strong>NVE, methicillin-resistant, vancomycin-susceptible (MIC ≤2 mg/L) rifampicin-susceptible Staphylococcus or penicillin allergy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin AND</td>
<td>1 g iv q12h</td>
<td>4</td>
<td>or dose according to local guidelines. Modify dose according to renal function and maintain pre-dose level 15–20 mg/L.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>300–600 mg q12h po</td>
<td>4</td>
<td>Use lower dose of rifampicin if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td><strong>NVE, methicillin-resistant, vancomycin-resistant (MIC &gt;2 mg/L), daptomycin-susceptible (MIC ≤1 mg/L) Staphylococcus spp. or patient unable to tolerate vancomycin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daptomycin AND</td>
<td>6 mg/kg q24h iv</td>
<td>4</td>
<td>Monitor creatine phosphokinase weekly. Adjust dose according to renal function.</td>
</tr>
<tr>
<td>Rifampicin OR</td>
<td>300–600 mg q12h po</td>
<td>4</td>
<td>Use lower dose of rifampicin if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 mg/kg iv, q12h</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PVE, methicillin, rifampicin-susceptible Staphylococcus spp.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>2 g every 4–6 h iv</td>
<td>6</td>
<td>Use q4h regimen if weight &gt;85 kg.</td>
</tr>
<tr>
<td>Rifampicin OR</td>
<td>300–600 mg q12h po</td>
<td>6</td>
<td>Use lower dose of rifampicin if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 mg/kg iv, q12h</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PVE, methicillin-resistant, vancomycin-susceptible (MIC ≤2 mg/L), Staphylococcus spp. or penicillin allergy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin AND</td>
<td>1 g iv q12h</td>
<td>6</td>
<td>or dose according to local guidelines. Modify dose according to renal function and maintain pre-dose level 15–20 mg/L.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>300–600 mg q12h po</td>
<td>6</td>
<td>Use lower dose of rifampicin if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 mg/kg iv, q12h</td>
<td>≥2</td>
<td>Continue gentamicin for the full course if there are no signs or symptoms of toxicity.</td>
</tr>
<tr>
<td><strong>PVE, methicillin-resistant, vancomycin-resistant (MIC &gt;2 mg/L), daptomycin-susceptible (MIC ≤1 mg/L) Staphylococcus spp. or patient unable to tolerate vancomycin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daptomycin AND</td>
<td>6 mg/kg q24h iv</td>
<td>6</td>
<td>Increase daptomycin dosing interval to 48 hourly if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>300–600 mg q12h po</td>
<td>6</td>
<td>Use lower dose of rifampicin if creatinine clearance &lt;30 mL/min.</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 mg/kg q12h iv</td>
<td>≥2</td>
<td>Continue gentamicin for the full course if there are no signs or symptoms of toxicity.</td>
</tr>
</tbody>
</table>

NVE, native valve endocarditis; PVE, prosthetic valve endocarditis; iv, intravenously; po, orally; q12h, every 12 h; q24h, every 24 h.

7. Staphylococcal endocarditis

See Table 3 for recommended regimens.

7.1 NVE

**Recommendation 7.1: First-line therapy for methicillin-susceptible staphylococci is 2 g of flucloxacillin every 6 h, increasing to 2 g every 4 h in patients weighing >85 kg. [A]**

This recommendation is unchanged from previous guidelines.

**Recommendation 7.2: Gentamicin should not be added to flucloxacillin for the initial treatment of native valve staphylococcal IE. [A]**

There is no evidence that the addition of gentamicin results in improved survival, reduced surgery or reduced complications. This recommendation is unchanged from previous guidelines, but since their publication, analysis of data from a randomized controlled trial has confirmed previous findings of increased nephrotoxicity in patients. There is no evidence that the addition of sodium fusidate or rifampicin to flucloxacillin offers any advantage in this setting.

**Recommendation 7.3: First-line therapy for methicillin-resistant staphylococci or in patients with penicillin allergy is vancomycin iv plus rifampicin [C].**

As vancomycin is less active than flucloxacillin, we recommend the addition of a second antibiotic to the treatment regimen; the recommendation to add rifampicin to vancomycin has not changed since previous recommendations. The addition of gentamicin was recommended previously in these guidelines; however, vancomycin and gentamicin are synergistically nephrotoxic, and the potential benefit of gentamicin may be outweighed by the risk of toxicity, particularly if higher trough levels of vancomycin are being used.

**Recommendation 7.4: For patients intolerant of vancomycin or with vancomycin-resistant staphylococci we recommend 6 mg/kg daptomycin every 24 h with another active agent. [A]**

One randomized controlled study has demonstrated non-inferiority of daptomycin when compared with standard therapy (flucloxacillin or vancomycin plus gentamicin) in the treatment of S. aureus bloodstream infections, including IE. Although this study included patients with IE, the number of patients was small. Of all the daptomycin-treated patients (120), 19 (15.8%) had persisting or relapsing bacteraemia and seven isolates had reduced susceptibility to daptomycin. Of the 28 IE patients treated with daptomycin, 3 developed daptomycin-resistant isolates on therapy (1 right-sided and 2 left-sided IE; none of these received concurrent gentamicin). Daptomycin treatment
failure for IE, associated with the development of resistance to daptomycin, is well described.65 –73 All but one of the separately reported cases of daptomycin resistance have occurred in patients treated with daptomycin monotherapy.63 –73 Nevertheless, daptomycin is more rapidly bactericidal than vancomycin, which makes it an attractive agent for the treatment of endocarditis. Current UK prescribing guidelines recommend 6 mg/kg once daily, but higher doses have been advocated by other authorities. Because rates of development of resistance are high and because of the serious implications of treatment failure, we recommend the addition of another active agent (e.g. rifampicin, gentamicin or linezolid, depending on susceptibility) to daptomycin, pending further information.

No new data have been reviewed to change previous recommendations regarding teicoplanin for staphylococcal IE. Linezolid has been used successfully to treat staphylococcal endocarditis in individual cases for whom conventional therapy has either been contraindicated or unsuccessful. Linezolid is a bacteriostatic agent and so we cannot recommend it as monotherapy.

7.2 PVE

Recommendation 7.5: First-line therapy for susceptible isolates is vancomycin, rifampicin and gentamicin. [C]

Recommendation 7.6: Daptomycin can be used in place of vancomycin for patients unresponsive to or intolerant of vancomycin or with vancomycin-resistant isolates. [C]

Recommendations for first-line therapy and penicillin allergy have not changed from previous guidelines. Daptomycin has been used successfully, in combination with other agents, to treat PVE caused by staphylococci, but published data are limited.73
PVE, or patients with secondary brain abscesses or vertebral osteomyelitis. [C]

Since the publication of the 2004 guidelines, the areas of further debate around the treatment of streptococcal endocarditis have included the role of gentamicin, the appropriate breakpoints for moderate and high-level penicillin resistance, and the treatment of patients with penicillin allergy.

The role of gentamicin has been questioned because of concerns of toxicity. A meta-analysis of the use of gentamicin only identified one randomized controlled trial for the treatment of streptococcal endocarditis and therefore concluded that there was insufficient evidence. A recent endocarditis study showed that a combination of gentamicin and a β-lactam led to a reduction in the estimated creatinine clearance compared with β-lactam monotherapy, but there was no association between the change in renal function during treatment and the post-discharge mortality for streptococcal or enterococcal endocarditis. The authors concluded that gentamicin did have a role in the treatment of endocarditis. The potential risk of aminoglycosides has to be balanced against the benefit of shorter treatment length for the very susceptible streptococci (see Table 4) and more effective treatment of moderately penicillin-resistant streptococci. (See also the discussion on reducing gentamicin toxicity under enterococcal endocarditis.)

There have been concerns that the prevalence of penicillin-resistant streptococci may be increasing. A recent BSAC study reviewed 2344 streptococci causing bacteraemia, from 2001 to 2006. No β-haemolytic streptococci (groups A, B, C and G) were resistant to penicillin (breakpoint of 0.125 mg/L), whereas rates of penicillin resistance for non-haemolytic and α-haemolytic streptococci varied between 13% and 17% each year, with no significant change over 6 years. Most resistant isolates had an MIC between 0.25 and 1 mg/L; none had an MIC >8 mg/L. All isolates were susceptible to vancomycin and teicoplanin (MIC ≤4 mg/L). A combination of 4–6 weeks of high-dose benzylpenicillin with 2 weeks of an aminoglycoside has been recommended for streptococci with moderate penicillin resistance. Moderate penicillin resistance was defined in the 2005 AHA guidelines as an MIC >0.125 and ≤0.5 mg/L. A treatment regimen for enterococci (e.g. 4–6 weeks of a penicillin plus an aminoglycoside) was advised for streptococci with an MIC >0.5 mg/L. In the more recent ESC guidelines, relative resistance to penicillin was defined as an MIC between 0.125 and 2 mg/L. In justification, the authors describe treatment of 60 patients with streptococcal endocarditis. If cases with inadequate information, those given additional antibiotics or those where the patient had valve replacement are excluded, there were 11 individuals infected with streptococci with MICs between 0.5 and 8 mg/L who were successfully treated with just 2 weeks of high-dose benzylpenicillin and aminoglycoside. While this appears encouraging, it is possible that the patients treated for the shorter period had good prognostic indicators or a very prompt response to treatment. In the absence of a randomized controlled trial, therefore, we continue to advise 4–6 weeks of high-dose benzylpenicillin with 2 weeks of an aminoglycoside for streptococci with a penicillin MIC >0.125 and ≤0.5 mg/L, and treatment for streptococci with an MIC >0.5 and ≤2 mg/L to follow the guidelines for enterococci.

Streptococci more commonly cause late- rather than early-onset PVE. There are limited clinical data on the treatment of this condition. Where a range of time for treatment length is given, we advise that the longer course is used for PVE.

Endocarditis caused by Abiotrophia and Granulicatella species (collectively referred to as nutritionally variant streptococci) has a high rate of complications and treatment failure. It is also difficult to reliably measure antibiotic susceptibility in vitro and tolerance is common. A retrospective case review published in 2007 described eight cases of endocarditis that were successfully treated with a combination of surgery, benzylpenicillin or vancomycin for 6 weeks combined with ≥2 weeks of gentamicin. We therefore advise that 4–6 weeks of the combination of benzylpenicillin/amoxicillin plus gentamicin is used to treat these microorganisms.

It is difficult to determine the appropriate breakpoint for ‘high-level’ penicillin resistance such that an alternative agent, such as vancomycin, should be used. Penicillin breakpoints quoted for infections other than IE are not helpful, as IE is treated with far higher penicillin doses than are used for most other infections and peak serum levels can be >100-fold greater than the MIC. In addition, combination with gentamicin is synergistic. The AHA guidelines advise treating streptococci with an MIC >0.5 mg/L according to the regimen for enterococci (e.g. 6 weeks penicillin plus gentamicin) and, by inference, the breakpoint for ‘high-level’ penicillin resistance for streptococci would be the same as the CLSI penicillin breakpoint for enterococci (≥16 mg/L). Accepting that there are still insufficient clinical data, the ESC suggest that vancomycin is used for streptococci with an MIC >4 mg/L. We have followed the ESC lead and adopted this advice.

There has been recent debate about the appropriate penicillin breakpoints for Streptococcus pneumoniae. We advise the use of the same endocarditis breakpoints as for other streptococci. As 28% of patients with pneumococcal endocarditis also have meningitis, we advise that the meningitis breakpoints should be used when meningitis is also present (i.e. a penicillin breakpoint of 0.06 mg/L and ceftriaxone 0.5 mg/L).

Vancomycin or teicoplanin are still the preferred treatment for patients with immediate-type (IgE-mediated) penicillin allergy. In the ESC guidelines, vancomycin plus gentamicin is recommended for allergic patients who are infected with relatively penicillin-resistant streptococci (MIC 0.125–2 mg/L), while vancomycin monotherapy is recommended for penicillin-susceptible isolates. We would question the logic of determining whether gentamicin should be added on the basis of penicillin resistance. Animal models have shown that the combination of vancomycin with gentamicin is better than vancomycin monotherapy, but a recent small clinical study and case report described successful vancomycin monotherapy for seven patients with streptococcal endocarditis, although two underwent surgery. As vancomycin-tolerant streptococci have been described with a vancomycin MBC well in excess of peak levels, it would seem prudent to treat penicillin-allergic patients with 4–6 weeks of vancomycin plus ≥2 weeks of gentamicin.

9. Enterococcal endocarditis

See Table 5 for recommended regimens.

Recommendation 9.1: First-line therapy for susceptible streptococci is amoxicillin or high-dose penicillin with gentamicin. [B]
Recommendation 9.2: Glycopeptides in combination with gentamicin are second-line therapy for susceptible enterococci. [B]

Recommendation 9.3: There should be a low threshold for stopping gentamicin in patients with deteriorating renal function or other signs of toxicity. [B]

Enterococci remain the third most common cause of IE after staphylococci and oral streptococci, accounting for 10% of episodes. There have been no randomized clinical trials or significant changes in epidemiology since the publication of the previous guidelines to justify major changes to the treatment recommendations. Our recommendations are consistent with ESC guidelines except for minor differences in the gentamicin dosing regimen and suggestions for resistant strains (see below).

The addition of gentamicin to a cell wall-acting agent is still recommended for enterococcal endocarditis, but this is based more on established practice rather than evidence of superiority of combination therapy over monotherapy. We remain concerned about the toxicity of gentamicin, particularly as the majority of enterococcal endocarditis occurs in older patients. The anecdotal experience of the Working Party members suggests that starting 1 mg/kg gentamicin twice a day achieves appropriate levels in most cases, but longer dosing intervals may be required in patients with pre-existing renal impairment and according to serum levels. Since shorter courses of aminoglycosides can still effect a clinical cure, we now recommend a low threshold for stopping aminoglycosides if renal function deteriorates or if signs of ototoxicity develop. Since there is no evidence that a short delay in the addition of an aminoglycoside to the primary treatment agent is detrimental to outcome, it would seem prudent to wait for the results of susceptibility testing before starting gentamicin to avoid the possibility of administering a potentially toxic antimicrobial until it has been proven that it has activity against the infecting microorganism.

There has been anecdotal success treating high-level aminoglycoside-resistant (HLAR) enterococcal endocarditis with penicillin and ceftriaxone combinations. However, in a non-randomized open-label multicentre evaluation of this combination, an in-hospital mortality rate of 23% was reported, which is much higher than the 11% seen in international studies. Given the lack of evidence that such penicillin with cephalosporin combination therapy is superior to monotherapy with penicillin, the current UK epidemic of C. difficile infection and increasing concerns about ESBL-producing microorganisms, the Working Party does not recommend the routine addition of ceftriaxone to a penicillin for HLAR enterococci.

10. HACEK endocarditis

Recommendation 10.1: Treatment should be with a β-lactamase-stable cephalosporin or amoxicillin if the isolate is susceptible. [B]

Recommendation 10.2: Gentamicin should only be added for the first 2 weeks of therapy. [C]

Recommendation 10.3: Ciprofloxacin can be considered an alternative agent. [C]

Recommendation 10.4: NVE should receive 4 weeks and PVE 6 weeks of treatment. [C]

The HACEK group of fastidious extracellular Gram-negative bacteria are uncommon and cause an estimated 3% of all
Table 6. Summary of treatment recommendations for Q fever

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Antimicrobial</th>
<th>Dose</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>doxycycline$^a$ and hydroxychloroquine$^b$</td>
<td>100 mg q12h po; 200 mg q8h po</td>
<td>both antibiotics for at least 18 months and 4 years; subsequent therapy for 3 years</td>
</tr>
<tr>
<td>2.</td>
<td>doxycycline$^a$ and ciprofloxacin</td>
<td>100 mg po; 200 mg q12h po</td>
<td>≥3 years</td>
</tr>
</tbody>
</table>

q8h, every 8 h; q12h, every 12 h; po, orally.
$^a$In slow responders, defined as <50% reduction in mean phase 1 titres, doxycycline dosing should be adjusted to achieve serum levels of ≤5 mg/L.$^{119}$
$^b$Plasma levels to be maintained at 0.8–1.2 mg/L. Monthly serum levels must be obtained and dose adjusted accordingly. Photosensitivity is common. Retinal accumulation necessitates regular examination.

Table 7. Summary of treatment recommendations for Bartonella IE

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/route</th>
<th>Duration (weeks)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin AND gentamicin</td>
<td>2 g q4h iv</td>
<td>6</td>
<td>if penicillin allergic use tetracycline regular serum levels are needed to guide maintenance dose</td>
</tr>
<tr>
<td>Doxycycline AND gentamicin</td>
<td>200 mg q24h po; 1 mg/kg q8h iv</td>
<td>4</td>
<td>po, orally; iv, intravenously; q4h, every 4 h; q8h, every 8 h; q24h, every 24 h.</td>
</tr>
</tbody>
</table>

chronic Bartonella infection.$^{121}$ Only aminoglycosides have bactericidal activity against Bartonella spp.,$^{122}$ although susceptibility to macrolides, rifampicin and tetracycline has been demonstrated.$^{123}$

11. Q fever

See Table 6 for recommended regimens.

**Recommendation 11.1**: A combination of doxycycline and hydroxychloroquine for ≥18 months provides bactericidal activity and adequate protection from relapse.$^{107}[B]$

**Recommendation 11.2**: Antibody titres should be determined every 6 months whilst on treatment and then every 3 months for a minimum of 2 years once treatment has been discontinued.$[B]$

**Recommendation 11.3**: Patients should be considered cured when IgG antibodies to C. burnetii phase I are <1:800 and phase I IgM and IgA antibodies are <1:50.$^{107}$

C. burnetii is an obligate intracellular pathogen and is the causative microorganism of Q fever. C. burnetii causes up to 3% of all cases of IE in England and Wales.$^{108}$ The estimated incidence of IE in those who contract Q fever ranges from 7% to 67%,$^{109}$ and is the primary manifestation of chronic infection.$^{111}$ Patients likely to develop Q-fever IE are those with predisposing valvular damage or prosthetic heart valves.$^{112,113}$ C. burnetii is the commonest cause of culture-negative IE.$^{114}$ Relative resistance to doxycycline has been reported recently and higher doses have been recommended in patients whose phase I antibody titres are slow to decrease.$^{115,116}$

12. Bartonella endocarditis

See Table 7 for recommended regimens.

**Recommendation 12.1**: Treatment should be with gentamicin in combination with a β-lactam or doxycycline for a minimum of 4 weeks.$^{117,118}$

Bartonella spp. are facultative intracellular Gram-negative aerobic bacteria that cause up to 3% of all cases of IE.$^{119}$ B. quintana can cause trench fever and IE, and is transmitted by the body louse. Predisposing factors to infection include homelessness and alcoholism.$^{119,120}$ B. henselae is the causative microorganism of cat-scratch fever and rarely IE. IE is a feature of HACEK IE and can be administered orally; it has therefore been included as an alternative agent for therapy.

13. Other Gram-negative bacteria

A wide range of other Gram-negative bacteria continue to cause a small proportion (≤5%) of IE.$^{124}$ Risk factors include intravenous drug use, end-stage liver disease, central venous catheters and old age. Members of the Enterobacteriaceae, Acinetobacter spp. and P. aeruginosa are virtually always implicated. Susceptibility patterns, such as the spread of ESBL-producing isolates, and multidrug- or pan-drug-resistant strains complicate therapy and preclude clear evidence-based recommendations for therapy. The Working Party continues to support the principle that combination therapy (where possible comprising a β-lactam (which could be amoxicillin, a cephalosporin or a carbapenem) and an aminoglycoside) may offer synergy and prevent the emergence of resistance, but acknowledges that there are a lack of supporting clinical data in this context. It seems reasonable to consider therapeutic ‘once-daily’ gentamicin dosing regimens (e.g. 7 mg/kg ‘Hartford’ dosing regimen) for the treatment of these infections, rather than the lower ‘synergistic’ dose recommended for IE caused by Gram-positive bacteria, because the post-dose levels recommended for the latter (3–5 mg/kg) are likely to be unreliable for Gram-negative sepsis. In the previous edition of these guidelines, high-dose therapy, based on careful in vitro susceptibility testing, and early consideration of surgery are recommended. It may not always be appropriate to add an aminoglycoside because of concerns about nephrotoxicity. Likewise, prolonged high-dose gentamicin carries a significant risk of nephrotoxicity and careful monitoring for toxicity, including audiometry, is advised for courses longer than 2 weeks.

14. Fungal endocarditis

See Table 8 for recommended regimens.

Fungi cause endocarditis in ~2%–4% of all endocarditis cases.$^{125}$ Of these, Candida albicans causes ~25% of cases, other Candida species cause ~25%, Aspergillus species (notably Aspergillus fumigatus, Aspergillus flavus and Aspergillus terreus) cause 25% and a wide variety of other fungi are implicated in
therapy, although each case should be considered on its own merits. In adults, the outcome following medical therapy alone is as successful as combined medical and surgical intervention; others indicate equivalent outcomes. In neonates, medical therapy alone is as successful as combined medical and surgical intervention. Optimal antifungal therapy is not clear, but voriconazole, with confirmation of susceptibility of the isolate to voriconazole and therapeutic drug monitoring, is first-line therapy with long-term suppression for fluconazole-resistant, voriconazole-susceptible isolates. Second-line therapy, or first line if azole resistance; should not be used for A. terreus or A. nidulans infection.

### Table 8. Summary of treatment recommendations for fungal endocarditis

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Dose/route</th>
<th>Serum levels required?</th>
<th>Role in treating Candida endocarditis</th>
<th>Role in treating Aspergillus endocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>400 mg daily, only reduced in severe renal failure/dialysis</td>
<td>no</td>
<td>long-term suppressive therapy</td>
<td>none</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>intravenous therapy preferred initially, licensed doses</td>
<td>yes, with dose modification important</td>
<td>long-term suppressive therapy for fluconazole-resistant, voriconazole-susceptible isolates</td>
<td>first-line therapy with long-term suppression</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>3 mg/kg/24 h (AmBisome)</td>
<td>no</td>
<td>second-line therapy</td>
<td>second-line therapy, or first line if azole resistance; should not be used for A. terreus or A. nidulans infection</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg/day (Abelcet)</td>
<td>no</td>
<td>second-line therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mg/kg/day (Fungizone)</td>
<td>no</td>
<td>second-line therapy</td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td>200 mg daily</td>
<td>no</td>
<td>first-line therapy</td>
<td>third- or fourth-line therapy</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>70 mg loading, 50–100 mg daily</td>
<td>no</td>
<td>first-line therapy</td>
<td>no role</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>licensed doses</td>
<td>no</td>
<td>first-line therapy</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>400 mg twice daily</td>
<td>yes</td>
<td>no role</td>
<td></td>
</tr>
<tr>
<td>Flucytosine</td>
<td>100 mg/kg/day in three doses, reduced with renal dysfunction</td>
<td>yes, with dose modification important</td>
<td>as combination therapy with amphotericin B</td>
<td>as combination therapy with amphotericin B</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>NA</td>
<td>NA</td>
<td>no role</td>
<td>no role</td>
</tr>
</tbody>
</table>

NA, not applicable.

the remaining 25% of cases. Fungal endocarditis is most common in patients with prosthetic valves, but also occurs in intravenous drug abusers, neonates and immunocompromised patients. Candida endocarditis is usually a healthcare-associated infection (87%), and ~75% of Aspergillus endocarditis cases follow some form of cardiac surgery and may occur in clusters related to contaminated operating room air or high spore counts in the ward environment. Almost all cases of Aspergillus endocarditis have occurred in adults, but premature neonates with candidaemia may also develop Candida endocarditis.

### 14.1 Candida endocarditis

**Recommendation 14.1:** Initial treatment should be with an echinocandin or amphotericin B (preferably a lipid preparation), and modified, once the species and susceptibility profile is known, if required. [C]

**Recommendation 14.2:** Surgical valve replacement is highly desirable, if technically feasible. [C]

The outcome following antifungal treatment for Candida endocarditis may have improved slightly over the past 5 years. Some reports indicate better outcomes following medical and surgical intervention; others indicate equivalent outcomes. In neonates, medical therapy alone is as successful as combined therapy, although each case should be considered on its merits. In adults, the outcome following medical therapy alone was as good as that following combined medical and surgical therapy. However, individual circumstances vary substantially and clinical judgement is required to assess the relative risks in each patient. The surgical excision of infected material may be critically important in patients with relatively resistant organisms, systemic emboli, valvular dysfunction or other complicating factors preventing adequate medical therapy, such as drug intolerance or significant renal dysfunction. For those infected with susceptible Candida isolates, antifungal treatment with lipid-associated amphotericin B or an echinocandin (most experience is with caspofungin) is first line. Many authorities recommend the addition of flucytosine to amphotericin B. Amphotericin B therapy is preferred to echinocandin therapy in those infected with Candida parapsilosis, Candida guilliermondii and Candida famata, as these organisms are intrinsically less susceptible to, and rarely killed by, the echinocandins. Echinocandin therapy is preferred in those with Candida krusei infection, as this organism is less susceptible to amphotericin B. Intravenous therapy should not be for <4 weeks and may need to be for much longer. Long-term oral fluconazole therapy, for those with susceptible organisms, is appropriate after prolonged intravenous therapy. In those with infected prosthetic material, fluconazole may need to be lifelong.

### 14.2 Aspergillus endocarditis

**Recommendation 14.3:** Initial treatment should be with voriconazole, with confirmation of susceptibility of the isolate to voriconazole and therapeutic drug monitoring. [C]

**Recommendation 14.4:** Surgical valve replacement is mandatory for survival. [B]

Surgical excision and valve replacement is important for a successful outcome in Aspergillus valvular endocarditis; exceptionally few patients have ever survived without surgical intervention. Optimal antifungal therapy is not clear, but voriconazole as first-line therapy is recommended for several reasons. In
an animal model of Aspergillus endocarditis, voriconazole at adequate doses was curative. Several case reports have indicated success with voriconazole. Voriconazole is the recommended primary therapy for other sites of invasive Aspergil-
lus. However, the pre-clinical data indicate that it is critical in Aspergillus endocarditis to achieve adequate plasma concentrations of voriconazole, that some patients cannot tolerate voriconazole and that some azole resistance has been described in A. fumigatus. In these circumstances lipid-associated amphotericin B would be appropriate, possibly with flucytosine. Both A. terreus and Aspergillus nidulans are amphotericin B resistant, in which case oral posaconazole therapy might be a better substitute for voriconazole than amphotericin B, if required. Echinocandins are not recommended as they are never fungicidal for Aspergillus species.

14.3 Endocarditis due to other fungi
A large number of other fungi have caused fungal endocarditis, including Histoplasma capsulatum, Penicillium spp., various Mucorales species, Trichosporon spp., Paecilomyces spp. and numerous other rare fungi. Overall, these rare fungi may account for as many as 25% of all mycological cases, but publication bias is probably partly responsible for this disproportionately high frequency compared with other forms of invasive fungal disease. Management requires optimizing antifungal therapy, recognizing a much higher proportion of intrinsic antifungal resistance amongst these fungi than among Aspergillus and Candida spp.

14.4 General recommendations
A positive culture result is highly desirable, so excised valves and tissue should be cultured for fungi as well as bacteria, and isolates should not be discarded. Susceptibility testing must be undertaken for any fungus causing endocarditis, including the determination of minimal fungicidal concentrations. Azole resistance in A. fumigatus and both echinocandin and azole resistance in Candida spp. are of particular concern. If fungi continue to be isolated from blood cultures obtained after 1 week of treatment, they should also be susceptibility tested, as resistance may emerge on therapy. Fungal blood cultures should continue to be taken for at least the first 2 weeks on therapy and if any deterioration occurs, after this. In cases where no cultures have been positive, but tissue is available, molecular methods of speciation should be used as histopathology interpretation is inadequate to guide therapy optimally. For drugs with variable bioavailability (especially the azoles and flucytosine), therapeutic drug monitoring is important. Key biomarkers (antigen, PCR, glucan, imaging to include vegetation size measurements and antibody) should be obtained before therapy to assist with monitoring antifungal therapy, including recognizing breakthrough infection.

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We thank Dr Vittoria Lutje for literature searches, Professor Marjan Jahangiri of St George’s Healthcare NHS Trust for her contribution and Mrs Angie Thompson for assistance with correction to the text.

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F. K. G. currently sits on the Advisory Boards of Merck and Astellas. She previously sat on the Advisory Boards of Novartis and Pfizer, and has received a travel grant from Roche. J. F. has received funding from Novartis comprising a speaker’s fee for the European Cystic Fibrosis conference and a consultancy fee for advice on Tobramycin Inhaled Powder. All other authors have none to declare.

References


Cubicin (daptomycin for injection) for the treatment of Staphylococcus aureus bacteraemia including those with known or suspected infective endocarditis http://www.fda.gov/ohrms/Dockets/AC/06/briefing/2006-4209B1_02_01-FDA-Background.pdf (15 September 2011, date last accessed).


M. E. Ellis, H. Al-Abdely, A. Sandridge, W. Greer, and W. Ventura

We analyzed 270 cases of fungal endocarditis (FE) that occurred over 30 years. Vascular lines, non-cardiac surgery, immunocompromise and injection drug abuse are increasing risk factors. Delayed or mistaken diagnosis (82% of patients), long duration of symptoms before hospitalization (mean ± standard deviation, 32 ± 39 days) and extracardiac manifestations were characteristic. From 1988 onwards, 72% of patients were diagnosed preoperatively, compared with 43% before 1988 (P = .0001). The fungi most commonly isolated were Candida albicans (24% of patients), non-albicans species of Candida (24%), Apergillus species (24%), and Histoplasma species (6%); recently-emerged fungi accounted for 25% of cases. The mortality rate was 72%. Survival rates were better among patients who received combined surgical-antifungal treatment, were infected with Candida, and had univalvular involvement. Improvement in the survival rate (from <20% before 1974 to 41% currently) coincided with the introduction of echocardiography and with improved diagnostic acumen. Fungal endocarditis recurs in 30% of survivors. It is recommended that fungal endocarditis be diagnosed early through heightened diagnostic acumen; that patients be treated with combined lipid-based amphotericin B and early surgery; and that patients be followed up for >4 years while on prophylactic antifungal therapy.

Previous reviews of fungal endocarditis (FE) published >20 years ago [1–5] indicated substantial related morbidity and mortality. Striking changes have since occurred in mycologic epidemiology, diagnostics, and therapeutics, which have made the challenge of management more dynamic. The objective of this study is to present an overview of the clinical profile, presumed risk factors, fungal organisms, therapeutic management, and outcome of well-documented cases of FE published between 1965–1995. To our knowledge, this is the most comprehensive case review in the last 20 years.

METHODS

In cooperation with a reference librarian, we conducted a literature search on the Ovid-Medline database using the key words "endocarditis," "mycoses," "fungi" (in general), and "fungi" (specific human pathogen names) to identify publications that describe FE in humans during the period 1965–1995. High-quality case reports containing adequate information were eligible for analysis if the article met the following criteria: it was written in English; it described human cases; it provided information relating to demography, risk factors, clinical features, mycologic results, findings of echocardiography, surgery, or autopsy (pooled cases with limited or summary findings or data or general reviews were not acceptable); it provided evidence of mycologic diagnosis (i.e., positive fungal culture from blood, cardiac vegetation, or distal embolus or histologic features of fungal disease); it used the Beth-Israel or Duke definitions for infective endocarditis; and it described the outcome. Care was
A patient could have to invasive fungal infection, such as immunosuppression [7], of infective endocarditis, such as valvular heart disease [6], and of a feature generally accepted to contribute to the development entry, and data cleaning was performed before analysis.

A presumed risk factor was defined as the documentation of a feature generally accepted to contribute to the development of infective endocarditis, such as valvular heart disease [6], and to invasive fungal infection, such as immunosuppression [7]. A patient could have ≥1 risk factor, and risk factors could be unrelated to each other (e.g., injection drug abuse and diabetes) or related (e.g., immunodeficiency disease, such as systemic lupus, and steroid therapy).

We estimated durations of survival using the Kaplan-Meier technique and compared them using a 2-sample log rank test. A logistic regression module of SPSS software was used to examine the influence of 19 risk factors, sex, and age on death. We did not perform an advanced in-depth analysis of confounders and interactions of risk factors and treatments.

RESULTS

A total of 490 articles were initially identified, but 270 were excluded because they were in a language other than English (n = 129), had adequate documentation (n = 102), were nonclinical publications (n = 32), or were general reviews (n = 7). Included in the analysis were 220 articles; 193 of these described a single patient, although 1 published in 1976 described 7. We also analyzed 270 case reports that met the inclusion criteria, but not all of them provided all the information we needed to complete the data form.

From these articles we obtained data on 184 male patients and 83 female patients (male-to-female ratio, 2.22), and 3 patients whose sex was not specified. The mean age (± SD) of adult patients was 44.3 ± 14.3 years (range, 13–77 years), and of children was 2.90 ± 3.81 years (range, 1 month to 12 years).

Risk factors. All but 6 patients had ≥1 risk factor. Fifty-six (21%) of 270 patients had 1 risk factor; 77 (29%) had 2 risk factors; 75 (28%) had 3; and 56 (21%) had 4–7. The average number of risk factors per patient was 2.5. The risk factors are summarized in table 1; they include previous valvular surgery, antibiotic use, rheumatic heart disease, and nonvalvular major surgery. We identified 82 unique patterns of risk factors and 20 patterns that were shared by ≥2 patients (table 2).

The spectrum of immunosuppressive conditions among the patients in the study included the following: infection with HIV, diabetes mellitus, malignancy, neutropenia, organ transplantation, and treatment with corticosteroids (42 of 45 patients who had immunosuppressive therapy as a stem risk factor) or with other immunosuppressive drugs (cyclosporin A, azathioprine, monoclonal CD3 antibody [OKT3], and cyclophosphamide).

Valvular disease and valvular surgery. Of the patients we studied, 174 (64%) had ≥1 “valvular” risk factor—that is, a history of previous valvular surgery, bacterial endocarditis, rheumatic heart disease, nonrheumatic heart disease, or prolapsed mitral valve. Of the 146 patients with previous valvular surgery, 23 (16%) had undergone the procedure >1 time; 135 had replacement valves (74 of which were with nonbiological materials); and 18 of 146 patients did not present with FE until >2 years later.

Anatomic site of FE. The anatomic site of the vegetations could be determined in 257 patients; the aorta was the most common site (table 3). Ring abscesses were associated with aortic valve FE in 25 of 55 patients classified as having FE of the aortic and another valve, but were associated with only 1 patient with mitral valve FE. Other sites included the pulmonary area (7 patients) and the endocardium (12 patients).

Of 112 patients who underwent surgery as part of the management for FE, there were 56 with sufficient published details to precisely correlate the anatomic location of the fungal vegetations noted at surgery with the site of previous valvular surgery. Twenty-seven (90%) of 30 patients who had previous aortic valvular surgery had FE that was confined to the aortic valve, whereas 3 (10%) had vegetations only on the mitral valve. Nine (64%) of 14 patients with previous mitral valve surgery were found to have FE on the same valve, but 5 (36%) had vegetations on the aortic valve only, and the valves operated on previously were free of FE. Of the 12 patients with previous surgery to other valves, fungal vegetations were found on the corresponding prosthetic valves in only 7 (58%).

We identified 36 patients without previous valve surgery whose only risk factor for right-side endocarditis (i.e., without existing known valvular disease) was injection drug abuse, total parenteral nutrition, or central iv line insertion. These patients might have been expected to be at risk for FE of tricuspid valves, pulmonary valves, or both. However, in this group of patients, FE occurred on these valves in only 8 (22%) of 36 patients (pulmonary, n = 2; tricuspid, n = 6).

Substantial changes in the percentage contribution of the most common risk factors were observed over the years. These are shown in the smoothed incidence curves in figure 1. The incidence of vascular lines, surgery other than cardiac surgery, immunocompromised patients, and injection drug abuse showed increases, in contrast to the decline in incidence of previous valve surgery, antibiotic use, rheumatic heart disease, previous bacterial endocarditis, and other valve disease.

Clinical features of presentation and diagnosis. Thirty-four distinct presenting features could be identified. The 6 most common were as follows: fever (n = 202), changing or new heart murmurs (n = 137), major peripheral embolization...
<table>
<thead>
<tr>
<th>Stem risk factor</th>
<th>Code</th>
<th>Stem risk factor</th>
<th>Other risk factors associated with stem risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous valve surgery(^{a,b})</td>
<td>18</td>
<td></td>
<td>146 (54) 251</td>
</tr>
<tr>
<td>Antibiotic use(^{a,c})</td>
<td>6</td>
<td></td>
<td>130 (48) 326</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>14</td>
<td></td>
<td>65 (24) 127</td>
</tr>
<tr>
<td>Surgery other than cardiac(^{d})</td>
<td>13</td>
<td></td>
<td>62 (23) 153</td>
</tr>
<tr>
<td>Vascular lines</td>
<td>11</td>
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<td>48 (18) 160</td>
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<td>Immunosuppressive treatment(^{a})</td>
<td>8</td>
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<td>45 (17) 96</td>
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<td>9</td>
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<td>Other valve disease</td>
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<td></td>
<td>35 (13) 66</td>
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<tr>
<td>Previous bacterial endocarditis(^{a,g})</td>
<td>17</td>
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<td>34 (13) 92</td>
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<td>5 (2) 22</td>
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<td>Urethral or peritoneal catheter</td>
<td>12</td>
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<td>Prolapsed mitral valve</td>
<td>15</td>
<td></td>
<td>3 (1) 7</td>
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<tr>
<td>Previous dental surgery</td>
<td>19</td>
<td></td>
<td>5 (2) 13</td>
</tr>
<tr>
<td>None</td>
<td>—</td>
<td></td>
<td>6 (2) —</td>
</tr>
</tbody>
</table>

\(^{a}\) Significant indicators of death.  
\(^{b}\) Of these patients, 76 (46%) of 149 were treated <3 months previously, 43 (30%) of 146 were treated 4–12 months previously, and 34 (23%) of 146 were treated >12 months previously.  
\(^{c}\) Penicillins, aminoglycosides, and cephalosporins were the most common; they were given for a mean of 55 (86.5) days in 56 patients.  
\(^{d}\) Abdominal surgery was performed for 30 (48%) of 62 patients and cardiothoracic surgery for 26 (42%) of 62. Of these, 25 (40%) of 62 patients had surgery within the previous 6 months.  
\(^{e}\) Of these patients, 9 (27%) of 34 were treated <3 months previously, 9 (27%) of 34 were treated 3–6 months previously, and 16 (47%) of 34 were treated >12 months previously.  
\(^{f}\) A total of 42 of 45 patients received corticosteroids.  
\(^{g}\) Patients may have >2 different immunocompromising risk factors (see table 2).  

\((n = 97)\), focal or generalized neurological features \((n = 83)\), heart failure \((n = 64)\), and dyspnea \((n = 61)\). Other symptoms included abdominal pain \((n = 26)\); arthralgia \((n = 8)\); arthritis \((n = 4)\); bloody stools \((n = 3)\); chest pain \((n = 26)\); finger clubbing \((n = 8)\); cough \((n = 22)\); hemoptysis \((n = 3)\); hepatomegaly \((n = 41)\); malaise \((n = 55)\); myalgia \((n = 12)\); rigors \((n = 29)\); skin petechiae \((n = 50)\); sweats \((n = 18)\); weight loss \((n = 25)\); arrhythmias \((n = 12)\); atrioventricular conduction defects \((n = 9)\); sudden death \((n = 6)\); septic shock \((n = 11)\); coagulopathy \((n = 7)\); jaundice \((n = 4)\); nausea, vomiting, or anorexia \((n = 21)\); hypotension \((n = 2)\); renal failure \((n = 4)\); and miscellaneous nonspecific symptoms \((n = 22)\). Classic signs of infective endocarditis were uncommon: 8 patients had finger clubbing, 11 had Osler’s nodes, 19 had splinter hemorrhages, 17 had Roth’s spots, and 40 had splenomegaly.

A total of 261 patients had ≥1 of the 6 most common symptoms. The mean number of symptoms was 6 per patient (range, 0–13 symptoms). For the 270 patients, there were 208 combinations of symptoms, 188 of which were unique. Thirteen patients had fever only; 10 had fever with major peripheral embolization; and 9 had fever with some other symptom.

Embolization of major arterial vessels that resulted in focal ischemia occurred in 154 sites in 122 (45%) of 270 patients (table 4). Major limb vessel occlusion of the femoral artery division was seen in 42 patients. Upper limb ischemic phenomena were seen in only 8 patients. Focal cerebrovascular symptomatic involvement was documented in 47 patients, and this was expressed mainly as hemiplegia, facial palsy, and amaurosis fugax or other transient ischemic attacks. A further 24 patients had nonfocal neurological features, namely confusion,
### Table 2. Pairwise risk factor combinations for patients with fungal endocarditis.

<table>
<thead>
<tr>
<th>Additional risk factor</th>
<th>Stem risk factor</th>
</tr>
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<tbody>
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<tr>
<td>36 1 0 1 0 5 0 1 3 0 1 0 1 2 1 4 8 11 0 1</td>
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<tr>
<td>3 0 1 0 5 0 1 0 0 1 0 0 0 0 0 1 0 0 0 2</td>
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</tr>
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<td>12 0 0 1 1 1 4 1 3 0 5 3 0 0 0 3 1 3</td>
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<tr>
<td>13 0 0 7 2 1 1 0 0 0 0 0 0 0 0 4 0</td>
<td></td>
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<tr>
<td>5 4 1 4 1 4 0 0 0 0 0 0 0 1 0 0 0 0 5</td>
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<td>130 18 30 28 5 38 1 39 31 0 18 26 64 4 6</td>
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<tr>
<td>21 4 9 0 18 0 15 1 1 1 0 2 0 0 7</td>
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</tr>
<tr>
<td>45 23 5 14 1 14 6 1 3 4 13 0 8</td>
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<td>2 0 0 0 0 0 0 1 0 12</td>
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<td>62 7 0 5 4 16 0 13</td>
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<td>65 0 1 12 54 2 14</td>
<td></td>
</tr>
<tr>
<td>3 0 1 0 15</td>
<td></td>
</tr>
<tr>
<td>35 4 22 1 16</td>
<td></td>
</tr>
<tr>
<td>35 25 1 17</td>
<td></td>
</tr>
<tr>
<td>146 3 18</td>
<td></td>
</tr>
<tr>
<td>5 19</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients with the specified risk factors (e.g., there were 12 patients with risk factor 3 [diabetes], of whom 7 also had risk factor 6 [antibiotic use], 1 had risk factor 7 [parenteral nutrition], etc.). Thus, the bottom cell in each column indicates the total number of patients with the risk factor.

### Table 3. Anatomic site of fungal vegetations in patients with fungal endocarditis.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic valve</td>
<td></td>
</tr>
<tr>
<td>Aortic valve alone</td>
<td>46 (18)</td>
</tr>
<tr>
<td>Aortic and mitral valves</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Aortic and other site</td>
<td>55 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>111 (44)</td>
</tr>
<tr>
<td>Mitral valve</td>
<td></td>
</tr>
<tr>
<td>Mitral valve alone</td>
<td>33 (13)</td>
</tr>
<tr>
<td>Mitral and other site</td>
<td>24 (9)</td>
</tr>
<tr>
<td>Mitral and aortic valves</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>67 (26)</td>
</tr>
<tr>
<td>Tricuspid site</td>
<td></td>
</tr>
<tr>
<td>Tricuspid valve alone</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Tricuspid and other site</td>
<td>8 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>47 (18)</td>
</tr>
<tr>
<td>Not specified</td>
<td>15 (6)</td>
</tr>
</tbody>
</table>

seizures, or altered consciousness. Thus a total of 71 (26%) of 270 patients had prominent central neurologic presentations.

FE was among the listed differential presenting diagnoses for only 48 (18%) of 270 patients. Subacute bacterial endocarditis was the most common initial misdiagnosis and was cited for 111 (41%) of 270 patients. A wide range of other incorrect diagnoses at presentation was mentioned for the remaining 111 patients, including fever of unknown origin, bacterial sepsis, valvular incompetence, renal failure, peripheral embolism, and stroke.

**Duration of symptoms before diagnosis.** Two hundred patients developed FE at home, and 69 patients developed the disease while hospitalized (for 1 patient, this information was not given). Of the 200 patients who developed FE at home, there were 129 patients for whom adequate information was reported on the duration of symptoms. The time from the first symptom to hospital admission ranged from 1 day to 1 year. The distribution showed peaks at regular intervals of 1 day, 1 week, 2 weeks, 3 weeks, 6 weeks, 2 months, and 3 months. The tail of the distribution extended past 4 and 5 months, with 4 patients each reporting symptoms for that period of time. There were 4 patients with symptoms that lasted 9 months to 1 year. If these 4 patients are excluded, the mean ± SD duration of symptoms was 32 ± 39 days; median, 21 days; n = 125). Another 69 patients were already hospitalized for the management of other illnesses; the duration of symptoms before diagnosis was described for 66 of these, and it ranged from 1 day to 1 year. This distribution was smoother—without peaks but with a gap between 5 months (n = 64) to 7 months followed by 1...


year \( n = 5 \). If the outliers at 7 months and 1 year are excluded, the mean time \( (\pm SD) \) from the first symptom to hospital admission was 34 ± 31 days (median, 27 days; \( n = 64 \)). Complete information regarding duration of hospitalization was available for 236 hospitalized patients. Hospitalization time was 1–395 days before the diagnosis of FE; 91% of the patients were hospitalized for up to 2 months before diagnosis. The mean time \( (\pm SD) \) to diagnosis for these 214 patients was 17 ± 16 days.

The diagnosis of FE was made preoperatively for 128 patients (47%), peripherally for 40 (15%), and was only made at postmortem examination for 92 (34%). The time was not clearly specified for 10 patients. However, over the years, there was an increasing proportion of the patients for whom the diagnosis was made earlier (i.e., preoperatively): 43% of patients before 1988 and 72% from 1988 onwards. This change in the timing of diagnosis was statistically significant \( (P = .0001) \).

**Echocardiography.** Echocardiography was used as a diagnostic tool for 105 patients (101 patients after 1975). There were 111 pretreatment examinations performed. For 82 of these 105 patients, the distinction between transthoracic and transesophageal modes was not specified. Eighty-six of 111 examinations of 84 patients showed evidence of FE (77% sensitive), and 25 (21 patients) did not (23% false negativity). Thirty-nine vegetations identified by echocardiography were described categorically for 37 patients as either small, medium, or large. Thirty-seven of these patients had large vegetations on \( \geq 1 \) valve. Two patients had large vegetations on 2 valves. The most common site for a vegetation seen via echocardiography was the aortic valve, and all of these were described as large. Only 35 of the vegetations were described categorically. Four vegetations were described both in millimeters and categorically: 2 tricuspid vegetations (40 and 100 mm), 1 mitral vegetation (20 mm), and 1 pulmonary vegetation (8 mm). Nine other patients had vegetations described only in millimeters, as follows: 2 aortic vegetations (3 and 8 mm); 4 mitral (2 that were 20 mm, 1 that was 14 mm, and 1 that was 5 mm); 2 tricuspid (15 and 10 mm); and 1 endocardium (10 mm).

**Laboratory data.** Chemistry and hematologic profiles were published for a minority of patients. The mean hemoglobin level \( (\pm SD) \) for 65 was 92 ± 36 g/dL; mean WBC of 106 patients was 14.5 ± 10.5 x 10^6 cells/L; and the erythrocyte sedimentation rate of 30 patients was 53 ± 26 mm/h for the first hour. Of 45 patients tested for hematuria, 19 had positive results.

**Source of mycologic diagnosis.** All 270 patients had either a blood culture, cardiac vegetation culture, or histologic examination of a valve performed. A positive mycologic diagnosis was made for 139 of 260 patients by blood cultures (54% sensitivity). Results of blood cultures were positive for specimens obtained preoperatively from 118 patients (85%), for specimens obtained postoperatively for 5 patients (4%), and for specimens

<table>
<thead>
<tr>
<th>Site</th>
<th>No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral</td>
<td>47 (17)</td>
</tr>
<tr>
<td>Femoral</td>
<td>42 (16)</td>
</tr>
<tr>
<td>Popliteal</td>
<td>12 (4)</td>
</tr>
<tr>
<td>Posterior tibial/dorsalis pedis</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Common iliac</td>
<td>8 (3)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Radial/brachial</td>
<td>8 (3)</td>
</tr>
<tr>
<td>Mesenteric/splenic/renal</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Coronary</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Imprecisely stated</td>
<td>6 (2)</td>
</tr>
</tbody>
</table>
obtained at postmortem examination for 13 patients (9%). Mycologic diagnosis was made for 143 of 197 patients by means of cardiac vegetation cultures (73% sensitivity) and for 173 of 183 patients by means of histologic examination of valve vegetations (95% sensitivity).

Of 270 patients, 145 had all 3 of these diagnostic tests performed, and 44 had positive results on all 3. Only 2 patients were negative by all 3 tests. For 21 patients, results were positive for culture of blood specimens and histologic examination of a valve, but negative for culture of cardiac vegetation specimens. For 4 patients, results of culture of blood specimens was positive but results of culture of cardiac vegetation specimens and histologic examination of a valve were negative. Table 5 presents 3-way correlations between these tests.

In addition, there were 77 patients with accessible peripheral large arterial vessel embolization; 65 of these underwent embolectomy. Histologic examination revealed that 41 of 65 patients (63% sensitivity) had microscopical features compatible with fungal infection.

For 27 patients (10%) with unexplained fever and no other features of FE, candidemia had been documented as part of a diagnostic work-up at a mean time of 15 weeks (range, 1–52 weeks) before diagnosis of FE. Eleven of these 27 patients (41%) received no systemic antifungal treatment. Serologic testing aided the diagnosis for only 46 patients (17%), and positive results were reported for 37 (80%) of 46.

**Pathogens.** All patients but 3 were reported as having a single fungal pathogen. These 3 had polymicrobial fungal infections: *Candida parapsilosis* and *Candida albicans*, *Candida tropicalis* and *C. parapsilosis*, and *C. albicans* and *Aspergillus* species. Table 6 presents data on the pathogens isolated by the year the study was published. *Candida* were isolated for 142 (53%) patients. The ratio between non-*albicans* species of *Candida* and *C. albicans* species was 1:2. *C. parapsilosis* comprised the majority of the identifiable non-*albicans* species of *Candida Aspergillus* species were identified for 66 (25%) patients, among whom *Aspergillus fumigatus* was identified for 25 (38%). *Histoplasma capsulatum* was the responsible pathogen for 15 (6%). The other pathogens identified are listed in the footnote to table 6.

Minor year-to-year variation in the relative proportion of different fungal species was found, but there was no overall change in the proportion of *Aspergillus* species to *Candida* species. There was a relative increase in the proportion of non-*albicans* species of *Candida* to *C. albicans* species over the 17 years, from 0.75 in 1965–1971 to 1.10 in 1988–1995. The significance of the change in ratio is limited by the small number of patients in the groups.

There is a striking increase in the category of other fungi, including dermatophytes from <10% to about a quarter of all fungi causing FE over the time of the study. With these 47 pathogens, there were strong risk factor associations for previous valve surgery (30 of 47), rheumatic heart disease (17 of 47), antibiotic use (12 of 47), surgery other than cardiac (9 of 47), and immunocompromised patients (8 of 47).

Thirty of the patients had concomitant bacterial infections, of whom 8 (3% of the total) had subacute bacterial endocarditis (SBE), as shown by examination of the valve by means of histological examination, culture, or both. The organisms were *Staphylococcus aureus* (2 patients), *Escherichia coli* (1 patient), *Serratia marcescens* (1 patient), *Pseudomonas aeruginosa* (1 patient), *Streptococcus viridans* (1 patient), *Staphylococcus epidermidis* (1 patient), and *Clostridium* species (1 patient).

**Antifungal and surgical strategies.** All patients received medical (antifungal) treatment or surgical treatment or combined medical and surgical treatment or no treatment. The number of patients who had either medical or surgical or medical and surgical treatment was 179; of these, 67 (37%) received systemic antifungal treatment only. Five (3%) received surgery only, and 107 (60%) had combined medical and surgical treatment. Ninety-one (34%) of 270 patients received no treatment for their FE, and 85 of these were diagnosed only at autopsy. In most cases, the correct diagnosis had not been made while the patient was alive, or the patients died at or shortly after admission to hospital.

Of the 112 patients who had surgery as part of their management, 19 (17%) underwent surgery because medical treatment with antifungal therapy failed, 37 (33%) because they had single indications, and 18 (16%) because they had multiple indications; for 38 (34%), the indication for surgery was not stipulated. The single indications were as follows: 11 for FE perse, 9 for the subsequent development of cardiac failure, 10 for subsequent embolization, 6 for subsequent hemodynamic instability, and 1 for persistent sepsis. The multiple indications were as follows: 1 patient had persistent sepsis and FE; 1 patient had cardiac failure and FE; 2 patients had persistent sepsis and hemodynamic instability; 2 patients had cardiac failure and persistent sepsis; 2 patients had persistent sepsis, FE, and subsequent embolization; 5 patients had cardiac failure and he-

<table>
<thead>
<tr>
<th>Patient group (n)</th>
<th>Blood culture</th>
<th>Culture of cardiac vegetation</th>
<th>Histologic examination of valve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (44)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (21)</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3 (4)</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4 (56)</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 (17)</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>6 (2)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**NOTE.** +, Fungus positively identified; −, no fungus identified.

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mododynamic instability; and 5 patients had FE and subsequent embolization. For 106 of the 107 patients who received both medical and surgical treatment, there was a clear statement of the time of administration of antifungal agents. Fifty-seven (54%) received antifungal agents both pre- and postoperatively, 10 (10%) preoperatively only, and 39 (36%) postoperatively only.

**Preoperative findings and surgical management.** The anatomic location of FE was described for 110 patients. The locations were as follows: aortic valve, 68 patients (62%; 35 on the aortic valve alone); mitral valve, 31 (25%; 17 on the mitral valve alone); tricuspid valve, 11 (10%; 8 on the tricuspid valve alone); and pulmonary valve, 2 (2%). Ring abscesses were noted in 18 patients (16%) but always in combination with some other valvular vegetation. Vegetations were described as “large” (but this was not quantified) for 60 (92%) of 65 patients for whom a comment on size was made. The actual sizes in millimeters for the vegetations were recorded for only 17 patients. The sizes were as follows: aortic valve, 9±50 mm; mitral valve, 29 (25%) but always in combination with some other valvular vegetation; tricuspid valve, 11 (10%) preoperatively only, and 39 (36%) postoperatively only.

**Table 6. Pathogens isolated from patients with fungal endocarditis, by year the results were published.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus species</td>
<td>18 (28)</td>
<td>20 (25)</td>
<td>14 (21)</td>
<td>14 (24)</td>
<td>66 (24)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>20 (31)</td>
<td>18 (23)</td>
<td>14 (21)</td>
<td>14 (24)</td>
<td>66 (24)</td>
</tr>
<tr>
<td>Non-albicans species of Candida</td>
<td>15 (24)</td>
<td>26 (32)</td>
<td>19 (28)</td>
<td>16 (28)</td>
<td>76 (28)</td>
</tr>
<tr>
<td>Histoplasma</td>
<td>6 (9)</td>
<td>4 (5)</td>
<td>4 (6)</td>
<td>1 (2)</td>
<td>15 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (8)</td>
<td>12 (15)</td>
<td>17 (25)</td>
<td>13 (22)</td>
<td>47 (17)</td>
</tr>
<tr>
<td>Total</td>
<td>64 (100)</td>
<td>80 (100)</td>
<td>68 (100)</td>
<td>58 (100)</td>
<td>270 (100)</td>
</tr>
</tbody>
</table>

**Ratio of:**

- Non-albicans species of Candida to C. albicans
  - 0.75

- Aspergillus to Candida
  - 0.75

NOTE. Data are no. (%) of pathogens isolated except as otherwise indicated.

- a Number of patients from which each pathogen was isolated was as follows: A. fumigatus, 25; A. flavus, 8; A. niger, 3; A. clavatus, 1; A. terreus, 5; A. auresus, 2; A. nidus, 1; Aspergillus unspecified, 11.
- b Number of patients from which each pathogen was isolated was as follows: C. glabrata/horulopsis, 10; C. tropicalis, 10; C. pseudotropicalis/kyster, 1; C. parapsilosis, 33; C. kruisei, 4; C. stellatoidea, 2; C. guillermondii, 4; C. parakruisei, 2; non-albicans species of Candida unspecified, 12.

- c Number of patients from which each pathogen was isolated was as follows: Mucor species, 3; Trichosporon beigelli, 1; Trichosporon species, 1; Cryptococcus neoformans, 3; Pseudallescheria boydii, 4; PhialophoraJeanselemis, 5; Curvularia lunata, 1; Trichophyton species, 2; Microsporum species, 1; Penicillium marneffei, 3; Fusarium species, 1; Paecilomyces species, 6; Penicillium chrysogenum, 1; Rhodotorula species, 1; Conidiobolus species, 1; Scedosporium species, 1; Engyodontium alba, 1; Wangiella dermatitidis, 1; Exophiala dermatitidis, 1; Saccharomyces species, 1; unspecified fungus, 6.

Survival rates associated with treatment, organism, risk factors, and valve site. The crude survival status (patient alive or dead as reported in each publication) was available for 269 patients: 195 (72%) patients died. The cause of death was directly related to FE for 151 (77%) of the 195 deaths. The percent of patients treated preoperatively, postoperatively, or both: 55 (25%) preoperatively only, 33 (15%) postoperatively only, and 39 (22%) patients, 5 of whom received amphotericin B and flucytosine. Seven (4%) received one of the following: rifampin, fluconazole, ketoconazole, terbinafine, miconazole, and itraconazole. 39 (22%) patients, 5 of whom received amphotericin B and flucytosine. Seven (4%) received one of the following: rifampin, fluconazole, ketoconazole, terbinafine, miconazole, and itraconazole.
before death and for whom the survival (defined as the number of days from first treatment for FE to time of last follow-up or death) was recorded. Patients receiving combined treatment with systemic antifungal agents and surgery tended to have an improved survival rate compared with those receiving medical treatment alone (figure 2A). Forty-five (55%) of 102 patients who received combined treatment modalities were alive at 1 year, compared with 21 (36%) of 58 patients who received only antifungal therapy. The differential higher survival was maintained for up to 3 years.

The proportion of patients who survived was analyzed according to the 3 most common pathogen groups (Aspergillus, C. albicans, and non-albicans species of Candida; figure 2A) and by treatment (antifungal agents alone, and antifungal agents plus surgical treatment; table 7). There were significantly more survivors for patients with Candida infections compared with those with Aspergillus when treated with antifungal agents alone ($P<.001$) and when treated with antifungal agents and surgical treatment ($P = .0001$). A significantly higher proportion of patients with Candida infections treated with antifungal agents and surgical treatment survived than did patients treated with antifungal agents alone ($P<.03$). No other differences

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Kaplan-Meier survival curves for (A) patient survival by antifungal therapy or combined valve removal and antifungal therapy and for (B) survival by fungal organism.
proved statistically significant. Survival rates for patients with histoplasmosis were 35% for those treated with antifungal agents and surgery and 63% for those treated with antifungal agents alone. The median duration of survival for patients with Aspergillus infection was 11 days; for those with Candida infection, 690 days; and for those with Histoplasma infection, 540 days (figure 2B).

Bivariate analyses were conducted on all 19 risk factors to examine their individual associations with survival ($\chi^2$ and Fischer’s exact tests). Previous valve surgery was found to have a statistically significant association with death ($P<.03$). However, a logistic regression analysis that used all 19 risk factors simultaneously found that antibiotic use, immunosuppressive treatment, and previous SBE were all statistically significant ($P<.05$), which suggests that they may be all indicators of death. Additional analysis also suggested that age might exert an influence that is different for men than for women.

The survival rate among patients who were given amphotericin B postoperatively was significantly higher than it was among patients who did not receive it: 47 (58%) of 85 patients compared with 6 (48%) of 21 ($P = .01$, Wilcoxon test), respectively, were alive at 1-year follow-up examinations. There was no significant difference in the survival rates between the group of patients who received amphotericin B before surgery and the group who did not.

Mortality was highest for patients with mitral valve FE either alone (26 [79%] of 33 patients died) or with concomitant aortic valve FE (6 [60%] of 10 patients died). Patients with solitary aortic valve FE had a 54% mortality rate (25 of 46). The survival rate was best among patients with tricuspid valve FE (8 [89%] of 9 patients survived). The median time of survival for patients with univalvular disease overall was 20 months, and was 5 months for patients with multivalvular FE ($P<.05$).

**Posttreatment complications and FE recurrence.** A total of 162 patients survived the initial treatment period. Emboli occurred in 41 (25%) of these 162 patients; 22 (54%) were cerebral, 7 (17%) were in a peripheral limb, 3 (7%) were pulmonary, 3 (7%) were coronary, and 2 (5%) were mesenteric. Major complications from treatment or FE itself were noted in 68 (42%) of 162 patients. Amphotericin B toxicity, flucytosine toxicity, or both was documented in 22 (32%) of 68 patients, prosthetic valve mechanical dysfunction in 11 (16%), nosocomial sepsis in 8 (12%), atrioventricular conduction defects in 7 (10%), and miscellaneous problems in 20 (29%).

FE recurred in 51 (29%) of 162 patients. In 5 (10%) of 51 patients, this was at $>2$ years of follow-up (treatment had previously been deemed successful for all 5 patients). Suboptimal antifungal therapy (including treatment with 5-flucytosine alone, resulting in documented emergence of resistant organisms and gross noncompliance) appeared to be present in 22 of 51 patients.

**DISCUSSION**

The identification of only 270 high-quality case reports in the English-language world literature over 30 years supports the notion that FE is a rare disease, with fungi responsible <10% of infective endocarditis cases. The objective of this retrospective meta-analysis of selected literature was to provide a template for managing FE by comprehensively describing important profile characteristics, determinants, course, and treatment. We believe it is the largest and most comprehensive review in the last 20 years; it focuses on well-described, authentic reports and is representative of published cases of FE. There is a small possibility that this article fails to reflect a wider picture of FE because of the bias inherent in selecting cases for publication. However, this is unlikely because of the generally accepted rarity of FE (a factor that promoted publication), the large time frame covered, and the low possibility of missing other quality cases in our search. Independent data extraction by 2 investigators

### Table 7. Survival rates for patients with fungal endocarditis, according to type of treatment and pathogen.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Treatment</th>
<th>n</th>
<th>Antifungal only</th>
<th>Antifungal and surgical</th>
<th>$P^a$</th>
<th>$P^b$</th>
<th>$P^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em></td>
<td></td>
<td>16</td>
<td>1/10 (10)</td>
<td>2/16 (13)</td>
<td>NS</td>
<td>.018</td>
<td>.0001</td>
</tr>
<tr>
<td><em>Candida</em></td>
<td></td>
<td>103</td>
<td>18/44 (41)</td>
<td>34/59 (58)</td>
<td>.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td></td>
<td>43</td>
<td>7/21 (33)</td>
<td>12/22 (55)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td></td>
<td>60</td>
<td>11/23 (48)</td>
<td>22/37 (60)</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Histoplasma</em></td>
<td></td>
<td>31</td>
<td>5/8 (63)</td>
<td>8/23 (35)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients who survived/no. of patients treated (%). NS, not significant.

- $^a$ Difference of outcome between antifungal treatment alone and combined antifungal and surgical treatment.
- $^b$ Difference of outcome between Aspergillus and all Candida species for combined antifungal and surgical treatment.
- $^c$ Difference of outcome between antifungal treatment alone and combined antifungal and surgical treatment.
minimized crucial errors such as survival duration, and where isolated incomplete information occurred, analyses reflected this.

A proposed major pathogenic component of FE, suggested by animal models, is a prerequisite for valvular anatomic abnormality, including traumatized endothelium [1, 5, 8–11], which mediates the formation of a fibrin/platelet bed that allows fungal adhesion. Our findings only give partial support for this hypothesis: only 64% of the patients had previous valve surgery, SBE, or rheumatic heart disease. The lack of concordance between the anatomic site of previous valve surgery and subsequent site of FE, and of predominantly left-side FE occurring in patients with predisposition to right-side endothelial trauma (e.g., injection drug abuse [12] and central lines) is further evidence that suggests pathetiologic factors other than those suggested from the animal studies are involved in FE genesis. Perhaps the vascular tropism of some fungi, particularly Aspergillus [13], may override the prerequisite for endothelial trauma in some instances.

Predisposition to an invasive fungal disease is the other important factor. We were able to identify several risk factors consistent with this: injection drug abuse (fungal contamination of drugs), immunosuppressive therapy, neutropenia, or malignancy (reduced anticonidial and antimycelial defenses and lack of integrity of mucosal surfaces), and total parenteral nutrition (contamination). It is remarkable that our review included no patient with hematologic malignancy—one of the highest risks for invasive fungal disease. This may be related to their profound thrombocytopenic status, because platelets play an integral role in the process of the fungus adhering to the vascular endothelium [14]. Recent experience confirms this rare association [15, 16]. Previous use of antibiotics was frequently observed to play a pivotal role (e.g., overgrowth of gastrointestinal Candida because of suppression of normal flora), an indirect role (e.g., use of central iv administration devices), or a surrogate role (e.g., as a marker for FE risk as a result of severe illness, immunosuppressive therapy, or abdominal surgery, because for patients in these groups, concomitant antibiotic use or placement of a central venous catheter are common).

Patients who have had undergone previous valve replacement or valve surgery tend to present with FE earlier than those who have had previous SBE. The late presentation (>12 months) of a substantial proportion of these patient groups is a salient reminder that such patients should be closely monitored for several years for early signs of FE.

The changes in risk factors over time, as displayed in figure 1, are notable. The most striking of these changes—steady increases in the incidence of use of vascular lines and non-cardiac surgery, and increases in the number of immunocompromised patients—reflect the changing hospital patient population; in-
specifications of glucan, and Aspergillus galactomannan agnostic tests. There have been major advances in detecting features, mycologic examination of accessible emboli, echocardiographic imaging, and fungal blood cultures, and (6) recent documented but untreated disease, (2) suspected diagnosis of SBE, (3) unexplained neurologic signs, (4) peripheral embolization (5) negative results of culture of peripheral blood and/or accessible emboli specimens or histologic examination of accessible emboli; and (c) positive findings from fungal antigen and antibody detection systems (which should be used routinely for diagnosis of high-risk patients in a validation setting).

Management: Early combination antifungal medication and valve replacement for optimal success; antifungal agents should be used early, in high dosages; amphotericin B is the drug of choice, but it should be administered as a lipid preparation to optimize continued uninterrupted therapy and reduce toxicity. Optimal dosage, timing, and duration of treatment is undetermined, but basic antifungal therapeutics direct that treatment is given preoperatively, perioperatively and postoperatively, aiming for an acute delivery load of 2–3 g. Careful, prolonged follow-up on secondary prophylaxis aided by serologic testing (requires validation) should be performed to detect the possibility of early fungal recurrence. Secondary prophylaxis should be for a minimum of 2 years, with fluconazole for susceptible Candida species and itraconazole-cyclodextrin or voriconazole for Aspergillus species.

Table 8. Recommendations for the diagnosis and management of fungal endocarditis (FE).

<table>
<thead>
<tr>
<th>Category</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspcion</td>
<td>FE should be highly suspected for middle-aged men with either (a) known preexisting valvular disease and asymptomatic fungemia; or (b) ≥1 major stem risk factor for FE (in particular, previous valve surgery, bacterial endocarditis, rheumatic heart disease) and fever, changed heart murmurs, major neural or neuroembolic phenomena and negative findings of bacterial culture</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Diagnosis should be made early by use of high diagnostic suspicion and acumen supported by the following: (a) transthoracic or transesophageal echocardiography that demonstrates large, bulky vegetations; (b) positive findings of culture of peripheral blood and/or accessible emboli specimens or histologic examination of accessible emboli; and (c) positive findings from fungal antigen and antibody detection systems (which should be used routinely for diagnosis of high-risk patients in a validation setting)</td>
</tr>
<tr>
<td>Management</td>
<td>Early combination antifungal medication and valve replacement for optimal success; antifungal agents should be used early, in high dosages; amphotericin B is the drug of choice, but it should be administered as a lipid preparation to optimize continued uninterrupted therapy and reduce toxicity. Optimal dosage, timing, and duration of treatment is undetermined, but basic antifungal therapeutics direct that treatment is given preoperatively, perioperatively and postoperatively, aiming for an acute delivery load of 2–3 g. Careful, prolonged follow-up on secondary prophylaxis aided by serologic testing (requires validation) should be performed to detect the possibility of early fungal recurrence. Secondary prophylaxis should be for a minimum of 2 years, with fluconazole for susceptible Candida species and itraconazole-cyclodextrin or voriconazole for Aspergillus species.</td>
</tr>
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</table>
a combined medical-surgical approach. Histological examination has revealed lesions of established FE comprising large vegetations that have protective biofilms [25, 26], which raises concern about whether antifungal drugs can be effectively delivered, and hence doubt about the efficacy of antifungal treatment alone. Indeed, documented cures of FE treated solely with systemic antifungal agents are rare [27]. These cases are usually in patients with Candida endocarditis [28, 29]. Recent publications from single centers advocate a combined medical-surgical approach. The surgical component includes radical surgical debridement and replacement of the valve with biologic material, whereas the medical component uses perioperative systemic antifungal agents and prolonged postoperative antifungal treatment [30–32].

Antifungal therapy could fail for other reasons. The prevalence of suboptimal dosing is underscored by the wide variation in amphotericin B dosage, the high frequency of reduced compliance with amphotericin B regimens because of toxicity, and treatment with 5-fluorocytosine alone. The availability of lipid-associated amphotericin B in recent years, which has a high therapeutic index, should obviate these therapeutic deficiencies. Lipid-associated amphotericin B allows the high and prolonged dosage necessary, on empirical grounds, to optimize the drug’s delivery into bulky vegetations, to adequately treat emboli, and to reduce seeding of target organs via the hematogenous route [33]. It is of some interest that combinations of antifungal agents were infrequently used. The importance of close follow-up after successful treatment of FE is shown by the late relapse of several patients who were initially deemed cured. More recent publications confirm the high rate of relapse (30%–40%) [30, 31], which can occur up to 9 years later [34], and the need for secondary antifungal prophylaxis.

Candida etiology seems to be associated with improved rates of survival when compared with Aspergillus etiology. A small series of 12 patients, most of whom had Candida FE and none Aspergillus, had an overall survival rate of 67% [31].

Our findings suggest a trend to improved outcome over recent years. There are at least 3 explanations for this trend. First, the introduction of echocardiography as a diagnostic tool in the mid-1970s and the recognition of the significance of large vegetations (37 of 39 patients in our review) have probably been major factors. Indeed, these developments coincided with the sudden improvement in the survival rate for patients with FE (from 13%–15% before 1974, to 24% at the time when echocardiography came into widespread use, to >30% after 1979). Second, our observation that diagnostic acumen for FE has significantly improved, such that more cases are diagnosed before surgery, is also notable, and it probably gained valuable therapeutic time for the patients. Finally, in recent years, supportive care of severely ill patients in intensive care units has improved in general. During the period of this study, there has been no widespread use of new antifungal drugs. The recent arrival of the lipid-associated compounds, such as lipid-associated amphotericin B (AmBisome; Gilead Sciences), may improve therapeutic outcome, if the experience with other invasive mycoses [34] is reproduced for FE.

Clarification of these crucial management issues by performance of randomized, prospective, comparative clinical trials in humans may be seriously compromised by the difficulties inherent in performing such studies given the rarity of FE, the demographic heterogeneity of the patient population, and the frequency of delayed diagnosis. A feasible alternative might be to notify a single data surveillance unit about any patients who have FE, and include high-quality clinical and management details to facilitate subsequent analysis. Expert panel-generated consensus management guidelines are required; these should address the choice of antifungal agents and their dosage, the timing of surgery, and follow-up. In the meantime, the essential therapeutic elements appear to be combined medical and surgical treatment that involves both pre- and postoperative lipid-associated amphotericin B (for improved compliance and to allow for higher doses), valve replacement as soon as the diagnosis is suspected, prolonged (perhaps lifelong) postoperative antifungal therapy, and prolonged medical surveillance to detect relapse. These recommendations are summarized in table 8.

References
Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America

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It is important to realize that guidelines cannot always account for individual variation among patients. They are not intended to supplant physician judgment with respect to particular patients or special clinical situations. IDSA considers adherence to these guidelines to be voluntary, with the ultimate determination regarding their application to be made by the physician in the light of each patient’s individual circumstances.

Keywords. candidemia; invasive candidiasis; fungal diagnostics; azoles; echinocandins.

EXECUTIVE SUMMARY

Background

Invasive infection due to Candida species is largely a condition associated with medical progress, and is widely recognized as a major cause of morbidity and mortality in the healthcare environment. There are at least 15 distinct Candida species that cause human disease, but >90% of invasive disease is caused by the 5 most common pathogens, C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei. Each of these organisms has unique virulence potential, antifungal susceptibility, and epidemiology, but taken as a whole, significant infections due to these organisms are generally referred to as invasive candidiasis. Mucosal Candida infections—especially those involving the oropharynx, esophagus, and vagina—are not considered to be classically invasive disease, but they are included in these guidelines. Since the last iteration of these guidelines in 2009 [1], there have been new data pertaining to diagnosis, prevention, and treatment for proven or suspected invasive candidiasis, leading to significant modifications in our treatment recommendations.

Summarized below are the 2016 revised recommendations for the management of candidiasis. Due to the guideline’s relevance to pediatrics, the guideline has been reviewed and endorsed by the American Academy of Pediatrics (AAP) and the Pediatric Infectious Diseases Society (PIDS). The Mycoses Study Group (MSG) has also endorsed these guidelines. The panel followed a guideline development process that has been adopted by the Infectious Diseases Society of America (IDSA), which includes a systematic method of grading both the quality of evidence (very low, low, moderate, and high) and the strength of the recommendation (weak or strong) [2] (Figure 1). [3] The guidelines are not intended to replace clinical judgment in the management of individual patients. A detailed description of the methods, background, and evidence summaries that support each recommendation can be found in the full text of the guideline.

I. What Is the Treatment for Candidemia in Nonneutropenic Patients?

Recommendations

1. An echinocandin (caspofungin: loading dose 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily) is recommended as initial therapy (strong recommendation; high-quality evidence).

2. Fluconazole, intravenous or oral, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily is an acceptable alternative to an echinocandin as initial therapy in selected patients, including those who are not critically ill and who are considered unlikely to have a fluconazole-resistant Candida species (strong recommendation; high-quality evidence).

3. Testing for azole susceptibility is recommended for all bloodstream and other clinically relevant Candida isolates. Testing for echinocandin susceptibility should be considered in patients who have had prior treatment with an echinocandin and among those who have infection with C. glabrata or C. parapsilosis (strong recommendation; low-quality evidence).
4. Transition from an echinocandin to fluconazole (usually within 5–7 days) is recommended for patients who are clinically stable, have isolates that are susceptible to fluconazole (eg, *C. albicans*), and have negative repeat blood cultures following initiation of antifungal therapy (strong recommendation; moderate-quality evidence).

5. For infection due to *C. glabrata*, transition to higher-dose fluconazole 800 mg (12 mg/kg) daily or voriconazole 200–300 (3–4 mg/kg) twice daily should only be considered among patients with fluconazole-susceptible or voriconazole-susceptible isolates (strong recommendation; low-quality evidence).

6. Lipid formulation amphotericin B (AmB) (3–5 mg/kg daily) is a reasonable alternative if there is intolerance, limited availability, or resistance to other antifungal agents (strong recommendation; high-quality evidence).

7. Transition from AmB to fluconazole is recommended after 5–7 days among patients who have isolates that are susceptible to fluconazole, who are clinically stable, and in whom repeat cultures on antifungal therapy are negative (strong recommendation; high-quality evidence).

8. Among patients with suspected azole- and echinocandin-resistant *Candida* infections, lipid formulation AmB (3–5 mg/kg daily) is recommended (strong recommendation; low-quality evidence).

9. Voriconazole 400 mg (6 mg/kg) twice daily for 2 doses, then 200 mg (3 mg/kg) twice daily is effective for candidemia, but offers little advantage over fluconazole as initial therapy (strong recommendation; moderate-quality evidence). Voriconazole is recommended as step-down oral therapy for selected cases of candidemia due to *C. krusei* (strong recommendation; low-quality evidence).

10. All nonneutropenic patients with candidemia should have a dilated ophthalmological examination, preferably performed by an ophthalmologist, within the first week after diagnosis (strong recommendation; low-quality evidence).

11. Follow-up blood cultures should be performed every day or every other day to establish the time point at which...
candidemia has been cleared (strong recommendation; low-quality evidence).

12. Recommended duration of therapy for candidemia without obvious metastatic complications is for 2 weeks after documented clearance of Candida species from the bloodstream and resolution of symptoms attributable to candidemia (strong recommendation; moderate-quality evidence).

II. Should Central Venous Catheters Be Removed in Nonneutropenic Patients With Candidemia?

**Recommendation**

13. Central venous catheters (CVCs) should be removed as early as possible in the course of candidemia when the source is presumed to be the CVC and the catheter can be removed safely; this decision should be individualized for each patient (strong recommendation; moderate-quality evidence).

III. What Is the Treatment for Candidemia in Neutropenic Patients?

**Recommendations**

14. An echinocandin (caspofungin: loading dose 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily) is recommended as initial therapy (strong recommendation; moderate-quality evidence).

15. Lipid formulation AmB, 3–5 mg/kg daily, is effective but less attractive alternative because of the potential for toxicity (strong recommendation; moderate-quality evidence).

16. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, is an alternative for patients who are not critically ill and have had no prior azole exposure (weak recommendation; low-quality evidence).

17. Fluconazole, 400 mg (6 mg/kg) daily, can be used for step-down therapy during persistent neutropenia in clinically stable patients who have susceptible isolates and documented bloodstream clearance (weak recommendation; low-quality evidence).

18. Voriconazole, 400 mg (6 mg/kg) twice daily for 2 doses, then 200–300 mg (3–4 mg/kg) twice daily, can be used in situations in which additional mold coverage is desired (weak recommendation; low-quality evidence). Voriconazole can also be used as step-down therapy during neutropenia in clinically stable patients who have had documented bloodstream clearance and isolates that are susceptible to voriconazole (weak recommendation; low-quality evidence).

19. For infections due to C. krusei, an echinocandin, lipid formulation AmB, or voriconazole is recommended (strong recommendation; low-quality evidence).

20. Recommended minimum duration of therapy for candidemia without metastatic complications is 2 weeks after documented clearance of Candida from the bloodstream, provided neutropenia and symptoms attributable to candidemia have resolved (strong recommendation; low-quality evidence).

21. Ophthalmological findings of choroidal and vitreal infection are minimal until recovery from neutropenia; therefore, dilated funduscopic examinations should be performed within the first week after recovery from neutropenia (strong recommendation; low-quality evidence).

22. In the neutropenic patient, sources of candidiasis other than a CVC (eg, gastrointestinal tract) predominate. Catheter removal should be considered on an individual basis (strong recommendation; low-quality evidence).

23. Granulocyte colony-stimulating factor (G-CSF)–mobilized granulocyte transfusions can be considered in cases of persistent candidemia with anticipated protracted neutropenia (weak recommendation; low-quality evidence).

IV. What Is the Treatment for Chronic Disseminated ( Hepatosplenic ) Candidiasis?

**Recommendations**

24. Initial therapy with lipid formulation AmB, 3–5 mg/kg daily OR an echinocandin (micafungin: 100 mg daily; caspofungin: 70-mg loading dose, then 50 mg daily; or anidulafungin: 200-mg loading dose, then 100 mg daily), for several weeks is recommended, followed by oral fluconazole, 400 mg (6 mg/kg) daily, for patients who are unlikely to have a fluconazole-resistant isolate (strong recommendation; low-quality evidence).

25. Therapy should continue until lesions resolve on repeat imaging, which is usually several months. Premature discontinuation of antifungal therapy can lead to relapse (strong recommendation; low-quality evidence).

26. If chemotherapy or hematopoietic cell transplantation is required, it should not be delayed because of the presence of chronic disseminated candidiasis, and antifungal therapy should be continued throughout the period of high risk to prevent relapse (strong recommendation; low-quality evidence).

27. For patients who have debilitating persistent fevers, short-term (1–2 weeks) treatment with nonsteroidal anti-inflammatory drugs or corticosteroids can be considered (weak recommendation; low-quality evidence).

V. What Is the Role of Empiric Treatment for Suspected Invasive Candidiasis in Nonneutropenic Patients in the Intensive Care Unit?

**Recommendations**

28. Empiric antifungal therapy should be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever and should be based on clinical assessment of risk factors, surrogate markers for invasive candidiasis, and/or culture data from nonsterile sites (strong recommendation; moderate-quality evidence). Empiric antifungal therapy should be started as soon as possible in patients who have the above risk factors and who have clinical signs of septic shock (strong recommendation; moderate-quality evidence).

29. Preferred empiric therapy for suspected candidiasis in nonneutropenic patients in the intensive care unit (ICU) is...
an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose of 200 mg, then 100 mg daily) (strong recommendation; moderate-quality evidence).

30. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, is an acceptable alternative for patients who have had no recent azole exposure and are not colonized with azole-resistant Candida species (strong recommendation; moderate-quality evidence).

31. Lipid formulation AmB, 3–5 mg/kg daily, is an alternative if there is intolerance to other antifungal agents (strong recommendation; low-quality evidence).

32. Recommended duration of empiric therapy for suspected invasive candidiasis in those patients who improve is 2 weeks, the same as for treatment of documented candidemia (weak recommendation; low-quality evidence).

33. For patients who have no clinical response to empiric antifungal therapy at 4–5 days and who do not have subsequent evidence of invasive candidiasis after the start of empiric therapy or have a negative non-culture-based diagnostic assay with a high negative predictive value, consideration should be given to stopping antifungal therapy (strong recommendation; low-quality evidence).

VI. Should Prophylaxis Be Used to Prevent Invasive Candidiasis in the Intensive Care Unit Setting?

Recommendations

34. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, could be used in high-risk patients in adult ICUs with a high rate (>5%) of invasive candidiasis (weak recommendation; moderate-quality evidence).

35. An alternative is to give an echinocandin (caspofungin: 70-mg loading dose, then 50 mg daily; anidulafungin: 200-mg loading dose and then 100 mg daily; or micafungin: 100 mg daily) (weak recommendation; low-quality evidence).

36. Daily bathing of ICU patients with chlorhexidine, which has been shown to decrease the incidence of bloodstream infections including candidemia, could be considered (weak recommendation; moderate-quality evidence).

VII. What Is the Treatment for Neonatal Candidiasis, Including Central Nervous System Infection?

What Is the Treatment for Invasive Candidiasis and Candidemia?

Recommendations

37. AmB deoxycholate, 1 mg/kg daily, is recommended for neonates with disseminated candidiasis (strong recommendation; moderate-quality evidence).

38. Fluconazole, 12 mg/kg intravenous or oral daily, is a reasonable alternative in patients who have not been on fluconazole prophylaxis (strong recommendation; moderate-quality evidence).

39. Lipid formulation AmB, 3–5 mg/kg daily, is an alternative, but should be used with caution, particularly in the presence of urinary tract involvement (weak recommendation; low-quality evidence).

40. Echinocandins should be used with caution and generally limited to salvage therapy or to situations in which resistance or toxicity preclude the use of AmB deoxycholate or fluconazole (weak recommendation; low-quality evidence).

41. A lumbar puncture and a dilated retinal examination are recommended in neonates with cultures positive for Candida species from blood and/or urine (strong recommendation; low-quality evidence).

42. Computed tomographic or ultrasound imaging of the genitourinary tract, liver, and spleen should be performed if blood cultures are persistently positive for Candida species (strong recommendation; low-quality evidence).

43. CVC removal is strongly recommended (strong recommendation; moderate-quality evidence).

44. The recommended duration of therapy for candidemia without obvious metastatic complications is for 2 weeks after documented clearance of Candida species from the bloodstream and resolution of signs attributable to candidemia (strong recommendation; low-quality evidence).

What Is the Treatment for Central Nervous System Infections in Neonates?

Recommendations

45. For initial treatment, AmB deoxycholate, 1 mg/kg intravenous daily, is recommended (strong recommendation; low-quality evidence).

46. An alternative regimen is liposomal AmB, 5 mg/kg daily (strong recommendation; low-quality evidence).

47. The addition of flucytosine, 25 mg/kg 4 times daily, may be considered as salvage therapy in patients who have not had a clinical response to initial AmB therapy, but adverse effects are frequent (weak recommendation; low-quality evidence).

48. For step-down treatment after the patient has responded to initial treatment, fluconazole, 12 mg/kg daily, is recommended for isolates that are susceptible to fluconazole (strong recommendation; low-quality evidence).

49. Therapy should continue until all signs, symptoms, and cerebrospinal fluid (CSF) and radiological abnormalities, if present, have resolved (strong recommendation; low-quality evidence).

50. Infected central nervous system (CNS) devices, including ventriculostomy drains and shunts, should be removed if at all possible (strong recommendation; low-quality evidence).

What Are the Recommendations for Prophylaxis in the Neonatal Intensive Care Unit Setting?

Recommendations

51. In nurseries with high rates (>10%) of invasive candidiasis, intravenous or oral fluconazole prophylaxis, 3–6 mg/kg twice
weekly for 6 weeks, in neonates with birth weights <1000 g is recommended (strong recommendation; high-quality evidence).
52. Oral nystatin, 100 000 units 3 times daily for 6 weeks, is an alternative to fluconazole in neonates with birth weights <1500 g in situations in which availability or resistance preclude the use of fluconazole (weak recommendation; moderate-quality evidence).
53. Oral bovine lactoferrin (100 mg/day) may be effective in neonates <1500 g but is not currently available in US hospitals (weak recommendation; moderate-quality evidence).

VIII. What Is the Treatment for Intra-abdominal Candidiasis?
Recommendations
54. Empiric antifungal therapy should be considered for patients with clinical evidence of intra-abdominal infection and significant risk factors for candidiasis, including recent abdominal surgery, anastomotic leaks, or necrotizing pancreatitis (strong recommendation; moderate-quality evidence).
55. Treatment of intra-abdominal candidiasis should include source control, with appropriate drainage and/or debridement (strong recommendation; moderate-quality evidence).
56. The choice of antifungal therapy is the same as for the treatment of candidemia or empiric therapy for nonneutropenic patients in the ICU (See sections I and V) (strong recommendation; moderate-quality evidence).
57. The duration of therapy should be determined by adequacy of source control and clinical response (strong recommendation; low-quality evidence).

IX. Does the Isolation of *Candida* Species From the Respiratory Tract Require Antifungal Therapy?
Recommendation
58. Growth of *Candida* from respiratory secretions usually indicates colonization and rarely requires treatment with antifungal therapy (strong recommendation; moderate-quality evidence).

X. What Is the Treatment for *Candida* Intravascular Infections, Including Endocarditis and Infections of Implantable Cardiac Devices?
What Is the Treatment for Candida Endocarditis?
Recommendations
59. For native valve endocarditis, lipid formulation AmB, 3–5 mg/kg daily, with or without flucytosine, 25 mg/kg 4 times daily, OR high-dose echinocandin (caspofungin 150 mg daily, micafungin 150 mg daily, or anidulafungin 200 mg daily) is recommended for initial therapy (strong recommendation; low-quality evidence).
60. Step-down therapy to fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended for patients who have susceptible *Candida* isolates, have demonstrated clinical stability, and have cleared *Candida* from the bloodstream (strong recommendation; low-quality evidence).
61. Oral voriconazole, 200–300 mg (3–4 mg/kg) twice daily, or posaconazole tablets, 300 mg daily, can be used as step-down therapy for isolates that are susceptible to those agents but not susceptible to fluconazole (weak recommendation; very low-quality evidence).
62. Valve replacement is recommended; treatment should continue for at least 6 weeks after surgery and for a longer duration in patients with perivalvular abscesses and other complications (strong recommendation; low-quality evidence).
63. For patients who cannot undergo valve replacement, long-term suppression with fluconazole, 400–800 mg (6–12 mg/kg) daily, if the isolate is susceptible, is recommended (strong recommendation; low-quality evidence).
64. For prosthetic valve endocarditis, the same antifungal regimens suggested for native valve endocarditis are recommended (strong recommendation; low-quality evidence). Chronic suppressive antifungal therapy with fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended to prevent recurrence (strong recommendation; low-quality evidence).

What Is the Treatment for Candida Infection of Implantable Cardiac Devices?
Recommendations
65. For pacemaker and implantable cardiac defibrillator infections, the entire device should be removed (strong recommendation; moderate-quality evidence).
66. Antifungal therapy is the same as that recommended for native valve endocarditis (strong recommendation; low-quality evidence).
67. For infections limited to generator pockets, 4 weeks of antifungal therapy after removal of the device is recommended (strong recommendation; low-quality evidence).
68. For infections involving the wires, at least 6 weeks of antifungal therapy after wire removal is recommended (strong recommendation; low-quality evidence).
69. For ventricular assist devices that cannot be removed, the antifungal regimen is the same as that recommended for native valve endocarditis (strong recommendation; low-quality evidence). Chronic suppressive therapy with fluconazole if the isolate is susceptible, for as long as the device remains in place is recommended (strong recommendation; low-quality evidence).

What Is the Treatment for Candida Suppurative Thrombophlebitis?
Recommendations
70. Catheter removal and incision and drainage or resection of the vein, if feasible, is recommended (strong recommendation; low-quality evidence).
71. Lipid formulation AmB, 3–5 mg/kg daily, OR fluconazole, 400–800 mg (6–12 mg/kg) daily, OR an echinocandin (caspofungin 150 mg daily, micafungin 150 mg daily, or anidulafungin 200 mg daily) for at least 2 weeks after candidemia
(if present) has cleared is recommended (strong recommendation; low-quality evidence).

72. Step-down therapy to fluconazole, 400–800 mg (6–12 mg/kg) daily, should be considered for patients who have initially responded to AmB or an echinocandin, are clinically stable, and have a fluconazole-susceptible isolate (strong recommendation; low-quality evidence).

73. Resolution of the thrombus can be used as evidence to discontinue antifungal therapy if clinical and culture data are supportive (strong recommendation; low-quality evidence).

XI. What Is the Treatment for Candida Osteoarticular Infections?

What Is the Treatment for Candida Osteomyelitis?

Recommendations

74. Fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months OR an echinocandin (caspofungin 50–70 mg daily, miconafungin 100 mg daily, or anidulafungin 100 mg daily) for at least 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months is recommended (strong recommendation; low-quality evidence).

75. Lipid formulation AmB, 3–5 mg/kg daily, for at least 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months is a less attractive alternative (weak recommendation; low-quality evidence).

76. Surgical debridement is recommended in selected cases (strong recommendation; low-quality evidence).

What Is the Treatment for Candida Septic Arthritis?

77. Fluconazole, 400 mg (6 mg/kg) daily, for 6 weeks OR an echinocandin (caspofungin 50–70 mg daily, miconafungin 100 mg daily, or anidulafungin 100 mg daily) for 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for at least 4 weeks is recommended (strong recommendation; low-quality evidence).

78. Lipid formulation AmB, 3–5 mg/kg daily, for 2 weeks, followed by fluconazole, 400 mg (6 mg/kg) daily, for at least 4 weeks is a less attractive alternative (weak recommendation; low-quality evidence).

79. Surgical drainage is indicated in all cases of septic arthritis (strong recommendation; moderate-quality evidence).

80. For septic arthritis involving a prosthetic device, device removal is recommended (strong recommendation; moderate-quality evidence).

81. If the prosthetic device cannot be removed, chronic suppression with fluconazole, 400 mg (6 mg/kg) daily, if the isolate is susceptible, is recommended (strong recommendation; low-quality evidence).

XII. What Is the Treatment for Candida Endophthalmitis?

What Is the General Approach to Candida Endophthalmitis?

Recommendations

82. All patients with candidemia should have a dilated retinal examination, preferably performed by an ophthalmologist, within the first week of therapy in nonneutropenic patients to establish if endophthalmitis is present (strong recommendation; low-quality evidence). For neutropenic patients, it is recommended to delay the examination until neutrophil recovery (strong recommendation; low-quality evidence).

83. The extent of ocular infection (chorioretinitis with or without macular involvement and with or without vitritis) should be determined by an ophthalmologist (strong recommendation; low-quality evidence).

84. Decisions regarding antifungal treatment and surgical intervention should be made jointly by an ophthalmologist and an infectious diseases physician (strong recommendation; low-quality evidence).

What Is the Treatment for Candida Chorioretinitis Without Vitritis?

Recommendations

85. For fluconazole-/voriconazole-susceptible isolates, fluconazole, loading dose, 800 mg (12 mg/kg), then 400–800 mg (6–12 mg/kg) daily OR voriconazole, loading dose 400 mg (6 mg/kg) intravenous twice daily for 2 doses, then 300 mg (4 mg/kg) intravenous or oral twice daily is recommended (strong recommendation; low-quality evidence).

86. For fluconazole-/voriconazole-resistant isolates, liposomal AmB, 3–5 mg/kg intravenous daily, with or without oral fluconazole, 25 mg/kg 4 times daily is recommended (strong recommendation; low-quality evidence).

87. With macular involvement, antifungal agents as noted above PLUS intravitreal injection of either AmB deoxycholate, 5–10 µg/0.1 mL sterile water, or voriconazole, 100 µg/0.1 mL sterile water or normal saline, to ensure a prompt high level of antifungal activity is recommended (strong recommendation; low-quality evidence).

88. The duration of treatment should be at least 4–6 weeks, with the final duration depending on resolution of the lesions as determined by repeated ophthalmological examinations (strong recommendation; low-quality evidence).

What Is the Treatment for Candida Chorioretinitis With Vitritis?

Recommendations

89. Antifungal therapy as detailed above for chorioretinitis without vitritis, PLUS intravitreal injection of either amphotericin B deoxycholate, 5–10 µg/0.1 mL sterile water, or voriconazole, 100 µg/0.1 mL sterile water or normal saline is recommended (strong recommendation; low-quality evidence).

90. Vitrectomy should be considered to decrease the burden of organisms and to allow the removal of fungal abscesses that are inaccessible to systemic antifungal agents (strong recommendation; low-quality evidence).

91. The duration of treatment should be at least 4–6 weeks, with the final duration dependent on resolution of the lesions.
as determined by repeated ophthalmological examinations (strong recommendation; low-quality evidence).

XIII. What is the Treatment for Central Nervous System Candidiasis?

Recommendations
92. For initial treatment, liposomal AmB, 5 mg/kg daily, with or without oral fluconazole, 25 mg/kg 4 times daily is recommended (strong recommendation; low-quality evidence).
93. For step-down therapy after the patient has responded to initial treatment, fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended (strong recommendation; low-quality evidence).
94. Therapy should continue until all signs and symptoms and CSF and radiological abnormalities have resolved (strong recommendation; low-quality evidence).
95. Infected CNS devices, including ventriculostomy drains, shunts, stimulators, prosthetic reconstructive devices, and biopolymer wafers that deliver chemotherapy should be removed if possible (strong recommendation; low-quality evidence).
96. For patients in whom a ventricular device cannot be removed, AmB deoxycholate could be administered through the device into the ventricle at a dosage ranging from 0.01 mg to 0.5 mg in 2 mL 5% dextrose in water (weak recommendation; low-quality evidence).

XIV. What is the Treatment for Urinary Tract Infections Due to Candida Species?

What is the Treatment for Asymptomatic Candiduria?

Recommendations
97. Elimination of predisposing factors, such as indwelling bladder catheters, is recommended whenever feasible (strong recommendation; low-quality evidence).
98. Treatment with antifungal agents is NOT recommended unless the patient belongs to a group at high risk for dissemination; high-risk patients include neutropenic patients, very low-birth-weight infants (<1500 g), and patients who will undergo urologic manipulation (strong recommendation; low-quality evidence).
99. Neutropenic patients and very low-birth-weight infants should be treated as recommended for candidemia (see sections III and VII) (strong recommendation; low-quality evidence).
100. Patients undergoing urologic procedures should be treated with oral fluconazole, 400 mg (6 mg/kg) daily, OR AmB deoxycholate, 0.3–0.6 mg/kg daily, for several days before and after the procedure (strong recommendation; low-quality evidence).

What is the Treatment for Symptomatic Candida Cystitis?

Recommendations
101. For fluconazole-susceptible organisms, oral fluconazole, 200 mg (3 mg/kg) daily for 2 weeks is recommended (strong recommendation; moderate-quality evidence).
102. For fluconazole-resistant C. glabrata, AmB deoxycholate, 0.3–0.6 mg/kg daily for 1–7 days OR oral fluconazole, 25 mg/kg 4 times daily for 7–10 days is recommended (strong recommendation; low-quality evidence).
103. For C. krusei, AmB deoxycholate, 0.3–0.6 mg/kg daily, for 1–7 days is recommended (strong recommendation; low-quality evidence).
104. Removal of an indwelling bladder catheter, if feasible, is strongly recommended (strong recommendation; low-quality evidence).
105. AmB deoxycholate bladder irrigation, 50 mg/L sterile water daily for 5 days, may be useful for treatment of cystitis due to fluconazole-resistant species, such as C. glabrata and C. krusei (weak recommendation; low-quality evidence).

What is the Treatment for Symptomatic Ascending Candida Pyelonephritis?

Recommendations
106. For fluconazole-susceptible organisms, oral fluconazole, 200–400 mg (3–6 mg/kg) daily for 2 weeks is recommended (strong recommendation; low-quality evidence).
107. For fluconazole-resistant C. glabrata, AmB deoxycholate, 0.3–0.6 mg/kg daily for 1–7 days with or without oral fluconazole, 25 mg/kg 4 times daily, is recommended (strong recommendation; low-quality evidence).
108. For fluconazole-resistant C. glabrata, monotherapy with oral fluconazole, 25 mg/kg 4 times daily for 2 weeks, could be considered (weak recommendation; low-quality evidence).
109. For C. krusei, AmB deoxycholate, 0.3–0.6 mg/kg daily, for 1–7 days is recommended (strong recommendation; low-quality evidence).
110. Elimination of urinary tract obstruction is strongly recommended (strong recommendation; low-quality evidence).
111. For patients who have nephrostomy tubes or stents in place, consider removal or replacement, if feasible (weak recommendation; low-quality evidence).

What is the Treatment for Candida Urinary Tract Infection Associated With Fungus Balls?

Recommendations
112. Surgical intervention is strongly recommended in adults (strong recommendation; low-quality evidence).
113. Antifungal treatment as noted above for cystitis or pyelonephritis is recommended (strong recommendation; low-quality evidence).
114. Irrigation through nephrostomy tubes, if present, with AmB deoxycholate, 25–50 mg in 200–500 mL sterile water, is recommended (strong recommendation; low-quality evidence).

XV. What is the Treatment for Vulvovaginal Candidiasis?

Recommendations
115. For the treatment of uncomplicated Candida vulvovaginalis, topical antifungal agents, with no one agent superior to
Recommendations

XVI. What Is the Treatment for Oropharyngeal Candidiasis?

Recommendations

122. For mild disease, clotrimazole troches, 10 mg 5 times daily, OR miconazole mucoadhesive buccal 50-mg tablet applied to the mucosal surface over the canine fossa once daily for 7–14 days are recommended (strong recommendation; high-quality evidence).

123. Alternatives for mild disease include nystatin suspension (100 000 U/mL) 4–6 mL 4 times daily, OR 1–2 nystatin pastilles (200 000 U each) 4 times daily, for 7–14 days (strong recommendation; moderate-quality evidence).

124. For moderate to severe disease, oral fluconazole, 100–200 mg daily, for 7–14 days is recommended (strong recommendation; high-quality evidence).

125. For fluconazole-refractory disease, itraconazole solution, 200 mg once daily OR posaconazole suspension, 400 mg twice daily for 3 days then 400 mg daily, for up to 28 days are recommended (strong recommendation; moderate-quality evidence).

126. Alternatives for fluconazole-refractory disease include voriconazole, 200 mg twice daily, OR AmB deoxycholate oral suspension, 100 mg/mL 4 times daily (strong recommendation; moderate-quality evidence).

127. Intravenous echinocandin (caspofungin: 70-mg loading dose, then 50 mg daily; micafungin: 100 mg daily; or anidulafungin: 200-mg loading dose, then 100 mg daily) OR intravenous AmB deoxycholate, 0.3 mg/kg daily, are other alternatives for refractory disease (weak recommendation; moderate-quality evidence).

128. Chronic suppressive therapy is usually unnecessary. If required for patients who have recurrent infection, fluconazole, 100 mg 3 times weekly, is recommended (strong recommendation; high-quality evidence).

129. For HIV-infected patients, antiretroviral therapy is strongly recommended to reduce the incidence of recurrent infections (strong recommendation; high-quality evidence).

130. For denture-related candidiasis, disinfection of the denture, in addition to antifungal therapy is recommended (strong recommendation; moderate-quality evidence).

XVII. What Is the Treatment for Esophageal Candidiasis?

Recommendations

131. Systemic antifungal therapy is always required. A diagnostic trial of antifungal therapy is appropriate before performing an endoscopic examination (strong recommendation; high-quality evidence).

132. Oral fluconazole, 200–400 mg (3–6 mg/kg) daily, for 14–21 days is recommended (strong recommendation; high-quality evidence).

133. For patients who cannot tolerate oral therapy, intravenous fluconazole, 400 mg (6 mg/kg) daily, OR an echinocandin (micafungin, 150 mg daily; caspofungin, 70-mg loading dose, then 50 mg daily, or anidulafungin, 200 mg daily) is recommended (strong recommendation; high-quality evidence).

134. A less preferred alternative for those who cannot tolerate oral therapy is AmB deoxycholate, 0.3–0.7 mg/kg daily (strong recommendation; moderate-quality evidence).

135. Consider de-escalating to oral therapy with fluconazole 200–400 mg (3–6 mg/kg) daily once the patient is able to tolerate oral intake (strong recommendation; moderate-quality evidence).

136. For fluconazole-refractory disease, itraconazole solution, 200 mg daily, OR voriconazole, 200 mg (3 mg/kg) twice daily either intravenous or oral, for 14–21 days is recommended (strong recommendation; high-quality evidence).

137. Alternatives for fluconazole-refractory disease include an echinocandin (micafungin: 150 mg daily; caspofungin: 70-mg loading dose, then 50 mg daily; or anidulafungin: 200 mg daily) for 14–21 days, OR AmB deoxycholate, 0.3–0.7 mg/kg daily, for 21 days (strong recommendation; high-quality evidence).

138. Posaconazole suspension, 400 mg twice daily, or extended-release tablets, 300 mg once daily, could be considered for fluconazole-refractory disease (weak recommendation; low-quality evidence).

139. For patients who have recurrent esophagitis, chronic suppressive therapy with fluconazole, 100–200 mg 3 times weekly, is recommended (strong recommendation; high-quality evidence).
INTRODUCTION

In the first section, the panel summarizes background information relevant to the topic. In the second section, the panel poses questions regarding the management of candidiasis, evaluates applicable clinical trial and observational data, and makes recommendations using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework [2]. The following 17 questions were answered:

I. What is the treatment for candidemia in nonneutropenic patients?
II. Should central venous catheters be removed in nonneutropenic patients with candidemia?
III. What is the treatment for candidemia in neutropenic patients?
IV. What is the treatment for chronic disseminated (hepatosplenic) candidiasis?
V. What is the role of empiric treatment for suspected invasive candidiasis in nonneutropenic patients in the intensive care unit?
VI. Should prophylaxis be used to prevent invasive candidiasis in the intensive care unit setting?
VII. What is the treatment for neonatal candidiasis, including central nervous system infection?
VIII. What is the treatment for intra-abdominal candidiasis?
IX. Does the isolation of Candida species from the respiratory tract require antifungal therapy?
X. What is the treatment for Candida intravascular infections, including endocarditis and infections of implantable cardiac devices?
XI. What is the treatment for Candida osteoarticular infections?
XII. What is the treatment for Candida endophthalmitis?
XIII. What is the treatment for central nervous system candidiasis?
XIV. What is the treatment for urinary tract infections due to Candida species?
XV. What is the treatment for vulvovaginal candidiasis?
XVI. What is the treatment for oropharyngeal candidiasis?
XVII. What is the treatment for esophageal candidiasis?

Infections due to Candida species are major causes of morbidity and mortality in humans, causing a diverse spectrum of clinical disease ranging from superficial and mucosal infections to invasive disease associated with candidemia and metastatic organ involvement. As an entity, candidemia is one of the most common healthcare-associated bloodstream infections in US hospitals, typically ranking as the third or fourth most common cause of healthcare-associated bloodstream infection. A recent multicenter point-prevalence survey identified Candida species as the most commonly isolated healthcare-associated bloodstream pathogen [4]. Among patients with candidemia and other forms of invasive candidiasis, non-albicans Candida species constitute approximately 50% of all relevant isolates, representing a steady trend in many regions throughout the world for more than a decade [5–12]

Among the many clinical manifestations of candidiasis, candidemia and invasive candidiasis have been given the most attention in clinical trials. Candidemia is associated with up to 47% attributable mortality [5–13], and this is even higher among persons with septic shock [14]. Several authors have demonstrated that mortality is closely linked to both timing of therapy and/or source control [14–19]. That is, earlier intervention with appropriate antifungal therapy and removal of a contaminated central venous catheter (CVC) or drainage of infected material is generally associated with better overall outcomes [14–19]. CVCs are commonly linked with candidemia, but catheters are not always the source, especially among neutropenic patients in whom the gastrointestinal tract is a common source. Most experts agree that thoughtful patient-specific management of CVCs is critical in the overall management of the infection [19].

The continued reliance on blood cultures, which are notoriously insensitive as markers of disease, remains a significant obstacle to early intervention for this condition. The development of reliable nonculture assays is critical to providing the opportunity for earlier intervention and more targeted antifungal therapy among large numbers of patients in whom traditional blood cultures are insensitive or provide untimely results [20].

Species distribution is also a significant challenge for all forms of candidiasis, and there is considerable geographic, center-to-center, and even unit-to-unit variability in the prevalence of pathogenic Candida species [8–12]. Indeed, candidiasis is not one but rather several diseases, with each Candida species presenting its own unique characteristics with respect to tissue tropism, propensity to cause invasive disease, virulence, and antifungal susceptibility. A working knowledge of the local epidemiology and rates of antifungal resistance is critical in making informed therapeutic decisions while awaiting culture and susceptibility data.

Despite the overall robust nature of the randomized controlled trials examining treatment of candidemia and other forms of invasive candidiasis [21–34], no single trial has demonstrated clear superiority of one therapeutic agent over another. Careful analysis of these clinical data sometimes leads to conflicting conclusions. For instance, the use of amphotericin B (AmB) plus fluconazole is as least as effective as higher-dose (800 mg daily) fluconazole given alone for patients with candidemia [22], but there is little role for this combination in current practice, especially as echinocandins are such a safe
and effective alternative. Similarly, voriconazole is as effective as the strategy of sequential AmB and fluconazole for candidemia, but few would choose voriconazole in this setting as there is little advantage and potentially greater toxicity associated with using this agent compared to other therapies [23].

The echinocandins have emerged as preferred agents for most episodes of candidemia and invasive candidiasis, with the exception of central nervous system (CNS), eye, and urinary tract infections due to these organisms. This preference is based on a strong safety profile, convenience, early fungicidal activity, a trend toward better outcomes based on data from individual studies and combined analyses of candidemia studies [19, 25], and the emergence of azole-resistant Candida species. The recent emergence of multidrug-resistant Candida species further complicates the selection of antifungal therapy for the immediate future [10, 12, 35–38] as there are no good prospective data to guide therapy.

There is an abundance of clinical data generated from large randomized clinical trials for candidemia, Candida esophagitis, oropharyngeal candidiasis, and prophylaxis studies in special populations, such as patients in intensive care units (ICUs), neonates, and selected transplant recipients, and these studies have led to important insights into optimal therapeutic approaches in these vulnerable populations. For those with less common manifestations of disease, such as osteomyelitis, endophthalmitis, and infective endocarditis, treatment recommendations are largely based on extrapolation from randomized studies of patients with other forms of disease, small retrospective series, and anecdotal reports. Thus, there is a critical need to assess these data in an ongoing manner to provide timely recommendations pertaining to the management of patients with these less common forms of candidiasis.

**METHODS**

**Panel Composition**

The most recent version of the Infectious Diseases Society of America (IDSA) guideline on the management of patients with candidiasis was published in 2009 [1]. For this update, the IDSA Standards and Practice Guidelines Committee (SPGC) convened a multidisciplinary panel of 12 experts in the management of patients with candidiasis. The panel consisted of 12 members of IDSA, and included 11 adult infectious diseases physicians and 1 pediatric infectious diseases physician. All panel members were selected on the basis of their expertise in clinical and/or laboratory mycology with a focus on candidiasis.

**Literature Review and Analysis**

Panel members were each assigned to review the recent literature for at least 1 topic, evaluate the evidence, determine the strength of recommendations, and develop written evidence in support of these recommendations. PubMed, which includes Medline (1946 to present), was searched to identify relevant studies for the Candida guideline PICO (population/patient, intervention/indicator, comparator/control, outcome) questions. Search strategies were developed and built by 2 independent health sciences librarians from the Health Sciences Library System, University of Pittsburgh. For each PICO question, the librarians developed the search strategies using PubMed’s command language and appropriate search fields. Medical Subject Headings (MeSH) terms and keywords were used for the main search concepts of each PICO question. Articles in all languages and all publication years were included. Initial searches were created and confirmed with input from the guideline committee chairs and group leaders from August to November 2013. The searches were finalized and delivered between late November 2013 and January 2014. After the literature searches were performed, authors continued to review the literature and added relevant articles as needed.

**Process Overview**

The panel met face-to-face twice and conducted a series of conference calls over a 2-year period. The panel reviewed and discussed all recommendations, their strength, and the quality of evidence. Discrepancies were discussed and resolved, and all final recommendations represent a consensus opinion of the entire panel. For the final version of these guidelines, the panel as a group reviewed all individual sections.

**Evidence Review: The GRADE Method**

GRADE is a systematic approach to guideline development that has been described in detail elsewhere [2, 39]. The IDSA adopted GRADE in 2008. In the GRADE system, the guideline panel assigns each recommendation with separate ratings for the underlying quality of evidence supporting the recommendation and for the strength with which the recommendation is made (Figure 1). Data from randomized controlled trials begin as “high” quality, and data from observational studies begin as “low” quality. However, the panel may judge that specific features of the data warrant decreasing or increasing the quality of evidence rating, and GRADE provides guidance on how such factors should be weighed [39]. The strength assigned to a recommendation chiefly reflects the panel’s confidence that the benefits of following the recommendation are likely to outweigh potential harms. While the quality of evidence is an important factor in choosing recommendation strength, it is not prescriptive.

**Guidelines and Conflicts of Interest**

The expert panel complied with the IDSA policy on conflicts of interest, which requires disclosure of any financial or other interest that may be construed as constituting an actual, potential, or apparent conflict. Panel members were provided IDSA’s conflicts of interest disclosure statement and were asked to identify ties to companies developing products that may be affected by promulgation of the guideline. Information was requested regarding employment, consultancies, stock ownership, honoraria, research
funding, expert testimony, and membership on company advisory committees. Decisions were made on a case-by-case basis as to whether an individual’s role should be limited as a result of a conflict. Potential conflicts of interests are listed in the Acknowledgments section.

Consensus Development Based on Evidence
The panel obtained feedback from 3 external peer reviewers. The guidelines were reviewed and endorsed by the MSG, the American Academy of Pediatrics (AAP) and the Pediatric Infectious Diseases Society (PIDS). The guideline was reviewed and approved by the IDSA SPGC and the IDSA Board of Directors prior to dissemination.

Revision Dates
At annual intervals, the panel chairs will be asked for their input on the need to update the guideline based on an examination of the current literature. The IDSA SPGC will consider this input and determine the necessity and timing of an update. If warranted, the entire panel or a subset thereof will be convened to discuss potential changes.

BACKGROUND
Antifungal Agents
Pharmacologic Considerations for Therapy for Candidiasis
Systemic antifungal agents shown to be effective for the treatment of invasive candidiasis comprise 4 major categories: the polyenes (amphotericin B [AmB] deoxycholate, liposomal AmB, AmB lipid complex [ABLC], and amphotericin B colloidal dispersion [ABCD, not available in the United States]), the triazoles (fluconazole, itraconazole, voriconazole, and posaconazole), the echinocandins (caspofungin, anidulafungin, and micafungin), and fluconazole. Data from a recently completed clinical trial comparing isavuconazole to an echinocandin for a clinical trial comparing isavuconazole to an echinocandin for systemic candidiasis are unavailable at this time. Clinicians should become familiar with strategies to optimize efficacy through an understanding of relevant pharmacokinetic properties.

Amphotericin B
Most experience with AmB is with the deoxycholate preparation. Three lipid formulations of AmB have been developed and approved for use in humans: ABLC, ABCD, and liposomal AmB. These agents possess the same spectrum of activity as AmB deoxycholate, but daily dosing regimens and toxicity profiles differ for each agent. The 3 lipid formulation AmB agents have different pharmacological properties and rates of treatment-related adverse events and should not be interchanged without careful consideration. In this document, a reference to AmB, without a specific dose or other discussion of form, should be taken to be a reference to the general use of any of the AmB preparations. For most forms of invasive candidiasis, the typical intravenous dosage for AmB deoxycholate is 0.5–0.7 mg/kg daily, but dosages as high as 1 mg/kg daily should be considered for invasive Candida infections caused by less susceptible species, such as C. glabrata and C. krusei. The typical dosage for lipid formulation AmB is 3–5 mg/kg daily when used for invasive candidiasis. Nephrotoxicity is the most common serious adverse effect associated with AmB deoxycholate therapy, resulting in acute kidney injury in up to 50% of recipients and an electrolyte-wasting tubular acidosis in a majority of patients [40, 41]. Lipid formulations of AmB are more expensive than AmB deoxycholate, but all have considerably less nephrotoxicity [42, 43]. Most observers agree that lipid formulations, with the exception of ABCD, have fewer infusion-related reactions than AmB deoxycholate. The impact of the pharmacokinetics and differences in toxicity of lipid formulations of AmB have not been formally examined in clinical trials. We are not aware of any forms of candidiasis for which lipid formulations of AmB are superior to AmB deoxycholate in terms of clinical efficacy. In addition, we are not aware of any situation in which lipid formulations should not be used, with the exception of urinary tract infections, because of reduced renal excretion of these formulations. Animal model studies suggest a pharmacokinetic and therapeutic advantage for liposomal AmB in the CNS [44]. Data demonstrating that AmB deoxycholate–induced nephrotoxicity is associated with a 6.6-fold increase in mortality have led many clinicians to use lipid formulations of AmB in proven or suspected candidiasis, especially among patients in a high-risk environment, such as an ICU [45].

Triazoles
Fluconazole, itraconazole, voriconazole, posaconazole, and a new expanded-spectrum triazole, isavuconazole, demonstrate similar activity against most Candida species [46–51]. Each of the azoles has less activity against C. glabrata and C. krusei than against other Candida species. All of theazole antifungals inhibit cytochrome P450 enzymes to some degree [52]. Thus, clinicians must carefully consider the influence on a patient’s drug regimen when adding or removing an azole. In large clinical trials, fluconazole demonstrated efficacy comparable to that of AmB deoxycholate for the treatment of candidemia [21, 22] and is also considered to be standard therapy for oropharyngeal, esophageal, and vaginal candidiasis, as well as urinary tract infections [53, 54]. Fluconazole is readily absorbed, with oral bioavailability resulting in concentrations equal to approximately 90% of those achieved by intravenous administration [55]. Absorption is not affected by food consumption, gastric pH, or disease state. Among the triazoles, fluconazole has the greatest penetration into the cerebrospinal fluid (CSF) and vitreous, achieving concentrations of >70% of those in serum [56–59]. For this reason, it is often used in the treatment of CNS and intraocular Candida infections. Fluconazole achieves urine concentrations that are 10–20 times the concentrations in serum and, thus, is the preferred treatment option for symptomatic cystitis [59]. For patients with invasive candidiasis, fluconazole
should be administered with an average loading dose of 800 mg (12 mg/kg), followed by an average daily dose of 400 mg (6 mg/kg). The higher-dose level (800 mg daily, 12 mg/kg) is often recommended for therapy of susceptible *C. glabrata* infections, but this has not been validated in clinical trials. Fluconazole elimination is almost entirely renal; thus, a dose reduction is needed in patients with creatinine clearance <50 mL/minute.

itraconazole is only available in oral formulations. It has not been well studied for invasive candidiasis, and is generally reserved for patients with mucosal candidiasis, especially those who have experienced treatment failure with fluconazole [60]. Gastrointestinal absorption is variable among patients and is greater for the oral solution compared with the capsule formulation. Histamine receptor antagonists and proton pump inhibitors result in decreased absorption of the capsule formulation, whereas acidic beverages enhance absorption [61]. Administration of the capsule formulation with food increases absorption, but the oral solution is better absorbed on an empty stomach [62]. Oral formulations are dosed in adults at 200 mg 3 times daily for 3 days, then 200 mg once or twice daily thereafter.

Voriconazole has demonstrated effectiveness for both mucosal and invasive candidiasis [23, 63]. Its clinical use has been primarily for step-down oral therapy in patients with infection due to *C. krusei* and fluconazole-resistant, voriconazole-susceptible *C. glabrata*. CSF and vitreous concentrations are >50% of serum concentration, and voriconazole has been shown to be efficacious in case series for these infection sites [64–66]. Voriconazole does not accumulate in active form in the urine and thus should not be used for urinary candidiasis. The oral bioavailability of voriconazole is excellent and is not affected by gastric pH, but it decreases when the drug is administered with food [67, 68]. In adults, the recommended oral dosing regimen for candidiasis includes a loading dose of 400 mg (6 mg/kg) twice daily for 2 doses, followed by 200–300 mg (3–4 mg/kg) twice daily.

Intravenous voriconazole is complexed to a cyclodextrin molecule; after 2 loading doses of 6 mg/kg every 12 hours, a maintenance dosage of 3–4 mg/kg every 12 hours is recommended. Because of the potential for cyclodextrin accumulation and possible nephrotoxicity among patients with significant renal dysfunction, intravenous voriconazole is not currently recommended for patients with a creatinine clearance <50 mL/minute. However, retrospective examination of intravenous voriconazole use in patients with varying degrees of renal function below this cutoff value has not identified toxic effects, mitigating some of these concerns [69, 70]. Oral voriconazole does not require dosage adjustment for renal insufficiency, but it is the only triazole that requires dosage reduction for patients with mild to moderate hepatic impairment [71].

Common polymorphisms in the gene encoding the primary metabolic enzyme for voriconazole result in wide variability of serum levels [72]. Drug–drug interactions are common with voriconazole and should be considered when initiating and discontinuing treatment with this compound [52]. Voriconazole has not been studied systematically in fluconazole-resistant *Candida* species, and with the exception of *C. krusei*, use is currently discouraged. Each of the triazoles can be associated with uncommon side effects. However, several effects are unique to voriconazole or more commonly associated with higher voriconazole concentrations, including hepatic injury, visual side effects, photosensitivity, periostitis, and CNS side effects [73–75].

Posaconazole does not have an indication for primary candidiasis therapy. It demonstrates in vitro activity against *Candida* species that is similar to that of voriconazole, but clinical data are inadequate to make an evidence-based recommendation for treatment of candidiasis other than oropharyngeal candidiasis [76]. Posaconazole is currently available as an extended-release tablet, an oral suspension, and an intravenous solution. The tablet formulation, given as 300 mg twice daily for 2 doses, then 300 mg daily produces predictable serum concentrations and excellent drug exposure and requires only once-daily dosing [77, 78]. The oral suspension has unpredictable bioavailability [79–81]. Intravenous posaconazole is given as 300 mg twice daily for 2 doses, then 300 mg daily.

Isavuconazole is a recently approved expanded-spectrum triazole antifungal with excellent in vitro activity against *Candida* species. Preliminary analysis of the recently completed large international double-blind trial comparing isavuconazole to an echinocandin for invasive candidiasis suggests that isavuconazole did not meet criteria for noninferiority (personal communication, Astellas US).

**Echinocandins**

Caspofungin, anidulafungin, and micafungin are available only as parenteral preparations [82–84]. The minimum inhibitory concentrations (MICs) of the echinocandins are low for most *Candida* species, including *C. glabrata* and *C. krusei* [48–50]. However, recent case series have described treatment failure associated with resistant strains of *C. glabrata* [85, 86]. *Candida parapsilosis* demonstrates innately higher MICs to the echinocandins than do most other *Candida* species, which raises the concern that *C. parapsilosis* may be less responsive to the echinocandins.

Each of these agents has been studied for the treatment of esophageal candidiasis [24, 87, 88] and invasive candidiasis [25–34], and each has demonstrated efficacy in these situations. Recent pooled analyses of almost exclusively nonneutropenic patients included in randomized invasive candidiasis treatment trials suggest a survival advantage associated with initial echinocandin therapy [19].

All echinocandins have minimal adverse effects. The pharmacologic properties in adults are also very similar, and each is administered once daily intravenously [82–84]. Echinocandins achieve therapeutic concentrations in all infection sites with the exception of the eye, CNS, and urine [59]. The
major route of elimination is nonenzymatic degradation. None of the echinocandins require dosage adjustment for renal insufficiency or dialysis. Both caspofungin and micafungin undergo minimal hepatic metabolism, but neither drug is a major substrate for cytochrome P450. Caspofungin is the only echinocandin for which dosage reduction is recommended for patients with moderate to severe hepatic dysfunction. The usual intravenous dosing regimens for invasive candidiasis are as follows: caspofungin: loading dose 70 mg, then 50 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily; and micafungin: 100 mg daily (no loading dose needed).

**Flucytosine**

Flucytosine demonstrates broad antifungal activity against most *Candida* species, with the exception of *C. krusei*. The compound is available in the United States only as an oral formulation. The drug has a short half-life (2.4–4.8 hours) and is ordinarily administered at a dosage of 25 mg/kg 4 times daily for patients with normal renal function. Flucytosine demonstrates excellent absorption after oral administration (80%–90%), and most of the drug is excreted unchanged (microbiologically active) in the urine [89, 90]; dose adjustment is necessary for patients with renal dysfunction [91, 92]. The compound exhibits high penetration into the CNS and eye. Concentration-dependent toxicity results in bone marrow suppression and hepatitis.

Flucytosine is usually given in combination with another antifungal agent due to a high rate of emergence of resistance during monotherapy [93]. The most common use of flucytosine in the setting of *Candida* infection is in combination with AmB for patients with more refractory infections, such as *Candida* endocarditis, meningitis, or endophthalmitis. Occasionally, it is used for the treatment of symptomatic urinary tract candidiasis due to fluconazole-resistant *C. glabrata* [94].

**Pediatric Dosing**

There is considerable variation in the pharmacokinetics of antifungal agents between adult and pediatric patients, and the data on dosing in pediatric patients are limited. The pharmacological properties of antifungal agents in children and infants have been reviewed in detail [95]. The optimal dose of AmB deoxycholate in neonates has not been clearly defined; a dosage of 1 mg/kg is generally used [96–98]. The safety, efficacy, area under the curve, and maximal concentration of ABLC 2–5 mg/kg/day are similar in adults and children [99]. The pharmacokinetics of liposomal AmB in neonates and children suggest that both volume and clearance are affected by weight [100].

Flucytosine clearance is directly proportional to glomerular filtration rate, and infants with a very low birth weight may accumulate high plasma concentrations because of poor renal function due to immaturity [101]. Thus, the use of flucytosine without careful monitoring of serum drug levels is discouraged in this group of patients.

Flucytosine pharmacokinetics vary with age, and the drug is rapidly cleared in children. Thus, a daily fluconazole dose of 12 mg/kg is necessary for neonates and children [102–105]. Voriconazole pharmacokinetics are also highly variable in children [106–108]. To attain plasma exposures comparable to those in adults receiving 4 mg/kg every 12 hours, a loading dose of intravenous voriconazole of 9 mg/kg twice daily, followed by 8 mg/kg twice daily is recommended in children. The recommended oral dose is 9 mg/kg twice daily (maximum dose 350 mg) [95, 107]. There are no data on voriconazole dosing in children <2 years old, and there are no pediatric studies examining the pharmacokinetics of the intravenous formulation, the oral suspension, or the extended-release tablets of posaconazole.

Caspofungin and micafungin are approved by the US Food and Drug Administration (FDA) for use in children. Caspofungin dosing is based on body surface area rather than weight. Dosing in children is a loading dose of 70 mg/m², followed by 50 mg/m²/day. Preliminary studies suggest an optimal dose of caspofungin in neonates of 25 mg/m²/day. The current recommendation for micafungin for invasive candidiasis is 2 mg/kg/day, with the option to increase to 4 mg/kg/day in children <40 kg. The optimal dose of micafungin in neonates is unknown, but likely to be 10 mg/kg/day or greater [109]. Anidulafungin should be dosed at 1.5 mg/kg/day for neonates and children [110–112].

**Considerations During Pregnancy**

AmB is the treatment of choice for invasive candidiasis in pregnant women [113]. Fluconazole, itraconazole, posaconazole, and isavuconazole should be avoided in pregnant women, especially those in the first trimester, because of the possibility of birth defects associated with their use. Voriconazole is contraindicated during pregnancy because of fetal abnormalities observed in animals. There are few data concerning the echinocandins; thus, their use is cautioned during pregnancy. Flucytosine is contraindicated during pregnancy because of fetal abnormalities observed in animals.

**Therapeutic Drug Monitoring**

Therapeutic drug monitoring (TDM) for itraconazole, voriconazole, posaconazole, and fluconazole has been shown to be useful for optimizing efficacy and limiting toxicity in patients receiving therapy for a variety of invasive fungal infections, including mucosal and invasive candidiasis [114]. The basis for TDM is widely variable concentrations among patients and a strong relationship between concentration and efficacy and/or toxicity.

For itraconazole, when measured by high-pressure liquid chromatography (HPLC), both itraconazole and its bioactive hydroxy-itraconazole metabolite are reported, the sum of which should be considered in assessing drug levels. Treatment success has been associated with concentrations ≥1 mg/L and toxicity with concentrations >5 mg/L. Bioassay levels are 3-
7-fold higher than those measured by HPLC. Because of nonlinear pharmacokinetics in adults and genetic differences in metabolism, there is both intrapatient and interpatient variability in serum voriconazole concentrations [115–118]. TDM should be considered for patients receiving voriconazole, because drug toxicity has been observed at higher serum concentrations and reduced clinical response has been observed at lower concentrations [117, 118]. The therapeutic trough concentration window for voriconazole is 1–5.5 mg/L. Few data are available to support a specific concentration to optimize posaconazole efficacy. Fluconosine monitoring is predominantly used to prevent concentration-associated toxicity. Peak concentrations <100 mg/L are recommended to avoid the predictable liver and bone marrow effects [119].

**Antifungal Susceptibility Testing**

Intensive efforts to develop standardized, reproducible, and relevant susceptibility testing methods for fungi have resulted in the development of the Clinical and Laboratory Standards Institute (CLSI) M27-A3 and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodologies for susceptibility testing of yeasts [120]. Interpretive breakpoints for susceptibility take into account the MIC, as well as pharmacokinetic/pharmacodynamic data and animal model data. They are reported for each species. Breakpoints have been established for most, but not all, drugs for the 5 most common *Candida* species [47, 50, 121, 122] (Table 1).

In many instances, clinical breakpoints have decreased from those used previously. For example, the prior *Candida* clinical breakpoint for susceptibility to fluconazole was ≤8 mg/L. With the new interpretation, the susceptible value has been reduced to ≤2 mg/L for *C. albicans*. For *C. glabrata*, there is no breakpoint established for susceptibility to fluconazole, itraconazole, posaconazole, or voriconazole (Table 1).

When there is no clinical breakpoint established, the epidemiologic cutoff value (ECV) based on an examination of the distribution of MICs within a species can be used. The ECV is defined as the MIC value that excludes non–wild type strains, notably isolates that are likely to contain a resistant mutant [50, 123]. The addition of the ECV method is particularly useful for detecting emergence of resistance in a *Candida* species at an institution.

The susceptibility of *Candida* to the currently available antifungal agents is generally predictable if the species of the infecting isolate is known. Currently, antifungal resistance in *C. albicans* is uncommon. However, individual isolates may not necessarily follow this general pattern [124]. Recent surveillance studies suggest that triazole resistance among *C. glabrata* isolates has increased to a degree that is difficult to rely upon these agents for therapy in the absence of susceptibility testing [12, 125, 126]. A similar trend has begun to emerge for a smaller proportion of *C. glabrata* isolates and the echinocandins [35, 85, 125]. The value of susceptibility testing for other *Candida* species is less clear, although resistance among *C. tropicalis* and *C. parapsilosis* has been reported from tertiary care institutions that have extensive use of antifungal agents [127, 128]. Because of these trends, susceptibility testing is increasingly used to guide the management of candidemia and invasive candidiasis.

### Diagnosis of Candidiasis

Cultures of blood or other samples collected under sterile conditions have long been considered diagnostic gold standards for invasive candidiasis. Nonculture diagnostic tests, such as

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**Table 1. Clinical Breakpoints for Antifungal Agents Against Common Candida Species**

<table>
<thead>
<tr>
<th>Candida Organism</th>
<th>Antifungal Agent</th>
<th>Clinical Breakpoint, µg/mL*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
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<tr>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>≤2</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>≤0.12</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
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<td></td>
<td>Posaconazole</td>
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<tr>
<td></td>
<td>Anidulafungin</td>
<td>≤0.25</td>
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<tr>
<td></td>
<td>Caspofungin</td>
<td>≤0.25</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
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<tr>
<td><em>C. glabrata</em></td>
<td>Fluconazole</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>≤2</td>
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<tr>
<td></td>
<td>Voriconazole</td>
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<tr>
<td></td>
<td>Posaconazole</td>
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<td></td>
<td>Anidulafungin</td>
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<td>Caspofungin</td>
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<td></td>
<td>Micafungin</td>
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<tr>
<td><em>C. tropicalis</em></td>
<td>Fluconazole</td>
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<td>Itraconazole</td>
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<td></td>
<td>Voriconazole</td>
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<td>Caspofungin</td>
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<td></td>
<td>Micafungin</td>
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<tr>
<td><em>C. krusei</em></td>
<td>Fluconazole</td>
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<tr>
<td></td>
<td>Itraconazole</td>
<td>≤0.5</td>
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<td></td>
<td>Voriconazole</td>
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<td>Micafungin</td>
<td>≤0.25</td>
</tr>
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</table>

Where no values are entered, there are insufficient data to establish clinical breakpoints.

Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible dose-dependent.

* Clinical breakpoints adopted by the Clinical and Laboratory Standards Institute.
antigen, antibody, or β-D-glucan detection assays, and polymerase chain reaction (PCR) are now entering clinical practice as adjuncts to cultures. If used and interpreted judiciously, these tests can identify more patients with invasive candidiasis and better direct antifungal therapy. To fully realize the benefits of combining culture and nonculture tests, however, clinicians must carefully consider the types of invasive candidiasis, understand the strengths and limitations of each assay, and interpret test results in the context of the clinical setting.

Use of Cultures in the Diagnosis of Invasive Candidiasis
Invasive candidiasis encompasses 3 entities: candidemia in the absence of deep-seated candidiasis, candidemia associated with deep-seated candidiasis, and deep-seated candidiasis in the absence of candidemia [20]. The distribution of these entities is likely to differ among centers; on balance, data suggest that the groups are approximately equal in size [129].

The overall sensitivity of blood cultures for diagnosing invasive candidiasis is roughly 50% [20]. The limit of detection for blood cultures is ≤1 colony-forming unit/mL [130, 131]. The limit of detection for cultures is at or below that of PCR [132–135]. As such, blood cultures should be positive during the vast majority of active Candida bloodstream infections. They may be negative in cases of extremely low-level candidemia, intermittent candidemia, deep-seated candidiasis that persists after sterilization of the bloodstream, or deep-seated candidiasis resulting from direct inoculation of Candida in the absence of candidemia. Blood cultures are limited by slow turnaround times (median time to positivity of 2–3 days, ranging from 1 to ≥7 days), and the fact that they may become positive relatively late in the disease course [130, 136]. Cultures of tissues or fluid recovered from infected sites during deep-seated candidiasis also exhibit poor sensitivity (often <50%) and slow turnaround times, and require invasive sampling procedures that may be dangerous or contraindicated due to underlying medical conditions [137].

Antigen and Antibody Detection
Candida antigen and anti-Candida antibody detection has gained greater acceptance in Europe than in the United States. In general, antigen detection is limited by rapid clearance from the bloodstream [138]. Concerns have been expressed about the reliability of antibody detection in immunosuppressed hosts, but assays have performed well in patients with neutropenia and cell-mediated immune defects (including hematopoietic cell and solid organ transplant recipients) [138, 139]. Serum immunoglobulin G (IgG) responses against specific antigens have typically performed better than immunoglobulin M (IgM) responses, suggesting that many patients mount amnestic responses or have ongoing, subclinical tissue invasion [139]. The best-studied test is a combined mannan/antimannan antibody assay, which is currently approved for use in Europe, but not the United States (Platelia Candida Ag and Ab; Bio-Rad). In a meta-analysis of 14 studies, the sensitivity/specificity for the diagnosis of invasive candidiasis of mannan and antiamannan IgG individually were 58%/93% and 59%/83%, respectively [140]. Values for the combined assay were 83% and 86%, with best performances for C. albicans, C. glabrata, and C. tropicalis infections. In one study of candidemia, at least one test was positive before blood culture in 73% of patients [141]. In a study of hepatosplenic candidiasis, at least one test was positive before radiographic changes in 86% of patients [142]. This assay is not used widely in the United States, and its role in the diagnosis and management of invasive candidiasis is unclear.

β-D-Glucan detection
β-D-glucan is a cell wall constituent of Candida species, Aspergillus species, Pneumocystis jiroveci, and several other fungi. A serum β-D-glucan assay (Fungitell; Associates of Cape Cod, East Falmouth, Massachusetts) has been approved by the FDA as an adjunct to cultures for the diagnosis of invasive fungal infections. True-positive results are not specific for invasive candidiasis, but rather suggest the possibility of an invasive fungal infection. For this reason, among patient populations that are also at risk for invasive mold infections, such as hematopoietic cell transplant recipients, β-D-glucan offers a theoretical advantage over more narrow assays for candidiasis. β-D-glucan detection can identify cases of invasive candidiasis days to weeks prior to positive blood cultures, and shorten the time to initiation of antifungal therapy [143]. Prophylactic or empiric antifungal treatment is likely to impact test performance. On the one hand, antifungal agents may reduce diagnostic sensitivity [144–146], but decreasing β-D-glucan levels may also correlate with responses to antifungal therapy [147].

In meta-analyses of β-D-glucan studies, the pooled sensitivity and specificity for diagnosing invasive candidiasis were 75%–80% and 80%, respectively [144–146]. A number of issues complicate the interpretation of these data, including uncertainties about the best cutoff value for a positive result, number of positive tests required to establish a diagnosis, and optimal timing and frequency of testing among at-risk patients. There is marked heterogeneity among studies in how they address these issues, as well as in patient and control populations, range and type of fungal pathogens targeted, invasive candidiasis disease entities, distributions of Candida species, prior antifungal use, specific β-D-glucan assays employed, and other aspects of study design and statistical interpretation.

The major concern about β-D-glucan detection is the potential for poor specificity and false positivity, which may be particularly problematic in the patient populations for which nonculture diagnostics would be most helpful. For example, false-positive results are rare in healthy controls, but decidedly more common among patients in an ICU [148]. Causes of false positivity include other systemic infections, such as
gram-positive and gram-negative bacteremia, certain antibiotics, such as intravenous amoxicillin-clavulenate (not available in the United States), hemodialysis, fungal colonization, receipt of albumin or immunoglobulin, use of surgical gauze or other material containing glucan, and mucositis or other disruptions of gastrointestinal mucosa [149–154]. The specificity of β-D-glucan can be improved by requiring consecutive positive results rather than a single result, but false positivity remains a significant limitation if the above-listed factors are common in the population tested. As an extreme example, the per-patient sensitivity/specificity and positive and negative predictive values of routine surveillance β-D-glucan testing in a recent study of lung transplant recipients were 64%/9% and 14%/50%, respectively [155]. Moreover, 90% of patients had at least one positive β-D-glucan result. Therefore, the test will be most useful if targeted to subgroups of patients whose clinical course or risk factors are particularly suggestive of invasive candidiasis or other fungal infection.

The role of β-D-glucan testing of samples other than serum in the diagnosis of invasive candidiasis is not established. Studies of β-D-glucan testing of CSF reported sensitivity and specificity of 100% and 95–98%, respectively, for the diagnosis of non-Candida fungal CNS infections [156, 157]. β-D-glucan detection was highly sensitive and specific in a rabbit model of hematogenous C. albicans meningoencephalitis [158]. Limited data suggest that positive predictive values of β-D-glucan in bronchoalveolar lavage fluid are poor for diagnosing fungal pneumonia [159]. There are case reports for testing of samples collected from other sites of invasive Candida infection [160].

Limited data exist pertaining to the usefulness of β-D-glucan testing in children [161]. The optimal threshold for positivity of β-D-glucan testing in children is not known. In studies of uninfected immunocompetent individuals, mean β-D-glucan levels are slightly higher in children than adults [162]. Currently, it is not recommended to use β-D-glucan testing to guide pediatric clinical decision making.

**Polymerase Chain Reaction**

Candida PCR shares many of the potential benefits and shortcomings of β-D-glucan detection. Compared to cultures, PCR assays of various blood fractions have been shown to shorten the time to diagnosis of invasive candidiasis and initiation of antifungal therapy [134, 135]. The pooled sensitivity and specificity of PCR for suspected invasive candidiasis in a recent meta-analysis were 95% and 92%, respectively [134]. In probable invasive candidiasis, sensitivity of PCR and blood cultures was 85% and 38%, respectively. The impact of antifungal agents on diagnostic sensitivity was unclear. Data among patients colonized with Candida were surprisingly limited, but there was a trend toward lower specificity.

A major limitation of PCR studies is the lack of standardized methodologies and multicenter validation of assay performance. A multicenter US study assessing the performance of a self-contained instrument that amplifies and detects Candida DNA by PCR and T2 magnetic resonance (T2 Biosystems, Lexington, Massachusetts), respectively, has been completed [163]. This assay is FDA approved, but its role in the early diagnosis and management of candidemia remains unclear until more data are available. PCR has potential advantages over β-D-glucan or antigen-antibody assays, including the capacity for species identification, detection of molecular markers for drug resistance, and multiplex formatting. In Europe, a whole-blood, multiplex real-time PCR assay (SeptiFast, Roche) that detects 19 bacteria and 6 fungi (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, and Aspergillus fumigatus) has been investigated in several studies of sepsis and neutropenic fever. Among patients with candidemia in one study, the sensitivity of the test was 94%; the only negative result was observed with C. famata candidemia [164]. The role of PCR in testing samples other than blood is not established.

**Nonculture Diagnostic Testing for Blood Culture-Negative Invasive Candidiasis**

The overwhelming majority of studies have examined nonculture diagnostics in the setting of candidemia. More limited data on deep-seated candidiasis demonstrate how these tests may identify cases that are currently missed by blood cultures. In a single-center study of prospectively enrolled patients, the sensitivities/specificities of the Fungitell β-D-glucan assay and a real-time quantitative PCR assay (ViraCor-IBT, Lee’s Summit, Missouri) for invasive candidiasis were 56%/73% and 80%/70%, respectively [132]. More importantly, the sensitivities of contemporaneously collected blood cultures, β-D-glucan assay, and PCR samples among patients with deep-seated candidiasis (mostly intra-abdominal candidiasis) were 21%, 67%, and 88%, respectively. The combination of either a positive blood culture or positive β-D-glucan assay had sensitivity for invasive candidiasis of 79%; a positive blood culture or positive PCR sample was 98% sensitive. A second study investigated the serum β-D-glucan assay, Candida score (a predictive score for invasive candidiasis based on clinical parameters and burden of Candida colonization), and Candida colonization indices (predictive scores based on burden of colonization) among prospectively enrolled patients who were in surgical ICUs at 2 hospitals and who were at particularly high risk for intra-abdominal candidiasis [143]. The sensitivity/specificity of 2 consecutive positive β-D-glucan results was 65%/78%. In contrast, the sensitivity of blood cultures was only 7%. In addition to identifying cases missed by blood cultures, the β-D-glucan assay was positive a median of 5 and 6 days prior to positive intra-abdominal cultures and institution of antifungal therapy, respectively. The sensitivities of Candida scores and colonization indices were comparable to β-D-glucan, but specificities were poorer (<43%).
The interpretation of specificity in these studies was complicated by the fact that negative controls were also at risk for invasive candidiasis. Therefore, it is unclear if positive test results for controls were false positives (as defined in the studies) or true positives that were missed due to the poor sensitivity of intra-abdominal and blood cultures. Indeed, this is a central challenge in assessing new diagnostics for invasive candidiasis: How can test performance be accurately measured when the gold standard is inadequate?

I. What Is the Treatment for Candidemia in Nonneutropenic Patients?

Recommendations

1. An echinocandin (caspofungin: loading dose 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily) is recommended as initial therapy (strong recommendation; high-quality evidence).

2. Fluconazole, intravenous or oral, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily is an acceptable alternative to an echinocandin as initial therapy in selected patients, including those who are not critically ill and who are considered unlikely to have a fluconazole-resistant Candida species (strong recommendation; high-quality evidence).

3. Testing for azole susceptibility is recommended for all bloodstream and other clinically relevant Candida isolates. Testing for echinocandin susceptibility should be considered in patients who have had prior treatment with an echinocandin and among those who have infection with C. glabrata or C. parapsilosis (strong recommendation; low-quality evidence).

4. Transition from an echinocandin to fluconazole (usually within 5–7 days) is recommended for patients who are clinically stable, have isolates that are susceptible to fluconazole (eg, C. albicans), and have negative repeat blood cultures following initiation of antifungal therapy (strong recommendation; moderate-quality evidence).

5. For infection due to C. glabrata, transition to higher-dose fluconazole 800 mg (12 mg/kg) daily or voriconazole 200–300 (3–4 mg/kg) twice daily should only be considered among patients with fluconazole-susceptible or voriconazole-susceptible isolates (strong recommendation; low-quality evidence).

6. Lipid formulation AmB (3–5 mg/kg daily) is recommended (strong recommendation; low-quality evidence).

9. Voriconazole 400 mg (6 mg/kg) twice daily for 2 doses, then 200 mg (3 mg/kg) twice daily is effective for candidemia, but offers little advantage over fluconazole as initial therapy (strong recommendation; moderate-quality evidence). Voriconazole is recommended as step-down oral therapy for selected cases of candidemia due to C. krusei (strong recommendation; low-quality evidence).

10. All nonneutropenic patients with candidemia should have a dilated ophthalmological examination, preferably performed by an ophthalmologist, within the first week after diagnosis (strong recommendation; low-quality evidence).

11. Follow-up blood cultures should be performed every day or every other day to establish the time point at which candidemia has been cleared (strong recommendation; low-quality evidence).

12. Recommended duration of therapy for candidemia without obvious metastatic complications is for 2 weeks after documented clearance of Candida species from the bloodstream and resolution of symptoms attributable to candidemia (strong recommendation; moderate-quality evidence).

Evidence Summary

Candidemia has emerged as one of the most common causes of healthcare-associated bloodstream infections, and in many US hospitals, candidemia represents the third or fourth most common hospital-acquired bloodstream isolate. In most clinical settings, C. albicans is the most commonly isolated species, but the non-albicans Candida species together represent approximately 50% of the bloodstream isolates, and this has been a growing trend in many hospitals throughout the world for more than a decade [8–12].

There are significant challenges in treating candidemia and invasive candidiasis. First, the infection is associated with high mortality. Earlier therapy is associated with better overall outcomes [14–18], but there remain significant limitations to early diagnosis. The development of rapid diagnostic assays has been slow; thus, clinicians continue to rely on cultures to establish a diagnosis [20]. Second, there is considerable geographic, center-to-center, and even unit-to-unit variability of species causing candidemia [12]; each Candida species presents its own unique challenges with respect to virulence, pathogenicity, and antifungal susceptibility. Third, despite the overall robust nature of the randomized controlled trials examining treatment of candidemia and other forms of invasive candidiasis, no single trial has demonstrated the clear superiority of one therapeutic agent over another [19, 21–34]. Fourth, the recent emergence of multidrug-resistant Candida species will complicate the selection of antifungal therapy in the immediate future [10, 12, 35–38].

The selection of any particular agent for the treatment of candidemia should take into account a history of recent azole or
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echinocandin exposure, a history of intolerance to an antifungal agent, the dominant Candida species and current susceptibility data in a particular clinical unit, severity of illness, relevant comorbidities, and evidence of involvement of the CNS, cardiac valves, and/or visceral organs. The risk of mortality among patients with candidemia ranges from 10% to 47% [6–8, 13], but the actual disease-associated mortality is more likely 10%–20%, with the risk of death being related to increasing age, higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, infecting Candida species, immunosuppressive agents, preexisting renal dysfunction, venous catheter retention, and antifungal selection [8, 19, 165–167]. Early initiation of effective antifungal therapy and source control is critical in the successful treatment of candidemia, as demonstrated by data suggesting significantly higher mortality rates among patients with candidemia in whom antifungal therapy was delayed or considered inadequate, and/or in whom source control was not promptly attained [14, 16–18, 168].

The echinocandins demonstrate significant fungicidal activity against most Candida species, and each of these agents has demonstrated success in approximately 70%–75% of patients in randomized, comparative clinical trials [24–28, 31, 32]. Despite the need for intravenous administration, their superb efficacy, favorable safety profile, limited drug interactions, and concerns about fluconazole resistance have led many experts to favor the echinocandins as initial therapy for most adult patients with candidemia. Few studies comparing different echinocandins have been performed [28, 169], but most experts agree that these agents are sufficiently similar to be considered interchangeable.

Only one study comparing an echinocandin to fluconazole has been performed, and the results from this study suggest a strong trend toward more favorable outcomes with anidulafungin compared with fluconazole as primary therapy for candidemia [27]. In a subanalysis of patients with C. albicans infections, there was a significant improvement in global response among those receiving anidulafungin [31]. In another subanalysis of critically ill patients from this trial, those receiving anidulafungin had significantly better responses at end of therapy compared with fluconazole-treated patients [170]. A combined analysis of 7 of the largest randomized clinical trials comparing treatment for candidemia and invasive candidiasis and involving almost 2000 patients found that initial therapy with an echinocandin was a significant predictor of survival [19]. This same analysis identified higher APACHE II score, older age, and infection with C. tropicalis to be associated with worse outcomes and higher mortality [19].

It has become common practice for clinicians treating patients with candidemia to initiate an echinocandin, then change to an oral azole (typically fluconazole) once the patient has become clinically stable [1]. A recent open-label noncomparative trial assessed outcomes of patients who were treated with anidulafungin for at least 5 days followed by step-down therapy to oral fluconazole or voriconazole (if the infecting organism was susceptible) when they were clinically stable and blood cultures had become negative [34]. There was no difference noted in outcomes among patients who continued on anidulafungin throughout the treatment course compared with those who were changed to an oral azole. Smaller pilot studies from Latin America and Asia demonstrated similar findings [33, 171]. Thus, on the basis of these data and other clinical trials [22, 23, 25, 26, 28, 33, 34, 171], the Expert Panel favors step-down therapy to fluconazole or voriconazole for patients who have improved clinically following initial therapy with an echinocandin, have documented clearance of Candida from the bloodstream, and who are infected with an organism that is susceptible to fluconazole (eg, C. albicans, C. parapsilosis, and C. tropicalis) or voriconazole (eg, C. krusei). This transition usually occurs within 5–7 days, but this time is variable and ultimately dependent on patient response and clinician preference.

In many parts of the world, based on success rates reported from well-designed clinical trials, fluconazole remains standard therapy for patients with candidemia [21–23, 27]. However, in light of recent data on the efficacy of echinocandins and increasing resistance to fluconazole, the Expert Panel believes that fluconazole should be considered first-line therapy only in patients who are hemodynamically stable, who have had no previous exposure to azoles, and who do not belong in a group at high risk for C. glabrata infection, including those who are elderly, have underlying malignancy, or are diabetic.

In previous iterations of these guidelines, the Expert Panel favored fluconazole over an echinocandin for treatment of candidemia due to C. parapsilosis based on reports of decreased in vitro activity of echinocandins against this species and of echinocandin resistance among some isolates [11, 12, 172–175]. In spite of these laboratory observations, there have been no clinical studies that have demonstrated superiority of fluconazole over the echinocandins for the treatment of C. parapsilosis infections. Moreover, recent observational data from Spain among almost 200 patients with candidemia due to C. parapsilosis suggested no difference in outcome among patients who received initial treatment with an echinocandin compared with those who received other regimens [176]. Any recommendation supporting fluconazole over an echinocandin is generally based on theoretical concerns rather than on observed therapeutic failure of the echinocandins in these patients.

Voriconazole was shown to be as effective for candidemia and invasive candidiasis as the comparator regimen of sequential therapy with AmB for 4–7 days followed by fluconazole [23]. Voriconazole possesses activity against most Candida species, including C. krusei [177, 178], but the need for more frequent administration, less predictable pharmacokinetics, more drug interactions, and poor tolerance to the drug make it less attractive for initial therapy. Parenteral voriconazole appears
to be safe when administered to those with baseline renal dysfunction, despite concerns based on possible nephrotoxicity of its vehicle (sulfobutylether β-cyclodextrin) [70]. Voriconazole does not provide predictable activity against fluconazole-resistant *C. glabrata* [47, 177–179]. It does, however, fill an important niche for patients who have fluconazole-resistant isolates of *C. krusei*, *C. guilliermondii*, or *C. glabrata* that are voriconazole susceptible and who are ready for transition from an echinocandin or AmB to oral therapy.

There is little role for oral itraconazole for the treatment of candidemia, given the similar antifungal spectrum, ease of administration, superior pharmacokinetics, and better tolerability of fluconazole. Posaconazole has excellent in vitro activity against most *Candida* species. The extended-release tablet and the intravenous formulation could prove useful in the future, but currently there is no role for posaconazole in the treatment of candidemia. The broad-spectrum azole isavuconazole demonstrates similar in vitro activity against *Candida* species, as do voriconazole and posaconazole, and could prove useful in the future [180].

AmB has broad activity against all *Candida* species with the exception of *C. lusitaniae*, which is frequently resistant. Lipid formulations of AmB are preferred to AmB deoxycylolate and should be considered when there is a history of intolerance to echinocandins and/or azoles, the infection is refractory to other therapy, the organism is resistant to other agents, or there is a suspicion of infection due to non-*Candida* yeasts, such as *Cryptococcus neofor mans* or *Histoplasma capsulatum*. Liposomal AmB, 3 mg/kg daily, has been shown to be as effective as micafungin for treatment of candidemia [26].

The emergence of echinocandin-resistant and echinocandin-/azole-resistant *Candida* isolates, especially *C. glabrata*, clearly has been documented, and this finding appears to be associated with worse clinical outcomes [10, 12, 35–37, 181, 182]. Fluconazole resistance is a frequent finding among echinocandin-resistant isolates [9, 10], further complicating therapeutic choices. There are currently no prospective data to inform a decision, but the Expert Panel favors lipid formulation AmB for treatment of patients with candidemia due to proven or suspected fluconazole and echinocandin-resistant (multidrug resistant) strains until more data become available.

Recent data suggest that as many as 16% of patients with candidemia have some manifestation of ocular involvement, and some of these patients will develop severe, sight-threatening endophthalmitis [70]. Thus, for all patients with candidemia, the Expert Panel strongly advises a dilated funduscopic examination, preferably performed by an ophthalmologist, within the first week after initiation of specific antifungal therapy. Some groups have suggested that it is possible to stratify patients according to risk in an effort to avoid performing ophthalmologic examinations on all candidemic patients [183]. This approach is possibly more cost-effective than examining all patients with candidemia, but the potential benefit of early identification of endophthalmitis and prevention of visual loss far outweighs the expense of performing a dilated funduscopic examination.

Follow-up blood cultures every day or every other day until demonstration of clearance of *Candida* from the bloodstream are helpful to establish the appropriate duration of antifungal therapy. If there are no metastatic complications of candidemia, the duration of therapy with systemic antifungal agents should be 14 days following documented clearance of *Candida* species from the bloodstream and resolution of signs and symptoms attributable to infection. This recommendation is based on the results of several prospective, randomized trials in which this rule has been universally and successfully applied, and it is generally associated with few complications and relapses [21–23, 26–28, 30, 32–34].

II. Should Central Venous Catheters Be Removed in Nonneutropenic Patients With Candidemia?

**Recommendation**

13. CVCs should be removed as early as possible in the course of candidemia when the source is presumed to be the CVC and the catheter can be removed safely; this decision should be individualized for each patient (strong recommendation; moderate-quality evidence).

**Evidence Summary**

Central venous catheters and other intravascular devices are important risk factors in the development and persistence of candidemia in nonneutropenic patients [5, 7–9, 184]. A CVC is present in at least 70% of nonneutropenic patients with candidemia at the time that the diagnostic blood culture is obtained [5, 7–9, 170, 184–187]. The relationship of candidemia to CVCs has been assumed on the basis of observation, clinical experience, and an understanding of the role of biofilm in the genesis of bloodstream infections [188, 189]. That candidemia in nonneutropenic patients is commonly due to contaminated CVCs is undeniable, but there remains controversy as to how best to distinguish a catheter-associated candidemia from one that is related to another source, such as the gastrointestinal tract.

There have been no prospective clinical studies designed to examine CVC management as a primary measurement related to outcome. Moreover, several retrospective analyses have led to very different conclusions regarding the necessity and timing of CVC removal in the candidemic patient [19, 190–193]. Thus, the controversy continues, with some groups arguing for a strictly individualized approach to each patient [190] and others for an approach that removes CVCs in all nonneutropenic candidemic patients in whom it is safe and feasible to do so [19]. No prospective study has demonstrated a survival benefit to early CVC removal in patients who have candidemia, but most studies have demonstrated a shorter duration of candidemia and/or a trend toward improved outcomes [14, 21–23, 27, 28, 168, 192–200]. The recent combined analysis of 7 candidemia trials...
observed a survival benefit among those who underwent CVC removal at some time during treatment for candidemia [19]. The survival benefit applied to patients across all levels of severity of illness as determined by APACHE II scores.

The Expert Panel members strongly believe that CVCs should be removed if this can be performed safely when candidemia is documented in the nonneutropenic patient. It is intuitive that each patient with candidemia must be managed individually with respect to CVC removal or retention, but on balance, the bulk of data supports an approach that leads to early removal among nonneutropenic patients in whom the catheter is a likely source of infection.

Among neutropenic patients, the role of the gastrointestinal tract as a source for disseminated candidiasis is evident from autopsy studies, but in an individual patient, it is difficult to determine the relative contributions of the gastrointestinal tract vs the CVC as the primary source of candidemia [195, 201]. An exception is made for candidemia due to C. parapsilosis, which is very frequently associated with CVCs [188, 189, 200, 202]. A recent retrospective analysis that included mostly nonneutropenic patients underscored the influence of early CVC removal, specifically among patients with C. parapsilosis bloodstream infection, on clinical outcome [176].

III. What Is the Treatment for Candidemia in Neutropenic Patients?

Recommendations

14. An echinocandin (caspofungin: loading dose 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose 200 mg, then 100 mg daily) is recommended as initial therapy (strong recommendation; moderate-quality evidence).
15. Lipid formulation AmB, 3–5 mg/kg daily, is an effective but less attractive alternative because of the potential for toxicity (strong recommendation; moderate-quality evidence).
16. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, is an alternative for patients who are not critically ill and have had no prior azole exposure (weak recommendation; low-quality evidence).
17. Fluconazole, 400 mg (6 mg/kg) daily, can be used for step-down therapy during persistent neutropenia in clinically stable patients who have susceptible isolates and documented bloodstream clearance (weak recommendation; low-quality evidence).
18. Voriconazole, 400 mg (6 mg/kg) twice daily for 2 doses, then 200–300 mg (3–4 mg/kg) twice daily, can be used in situations in which additional mold coverage is desired (weak recommendation; low-quality evidence). Voriconazole can also be used as step-down therapy during neutropenia in clinically stable patients who have had documented bloodstream clearance and isolates that are susceptible to voriconazole (weak recommendation; low-quality evidence).
19. For infections due to C. krusei, an echinocandin, lipid formulation AmB, or voriconazole is recommended (strong recommendation; low-quality evidence).
20. Recommended minimum duration of therapy for candidemia without metastatic complications is 2 weeks after documented clearance of Candida from the bloodstream, provided neutropenia and symptoms attributable to candidemia have resolved (strong recommendation; low-quality evidence).
21. Ophthalmological findings of choroidal and vitreal infection are minimal until recovery from neutropenia; therefore, dilated funduscopic examinations should be performed within the first week after recovery from neutropenia (strong recommendation; low-quality evidence).
22. In the neutropenic patient, sources of candidiasis other than a CVC (eg, gastrointestinal tract) predominate. Catheter removal should be considered on an individual basis (strong recommendation; low-quality evidence).
23. Granulocyte colony-stimulating factor (G-CSF)–mobilized granulocyte transfusions can be considered in cases of persistent candidemia with anticipated protracted neutropenia (weak recommendation; low-quality evidence).

Evidence Summary

Candidemia that develops in neutropenic patients is a life-threatening infection associated with acute disseminated candidiasis, a sepsis-like syndrome, multiorgan failure, and death. Outcomes are particularly poor in people with protracted neutropenia, such as that which develops after induction therapy for hematologic malignancies [190, 203, 204]. Candidemia associated with C. tropicalis is associated with particularly poor outcomes in neutropenic hosts. Chronic disseminated candidiasis (hepatosplenic candidiasis) can ensue as a complication of candidemia in neutropenic patients, especially when patients with gastrointestinal tract mucositis do not receive antifungal prophylaxis. There are no adequately powered randomized controlled trials of treatment of candidemia in neutropenic patients. The data are largely derived from single-arm studies, small subsets of randomized controlled studies that have enrolled mostly nonneutropenic patients, and pooled outcomes from randomized trials [205, 206].

Historically, candidemia in neutropenic patients was treated with an AmB formulation. The availability of voriconazole and the echinocandins has led to greater use of these agents, but without compelling clinical data. The extensive use of fluconazole for prophylaxis to prevent invasive candidiasis in neutropenic patients and the lack of meaningful prospective data has led to a diminished therapeutic role for this agent among these patients, except for use as maintenance, or step-down therapy after organism species and susceptibilities are obtained in clinically stable patients [207].

The numbers of neutropenic patients included in candidemia treatment studies are small. In these trials, 50% of caspofungin recipients vs 40% of AmB deoxycholate recipients [25], 68% of micafungin recipients vs 61% of liposomal AmB recipients [26], and 69% of micafungin recipients vs 64% of caspofungin recipients observed a survival benefit among those who underwent CVC removal at some time during treatment for candidemia [19]. The survival benefit applied to patients across all levels of severity of illness as determined by APACHE II scores.

The Expert Panel members strongly believe that CVCs should be removed if this can be performed safely when candidemia is documented in the nonneutropenic patient. It is intuitive that each patient with candidemia must be managed individually with respect to CVC removal or retention, but on balance, the bulk of data supports an approach that leads to early removal among nonneutropenic patients in whom the catheter is a likely source of infection.

Among neutropenic patients, the role of the gastrointestinal tract as a source for disseminated candidiasis is evident from autopsy studies, but in an individual patient, it is difficult to determine the relative contributions of the gastrointestinal tract vs the CVC as the primary source of candidemia [195, 201]. An exception is made for candidemia due to C. parapsilosis, which is very frequently associated with CVCs [188, 189, 200, 202]. A recent retrospective analysis that included mostly nonneutropenic patients underscored the influence of early CVC removal, specifically among patients with C. parapsilosis bloodstream infection, on clinical outcome [176].
recipients [28] with neutropenia at onset of therapy were successfully treated. The randomized controlled trial of anidulafungin vs fluconazole enrolled too few neutropenic patients with candidemia to generate meaningful data regarding efficacy [27]. In 2 retrospective studies, successful outcomes for primary treatment of neutropenic patients were reported in 64% of those receiving AmB deoxycholate, 64% of those receiving fluconazole, and 68% of those receiving caspofungin [29,208].

Additional insights can be gleaned from data derived from studies of empiric antifungal therapy involving febrile patients with neutropenia who had candidemia at baseline. In these studies, baseline candidemia was cleared in 73% of those treated with AmB deoxycholate vs 82% of those treated with liposomal AmB [209] and in 67% of those treated with caspofungin vs 50% of those treated with liposomal AmB [210]. Data from a large randomized trial also suggest that voriconazole is a reasonable choice for febrile patients with neutropenia and suspected invasive candidiasis for whom additional mold coverage is desired [211].

A systematic review was conducted to analyze available data generated in treatment trials and empiric therapy trials that enrolled neutropenic patients [205]. This included 17 trials that randomized 342 neutropenic patients with documented invasive candidiasis. Pooling of results favored use of nonpolyenes to AmB-containing comparators. Another pooled analysis that summarized results of treating with micafungin or comparators (liposomal AmB or caspofungin) for candidemia in the setting of malignancy-associated neutropenia from 2 randomized trials demonstrated success rates ranging from 53% to 85%, but no significant differences among treatment groups [206].

On the basis of these limited data, the success rates of antifungal therapy for candidemia in patients with neutropenia do not appear to be substantially different from those reported in the large randomized trials of nonneutropenic patients. However, conclusions may be limited by significant enrollment bias of selected patients. Although these data do not suggest less favorable outcomes associated with fluconazole and voriconazole, many experts prefer lipid formulation AmB or an echinocandin, which are fungicidal, as first-line agents. Similar to the approach in nonneutropenic patients, the recommended duration of therapy for candidemia in neutropenic patients is for 14 days after resolution of attributable signs and symptoms and clearance of the bloodstream of Candida species, provided that there has been recovery from neutropenia. When neutropenia is protracted, an antifungal drug should be continued until engraftment. This recommendation is based on limited data from prospective randomized trials and has been associated with few complications and relapses [209,210].

The management of intravascular catheters in neutropenic patients with candidemia is less straightforward than in their non-neutropenic counterparts. Distinguishing gut-associated from vascular catheter-associated candidemia can be difficult in these patients [201]. The data for catheter removal are less compelling, and catheter removal often creates significant intravenous access problems. An analysis of 842 patients enrolled in 2 phase 3 treatment trials failed to demonstrate significant clinical benefits of catheter removal in multivariable analyses that adjusted for other measures of prognostic significance [190]. The Expert Panel suggests that catheter removal should be considered on an individual basis, taking into account feasibility and risk of removal.

An extremely important factor influencing the outcome of candidemia in neutropenic patients is the recovery of neutrophils during therapy. In multiple cohort studies of patients with cancer who had candidemia, and pooled analyses of randomized trials, persistent neutropenia was associated with a greater chance of treatment failure [190,203,204,212]. This has led to improvement of strategies to harvest granulocytes from donors (including community volunteers), using G-CSF mobilization, which has been shown to be safe and feasible [213]. Analysis of subsets of people within phase 1/2 granulocyte transfusion studies, retrospective observations, and small cohort studies suggest that G-CSF–mobilized granulocyte transfusions may be of benefit in patients with persistent candidemia and prolonged neutropenia [213–215]. In a randomized controlled trial, granulocyte infusions were associated with few toxicities, but small numbers of patients in infection subgroups limited conclusions of efficacy [216]. The panel recommends consideration of granulocyte infusions in select situations, when such technology is feasible.

IV. What Is the Treatment for Chronic Disseminated (Hepatosplenic) Candidiasis?

Recommendations

24. Initial therapy with lipid formulation AmB, 3–5 mg/kg daily OR an echinocandin (micafungin: 100 mg daily; caspofungin: 70-mg loading dose, then 50 mg daily; or anidulafungin: 200-mg loading dose, then 100 mg daily), for several weeks is recommended, followed by oral fluconazole, 400 mg (6 mg/kg) daily, for patients who are unlikely to have a fluconazole-resistant isolate (strong recommendation; low-quality evidence). Therapy should continue until lesions resolve on repeat imaging, which is usually several months. Premature discontinuation of antifungal therapy can lead to relapse (strong recommendation; low-quality evidence).

25. If chemotherapy or hematopoietic cell transplantation is required, it should not be delayed because of the presence of chronic disseminated candidiasis, and antifungal therapy should be continued throughout the period of high risk to prevent relapse (strong recommendation; low-quality evidence).

26. For patients who have debilitating persistent fevers, short term (1–2 weeks) treatment with nonsteroidal anti-inflammatory drugs or corticosteroids can be considered (weak recommendation; low-quality evidence).

Evidence Summary

Chronic disseminated candidiasis is an uncommon syndrome seen almost entirely in patients who have hematologic...
malignancies and who have just recovered from neutropenia [217–219]. Candida albicans is the species most commonly isolated, but C. tropicalis, C. glabrata, C. krusei, and other Candida species also have been implicated. Fever, right upper quadrant discomfort, nausea, and elevation of liver enzymes occur following return of neutrophils and persist for months unless treatment is initiated. Contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography-CT (PET-CT), and sometimes ultrasound have all been shown to be useful for diagnosis and for follow-up [217, 218, 220, 221]. Biopsy of lesions may reveal budding yeasts and hyphae, but organisms may not be seen on biopsy specimens and often do not grow in culture, leading some to suggest that chronic disseminated candidiasis represents an immune reconstitution syndrome [219].

Approaches to the treatment of chronic disseminated candidiasis are based on anecdotal case reports and open-label series. Early experience with AmB was discouraging; as many as one-third of patients died within 3 months with active infection, and the overall mortality was 74% [222]. With the use of newer antifungal agents, mortality has decreased to 21% overall and is highly linked to relapse of leukemia [223]. Lipid formulations of AmB have proved more efficacious, perhaps related to better tissue concentrations [217, 218, 224, 225]. Fluconazole alone or following AmB induction has been shown to be effective [226, 227]. Increasingly, patients are receiving fluconazole prophylaxis, and thus have an increased risk of developing infection with a fluconazole-resistant organism. In this population, a broader-spectrumazole or an echinocandin is more appropriate therapy. Only a few reports note experience with voriconazole or posaconazole for this condition, but echinocandins are increasingly used to treat this infection [219, 223, 228–231].

Antifungal therapy should be given until all lesions have resolved radiographically in order to prevent relapse. MRI or PET-CT appear to be the most sensitive follow-up modalities, but are expensive [220, 221]; standard contrast-enhanced CT is less expensive and is adequate for follow-up. Additional chemotherapy and hematopoietic cell transplant should be pursued when clinically appropriate and not delayed because of candidiasis. However, antifungal therapy must be continued during the period of immunosuppression to prevent relapse of infection [219, 223, 228–231].

There is evidence that this syndrome could possibly be a form of immune reconstitution and that corticosteroids or anti-inflammatory agents might have a role in selected patients. Several investigators have reported rapid defervescence and improvement in liver enzyme tests when corticosteroids have been given in conjunction with antifungal agents [219, 223, 232, 233]. The dosage of corticosteroids has generally been 0.5–1 mg/kg daily of oral prednisone. The duration of steroid treatment, although highly variable, in most cases has been several weeks, given as a tapering dose [232, 233]. However, the role of corticosteroids in this disease is still not clear.

V. What Is the Role of Empiric Treatment for Suspected Invasive Candidiasis in Nonneutropenic Patients in the Intensive Care Unit? Recommendations

28. Empiric antifungal therapy should be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever and should be based on clinical assessment of risk factors, surrogate markers for invasive candidiasis, and/or culture data from nonsterile sites (strong recommendation; moderate-quality evidence). Empiric antifungal therapy should be started as soon as possible in patients who have the above risk factors and who have clinical signs of septic shock (strong recommendation; moderate-quality evidence).

29. Preferred empiric therapy for suspected candidiasis in nonneutropenic patients in the ICU is an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg daily; micafungin: 100 mg daily; anidulafungin: loading dose of 200 mg, then 100 mg daily) (strong recommendation; moderate-quality evidence).

30. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, is an acceptable alternative for patients who have had no recent azole exposure and are not colonized with azole-resistant Candida species (strong recommendation; moderate-quality evidence).

31. Lipid formulation AmB, 3–5 mg/kg daily, is an alternative if there is intolerance to other antifungal agents (strong recommendation; low-quality evidence).

32. Recommended duration of empiric therapy for suspected invasive candidiasis in those patients who improve is 2 weeks, the same as for treatment of documented candidemia (weak recommendation; low-quality evidence).

33. For patients who have no clinical response to empiric antifungal therapy at 4–5 days and who do not have subsequent evidence of invasive candidiasis after the start of empiric therapy or have a negative non-culture-based diagnostic assay with a high negative predictive value, consideration should be given to stopping antifungal therapy (strong recommendation; low-quality evidence).

Evidence Summary

Candida species are an increasing cause of invasive infection in nonneutropenic patients in the ICU; half to two-thirds of all episodes of candidemia occur in an ICU [5, 14, 167, 170, 234]. Candida bloodstream infections are associated with increased ICU and hospital stay [129, 235]. Most estimates of attributable mortality rates for invasive candidiasis in this setting are 30%–40% [167, 170]. In those patients who have septic shock due to Candida species and who do not have adequate source control or antifungal therapy begun within 24 hours, the mortality approaches 100% [14]. Prompt initiation of appropriate antifungal...
therapy has been associated with as much as a 50% reduction in mortality [14, 17, 18, 236]. Prompt and appropriate antifungal therapy is often delayed because of the relative insensitivity of blood cultures, the time needed for blood cultures to yield growth, the possibility of negative blood cultures with invasive abdominal candidiasis, and the lack of specific clinical signs and symptoms. Strategies for initiating empiric antifungal therapy include an evaluation of risk factors and use of surrogate markers.

Optimal utilization of risk factors and colonization status to derive clinical scoring systems and the interpretation of non-culture-based diagnostic tests to identify patients with invasive candidiasis to initiate early empiric antifungal therapy have been the subjects of many investigations. Retrospective and single-center studies have yielded conflicting results, depending on unique patient populations. Well-designed prospective clinical trials in this area have been difficult to perform, and many unanswered questions remain.

Risk factors for development of invasive candidiasis include Candida colonization, severity of illness, exposure to broad-spectrum antibiotics, recent major surgery, particularly abdominal surgery, necrotizing pancreatitis, dialysis, parenteral nutrition, corticosteroids, and the use of CVCs [237, 238]. Empiric therapy based solely on colonization with Candida species appears inadequate [16, 239]. Prospective studies evaluating the extent of Candida colonization with scores or indices have not been shown to change management, and they are labor intensive and expensive [234].

Several studies have looked at prediction models to identify patients at highest risk. These studies are characterized by high specificity, but low sensitivity, thus missing many patients with candidiasis [240–242]. A subset of postoperative patients, particularly those with recurrent gastrointestinal perforation, anastomotic leaks, or acute necrotizing pancreatitis may be at uniquely high risk for candidiasis [238, 240, 243, 244]. The most important combination of factors in an individual patient has not been established.

Surrogate markers that have been evaluated in the ICU setting include β-D-glucan, mannan-antimannan antibodies, and PCR testing. β-D-glucan appears to be more sensitive than Candida colonization scores or indices, but appears to have low positive predictive value [245–248]. False-positive results are a problem, as noted in the Background section. The optimal timing and number of samples is unknown. In a recent prophylaxis trial of high-risk ICU patients, β-D-glucan testing performed twice weekly identified 87% of patients with proven candidiasis [249]. Small studies basing preemptive therapy on β-D-glucan testing suggest that the high negative predictive value of this test could be useful in excluding invasive candidiasis in the ICU setting [151, 248, 250–252].

Combined mannan-antimannan testing has variable sensitivity and specificity [142, 253]. Real-time PCR appears to have similar sensitivity to β-D-glucan for the diagnosis of candidemia, but may be more sensitive for the diagnosis of other forms of invasive candidiasis [132]. Tests using magnetic biosensor technology for the rapid detection of Candida species from whole-blood samples (T2 Biosystems) are also promising [163]. Recommendations for the clinical use of these tests are challenging without robust data in the at-risk ICU population.

Limited clinical studies have evaluated the efficacy of empiric strategies. Retrospective studies indicate potential for higher survival when empiric antifungal therapy is given to high-risk patients [254]. Prospective clinical trials of empiric antifungal therapy in the ICU are difficult to conduct and have yielded conflicting results. Selected older studies, including those in specific patient populations, such as those with prior gastrointestinal surgery or bowel perforation, demonstrated potential benefit [255, 256]. In a randomized clinical trial of ICU patients at risk for invasive candidiasis and with unexplained fever, empiric fluconazole (800 mg daily for 14 days) was not associated with better outcomes when compared with placebo [257]. A recent study comparing caspofungin to placebo among ICU patients with signs of infection, Candida colonization, and clinical risk factors for invasive candidiasis was stopped prematurely due to poor patient accrual, confirming the difficulty in conducting these trials [249].

Widespread use of antifungal agents must be balanced against the cost, the risk of toxicity, and the emergence of resistance. None of the existing clinical trials have been adequately powered to assess the risk of the emergence of azole or echinocandin resistance. Empiric antifungal therapy should be considered in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever. Preference should be given to an echinocandin in hemodynamically unstable patients, those previously exposed to an azole, and in those colonized with azole-resistant Candida species. Fluconazole may be considered in hemodynamically stable patients who are colonized with azole-susceptible Candida species or who have no prior exposure to azoles. There are no data guiding the appropriate duration of empiric antifungal therapy among patients who have a clinical response to therapy, but it is logical that it should not differ from the treatment of documented candidemia. Conversely, therapy can be stopped after several days in the absence of clinical response if cultures and surrogate markers are negative.

VI. Should Prophylaxis Be Used to Prevent Invasive Candidiasis in the ICU Setting?

Recommendations
34. Fluconazole, 800-mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily, could be used in high-risk patients in adult ICUs with a high rate (>5%) of invasive candidiasis (weak recommendation; moderate-quality evidence).
35. An alternative is to give an echinocandin (caspofungin: 70-mg loading dose, then 50 mg daily; anidulafungin: 200-mg
Evidence Summary

Time to appropriate therapy in candidemia appears to have a significant impact on the outcome of patients with this infection [14, 17, 18]. However, insensitivity and significant delays using culture techniques, as well as limitations of rapid diagnostic tests, remain for this common cause of bloodstream infection among patients in the ICU [258, 259]. A safe and effective prophylactic strategy to prevent candidemia among high-risk patients could be of great benefit [260]. The approach to prophylaxis has been either broad, in which all patients within the ICU setting are treated [261, 262], or selective, in which only specific high-risk groups of patients are targeted for prophylaxis [249, 263, 264].

For ICUs that show very high rates of invasive candidiasis, in excess of the expected rates of <5% of patients, antifungal prophylaxis may be warranted in selected patients who are at highest risk [260]. Two randomized, placebo-controlled trials have shown a reduction in the incidence of invasive candidiasis in single units or single hospitals when fluconazole prophylaxis was used broadly in the ICU; one study targeted all patients in a surgical ICU [262] and, in the other, all patients receiving mechanical ventilation [261]. In both studies, Candida urinary tract infections, as well as invasive candidiasis and candidemia, were included as endpoints.

In a blinded placebo-controlled trial that enrolled a small number of patients, fluconazole prophylaxis was shown to decrease Candida intra-abdominal infections in high-risk patients in the surgical ICU [263]. A noncomparative, open-label trial using caspofungin prophylaxis in a small number of similar high-risk surgical patients also showed benefit [264]. A recent multicenter placebo-controlled, blinded clinical trial of caspofungin prophylaxis targeting only those ICU patients who met specific criteria for high risk for invasive candidiasis showed a trend toward reduction of invasive candidiasis, but was limited by the sample size [249].

Several meta-analyses have assessed the issue of fluconazole prophylaxis in ICU patients [265–268]. Not surprisingly, there were methodological differences among the studies, and there was variation among the study populations. All 4 meta-analyses showed that fluconazole prophylaxis was associated with a reduction in invasive candidiasis, but only 2 showed a reduction in candidemia [267, 268]. Importantly, only one analysis showed a reduction in mortality from invasive candidiasis [268]. None of the meta-analyses assessed the issues of adverse effects of antifungal agents, the emergence of resistance to fluconazole, or major ecological shifts in Candida species, topics of great importance in the ICU setting. A Cochrane analysis confirmed the importance of focusing prophylactic efforts on high-risk patients, noting that the number needed to treat to prevent one case of invasive candidiasis in the ICU setting varied from 9 in high-risk patients to 188 in low-risk patients [269].

Few data exist on risk factors for candidemia in pediatric intensive care unit (PICU) patients. A population-based, case-control study conducted in a large tertiary care pediatric center found an incidence of candidemia of 3.5 per 1000 PICU admissions [270]. The presence of a CVC, a diagnosis of malignancy, and receipt of either vancomycin or an antianaerobic antimicrobial agent for >3 days were independently associated with the development of candidemia. Children who had ≥3 of these risk factors in different combinations had a predicted probability of developing candidemia of between 10% and 46%.

Data are accruing on the use of skin decolonization with antiseptic agents in the ICU to decrease bloodstream infections, including those caused by Candida species [271–274]. Several multicenter randomized clinical trials have shown that daily bathing of ICU patients with chlorhexidine decreases the incidence of both catheter-associated and non-catheter-associated hospital-acquired bloodstream infections [271–273]. These studies were aimed primarily at evaluating the impact on multidrug-resistant bacterial infections and provide few data on Candida infections. However, at least one of these trials found a significant reduction in catheter associated Candida bloodstream infections [272]. A meta-analysis on the effects of daily chlorhexidine bathing included 10 studies performed in an ICU setting, only one of which was a randomized controlled trial. The conclusion was that chlorhexidine bathing reduced the incidence of bloodstream infections, including catheter-associated bacterial infections [274]. Although not proven to prevent candidemia, there is little risk to the use of chlorhexidine in ICU patients, and this practice may prove beneficial.

VII. What Is the Treatment for Neonatal Candidiasis, Including Central Nervous System Infection? What is the Treatment for Neonatal Invasive Candidiasis and Candidemia?

Recommendations

37. AmB deoxycholate, 1 mg/kg daily, is recommended for neonates with disseminated candidiasis (strong recommendation; moderate-quality evidence).

38. Fluconazole, 12 mg/kg intravenous or oral daily, is a reasonable alternative in patients who have not been on fluconazole prophylaxis (strong recommendation; moderate-quality evidence).

39. Lipid formulation AmB, 3–5 mg/kg daily, is an alternative, but should be used with caution, particularly in the presence of urinary tract involvement (weak recommendation; low-quality evidence).
80% candidiasis has decreased dramatically over the past decade in the United States [275]. However, the incidence of neonatal candidiasis occurs predominately in the neonatal intensive care unit (NICU). Neonatal candidiasis is associated with significant risk of death, neurodevelopmental impairment in extremely low-birth-weight infants who weigh ≤1000 g, and increased healthcare costs [279–284]. The primary risk factor for neonatal candidiasis is prematurity with those neonates who have an extremely low birth weight at greatest risk. These infants are at high risk to have CNS involvement as a complication of candidemia [285, 286]. Candida albicans and C. parapsilosis account for 80%–90% of neonatal invasive candidiasis [278, 287].

Neonatal candidiasis differs from invasive disease in older patients in that neonates are more likely to present with nonspecific or subtle signs and symptoms of infection. Candida species invade virtually all tissues, including the retina, brain, heart, lung, liver, spleen, and joints [288]. Endocarditis is an uncommon complication of candidiasis in neonates. Although meningitis is seen frequently in association with candidemia, approximately half of neonates with Candida meningitis do not have a positive blood culture [285]. CNS disease in the neonate typically manifests as meningoencephalitis and should be assumed to be present in the neonate who has candidemia and signs and symptoms suggesting meningoencephalitis, as CSF findings of Candida infection may be unreliable. Neurodevelopmental impairment is common in survivors; therefore, careful follow-up of neurodevelopmental parameters is important [279, 281, 282, 284].

Recent studies have highlighted the significance of candiduria in the absence of candidemia in this population [281]. Extremely low-birth-weight infants with candiduria are at a substantial risk of death or neurodevelopmental impairment. Candiduria in this population should prompt an evaluation (blood cultures, lumbar puncture, and abdominal ultrasound) for disseminated Candida infection and warrants treatment.

Evidence Summary

Neonatal candidiasis occurs predominately in the neonatal intensive care unit (NICU). Candida species are the third most common pathogen associated with bloodstream infection in NICUs in the United States [275]. However, the incidence of neonatal candidiasis has decreased dramatically over the past decade [276–278]. Neonatal candidiasis is associated with significant risk of death, neurodevelopmental impairment, and increased healthcare costs [279–284]. The primary risk factor for neonatal candidiasis is prematurity with those neonates who have an extremely low birth weight at greatest risk. These infants are at high risk to have CNS involvement as a complication of candidemia [285, 286]. Candida albicans and C. parapsilosis account for 80%–90% of neonatal invasive candidiasis [278, 287].

What Is the Treatment for Central Nervous System Infections in Neonates?

Recommendations

45. For initial treatment, AmB deoxycholate, 1 mg/kg intravenously daily, is recommended (strong recommendation; low-quality evidence).
46. An alternative regimen is liposomal AmB, 5 mg/kg daily (strong recommendation; low-quality evidence).
47. The addition of flucytosine, 25 mg/kg 4 times daily, may be considered as salvage therapy in patients who have not had a clinical response to initial AmB therapy, but adverse effects are frequent (weak recommendation; low-quality evidence).
48. For step-down treatment after the patient has responded to initial treatment, fluconazole, 12 mg/kg daily, is recommended for isolates that are susceptible to fluconazole (strong recommendation; low-quality evidence).
49. Therapy should continue until all signs, symptoms, and CSF and radiological abnormalities, if present, have resolved (strong recommendation; low-quality evidence).
50. Infected CNS devices, including ventriculostomy drains and shunts, should be removed if at all possible (strong recommendation; low-quality evidence).

**Evidence Summary**

There are limited data to guide therapy for CNS *Candida* infections in the neonate. All AmB preparations, including the lipid formulations, penetrate the CNS and have fungicidal activity in the CNS [44]. AmB deoxycholate and liposomal AmB were found to have greater antifungal efficacy when studied in a rabbit model of *Candida* meningoencephalitis compared with the other formulations [44]. The clinician must weigh the benefits and drawbacks of using liposomal AmB with its good CSF penetration but poor urine levels vs using AmB deoxycholate with less good CSF levels but better urine levels.

The benefit of adding flucytosine for neonates with CNS candidiasis is uncertain. In the largest prospective study evaluating treatment outcomes of CNS candidiasis in neonates, the median time to clear CSF was longer for those who received flucytosine plus AmB deoxycholate (17.5 days; 6 infants), compared with those who received only AmB deoxycholate (6 days; 18 infants) [279]. In addition, flucytosine is poorly tolerated, and gastrointestinal side effects may hinder oral feeding in neonates. In general, flucytosine is used only in neonates who have not responded to AmB alone.

Data supporting the use of echinocandins in neonates are emerging; however, several key issues require further clarification. The optimal dose of echinocandins in neonates remains uncertain [109, 284, 293–297]. Furthermore, there are concerns regarding the penetration of echinocandins into the CSF. Echinocandins appear to penetrate brain tissue, but not the CSF, and achieve concentrations in brain shown to be effective in animal models when dosages higher than those recommended for humans have been used [298, 299]. Limited clinical data suggest that the echinocandins may be effective for the treatment of CNS infections in neonates, but are not adequate to recommend their use at this time [293].

**What Are the Recommendations for Prophylaxis in the Neonatal Intensive Care Unit Setting?**

51. In nurseries with high rates (>10%) of invasive candidiasis, intravenous or oral fluconazole prophylaxis, 3–6 mg/kg twice weekly for 6 weeks, in neonates with birth weights <1000 g is recommended (strong recommendation; high-quality evidence).
52. Oral nystatin, 100 000 units 3 times daily for 6 weeks, is an alternative to fluconazole in neonates with birth weights <1500 g in situations in which availability or resistance preclude the use of fluconazole (weak recommendation; moderate-quality evidence).
53. Oral bovine lactoferrin (100 mg/day) may be effective in neonates <1500 g but is not currently available in US hospitals (weak recommendation; moderate-quality evidence).

**Evidence Summary**

Numerous studies examining fluconazole prophylaxis for the prevention of invasive candidiasis in neonates have consistently demonstrated efficacy and possibly reduced mortality [300–310]. Fluconazole, 3 mg/kg or 6 mg/kg twice weekly, significantly reduced rates of invasive candidiasis in premature neonates weighing <1000 g in nurseries with a very high incidence of *Candida* infections [300, 302]. A 2007 Cochrane review of clinical trials of fluconazole prophylaxis demonstrated efficacy, with a typical relative risk of 0.23 and number needed to treat of 9. The number needed to treat varied substantially depending on the incidence of invasive candidiasis in a particular ICU. The majority of studies have demonstrated the safety of fluconazole prophylaxis and lack of emergence of resistance.

Enteral or orally administered nystatin has been shown to be effective in reducing invasive candidiasis in preterm infants [303, 311–313]. In one study, nystatin prophylaxis was also associated with a reduction in all-cause mortality [313]. However, there remains a paucity of data on nystatin prophylaxis in infants <750 grams (the group at highest risk), and nystatin may not always be able to be administered when there is an ileus, gastrointestinal disease, feeding intolerance, or hemodynamic instability. These clinical situations are very common in low-gestational-age premature infants and limit the broad applicability of nystatin prophylaxis as a preventive strategy.

Lactoferrin is a mammalian milk glycoprotein involved in innate immunity. In a randomized trial of bovine lactoferrin in infants <1500 g, the incidence of late-onset sepsis was significantly lower in the lactoferrin group than in the placebo group [314]. A secondary analysis of the clinical trial showed that lactoferrin also reduced the incidence of invasive fungal infections compared with placebo [314]. Further confirmation of the efficacy and safety of oral bovine lactoferrin for the prevention of invasive candidiasis is needed, especially in infants <750 g, because there were only a few neonates in this category in this trial.
VIII. What is the Treatment for Intra-abdominal Candidiasis?

Recommendations

54. Empiric antifungal therapy should be considered for patients with clinical evidence of intra-abdominal infection and significant risk factors for candidiasis, including recent abdominal surgery, anastomotic leaks, or necrotizing pancreatitis (strong recommendation; moderate-quality evidence).

55. Treatment of intra-abdominal candidiasis should include source control, with appropriate drainage and/or debridement (strong recommendation; moderate-quality evidence).

56. The choice of antifungal therapy is the same as for the treatment of candidemia or empiric therapy for nonneutropenic patients in the ICU (See sections I and V) (strong recommendation; moderate-quality evidence).

57. The duration of therapy should be determined by adequacy of source control and clinical response (strong recommendation; low-quality evidence).

Evidence Summary

Intra-abdominal candidiasis in patients who have had recent abdominal surgery or intra-abdominal events refers to a heterogeneous group of infections that includes peritonitis, abdominal abscess, and purulent or necrotic infection at sites of gastrointestinal perforation or anastomotic leak. Up to 40% of patients with secondary or tertiary peritonitis, as defined by a multinational consensus panel, may develop intra-abdominal candidiasis with a high mortality rate [243, 244, 315, 316]. A subset of postsurgical patients, particularly those with recurrent gastroduodenal perforation, anastomotic leaks, or acute necrotizing pancreatitis, are at uniquely high risk for invasive candidiasis [243, 244, 263, 316–320]. In other settings, such as perforated appendicitis, invasive candidiasis appears to be a rare complication [316, 319]. Infections are often polymicrobial, with yeast noted in as high as 20% of all cases and 40% in patients with a recent gastroduodenal perforation [319, 320].

Diagnosis is hampered by the lack of specific clinical signs and symptoms. Blood cultures are often negative [321]. A laboratory report of yeast isolated from an abdominal specimen must be evaluated to distinguish between contamination, colonization, and invasive infection. Swabs of superficial wounds and specimens taken from intra-abdominal catheters that have been in place for >24 hours do not provide useful information and should not be performed. In contrast, the presence of yeast obtained from normally sterile intra-abdominal specimens (operative room specimens, and/or drains that have been placed within 24 hours) in patients with clinical evidence for infection should be considered indicative of intra-abdominal candidiasis.

The role of surrogate markers and Candida risk scores in this setting has not been established. There are limited data on the utility of using β-D-glucan in postsurgical patients with suspected intra-abdominal candidiasis. In one study, β-D-glucan had a 72% positive predictive value and an 80% negative predictive value for distinguishing colonization from intra-abdominal invasive candidiasis and performed better than Candida colonization scores or indices [143].

Clinical evidence for the use of antifungal therapy for patients with suspected intra-abdominal invasive candidiasis is limited. Most studies are small, uncontrolled, single-center, or performed in specific populations. Patients who have Candida species isolated from normally sterile abdominal cultures or drains placed within 24 hours and who have clinical evidence of infection should be treated for intra-abdominal candidiasis. Patients who have had gastroduodenal perforations, anastomotic leaks, necrotizing pancreatitis, or other intra-abdominal events without the isolation of Candida species and who are doing poorly despite treatment for bacterial infections may benefit from empiric antifungal therapy. Several meta-analyses of antifungal prophylaxis in high-risk surgical ICU patients have yielded conflicting results [265–268]. Source control with adequate drainage and/or debridement is an important part of therapy of intra-abdominal candidiasis [14]. The choice of antifungal agent should be guided by the Candida species isolated and knowledge of the local epidemiology, including antifungal susceptibility patterns. Duration of antifungal therapy should be guided by clinical response and the adequacy of source control.

IX. Does the Isolation of Candida Species from the Respiratory Tract Require Antifungal Therapy?

Recommendation

58. Growth of Candida from respiratory secretions usually indicates colonization and rarely requires treatment with antifungal therapy (strong recommendation; moderate-quality evidence).

Evidence Summary

The isolation of Candida species from the respiratory tract is commonly encountered among patients who are in the ICU and are intubated or have a chronic tracheostomy. This almost always reflects colonization of the airways and not infection. Candida pneumonia and lung abscess are very uncommon [322, 323]. Only rarely after aspiration of oropharyngeal material has primary Candida pneumonia or abscess been documented [324, 325]. Pneumonia due to Candida species is generally limited to severely immunocompromised patients who develop infection following hematogenous spread to the lungs. CT scan of the thorax usually shows multiple pulmonary nodules. Isolation of Candida species from respiratory samples in a patient who is severely immunosuppressed should trigger a search for evidence of invasive candidiasis.

Although the diagnosis of Candida pneumonia is supported by isolation of the organism from a bronchoalveolar lavage (BAL) specimen, a firm diagnosis requires histopathological evidence of invasive disease. Multiple prospective and retrospective autopsy studies consistently demonstrate the poor predictive...
value of the growth of Candida from respiratory secretions, including BAL fluid [326–328]. In one prospective study, none of 77 patients who died in an ICU and who had clinical and radiologic evidence of pneumonia and a positive culture for Candida species from BAL or sputum demonstrated evidence of Candida pneumonia at autopsy [328]. Because of the rarity of Candida pneumonia, the extremely common finding of Candida in respiratory secretions, and the lack of specificity of this finding [329–331], a decision to initiate antifungal therapy should not be made on the basis of respiratory tract culture results alone.

Recent observations suggest that colonization of the airway with Candida species is associated with the development of bacterial colonization and pneumonia [332–336]. Candida airway colonization was also associated with worse clinical outcomes and higher mortality in these studies. However, it is not clear if Candida airway colonization has a causal relationship to poorer outcomes or is simply a marker of disease severity.

X. What Is the Treatment for Candida Intravascular Infections, Including Endocarditis and Infections of Implantable Cardiac Devices? What Is the Treatment for Candida Endocarditis?

Recommendations

59. For native valve endocarditis, lipid formulation AmB, 3–5 mg/kg daily, with or without fluconazole, 25 mg/kg 4 times daily, OR high-dose echinocandin (caspofungin 150 mg daily, micafungin 150 mg daily, or anidulafungin 200 mg daily) is recommended for initial therapy (strong recommendation; low-quality evidence).

60. Step-down therapy to fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended for patients who have fluconazole-susceptible Candida isolates, have demonstrated clinical stability, and have cleared Candida from the bloodstream (strong recommendation; low-quality evidence).

61. Oral voriconazole, 200–300 mg (3–4 mg/kg) twice daily, or posaconazole tablets, 300 mg daily, can be used as step-down therapy for isolates that are susceptible to those agents but not susceptible to fluconazole (weak recommendation; very low-quality evidence).

62. Valve replacement is recommended; treatment should continue for at least 6 weeks after surgery and for a longer duration in patients with perivalvular abscesses and other complications (strong recommendation; low-quality evidence).

63. For patients who cannot undergo valve replacement, long-term suppression with fluconazole, 400–800 mg (6–12 mg/kg) daily, if the isolate is susceptible, is recommended (strong recommendation; low-quality evidence).

64. For prosthetic valve endocarditis, the same antifungal regimens suggested for native valve endocarditis are recommended (strong recommendation; low-quality evidence). Chronic suppressive antifungal therapy with fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended to prevent recurrence (strong recommendation; low-quality evidence).

Evidence Summary

The incidence of Candida endocarditis has increased concurrent with the general increase in Candida infections [337]. Endocarditis should be suspected when blood cultures are persistently positive, when a patient with candidemia has persistent fever despite appropriate treatment, or when a new heart murmur, heart failure, or embolic phenomena occur in the setting of candidemia [338]. Most cases occur following cardiac valvular surgery, but other risk factors include injection drug use, cancer chemotherapy, prolonged presence of CVCs, and prior bacterial endocarditis. The signs, symptoms, and complications are generally similar to those of bacterial endocarditis, except for the frequent occurrence of large emboli to major vessels. Cases are fairly evenly divided between C. albicans and non-albicans Candida species [339].

Medical therapy of Candida endocarditis has occasionally been curative [340–348], but the optimum therapy for both native and prosthetic valve endocarditis in adults is a combination of valve replacement and a long course of antifungal therapy based on case reports, case series, cohort studies, a meta-analysis, and clinical experience [339, 349]. Valve repair and vegetectomy are alternatives to valve replacement. Most of the cases reported in the literature have been treated with AmB deoxycholate, with or without fluconazole [339, 342, 349–355]. Fluconazole monotherapy is associated with an unacceptably high rate of relapse and mortality [354]. However, fluconazole is useful for step-down therapy.

AmB deoxycholate and azoles have decreased activity when compared with echinocandins against biofilms formed by Candida in vitro, and they penetrate poorly into vegetations. Echinocandins and lipid formulations of AmB demonstrate more potent activity against Candida biofilms [356]. A prospective, open-label clinical trial, cohort studies, and several case reports show a role for the echinocandins in the treatment of endocarditis [228, 346, 348, 357–365]. Higher dosages of the echinocandins are thought to be necessary to treat endocarditis [228, 365]. Caspofungin has been used as monotherapy and in combination with AmB, azoles, or fluconazole in single case reports, but data are limited for the other echinocandins [346, 360, 361, 363, 365, 366].

Lifelong suppressive therapy with fluconazole has been used successfully after a course of primary therapy in patients for whom cardiac surgery is contraindicated; it has also been advocated to prevent late recurrence of Candida prosthetic valve endocarditis [360, 367, 368]. Because Candida endocarditis has a propensity to relapse months to years later, follow-up should be maintained for several years after treatment [350, 351].

What Is the Treatment for Candida Infection of Implantable Cardiac Devices?

Recommendations

65. For pacemaker and implantable cardiac defibrillator infections, the entire device should be removed (strong recommendation; moderate-quality evidence).
66. Antifungal therapy is the same as that recommended for native valve endocarditis (strong recommendation; low-quality evidence).

67. For infections limited to generator pockets, 4 weeks of antifungal therapy after removal of the device is recommended (strong recommendation; low-quality evidence).

68. For infections involving the wires, at least 6 weeks of antifungal therapy after wire removal is recommended (strong recommendation; low-quality evidence).

69. For ventricular assist devices that cannot be removed, the antifungal regimen is the same as that recommended for native valve endocarditis (strong recommendation; low-quality evidence). Chronic suppressive therapy with fluconazole, if the isolate is susceptible, for as long as the device remains in place is recommended (strong recommendation; low-quality evidence).

Evidence Summary

There are a few case reports and a single retrospective review of Candida infections of pacemakers and cardiac defibrillators [369–374]. The entire device should be removed and antifungal therapy given for 4–6 weeks depending on whether the infection involves the wires in addition to the generator pocket [369, 371–374]. Medical therapy alone has failed [370].

There are isolated case reports and a few case series on Candida infections of ventricular assist devices [375–378]. The Expert Panel believes that suppressive azole therapy after a full course of initial antifungal therapy is warranted. Many of these devices cannot be removed and suppression will be lifelong. The role of antifungal prophylaxis to prevent infection in all patients receiving an assist device remains controversial [378].

What Is the Treatment for Candida Suppurative Thrombophlebitis?

Recommendations

70. Catheter removal and incision and drainage or resection of the vein, if feasible, is recommended (strong recommendation; low-quality evidence).

71. Lipid formulation AmB, 3–5 mg/kg daily, or fluconazole, 400–800 mg (6–12 mg/kg) daily, OR an echinocandin (caspofungin 50–70 mg daily, micafungin 100 mg daily, or anidulafungin 100 mg daily) for at least 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months is recommended (strong recommendation; low-quality evidence).

72. Step-down therapy to fluconazole, 400–800 mg (6–12 mg/kg) daily, should be considered for patients who have initially responded to AmB or an echinocandin, are clinically stable, and have a fluconazole-susceptible isolate (strong recommendation; low-quality evidence).

73. Resolution of the thrombus can be used as evidence to discontinue antifungal therapy if clinical and culture data are supportive (strong recommendation; low-quality evidence).

Evidence Summary

Most experience treating suppurative thrombophlebitis has been with AmB deoxycholate. Fluconazole and caspofungin have also been successful in some cases [379–381], but other agents used for primary treatment of candidemia, including echinocandins and voriconazole, should be effective [382]. Higher-than-usual doses of echinocandins should be used, similar to therapy for endocarditis.

Surgical excision of the vein plays an important role in the treatment of peripheral-vein Candida thrombophlebitis. When a central vein is involved, surgery is usually not an option. In some cases, systemic anticoagulation or thrombolytic therapy has been used as adjunctive therapy, but there are insufficient data to recommend their use. Thrombolytic therapy, in conjunction with antifungal therapy, has been used successfully in the management of an infected thrombus attached to a CVC in a patient with persistent candidemia [381].

XI. What Is the Treatment for Candida Osteoarticular Infections?

What Is the Treatment for Candida Osteomyelitis?

Recommendations

74. Fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months OR an echinocandin (caspofungin 50–70 mg daily, micafungin 100 mg daily, or anidulafungin 100 mg daily) for at least 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months is recommended (strong recommendation; low-quality evidence).

75. Lipid formulation AmB, 3–5 mg/kg daily, for at least 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for 6–12 months is a less attractive alternative (weak recommendation; low-quality evidence).

76. Surgical debridement is recommended in selected cases (strong recommendation; low-quality evidence).

Evidence Summary

Most patients with osteomyelitis present with a subacute to chronic course [383, 384]. The most common mechanism of infection is hematogenous dissemination, but direct inoculation and contiguous spread of infection also occur. Involvement of 2 or more bones is common, and therefore, when a single focus of infection is identified, there should be a search for other sites of involvement. The axial skeleton, especially the spine, is the most common site of involvement in adults; in children, the long bones are more commonly involved [228, 384–388]. Neither the clinical picture nor the findings on radiographic imaging are specific for Candida infection. Candida albicans remains the dominant pathogen. However, 2 retrospective reviews of a large number of cases found that non-albicans Candida were an increasingly frequent cause of Candida osteomyelitis and mixed infections with bacteria, especially Staphylococcus aureus, were not uncommon, underscoring the need for biopsy and culture [384, 389].
Treatment recommendations are based on case reports and case series. Historically, AmB deoxycholate has been the most commonly used agent [388]. Recent literature favors the use of fluconazole or an echinocandin over AmB [228, 384–386]. Fluconazole has been used successfully as initial therapy for patients who have susceptible isolates, but treatment failures have also been reported [390–393]. There are case reports of the successful treatment of osteomyelitis with itraconazole, voriconazole, posaconazole, and caspofungin [228, 229, 394–396].

Cure rates appear to be significantly higher when an antifungal agent is administered for at least 6 months [384, 385]. The addition of AmB deoxycholate or fluconazole to bone cement has been suggested to be of value as adjunctive therapy in complicated cases and appears to be safe, but this practice is controversial [397, 398].

Surgical debridement is frequently performed in conjunction with antifungal therapy. Some reports have found surgical therapy important for Candida vertebral osteomyelitis [387], but others have not found that to be the case [388]. Surgery is indicated in patients who have neurological deficits, spinal instability, large abscesses, or persistent or worsening symptoms during therapy [384].

On the basis of a small number of cases, Candida mediastinitis and sternal osteomyelitis in patients who have undergone sternotomy can be treated successfully with surgical debridement followed by either AmB or fluconazole [391, 399]. Irrigation of the mediastinal space with AmB is not recommended, because it can cause irritation. Antifungal therapy of several months’ duration, similar to that needed for osteomyelitis at other sites, is appropriate.

**What Is the Treatment for Candida Septic Arthritis?**

77. Fluconazole, 400 mg (6 mg/kg) daily, for 6 weeks OR an echinocandin (caspofungin 50–70 mg daily, micafungin 100 mg daily, or anidulafungin 100 mg daily) for 2 weeks followed by fluconazole, 400 mg (6 mg/kg) daily, for at least 4 weeks is recommended (strong recommendation; low-quality evidence).

78. Lipid formulation AmB, 3–5 mg/kg daily, for 2 weeks, followed by fluconazole, 400 mg (6 mg/kg) daily, for at least 4 weeks is a less attractive alternative (weak recommendation; low-quality evidence).

79. Surgical drainage is indicated in all cases of septic arthritis (strong recommendation; moderate-quality evidence).

80. For septic arthritis involving a prosthetic device, device removal is recommended (strong recommendation; moderate-quality evidence).

81. If the prosthetic device cannot be removed, chronic suppression with fluconazole, 400 mg (6 mg/kg) daily, if the isolate is susceptible, is recommended (strong recommendation; low-quality evidence).

**Evidence Summary**

Adequate drainage is critical to successful therapy of Candida arthritis. In particular, Candida arthritis of the hip requires open surgical drainage. Case reports have documented cures with AmB, fluconazole, and caspofungin in combination with adequate drainage [400–402]. Administration of either AmB or fluconazole produces substantial synovial fluid levels, so that intra-articular injection of antifungal agents is not necessary.

Candida prosthetic joint infection generally requires resection arthroplasty, although success with medical therapy alone has been described rarely [403, 404]. The combination of removal and reimplantation of the prosthesis in 2 stages separated by 3–6 months and a prolonged period of antifungal therapy for at least 12 weeks after the resection arthroplasty and at least 6 weeks after prosthetic implantation is suggested on the basis of limited data [405–407]. The efficacy of antifungal-loaded cement spacers is controversial [408]. If the prosthetic device cannot be removed, chronic suppression with an antifungal agent, usually fluconazole, is necessary.

**XII. What Is the Treatment for Candida Endophthalmitis?**

**What Is the General Approach to Candida Endophthalmitis?**

**Recommendations**

82. All patients with candidemia should have a dilated retinal examination, preferably performed by an ophthalmologist, within the first week of therapy in nonneutropenic patients to establish if endophthalmitis is present (strong recommendation; low-quality evidence). For neutropenic patients, it is recommended to delay the examination until neutrophil recovery (strong recommendation; low-quality evidence).

83. The extent of ocular infection (chorioretinitis with or without macular involvement and with or without vitritis) should be determined by an ophthalmologist (strong recommendation; low-quality evidence).

84. Decisions regarding antifungal treatment and surgical intervention should be made jointly by an ophthalmologist and an infectious diseases physician (strong recommendation; low-quality evidence).

**Evidence Summary**

Endophthalmitis refers to infections within the eye, usually involving the posterior chamber and sometimes also the anterior chamber. Candida endophthalmitis can be exogenous, initially affecting the anterior chamber and occurring following trauma or a surgical procedure. More often, Candida species cause endogenous infection in which the organism reaches the posterior chamber of the eye via hematogenous spread. Endogenous infections can be manifested as isolated chorioretinitis or as chorioretinitis with extension into the vitreous, leading to vitritis [409–412]. Candida albicans is the species most commonly responsible for endogenous endophthalmitis, but all Candida species that cause candidemia have been reported to cause...
this complication [411–414]. Outcomes in terms of visual acuity depend on the extent of visual loss at the time of presentation and macular involvement [415].

Several basic principles are important in the approach to treatment of Candida infections of the eye. It should first be determined whether infection involves the anterior and/or posterior segment of the eye and whether the macula or vitreous are involved [70, 416–418]. Achieving adequate concentrations of the appropriate antifungal agent in the area of the eye that is infected is crucial to success [419, 420]. Infections involving the choroidal layer are more easily treated because this area of the posterior chamber is highly vascular; many systemic antifungal agents likely reach adequate concentrations within the choroid and the retina [420–422]. The antifungal susceptibilities of the infecting species are important. Species that are susceptible to fluconazole or voriconazole are more easily treated because these agents achieve adequate concentrations in the posterior segment of the eye, including the vitreous [419, 420, 422]. Treatment must be systemic to treat candidemia and other organ involvement, if present, in addition to the ocular infection.

Sight-threatening lesions near the macula or invasion into the vitreous usually necessitate intravitreal injection of antifungal agents, usually AmB deoxycholate or voriconazole, with or without vitrectomy, in addition to systemic antifungal agents [412, 419, 422–425]. The ophthalmologist plays a key role in following the course of endogenous Candida endophthalmitis, deciding when and if to perform intravitreal injections and vitrectomy.

The approach to the patient who has candidemia has evolved over time, and standard practice now includes consultation with an ophthalmologist to do a dilated retinal examination. The basis for the recommendation to perform an ophthalmological evaluation is not a result of randomized controlled trials showing the benefits of such an assessment, but rather clinical judgment that the result of missing and not appropriately treating Candida endophthalmitis could be of great consequence to the patient. The issue of whether an ophthalmological examination of all candidemic patients is cost-effective has been raised [183, 426]. The members of the Expert Panel believe that the risk of missing Candida endophthalmitis outweighs the cost of obtaining an ophthalmological examination. We are concerned about the greater risk of loss of visual acuity in patients who are examined only after manifesting ocular symptoms [415], and note that other centers report higher rates of endophthalmitis than reports from the centers cited by those who question the routine use of ocular examination [417, 418, 421].

What Is the Treatment for Candida Chorioretinitis Without Vitritis?

Recommendations

85. For fluconazole-/voriconazole-susceptible isolates, fluconazole, loading dose, 800 mg (12 mg/kg), then 400–800 mg (6–12 mg/kg) daily OR voriconazole, loading dose 400 mg (6 mg/kg) intravenous twice daily for 2 doses, then 300 mg (4 mg/kg) intravenous or oral twice daily is recommended (strong recommendation; low-quality evidence).

86. For fluconazole-/voriconazole-resistant isolates, liposomal AmB, 3–5 mg/kg intravenous daily, with or without oral fluconazole, 25 mg/kg 4 times daily is recommended (strong recommendation; low-quality evidence).

87. With macular involvement, antifungal agents as noted above PLUS intravitreal injection of either AmB deoxycholate, 5–10 µg/0.1 mL sterile water, or voriconazole, 100 µg/0.1 mL sterile water or normal saline, to ensure a prompt high level of antifungal activity is recommended (strong recommendation; low-quality evidence).

88. The duration of treatment should be at least 4–6 weeks, with the final duration depending on resolution of the lesions as determined by repeated ophthalmological examinations (strong recommendation; low-quality evidence).

Evidence Summary

The greatest clinical experience for treatment of Candida endophthalmitis has been with intravenous AmB deoxycholate, only because it has been available for the longest time. However, this agent does not achieve adequate concentrations in the posterior chamber [419, 420, 427, 428]. In animal experiments in inflamed eyes, liposomal AmB achieved higher concentrations in the eye than either AmB deoxycholate or AmB lipid complex [427]. A few patients have been treated successfully with lipid formulations of AmB, but concentrations in the vitreous in humans have not been reported [429].

Flucytosine provides adjunctive synergistic activity when used with AmB; it should not be used as monotherapy because of development of resistance and reports of decreased efficacy in animal models [428]. It attains excellent levels in the ocular compartments, including the vitreous [412, 430]. Toxicity is common, and flucytosine serum levels must be monitored weekly to prevent dose-related toxicity.

Fluconazole is frequently used for the treatment of Candida endophthalmitis. In experimental animals, this agent achieves excellent concentrations throughout the eye, including the vitreous [428]. In humans, concentrations in the vitreous are approximately 70% of those in the serum [57]. Clinical and microbiological response rates in animals with experimental infection are somewhat conflicting, with most reports showing efficacy of fluconazole, but some noting better efficacy with AmB than fluconazole [428, 431, 432]. Early reports in humans noted the efficacy of fluconazole, but some patients had received intravitreal injection of antifungal agents, as well as systemic fluconazole [433, 434]. Despite the fact that no large published series has defined the efficacy of fluconazole therapy, this agent is routinely used for the treatment of Candida endophthalmitis [410, 411, 415, 421].

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Voriconazole has played an increasing role in the treatment of endophthalmitis [419]. Concentrations in the vitreous in humans are approximately 40% of serum concentrations; the drug is relatively safe, and, like fluconazole, can be given by the oral or intravenous route [435–438]. It is more active than fluconazole against *C. glabrata*, although resistance is increasing and may preclude its use for some patients; it is uniformly active against *C. krusei*. Efficacy of voriconazole in treating Candida endophthalmitis has been documented, but not compared with fluconazole [429, 436, 438]. Serum and (presumably) intracellular concentrations of voriconazole are quite variable, and serum trough levels should be routinely monitored to achieve concentrations between 2 µg/mL and 5 µg/mL to enhance efficacy and avoid toxicity [118].

There are few data regarding the use of posaconazole for Candida endophthalmitis. Intraocular penetration is poor, this agent has been used in very few patients, and it is not approved for the treatment of candidemia [419].

Echinocandins are first-line agents for the treatment of candidemia. Whether they can effectively treat chorioretinitis without vitreous involvement cannot be answered with the data available. Penetration of all echinocandins into the different chambers of the eye is poor, and is especially poor in the vitreous [412, 419, 420]. When levels have been achieved in experimental animal models and in one study in humans with micafungin, the dosages needed have been higher than those currently licensed for use [112, 439–443]. Only a few case reports of the use of an echinocandin as monotherapy have been published, and the results are contradictory [444, 445]. With the availability of other agents that achieve adequate concentrations in the vitreous, there is little reason to recommend the use of echinocandins for Candida endophthalmitis.

Because involvement of the macula is sight-threatening and concentrations of antifungal agents in the posterior chamber do not immediately reach therapeutic levels, many ophthalmologists perform an intravitreal injection of either AmB deoxycholate or voriconazole to quickly achieve high antifungal activity in the posterior chamber. AmB is the agent that has been used most often for intravitreal injection [422, 423]. A dosage of 5–10 µg given in 0.1 mL sterile water is generally safe [419]. Intravitreal injection of lipid formulations of AmB has been compared with AmB deoxycholate in rabbits; all formulations showed toxicity at higher doses, but at 10 µg, the least toxic was liposomal AmB [446], confirming a prior study using a noncommercial liposomal formulation [447].

Voriconazole is increasingly used for intravitreal injection for both Candida and mold endophthalmitis [438, 448]. It has been shown to be safe in animal eyes at concentrations <250 µg/mL [449]. The usual dose given to humans is 100 µg in 0.1 mL sterile water or normal saline (achieving a final concentration of 25 µg/mL) [419, 438]. In vitrectomized eyes, the half-life of both AmB and voriconazole is shortened, and repeated injections may be required [450, 451].

**What Is the Treatment for Candida Chorioretinitis With Vitritis?**

**Recommendations**

89. Antifungal therapy as detailed above for chorioretinitis without vitritis, PLUS intravitreal injection of either amphotericin B deoxycholate, 5–10 µg/0.1 mL sterile water, or voriconazole, 100 µg/0.1 mL sterile water or normal saline, is recommended (strong recommendation; low-quality evidence).

90. Vitrectomy should be considered to decrease the burden of organisms and to allow the removal of fungal abscesses that are inaccessible to systemic antifungal agents (strong recommendation; low-quality evidence).

91. The duration of treatment should be at least 4–6 weeks, with the final duration dependent on resolution of the lesions as determined by repeated ophthalmological examinations (strong recommendation; low-quality evidence).

**Evidence Summary**

*Candida* endophthalmitis that has extended into the vitreous results in worse visual outcomes than chorioretinitis without vitritis [415]. This may be related to the inability of many antifungal agents to achieve adequate concentrations in the vitreous body. Poor outcomes could also be due to an increased burden of organisms in the posterior chamber or the existence of an abscess that cannot be visualized through the vitreal haziness. Additionally, in cases of endophthalmitis in which fungemia is not documented and the organism is unknown, vitrectomy provides material for culture that is superior to needle aspiration and allows the proper antifungal agent to be used [422, 424].

The treatment when vitritis is documented is similar to that recommended for chorioretinitis without vitreal involvement, with the added recommendations to (1) inject either AmB deoxycholate or voriconazole into the vitreous to achieve high drug concentrations in the posterior chamber and to (2) consider performing a pars plana vitrectomy. Several small series have noted success in patients in whom early pars plana vitrectomy was accomplished [415, 423, 424, 452]. Removal of the vitreous is usually accompanied by intravitreal injection of antifungal agents, and as noted above, the half-life of injected antifungal agents is shortened with vitrectomy [450, 451]. The risk of retinal detachment, a severe late complication of endophthalmitis with vitreal involvement, is decreased with early vitrectomy [412, 415]. To have the best outcomes, *Candida* endophthalmitis with vitritis must be managed with close cooperation between ophthalmologists and infectious diseases specialists.

**XIII. What Is the Treatment for Central Nervous System Candidiasis?**

**Recommendations**

92. For initial treatment, liposomal AmB, 5 mg/kg daily, with or without oral fluconosine, 25 mg/kg 4 times daily, is recommended (strong recommendation; low-quality evidence).
93. For step-down therapy after the patient has responded to initial treatment, fluconazole, 400–800 mg (6–12 mg/kg) daily, is recommended (strong recommendation; low-quality evidence).

94. Therapy should continue until all signs and symptoms and CSF and radiological abnormalities have resolved (strong recommendation; low-quality evidence).

95. Infected CNS devices, including ventriculostomy drains, shunts, stimulators, prosthetic reconstructive devices, and biopolymer wafers that deliver chemotherapy, should be removed if possible (strong recommendation; low-quality evidence).

96. For patients in whom a ventricular device cannot be removed, AmB deoxycholate could be administered through the device into the ventricle at a dosage ranging from 0.01 mg to 0.5 mg in 2 mL 5% dextrose in water (weak recommendation; low-quality evidence).

Evidence Summary

CNS Candida infections can occur as a manifestation of disseminated candidiasis, as a complication of a neurosurgical procedure, especially when an intracranial device is inserted, or rarely as an isolated chronic infection [453–462]. Meningitis is the most common presentation, but multiple small abscesses throughout the brain parenchyma, large solitary brain abscesses, and epidural abscesses have been reported [462]. Low-birthweight neonates are at high risk to have CNS infection as a complication of candidemia; neonatal CNS candidiasis is dealt with in the section on neonatal Candida infections. Most infections are due to C. albicans, with few reports of C. glabrata and other species causing infection [453–457, 459, 461, 462]. Treatment is based on the antifungal susceptibilities of the infecting species and the ability of the antifungal agent to achieve appropriate concentrations in the CSF and brain.

No randomized controlled trials have been performed to evaluate the most appropriate treatment for these uncommon infections. Single cases and small series are reported. Most experience has accrued with the use of AmB deoxycholate, with or without flucytosine [453–455, 457, 459, 460, 462]. Liposomal AmB (AmBisome) has been found to attain higher levels in the brain than amphotericin B lipid complex (ABLC) or AmB deoxycholate in a rabbit model of Candida meningencephalitis [44].

The combination of AmB and flucytosine is recommended because of the in vitro synergism noted with the combination and the excellent CSF concentrations achieved by flucytosine. However, flucytosine’s toxic effects on bone marrow and liver must be carefully monitored, preferably with frequent serum flucytosine levels. The optimal length of therapy with AmB alone or in combination with flucytosine has not been studied. Several weeks of therapy are suggested before transitioning to oral azole therapy.

Fluconazole achieves excellent levels in CSF and brain tissue and has proved useful as step-down therapy [453, 454, 459]. Fluconazole also has been used as monotherapy; both success and failure have been noted, and for this reason it is not recommended as first-line therapy [453, 454, 463–465]. Fluconazole combined with flucytosine has been reported to cure Candida meningitis in a few patients [459], and this is a possible regimen for step-down therapy. There are no reports of the use of voriconazole or posaconazole for CNS candidiasis. Voriconazole achieves excellent levels in CSF, and should be considered for the rare case of C. glabrata that is not voriconazole resistant or C. krusei meningitis after initial treatment with AmB with or without flucytosine. Posaconazole does not reach adequate concentrations in the CSF, and this agent is not recommended.

Echinocandins have been used infrequently for CNS candidiasis. There are case reports noting success [466], but CNS breakthrough infections in patients receiving an echinocandin for candidemia have been reported [467]. There are experimental animal data noting that anidulafungin and micafungin can successfully treat C. albicans meningitis, but the doses required in humans are much higher than currently recommended for candidemia [296, 299]. At present, echinocandins are not recommended for CNS candidiasis.

Infected CNS devices should be removed to eradicate Candida. Most experience has been with external ventricular drains and ventriculoperitoneal shunts that have become infected with Candida species [460, 463]. In recent years, infected devices include deep brain stimulators and Gliadel biopolymer wafers that have been placed into the site of a brain tumor to deliver chemotherapy locally. Although difficult to remove, experience has shown that these devices must be taken out for cure of the infection [456, 468, 469].

Intraventricular administration of antifungal agents is not usually necessary for treatment of CNS Candida infections. In patients in whom the removal of a ventricular shunt or external ventriculostomy drain is too risky because of significantly elevated intracranial pressure, or among patients who have not responded to systemic antifungal therapy, intraventricular AmB deoxycholate has proved useful [453, 454, 460, 463, 469]. The dose of intraventricular AmB deoxycholate is not standardized, and recommendations vary from 0.01 mg to 1 mg in 2 mL of 5% dextrose in water daily [455, 463, 466, 469]. Toxicity—mainly headache, nausea, and vomiting—is a limiting factor when administering AmB by this route [454, 463].

XIV. What Is the Treatment for Urinary Tract Infections Due to Candida Species?

What Is the Treatment for Asymptomatic Candiduria?

Recommendations

97. Elimination of predisposing factors, such as indwelling bladder catheters, is recommended whenever feasible (strong recommendation; low-quality evidence).

98. Treatment with antifungal agents is NOT recommended unless the patient belongs to a group at high risk for
dissemination; high-risk patients include neutropenic patients, very low-birth-weight infants (<1500 g), and patients who will undergo urologic manipulation (strong recommendation; low-quality evidence).

99. Neutropenic patients and very low-birth-weight infants should be treated as recommended for candidemia (see sections III and VII) (strong recommendation; low-quality evidence).

100. Patients undergoing urologic procedures should be treated with oral fluconazole, 400 mg (6 mg/kg) daily, OR AmB deoxycholate, 0.3–0.6 mg/kg daily, for several days before and after the procedure (strong recommendation; low-quality evidence).

Evidence Summary

The presence of candiduria is the usual trigger for a physician to consider whether a patient has a urinary tract infection due to Candida species. The patients at most risk for candiduria are those who are elderly, female, diabetic, have indwelling urinary devices, are taking antibiotics, and have had prior surgical procedures [470–475]. In the asymptomatic patient, candiduria almost always represents colonization, and elimination of underlying risk factors, such as indwelling catheters, is often adequate to eradicate candiduria [471].

Multiple studies have noted that candiduria does not commonly lead to candidemia [471, 472, 476–480]. Several of these studies have shown that candiduria is a marker for greater mortality, but death is not related to Candida infection and treatment for Candida infection does not change mortality rates [476, 480, 481]. A prospective study in renal transplant recipients found that although mortality was higher in patients who had candiduria, treatment did not improve outcomes, suggesting again that candiduria is a marker for severity of underlying illness [482].

Several conditions require an aggressive approach to candiduria in asymptomatic patients. These include neonates with very low birth weight, who are at risk for invasive candidiasis that often involves the urinary tract [281, 483]. Many physicians who care for neutropenic patients treat those who have fever and candiduria because the candiduria may indicate invasive candidiasis. However, a recent study from a cancer hospital of a small number of patients, 25% of whom were neutropenic, found that these patients did not develop candidemia or other complications of candiduria [484]. Several reports have documented a high rate of candidemia when patients undergo urinary tract instrumentation [485, 486], which has led to recommendations to treat with antifungal agents periprocedure.

What Is the Treatment for Symptomatic Candida Cystitis?

Recommendations

101. For fluconazole-susceptible organisms, oral fluconazole, 200 mg (3 mg/kg) daily for 2 weeks is recommended (strong recommendation; moderate-quality evidence).

102. For fluconazole-resistant C. glabrata, AmB deoxycholate, 0.3–0.6 mg/kg daily for 1–7 days OR oral flucytosine, 25 mg/kg 4 times daily for 7–10 days is recommended (strong recommendation; low-quality evidence).

103. For C. kruzei, AmB deoxycholate, 0.3–0.6 mg/kg daily, for 1–7 days is recommended (strong recommendation; low-quality evidence).

104. Removal of an indwelling bladder catheter, if feasible, is strongly recommended (strong recommendation; low-quality evidence).

105. AmB deoxycholate bladder irrigation, 50 mg/L sterile water daily for 5 days, may be useful for treatment of cystitis due to fluconazole-resistant species, such as C. glabrata and C. krusei (weak recommendation; low-quality evidence).

What Is the Treatment for Symptomatic Ascending Candida Pyelonephritis?

Recommendations

106. For fluconazole-susceptible organisms, oral fluconazole, 200–400 mg (3–6 mg/kg) daily for 2 weeks, is recommended (strong recommendation; low-quality evidence).

107. For fluconazole-resistant C. glabrata, AmB deoxycholate, 0.3–0.6 mg/kg daily for 1–7 days, with or without oral flucytosine, 25 mg/kg 4 times daily, is recommended (strong recommendation; low-quality evidence).

108. For fluconazole-resistant C. glabrata, monotherapy with oral flucytosine, 25 mg/kg 4 times daily for 2 weeks, could be considered (weak recommendation; low-quality evidence).

109. For C. krusei, AmB deoxycholate, 0.3–0.6 mg/kg daily, for 1–7 days is recommended (strong recommendation; low-quality evidence).

110. Elimination of urinary tract obstruction is strongly recommended (strong recommendation; low-quality evidence).

111. For patients who have nephrostomy tubes or stents in place, consider removal or replacement, if feasible (weak recommendation; low-quality evidence).

Evidence Summary

Candida UTI can develop by 2 different routes [487]. Most symptomatic UTIs evolve as an ascending infection beginning in the lower urinary tract, similar to the pathogenesis of bacterial UTI. Patients with ascending infection can have symptoms of cystitis or pyelonephritis. The other route of infection occurs as a consequence of hematogenous spread to the kidneys in a patient who has candidemia. These patients usually have no urinary tract symptoms or signs, and are treated for candidemia.

Diagnostic tests on urine often are not helpful in differentiating colonization from infection or in pinpointing the involved site within the urinary tract [488, 489]. For example, pyuria in a patient with an indwelling bladder catheter cannot differentiate Candida infection from colonization. Similarly, the colony
count in the urine, especially when a catheter is present, cannot be used to define infection [488, 489]. Imaging of the urinary tract by ultrasound or CT scanning is helpful in defining structural abnormalities, hydronephrosis, abscesses, emphysematous pyelonephritis, and fungus ball formation [490–492]. Aggregation of mycelia and yeasts (fungus balls) in bladder or kidney leads to obstruction and precludes successful treatment of infection with antifungal agents alone [94]. Rarely, Candida species cause localized infections in prostate, epididymis, or testicles [491, 493–495].

Several basic principles are important in the approach to treatment of Candida UTI. The ability of the antifungal agent to achieve adequate concentrations in the urine is as important as the antifungal susceptibilities of the infecting species [94]. Candida albicans, the most common cause of fungal UTI, is relatively easy to treat because it is susceptible to fluconazole, which achieves high concentrations in the urine. In contrast, UTIs due to fluconazole-resistant C. glabrata and C. krusei can be extremely difficult to treat.

Fluconazole is the drug of choice for treating Candida UTI. It was shown to be effective in eradicating candiduria in the only randomized, double-blind, placebo-controlled trial that has been conducted in patients with candiduria [496]. It is important to note that the patients in this trial were asymptomatic or had minimal symptoms of cystitis. Fluconazole is available as an oral formulation, is excreted into the urine in its active form, and easily achieves urine levels exceeding the MIC for most Candida isolates [94].

Flucytosine demonstrates good activity against many Candida species, with the exception of C. krusei, and is excreted as active drug in the urine [94]. Treatment with flucytosine is limited by its toxicity and the development of resistance when it is used as a single agent.

AmB deoxycholate is active against most Candida species (although some C. krusei isolates are resistant) and achieves concentrations in the urine that exceed the MICs for most isolates, and even low doses have been shown to be effective in treating Candida UTI [497]. The major drawbacks are the need for intravenous administration and toxicity. The lipid formulations of AmB appear to not achieve urine concentrations that are adequate to treat UTI and should not be used [498].

All other antifungal drugs, including the other azole agents and echinocandins, have minimal excretion of active drug into the urine and generally are ineffective in treating Candida UTI [94]. However, there are several reports of patients in whom echinocandins were used, primarily because of UTI due to fluconazole-resistant organisms, and both success and failure were reported [499–502]. Infection localized to the kidney, as occurs with hematogenous spread, probably can be treated with echinocandins because tissue concentrations are adequate even though these agents do not achieve adequate urine concentrations [499].

Irrigation of the bladder with AmB deoxycholate resolves candiduria in 80%–90% of patients, as shown in several open-label trials, but in those studies, recurrent candiduria within several weeks was very common [503–505]. This approach is useful only for bladder infections and generally is discouraged, especially in patients who would not require an indwelling catheter for any other reason [94, 506, 507]. Cystitis due to C. glabrata or C. krusei can sometimes be treated with amphotericin B bladder irrigation and endoscopic removal of any obstructing lesions [94].

What Is the Treatment for Candida Urinary Tract Infection Associated With Fungus Balls?

Recommendations

112. Surgical intervention is strongly recommended in adults (strong recommendation; low-quality evidence).

113. Antifungal treatment as noted above for cystitis or pyelonephritis is recommended (strong recommendation; low-quality evidence).

114. Irrigation through nephrostomy tubes, if present, with AmB deoxycholate, 25–50 mg in 200–500 mL sterile water, is recommended (strong recommendation; low-quality evidence).

Evidence Summary

Fungus balls are an uncommon complication of Candida UTI except in neonates, in whom fungus ball formation in the collecting system commonly occurs as a manifestation of disseminated candidiasis [483]. In adults, surgical or endoscopic removal of the obstructing mycelial mass is central to successful treatment [94, 508, 509]. In neonates, some series documented resolution of fungus balls with antifungal treatment alone [510], but others found that endoscopic removal was necessary [511, 512]. There are anecdotal reports of a variety of techniques used to remove fungus balls from the renal pelvis; these include endoscopic removal via a percutaneous nephrostomy tube, infusion of streptokinase locally, and irrigation with antifungal agents through a nephrostomy tube [511–513]. Fungus balls in the bladder and lower ureter usually can be removed endoscopically [509].

XV. What Is the Treatment for Vulvovaginal Candidiasis?

Recommendations

115. For the treatment of uncomplicated Candida vulvovaginitis, topical antifungal agents, with no one agent superior to another, are recommended (strong recommendation; high-quality evidence).

116. Alternatively, for the treatment of uncomplicated Candida vulvovaginitis, a single 150-mg oral dose of fluconazole is recommended (strong recommendation; high-quality evidence).

117. For severe acute Candida vulvovaginitis, fluconazole 150 mg, given every 72 hours for a total of 2 or 3 doses, is recommended (strong recommendation; high-quality evidence).
118. For *C. glabrata* vulvovaginitis that is unresponsive to oral azoles, topical intravaginal boric acid, administered in a gelatin capsule, 600 mg daily, for 14 days is an alternative (strong recommendation; low-quality evidence).

119. Another alternative agent for *C. glabrata* infection is nystatin intravaginal suppositories, 100 000 units daily for 14 days (strong recommendation; low-quality evidence).

120. A third option for *C. glabrata* infection is topical 17% fluocytosine cream alone or in combination with 3% AmB cream administered daily for 14 days (weak recommendation; low-quality evidence).

121. For recurring vulvovaginal candidiasis, 10–14 days of induction therapy with a topical agent or oral fluconazole, followed by fluconazole, 150 mg weekly for 6 months, is recommended (strong recommendation; high-quality evidence).

**Evidence Summary**

Vulvovaginal candidiasis can be classified as either uncomplicated, which is present in about 90% of cases, or complicated, which accounts for only about 10% of cases, on the basis of clinical presentation, microbiological findings, host factors, and response to therapy [514]. Complicated vulvovaginal candidiasis is defined as severe or recurrent disease, infection due to non-*albicans* species, and/or infection in an abnormal host. *Candida albicans* is the usual pathogen, but other *Candida* species can also cause this infection.

A diagnosis of vulvovaginal candidiasis can usually be made clinically when a woman presents with symptoms of pruritus, irritation, vaginal soreness, external dysuria, and dyspareunia, often accompanied by a change in vaginal discharge. Signs include vulvar edema, erythema, excoriation, fissures, and a white, thick, curdlike vaginal discharge. Unfortunately, these symptoms and signs are nonspecific and can be the result of a variety of infectious and noninfectious etiologies. Before proceeding with empiric antifungal therapy, the diagnosis should be confirmed by a wet-mount preparation with use of saline and 10% potassium hydroxide to demonstrate the presence of yeast or hyphae and a normal pH (4.0–4.5). For those with negative findings, vaginal cultures for *Candida* should be obtained.

A variety of topical and systemic oral agents are available for treatment of vulvovaginal candidiasis. No evidence exists to show the superiority of any one topical regimen [515, 516], and oral and topical antifungal formulations have been shown to achieve entirely equivalent results [517]. Uncomplicated infection can be effectively treated with either single-dose fluconazole or short-course fluconazole for 3 days, both of which achieve >90% response [517, 518]. Treatment of vulvovaginal candidiasis should not differ on the basis of human immunodeficiency virus (HIV) infection status; identical response rates are anticipated for HIV-positive and HIV-negative women.

Complicated vulvovaginal candidiasis requires that therapy be administered intravaginally with topical agents for 5–7 days or orally with fluconazole 150 mg every 72 hours for 3 doses [54, 514]. Most *Candida* species, with the exception of *C. krusei* and *C. glabrata*, respond to oral fluconazole. *Candida krusei* responds to all topical antifungal agents. However, treatment of *C. glabrata* vulvovaginal candidiasis is problematic [514, 516]. The most important decision to make is whether the presence of *C. glabrata* in vaginal cultures reflects colonization in a patient who has another disease, or whether it indicates true infection requiring treatment. Azole therapy, including voriconazole, is frequently unsuccessful [519]. A variety of local regimens have sometimes proved effective. These include boric acid contained in gelatin capsules and nystatin intravaginal suppositories [520]. Topical 17% fluocytosine cream can be used alone or in combination with 3% AmB cream in recalcitrant cases [520, 521]. These topical formulations, as well as boric acid gelatin capsules, must be compounded by a pharmacist for specific patient use. Azole-resistant *C. albicans* infections are extremely rare. However, recent evidence has emerged documenting fluconazole and azole class resistance in women following prolonged azole exposure [522].

Recurrent vulvovaginal candidiasis, defined as ≥4 episodes of symptomatic infection within one year, is usually caused by azole-susceptible *C. albicans* [514, 523]. Contributing factors, such as diabetes, are rarely found. Treatment should begin with induction therapy with a topical agent or oral fluconazole for 10–14 days, followed by a maintenance azole regimen for at least 6 months [523–525]. The most convenient and well-tolerated regimen is 150 mg fluconazole once weekly. This regimen achieves control of symptoms in >90% of patients [523]. After cessation of maintenance therapy, a 40%–50% recurrence rate can be anticipated. If fluconazole therapy is not feasible, topical clotrimazole cream, 200 mg twice weekly, clotrimazole vaginal suppository 500 mg once weekly, or other intermittent oral or topical antifungal treatment is recommended [526, 527].

**XVI. What Is the Treatment for Oropharyngeal Candidiasis?**

**Recommendations**

122. For mild disease, clotrimazole troches, 10 mg 5 times daily, OR miconazole mucoadhesive buccal 50 mg tablet applied to the mucosal surface over the canine fossa once daily for 7–14 days, are recommended (strong recommendation; high-quality evidence).

123. Alternatives for mild disease include nystatin suspension (100 000 U/mL) 4–6 mL 4 times daily, OR 1–2 nystatin pastilles (200 000 U each) 4 times daily, for 7–14 days (strong recommendation; moderate-quality evidence).

124. For moderate to severe disease, oral fluconazole, 100–200 mg daily, for 7–14 days is recommended (strong recommendation; high-quality evidence).

125. For fluconazole-refractory disease, itraconazole solution, 200 mg once daily OR posaconazole suspension, 400 mg twice daily for 3 days then 400 mg daily, for up to 28 days,
126. Alternatives for fluconazole-refractory disease include voriconazole, 200 mg twice daily, OR AmB deoxycholate oral suspension, 100 mg/mL 4 times daily (strong recommendation; moderate-quality evidence).

127. Intravenous echinocandin (caspofungin: 70-mg loading dose, then 50 mg daily; micafungin: 100 mg daily; or anidulafungin: 200-mg loading dose, then 100 mg daily) OR intravenous AmB deoxycholate, 0.3 mg/kg daily, are other alternatives for refractory disease (weak recommendation; moderate-quality evidence).

128. Chronic suppressive therapy is usually unnecessary. If required for patients who have recurrent infection, fluconazole, 100 mg 3 times weekly, is recommended (strong recommendation; high-quality evidence).

129. For HIV-infected patients, antiretroviral therapy is strongly recommended to reduce the incidence of recurrent infections (strong recommendation; high-quality evidence).

130. For denture-related candidiasis, disinfection of the denture, in addition to antifungal therapy, is recommended (strong recommendation; moderate-quality evidence).

Evidence Summary

Oropharyngeal and esophageal candidiasis occur in association with HIV infection, diabetes, leukemia and other malignancies, steroid use, radiation therapy, antimicrobial therapy, and denture use [528, 529], and their occurrence is recognized as an indicator of immune dysfunction. In HIV-infected patients, oropharyngeal candidiasis is most often observed in patients with CD4 counts <200 cells/µL [528–530]. The advent of effective antiretroviral therapy has led to a dramatic decline in the prevalence of oropharyngeal candidiasis and a marked diminution in cases of refractory disease [531].

Fluconazole or multiazole resistance is predominantly the consequence of previous repeated and long-term exposure to fluconazole or other azoles [530–533]. Especially in patients with advanced immunosuppression and low CD4 counts, C. albicans resistance has been described, as has gradual emergence of non-albicans Candida species, particularly C. glabrata, as a cause of refractory mucosal candidiasis [532, 533].

Most cases of oropharyngeal candidiasis are caused by C. albicans, either alone or in mixed infections. Symptomatic infections caused by C. glabrata, C. dubliniensis, and C. krusei alone have been described [532–534]. Multiple randomized prospective studies of oropharyngeal candidiasis have been performed involving patients with AIDS and patients with cancer. Most patients will respond initially to topical therapy [532, 535, 536]. In HIV-infected patients, symptomatic relapses occur sooner and more frequently with topical therapy than with fluconazole [535]. In a multicenter randomized study among HIV-infected individuals, 50-mg mucoadhesive buccal tablets of miconazole applied once daily to the mucosal surface over the canine fossa were as effective as 10-mg clotrimazole troches used 5 times daily [537].

Fluconazole tablets and itraconazole solution are superior to ketoconazole and itraconazole capsules [538–540]. Local effects of oral solutions may be as important as the systemic effects. Posaconazole suspension is also as efficacious as fluconazole in patients with AIDS [541]. Posaconazole, 100-mg delayed release tablets, given as 300 mg daily as a single dose, are FDA approved for the prophylaxis of fungal infections in high-risk patients. The tablets provide a stable bioavailability (approximately 55%), once-daily dosing, and the convenience of less stringent food requirements for absorption. This formulation has not been fully evaluated for mucosal candidiasis, but, with further study, could replace the oral suspension for this purpose.

Recurrent infections typically occur in patients who have persistent immunosuppression, especially those who have AIDS and low CD4 cell counts (<50 cells/µL) [530–533]. Long-term suppressive therapy with fluconazole has been shown to be effective in the prevention of oropharyngeal candidiasis [53, 542, 543]. In a large multicenter study of HIV-infected patients, long-term suppressive therapy with fluconazole was compared with the episodic use of fluconazole in response to symptomatic disease. Continuous suppressive therapy reduced the relapse rate more effectively than did intermittent therapy, but was associated with increased in vitro resistance. The frequency of refractory disease was the same for both groups [53]. Oral AmB deoxycholate, nystatin solution, and itraconazole capsules are less effective than fluconazole in preventing oropharyngeal candidiasis [544, 545].

Fluconazole-refractory infections should be treated initially with itraconazole solution; between 64% and 80% of patients will respond to this therapy [546, 547]. Posaconazole suspension is efficacious in approximately 75% of patients with refractory oropharyngeal or esophageal candidiasis [548], and voriconazole also is efficacious for fluconazole-refractory infections [549]. Intravenous caspofungin, micafungin, and anidulafungin have been shown to be effective alternatives toazole agents for refractory candidiasis [24, 87, 88, 550]. Oral or intravenous AmB deoxycholate is also effective in some patients; however, a pharmacist must compound the oral formulation [551]. Immunomodulation with adjunctive granulocyte-macrophage colony-stimulating factor or interferon-γ have been occasionally used in the management of refractory oral and esophageal candidiasis [552, 553].

Decreasing rates of oral carriage of Candida species and a reduced frequency of symptomatic oropharyngeal candidiasis are seen among HIV-infected patients on effective antiretroviral therapy [554]. Thus, antiretroviral therapy should be used whenever possible for HIV-infected patients with oropharyngeal or esophageal candidiasis.

Chronic mucocutaneous candidiasis is a rare condition that is characterized by chronic, persistent onychomycosis and/or
mucocutaneous lesions due to *Candida* species. Some patients have a thymoma or autoimmune polyendocrinopathy syndrome type 1 [555]. Fluconazole should be used as initial therapy for candidiasis in these patients. Response to antifungal therapy may be delayed when there is extensive skin or nail involvement. Because of the intrinsic immunodeficiency, most patients require chronic suppressive antifungal therapy and frequently develop azole-refractory infections [556]. Patients with fluconazole-refractory *Candida* infections should be treated the same as patients with AIDS who develop azole refractory infections [528].

### XVII. What Is the Treatment for Esophageal Candidiasis?

#### Recommendations

131. Systemic antifungal therapy is always required. A diagnostic trial of antifungal therapy is appropriate before performing an endoscopic examination (strong recommendation; high-quality evidence).

132. Oral fluconazole, 200–400 mg (3–6 mg/kg) daily, for 14–21 days is recommended (strong recommendation; high-quality evidence).

133. For patients who cannot tolerate oral therapy, intravenous fluconazole, 400 mg (6 mg/kg) daily, OR an echinocandin (micafungin: 150 mg daily; caspofungin: 70-mg loading dose, then 50 mg daily; or anidulafungin: 200 mg daily) is recommended (strong recommendation; high-quality evidence).

134. A less preferred alternative for those who cannot tolerate oral therapy is AmB deoxycholate, 0.3–0.7 mg/kg daily (strong recommendation; moderate-quality evidence).

135. Consider de-escalating to oral therapy with fluconazole 200–400 mg (3–6 mg/kg) daily once the patient is able to tolerate oral intake (strong recommendation; moderate-quality evidence).

136. For fluconazole-refractory disease, itraconazole solution, 200 mg daily, OR voriconazole, 200 mg (3 mg/kg) twice daily either intravenous or oral, for 14–21 days is recommended (strong recommendation; high-quality evidence).

137. Alternatives for fluconazole-refractory disease include an echinocandin (micafungin: 150 mg daily; caspofungin: 70-mg loading dose, then 50 mg daily; or anidulafungin: 200 mg daily) for 14–21 days, OR AmB deoxycholate, 0.3–0.7 mg/kg daily, for 21 days (strong recommendation; high-quality evidence).

138. Posaconazole suspension, 400 mg twice daily, or extended-release tablets, 300 mg once daily, could be considered for fluconazole-refractory disease (weak recommendation; low-quality evidence).

139. For patients who have recurrent esophagitis, chronic suppressive therapy with fluconazole, 100–200 mg 3 times weekly, is recommended (strong recommendation; high-quality evidence).

140. For HIV-infected patients, antiretroviral therapy is strongly recommended to reduce the incidence of recurrent infections (strong recommendation; high-quality evidence).

#### Evidence Summary

Esophageal candidiasis typically occurs at lower CD4 counts than oropharyngeal disease [528–530]. The advent of effective antiretroviral therapy has led to a dramatic decline in the prevalence of esophageal candidiasis and a marked diminution in cases of refractory disease [531]. Most cases of esophageal candidiasis are caused by *C. albicans*. However, symptomatic infections caused by *C. glabrata*, *C. dubliniensis*, and *C. krusei* have been described [534].

The presence of oropharyngeal candidiasis and dysphagia or odynophagia in an immunocompromised host is frequently predictive of esophageal candidiasis, although esophageal candidiasis can present as odynophagia without concomitant oropharyngeal candidiasis. A therapeutic trial with fluconazole for patients with presumed esophageal candidiasis is a cost-effective alternative to endoscopic examination. In general, most patients with esophageal candidiasis will have improvement or resolution of their symptoms within 7 days after the initiation of antifungal therapy [557].

Fluconazole is superior to ketoconazole, itraconazole capsules, and fluconazole, and is comparable to itraconazole solution for the treatment of esophageal candidiasis [558, 559]; up to 80% of patients with fluconazole-refractory infections will respond to itraconazole solution [547]. Voriconazole is as efficacious as fluconazole and has shown success in the treatment of fluconazole-refractory mucosal candidiasis [63, 549].

The echinocandins are as effective as fluconazole but are associated with higher relapse rates than those observed with fluconazole [24, 87, 88, 550]. Thus, higher doses of echinocandins are recommended for use for esophageal disease than are used for candidemia to decrease relapses. Higher doses have been studied for micafungin [560]. Fluconazole-refractory disease responds to caspofungin, and it is likely that micafungin and anidulafungin are as effective as caspofungin. In patients with advanced AIDS, recurrent infections are common, and long-term suppressive therapy with fluconazole is effective in decreasing the recurrence rates [53]. The use of effective antiretroviral therapy has dramatically decreased the incidence of esophageal candidiasis in HIV-infected patients.

#### Notes

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Potential conflicts of interest. The following list is a reflection of what has been reported to IDSA. To provide thorough transparency, IDSA requires full disclosure of all relationships, regardless of relevancy to the guideline topic. Evaluation of such relationships as potential conflicts of interest (COI) is determined by a review process that includes assessment by the SPGC Chair, the SPGC liaison to the development panel, and the Board of Directors liaison to the SPGC and, if necessary, the COI Task Force of the Board. This assessment of disclosed relationships for possible COI will be based on the relative weight of the financial relationship (ie, monetary amount) and the relevance of the relationship (ie, the degree to which an association might reasonably be interpreted by an independent observer as related to the topic or recommendation of consideration). The reader of these guidelines should be mindful of this when the list of disclosures is reviewed. For activities outside of the submitted work, P. G. P. served as a consultant to Merck, Astellas, (past), Gilead, T2 Biosystems, Scynexis, Viamet, IMMY Diagnostics, and Pfizer (past) and has received research grants from T2 Biosystems, Gilead, Merck, Scynexis, and IMMY. For activities outside of the submitted work, C. A. K. has received research grants from VA Cooperative Studies, Merck, the Centers for Disease Control and Prevention (CDC) and The National Institute on Aging (all past), and has received royalties from UpToDate. For activities outside of the submitted work, D. A. has served a consultant to Merck, Astellas, Pfizer, Seachaid, Mayne, Roche, Theravance, Viamet, and Scynexis and has received research grants from Merck, Pfizer, MSG, Actellion, Theravance, Scynexis, and Astellas. For activities outside of the submitted work, C. J. C. has consulted for Merck, and received research grants from Pfizer, Merck, Astellas, CSL Behring, and T2 Diagnostics. For activities outside of the submitted work, K. A. M. has received research grants from Pfizer, Merck, Astellas, the National Institutes of Health (NIH) and served as a consultant for Astellas, Chimerix, Cidara, Genentech, Merck, Revolution Medicines, and Theravance. She has a licensed patent to MycoMed Technologies. For activities outside of the submitted work, L. O.-Z. has served as a consultant to Viracor (past), Novadigm (past), Pfizer (past), Astellas, Cidara, Scynexis, and Merck and has received research grants from Merck (past), Astellas, Pfizer (past), ImmuneX, Associates of Cape Cod (past), and T2 Biosystems, and has been on the speakers’ bureau for Merck and Pfizer. For activities outside of the submitted work, A. C. R. has received research grants from Merck and T2 Biosystems, and royalties from UpToDate. For activities outside of the submitted work, J. A. V. has served as a consultant for Astellas, Forest, served on promotional speakers’ bureau for Astellas, Pfizer, Forest, and Astra Zeneca, and has received research grants from Astellas, Pfizer, Merck, MSG, T2 Biosystems, and NIH/National Institute of Dental and Craniofacial Research. For activities outside of the submitted work, T. J. W. has served as a consultant for Astellas, Drais (past), Novartis, Pfizer, Methylgene, SigmaTau, Merck, ContraFect Trius, and has received research grants from SOS Kids Foundation, Sharpe Family Foundation, Astellas, Cubist, Theravance, Medicines Company, Actavis, Pfizer, Merck, Novartis, ContraFect, and The Schueler Foundation. For activities outside of the submitted work, T. E. Z. has served as a consultant for Astellas, Pfizer, Merck, and Cubist (past) and has received grants from Merck (past), and (past), Agency for Health Research and Quality, CDC, NIH, and the Thrasher Foundation. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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A meta-analysis of medical versus surgical therapy for *Candida* endocarditis

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Abstract  Objectives. The optimal management of *Candida* infective endocarditis (IE) is unknown.

Methods. We reviewed all 879 cases of *Candida* IE reported from 1966-2002 in the peer-reviewed literature to better understand the role of medical and surgical therapies. This review included 163 patients from 105 reports that met our inclusion criteria: 31 cases treated with antifungal monotherapy, 25 cases treated with medical antifungal combination therapy, and 107 cases treated with adjunctive surgical plus medical antifungal therapy. We also used meta-analytic techniques to evaluate 22 observational case-series (72 patients) of the 105 reports with two or more patients with definite *Candida* IE.

Results. We found that in patients who underwent adjunctive surgery there was a lower reported proportion of deaths [prevalence odds ratio (POR) = 0.56; 95% confidence interval (CI) = 0.16, 1.99]. Higher mortality was noted in patients treated prior to 1980 (POR = 2.03; 95% CI = 0.55, 7.61), treated with antifungal monotherapy (POR = 1.49; 95% CI = 0.39, 5.81), infected with *Candida parapsilosis* (POR = 1.51; 95% CI = 0.41, 5.52), or with left-sided endocarditis (POR = 2.36; 95% CI = 0.55, 10.07).

Conclusions. Medical antifungal therapy of *Candida* IE is poorly characterized, and recent antifungal developments lend promise for those patients who cannot undergo surgery.
Introduction

Fungal infective endocarditis (IE) is increasing in incidence; fungi now comprise between 1 and 10% of organisms isolated in IE, including approximately 10% of prosthetic valve endocarditis cases. In recent reviews of fungal endocarditis, 53–68% were Candida species and Candida albicans was the most common. Surgical intervention over the last several decades has decreased overall mortality of infective endocarditis, and surgery is generally regarded as a standard treatment for fungal IE because of the risk of septic embolization and the difficulty in sterilizing a fungal vegetation. The American College of Cardiology (ACC) and the American Heart Association (AHA) published guidelines list ‘fungal endocarditis’ as an indication for surgery as defined by a Class I recommendation—‘conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective’. Unfortunately, some patients are poor surgical candidates. Furthermore, the requirement for surgery for Candida IE has never been formally tested, which is particularly relevant as new fungicidal antifungal agents now become available.

The most recent 2004 Infectious Diseases Society of America (IDSA) guidelines for management of Candida IE state that ‘both native-valve and prosthetic-valve infection should be managed with surgical replacement of the infected valve’. The key antifungal recommendations from the IDSA are ‘medical therapy with amphotericin B with or without flucytosine at maximal tolerated doses has most often been used. If valve replacement is not possible, long-term (possibly life-long) suppressive therapy with flucytosine may be used’. These IDSA guidelines implicitly state that the evidence for these recommendations is derived only from individual case reports and case series and no prospective or randomized trials have been performed.

Most patients with Candida IE treated without surgery have traditionally received extended courses of amphotericin B deoxycholate. Such medical management of Candida IE has been associated with high rates of death and amphotericin B-induced nephrotoxicity, making it an unappealing treatment option. However, these observations were reported prior to the recent availability of safe, potent antifungal agents, including the echinocandins and second generation azoles. As a result, the optimal medical management of patients with Candida IE in whom cardiac surgery is not an option is unknown. In the current report, we systematically review the published literature of medical and surgical management of Candida IE and provide data to question the untested yet accepted practice of required surgical intervention for Candida IE in this new era of safer, potent antifungals.

Patients and methods

Review of literature

We conducted a MEDLINE search using the keywords ‘Candida’ and ‘endocarditis’ and text word searching. All English language clinical reports of medical or medical with adjunctive surgical therapy for patients with Candida IE from January 1, 1966 to December 31, 2002 were reviewed. Publications cited in these reports and abstracts from recent (2000-2003) scientific meetings (Interscience Conference on Antimicrobial Agents and Chemotherapy, Infectious Diseases Society of America) were also considered for inclusion in the current analysis. In order to increase reliability, a single reviewer (WJS) abstracted data from all included publications using a data collection form developed specifically for the current analysis.

Inclusion and exclusion criteria

Cases were included in the current analysis if they (a) met criteria for definite Candida IE according to the modified Duke criteria, and (b) contained specific information regarding therapy and outcome. Cases were excluded if they (a) received <7 days of systemic antifungal therapy, (b) involved a permanent pacemaker or defibrillator, or (c) presented with a cardiac mass without apparent valvular involvement.

Study definitions

Three treatment categories were included in the current analysis: medical antifungal monotherapy, medical antifungal combination therapy, and adjunctive surgical plus medical antifungal therapy. Medical antifungal monotherapy was defined as patients who were treated with a single antifungal agent at one time, and did not undergo surgery. Medical antifungal combination therapy was defined as patients who were treated with two or more antifungal agents concurrently, and did not undergo surgery. Adjunctive surgical plus medical therapy was defined as patients who underwent

...
surgery for IE as well as received either antifungal monotherapy or antifungal combination therapy.

Analysis plan

Data from included cases were presented in two stages. The first stage of data presentation consisted of a detailed summary of all Candida IE cases (1966-2002) identified in the literature which met study inclusion criteria. Mortality was deemed to be due to fungal infection in the majority of reviewed cases, but due to the reporting style of many of the individual retrospective cases this could not be verified. In the next stage, a meta-analysis of all reports meeting study inclusion criteria and describing ≥2 cases of definite Candida IE was performed. The following variables were considered within the meta-analysis as dichotomous variables: year of publication (prior versus subsequent to 1980), left- versus right-sided valvular involvement, use of adjunctive surgery, use of antifungal monotherapy, and infection with C. parapsilosis. The year 1980 was selected due to the widespread clinical use of transthoracic echocardiography for the diagnosis of endocarditis beginning in this decade. We used as reference categories the group with less than 100% of patients in the series with adjunctive surgery, antifungal monotherapy, infection with C. parapsilosis, and left-sided endocarditis.

We transformed risk-factor specific mortality rates into their respective logits (natural log of the probability divided by 1-probability) to take advantage of the properties of the logit (normal distribution and stable variance) transformation. We then evaluated the data with the Egger test.13 In order to address heterogeneity, we compared average prevalence estimates between groups of studies using random-effects meta-regression.14 The dependent variable was the logit of the prevalence of mortality and the independent variables were year of study publication, use of surgery, medical antifungal monotherapy, affected heart side of IE, and isolation of C. parapsilosis.

The regressions were fit with random-effects weights (reciprocal sum of the estimated within-study and among-study variances) using restricted maximum likelihood estimation of the between-study variance to account for heterogeneity. For each independent variable, we compared studies and present the 95% confidence intervals (CI) of the prevalence odds ratios (POR). The meta-regression techniques presented are analogous to logistic regression. However, because most reviewed studies are primarily observational or explanatory, p-values are not presented, and formal inferences are not drawn from the presented 95% confidence intervals (CI).

Results

A total of 879 cases of Candida endocarditis in 418 reports were reviewed. Of these, 163 cases of definite Candida endocarditis from 105 reports met inclusion criteria (Appendices A–C). Those cases were divided as treatment with medical antifungal monotherapy alone (n=31), treatment with medical antifungal combination therapy (n=25), and treatment with adjunctive surgical plus medical antifungal therapy (n=107).

Summary of reported cases

Because we observed a greater association with reported mortality in patients treated prior to 1980 (POR 2.03) in our meta-analysis, and considering the advances in echocardiographic diagnostic techniques as well as the frequency of adjunctive surgical intervention after that time period, in order to better reflect current medical practice only results from the 92 patients reported after 1980 are described in greater detail below.

These 92 cases were grouped as follows: medical antifungal monotherapy (n=15), medical antifungal combination therapy (n=19), and adjunctive surgical plus medical antifungal therapy (n=58). After 1980, C albicans and C. parapsilosis were the most common Candida species isolated. In the medical antifungal monotherapy cohort, 9 (60%) patients were infected with C. albicans, 3 (20%) were infected with C. parapsilosis, and 3 (20%) were infected with other Candida species. In the medical antifungal combination therapy cohort, 9 (47.4%) patients were infected with C. albicans, 8 (42.1%) with C. parapsilosis, and 2 (10.5%) with other Candida species. Finally, in the adjunctive surgical plus medical antifungal cohort, 25 (43.1%) patients were infected with C. albicans, 21 (36.2%) with C. parapsilosis, and 12 (20.7%) with other Candida species.

The majority of reported cases of Candida IE were left-sided disease. After 1980, in the medical antifungal monotherapy cohort there were 80% (12/15) reported with left-sided infection, 63.1% (12/19) with left-sided infection in the medical antifungal combination cohort, and 82.7% (48/58) with left-sided IE in the adjunctive surgical plus...
Candida endocarditis therapy

The majority of cases were also native valve IE. After 1980, in the medical antifungal monotherapy cohort there were 73.3% (11/15) reported with native valve infection, 63.1% (12/19) with native valve infection in the medical antifungal combination cohort, 62.1% (36/58) with native valve IE in the adjunctive surgical plus antifungal therapy group.

The most common antifungal therapy reported after 1980 remained amphotericin B, generally reported at doses of 0.5–1.0 mg/kg/day. Amphotericin B was reported as treatment in 53.3% (8/15) of patients with medical antifungal monotherapy, and 48.3% (28/58) of patients with adjunctive surgical plus antifungal therapy. Combination antifungal therapy with amphotericin B + flucytosine was used in 73.7% (14/19) of patients with medical antifungal combination therapy, and 36.2% (21/58) of patients with adjunctive surgical plus antifungal therapy.

Patient outcome in the cases reported after 1980 was dichotomized based on reported overall patient mortality. In the medical antifungal monotherapy cohort there were 6/11 reported survivors with native valve endocarditis and 2/4 reported survivors with prosthetic valve endocarditis. In the combination antifungal therapy cohort there were 8/12 reported survivors with native valve endocarditis and 4/7 reported survivors with prosthetic valve endocarditis. In the adjunctive surgical plus antifungal therapy cohort there were 24/36 reported survivors with native valve endocarditis and 19/22 reported survivors with prosthetic valve endocarditis.

The findings in this investigation suggest that antifungal monotherapy (primarily with amphotericin B) without adjunctive surgery was associated with the poorest patient outcome. Interestingly, the clinical outcomes were similar for those patients receiving combination antifungal agents without surgery and patients receiving adjunctive surgical intervention. This observation suggests that while surgical therapy remains the cornerstone of therapy for most patients with Candida IE, combination antifungal therapy may provide an incremental advantage to monotherapy for those patients in whom surgery is not an option.

Several patient characteristics were associated with mortality in the meta-analysis. Left-sided involvement, infection prior to 1980, and antifungal monotherapy were associated with patient mortality. Patients reported prior to 1980 had a higher reported mortality (POR=2.03), which could be related to delays in diagnosis due to a lack of echocardiography or the reported use of generally lower doses of amphotericin B (<1 mg/kg/day) in cases during that time period. Interestingly, surgery appeared to confer a protective effect to patients with Candida IE, as series in which all patients underwent surgery had lower mortality rates (POR=0.56).

Although historically amphotericin B has been considered the ‘gold standard’ for antifungal therapy for decades, a growing body of evidence suggests that it may not be an optimal therapy for Candida IE. Amphotericin B fails to penetrate well into fibrin clots and vegetations, leading to lower in vivo activity than predicted by in vitro testing.

Discussion

Candida IE has been reviewed and in several studies the overall mortality is approximately 80%. Some studies noted no difference in mortality with antifungal treatment versus surgical intervention, but mortality did decrease in those patients who underwent both surgical replacement and antifungal therapy. This analysis is the largest literature review of Candida IE treatment and outcome, presenting the cases as both a descriptive literature review (Tables 1 and 2, Appendices A–C) and a meta-analysis of evaluable cases (Table 3).
Both fluconazole and flucytosine also have important limitations. Fluconazole is fungistatic and has been shown to be inferior compared to amphotericin B in IE animal models.\textsuperscript{26,27} Flucytosine monotherapy is also ineffective\textsuperscript{27,28} and rapidly results in drug resistance. The echinocandins are new fungicidal antifungals with excellent activity against \textit{Candida} biofilms.\textsuperscript{29–31} While there are little clinical data on echinocandin activity against \textit{Candida} endocarditis, success in a biofilm model may possibly mimic the vegetation environment of endocarditis.\textsuperscript{31}

The findings of the current review suggest that combination antifungal therapies, primarily amphotericin B + flucytosine, might be associated with better clinical outcomes compared with antifungal monotherapy. The findings also suggest that in select patients in whom surgical therapy is not an alternative, combination therapy can optimize the chance for treatment success. Whether the availability of new rapidly fungicidal agents such as echinocandins can make it possible to treat this lethal infection in select patients with medical therapy alone is unknown.

This literature review and meta-analysis have the limitations of any retrospective analyses of a large number of heterogeneous, uncontrolled observational studies and case reports. We attempted to standardize the diagnosis by using only definitive IE cases according to the modified Duke criteria,\textsuperscript{12} and a case was disregarded if we could not reliably conclude it was truly definite IE.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Details of 92 reported cases of \textit{Candida} infective endocarditis (1980–2002)</th>
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<tbody>
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<td>Variable</td>
<td>No. cases</td>
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<td>Location of cardiac valve</td>
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<td>Left-sided</td>
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<td>Type of infected valve</td>
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<td>Amphotericin B + flucytosine</td>
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<td>Amphotericin B + rifampin + flucytosine</td>
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<td>Amphotericin B</td>
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<tr>
<td>Amphotericin B + fluconazole + flucytosine</td>
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However, we are dependent on the individual investigator’s reports for other variables of patient care including the inherent differences in the hosts, antifungal dosing, supportive care management, and investigator-assessed outcome. Importantly, the reported outcomes are subjective and based on the individual investigator’s own biases, thereby making it impossible to draw firm recommendations from the descriptive results. There is also potential for significant selection bias for better outcome in the choice of patients who are reported in the literature. Additionally, there is possible bias in those patients who might be considered surgical candidates, with the sickest patients often excluded from surgical options.

While the decision to exclude cases not receiving at least 7 days of antifungal therapy biases the review by excluding early episodes of failed medical therapy, we felt it would create worse bias by including the myriad of cases where a dying patient was given 1–2 days of an antifungal and that patient’s death deemed due to antifungal therapy failure. On the other hand, there was little mention in the individual reports of long-term suppressive antifungal therapy, so it is impossible to analyse how that may have impacted treatment outcome. Additionally, issues of valvular dysfunction have not been considered, and particularly how it might relate to medical versus surgical therapy. Finally, for most cases the outcome was reported after

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<th>Table 2</th>
<th>Patient age and pathogen for 92 reported cases of <em>Candida</em> infective endocarditis reviewed (1980-2002)</th>
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<th>Table 3</th>
<th>Meta-regression analysis of <em>Candida</em> infective endocarditis (1966-2002) using mortality as outcome (n=22 studies; 72 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable</td>
<td>Prevalence odds ratio</td>
</tr>
<tr>
<td>Left-sided <em>Candida</em> endocarditis</td>
<td>2.36</td>
</tr>
<tr>
<td>Studies prior to 1980</td>
<td>2.03</td>
</tr>
<tr>
<td>Infection with <em>C. parapsilosis</em></td>
<td>1.51</td>
</tr>
<tr>
<td>Antifungal monotherapy</td>
<td>1.49</td>
</tr>
<tr>
<td>Adjunctive surgery</td>
<td>0.56</td>
</tr>
</tbody>
</table>
some post-therapy follow-up but for some the exact duration was not specified, making it impossible to incorporate that aspect into any meaningful analysis.

In conclusion, *Candida* IE is a difficult disease to treat effectively and the present guidelines acknowledge a weak evidence-based foundation. Clinical care has changed dramatically over the review period, but the general perception of *Candida* IE therapy seems to be unchanged based on the lack of prospective studies and controlled clinical trials, as demonstrated by unchanging published recommendations.\textsuperscript{10,11} Antifungal monotherapy has led to relatively poor outcomes, while combination antifungal therapy alone appears to possibly approach the success of adjunctive surgery. With the recent availability of less-toxic agents such as the echinocandins or the extended spectrum triazoles, the medical options for *Candida* IE have increased. The optimal antifungal regimen for *Candida* IE is not clear, and no specific regimen can be firmly recommended. However, medical antifungal therapy of *Candida* IE deserves study. With newer fungicidal agents now available and the development of a multicentre collaborative effort such as the 23-site International Collaboration on Endocarditis,\textsuperscript{32} we are poised for a prospective pilot study to determine optimal antifungal therapy and question the primary requirement for surgery.

**Acknowledgements**

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**Appendix A: Previously reported cases of medical antifungal monotherapy for definite cases of *Candida* endocarditis (1966-2002; n = 31 cases)**

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Medical treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Rheumatic heart disease</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>50 days</td>
<td>Alive</td>
<td>1962</td>
<td>33</td>
</tr>
<tr>
<td>34</td>
<td>Mitral valve commissurotomy</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>11 weeks</td>
<td>Expired</td>
<td>1966</td>
<td>34</td>
</tr>
<tr>
<td>73</td>
<td>None</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB, then flucytosine</td>
<td>1 week, then 40 days</td>
<td>Alive</td>
<td>1971</td>
<td>35</td>
</tr>
<tr>
<td>6 weeks</td>
<td>Cushing’s syndrome</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>NR</td>
<td>Expired</td>
<td>1971</td>
<td>36</td>
</tr>
<tr>
<td>53</td>
<td>Aortic valve debridement</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB, then flucytosine, then clotrimazole Nystatin</td>
<td>9 months</td>
<td>Expired</td>
<td>1972</td>
<td>37</td>
</tr>
<tr>
<td>53</td>
<td>Tricuspid valve trauma</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>Nystatin</td>
<td>12 days</td>
<td>Expired</td>
<td>1973</td>
<td>38</td>
</tr>
<tr>
<td>60</td>
<td>Aortic, tricuspid, mitral valve replacement</td>
<td>(P) Aortic, tricuspid, mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>46 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>62</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>14 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>29</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1968</td>
<td>40</td>
</tr>
<tr>
<td>26</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>4 months</td>
<td>Expired</td>
<td>1971</td>
<td>41</td>
</tr>
<tr>
<td>58</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>NR</td>
<td>Expired</td>
<td>1971</td>
<td>41</td>
</tr>
<tr>
<td>57</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB, then flucytosine</td>
<td>2 weeks, then 11 weeks</td>
<td>Expired</td>
<td>1971</td>
<td>35</td>
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</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Medical treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>Aortic, tricuspid, mitral valve replacement</td>
<td>Aortic, tricuspid, (P) mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>46 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>62</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>14 days</td>
<td>Expired</td>
<td>1973</td>
<td>39</td>
</tr>
<tr>
<td>60</td>
<td>Aortic, tricuspid, mitral valve replacement, radiation therapy</td>
<td>Aortic, tricuspid, (P) mitral</td>
<td><em>C. albicans</em></td>
<td>Flucytosine</td>
<td>69 days</td>
<td>Expired</td>
<td>1975</td>
<td>42</td>
</tr>
<tr>
<td>48</td>
<td>Mitral, aortic valve replacement</td>
<td>Mitral, (P) aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>37 days</td>
<td>Expired</td>
<td>1975</td>
<td>42</td>
</tr>
<tr>
<td>83</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>58 days</td>
<td>Expired</td>
<td>1989</td>
<td>43</td>
</tr>
<tr>
<td>64</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>Fluconazole</td>
<td>50 days</td>
<td>Expired</td>
<td>1991</td>
<td>44</td>
</tr>
<tr>
<td>64</td>
<td>Abdominal surgery</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>Fluconazole</td>
<td>50 days</td>
<td>Expired</td>
<td>1991</td>
<td>44</td>
</tr>
<tr>
<td>43 days</td>
<td>29 week gestation</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>75 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
</tr>
<tr>
<td>47</td>
<td>Broad spectrum antibiotics HIV</td>
<td>(N) Mitral, tricuspid</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>17 months</td>
<td>Alive</td>
<td>1995</td>
<td>46</td>
</tr>
<tr>
<td>44</td>
<td>Mitral valve commissurotomy</td>
<td>(N) Mitral</td>
<td><em>C. zeylanoides</em></td>
<td>AmB</td>
<td>2 g</td>
<td>Alive</td>
<td>1996</td>
<td>47</td>
</tr>
<tr>
<td>51</td>
<td>Mitral valve replacement</td>
<td>Mitral</td>
<td><em>C. albicans</em></td>
<td>Fluconytosine</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>8</td>
</tr>
<tr>
<td>12 days</td>
<td>32 weeks gestation</td>
<td>(N) Tricuspid, Pulmonary</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>7 days</td>
<td>Expired</td>
<td>1996</td>
<td>48</td>
</tr>
<tr>
<td>14</td>
<td>Liver transplantation Prematurity</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>8 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>25 days</td>
<td>Abdominal surgery, hyper-alimentation</td>
<td>(N) Pulmonary</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>20 days</td>
<td>Expired</td>
<td>2000</td>
<td>50</td>
</tr>
<tr>
<td>72</td>
<td>Abdominal surgery, hyper-alimentation</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>AmB, then fluconazole</td>
<td>16 weeks, then NR</td>
<td>Alive</td>
<td>2001</td>
<td>51</td>
</tr>
<tr>
<td>74</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>Fluconazole</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1997</td>
<td>52</td>
</tr>
<tr>
<td>56</td>
<td>Rheumatic heart disease, mitral valve replacement</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB, then fluconazole</td>
<td>8 weeks, then 1 year</td>
<td>Alive</td>
<td>2001</td>
<td>53</td>
</tr>
<tr>
<td>58</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB, then fluconazole</td>
<td>26 months</td>
<td>Alive</td>
<td>1993</td>
<td>54</td>
</tr>
<tr>
<td>45</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>Fluconazole, then AmB, then ABLC</td>
<td>12 months</td>
<td>Expired</td>
<td>1995</td>
<td>55</td>
</tr>
</tbody>
</table>

AmB, amphotericin B; ABLC, amphotericin B lipid complex; IVDA, intravenous drug abuser; HIV, human immunodeficiency virus; NR, not recorded.
Appendix B: Previously reported cases of medical combination antifungal therapies for definite cases of *Candida* endocarditis (1966–2002; *n* = 25 cases)

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Heroin abuse, aortic patch infection</td>
<td>(N) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + clotrimazole, flucytosine</td>
<td>7 days</td>
<td>Expired</td>
<td>1974</td>
<td>56</td>
</tr>
<tr>
<td>57</td>
<td>Alcoholism, malnutrition</td>
<td>(N) Aortic</td>
<td><em>C. glabrata</em></td>
<td>AmB + flucytosine</td>
<td>3 months</td>
<td>Alive</td>
<td>1975</td>
<td>19</td>
</tr>
<tr>
<td>42</td>
<td>Heroin abuse</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>4 months</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
</tr>
<tr>
<td>37 days</td>
<td>34 week gestation, TEF, PDA, VSD</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>7 days</td>
<td>Expired</td>
<td>1977</td>
<td>57</td>
</tr>
<tr>
<td>43</td>
<td>Heroin abuse, mitral valve regurgitation</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine, then AmB for 7 weeks</td>
<td>7 weeks</td>
<td>Alive</td>
<td>1978</td>
<td>58</td>
</tr>
<tr>
<td>17</td>
<td>Rheumatic heart disease, heroin addict, mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>7 days</td>
<td>Expired</td>
<td>1976</td>
<td>18</td>
</tr>
<tr>
<td>36</td>
<td>Heroin abuse</td>
<td>(N) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine, then flucytosine for life</td>
<td>3 weeks</td>
<td>Alive</td>
<td>1980</td>
<td>59</td>
</tr>
<tr>
<td>6 days</td>
<td>29 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine</td>
<td>8 days</td>
<td>Expired</td>
<td>1983</td>
<td>60</td>
</tr>
<tr>
<td>12 days</td>
<td>27 week gestation</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>102 days</td>
<td>Alive</td>
<td>1990</td>
<td>61</td>
</tr>
<tr>
<td>73 days</td>
<td>24 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>98 days</td>
<td>Expired</td>
<td>1991</td>
<td>62</td>
</tr>
<tr>
<td>91 days</td>
<td>Trisomy 21, ASD, PDA, pulmonic, mitral</td>
<td>(N) Tricuspid</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine, then AmB for 4 weeks</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1991</td>
<td>62</td>
</tr>
<tr>
<td>73 days</td>
<td>24 week gestation</td>
<td>(N) Pulmonic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB + flucytosine</td>
<td>98 days</td>
<td>Alive</td>
<td>1991</td>
<td>62</td>
</tr>
<tr>
<td>26 days</td>
<td>Apneic episode, central venous catheter</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB + Rifampin + flucytosine</td>
<td>30 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
</tr>
<tr>
<td>29 days</td>
<td>26 week gestation</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB + flucytosine, then AmB for 4 weeks</td>
<td>4 days</td>
<td>Alive</td>
<td>1991</td>
<td>45</td>
</tr>
<tr>
<td>73</td>
<td>Candidemia three months earlier, not treated</td>
<td>(N) Aortic</td>
<td><em>C. tropicalis</em></td>
<td>AmB + flucytosine</td>
<td>NR</td>
<td>Expired</td>
<td>1998</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>Acute lymphoblastic leukemia</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB + fluconazole, then fluconazole for 2 weeks</td>
<td>3 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>64</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Tetralogy of fallot</td>
<td>(N) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then fluconazole for life</td>
<td>2 weeks</td>
<td>Alive</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>16</td>
<td>Renal transplantation</td>
<td>(N) Mitral, aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine + fluconazole</td>
<td>5 months</td>
<td>Expired</td>
<td>1999</td>
<td>49</td>
</tr>
<tr>
<td>37</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine, then fluconazole</td>
<td>NR</td>
<td>Alive</td>
<td>1988</td>
<td>65</td>
</tr>
<tr>
<td>58</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ fluconazole, then fluconazole</td>
<td>2 weeks</td>
<td>Expired</td>
<td>1993</td>
<td>66</td>
</tr>
<tr>
<td>57</td>
<td>Mitral valve prosthesis</td>
<td>(P) Mitral</td>
<td><em>C. albicans</em></td>
<td>AmB+ fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1994</td>
<td>67</td>
</tr>
<tr>
<td>61</td>
<td>Aortic valve prosthesis</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine, then fluconazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>68</td>
</tr>
<tr>
<td>61</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB+ flucytosine</td>
<td>5 weeks</td>
<td>Alive</td>
<td>1996</td>
<td>8</td>
</tr>
<tr>
<td>41</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. lusitaniae</em></td>
<td>AmB+ flucytosine, fluconazole + flucytosine, then fluconazole 4 months, then AmB+ flucytosine for relapse for 3 months</td>
<td>5 weeks</td>
<td>Expired</td>
<td>1998</td>
<td>69</td>
</tr>
<tr>
<td>51</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td><em>C. parapsilosis</em></td>
<td>AmB+ flucytosine (7 days) then caspofungin + fluconazole (6 weeks), then caspofungin then ABLC</td>
<td>7 weeks, 6 weeks, 20 days, 2 g</td>
<td>Expired</td>
<td>2002</td>
<td>70</td>
</tr>
</tbody>
</table>

NR, not recorded; AmB, amphotericin B; TEF, tracheal-esophageal fistula; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus.
### Appendix C: Previously reported cases of adjunctive surgical and medical antifungal therapies for definite cases of *Candida* endocarditis (1966-2002; n = 107 cases)

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Underlying risk factor</th>
<th>Affected valve (native or prosthetic)</th>
<th>Organism</th>
<th>Medical treatment</th>
<th>Duration</th>
<th>Outcome</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Bacterial endocarditis</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>9 months</td>
<td>Expired</td>
<td>1968</td>
<td>71</td>
</tr>
<tr>
<td>34</td>
<td>None</td>
<td>(N) Aortic</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>12 weeks</td>
<td>Alive</td>
<td>1968</td>
<td>71</td>
</tr>
<tr>
<td>29</td>
<td>Rheumatic heart disease</td>
<td>(N) Aortic</td>
<td><em>C. parakruzei</em></td>
<td>AmB</td>
<td>2 months</td>
<td>Alive</td>
<td>1968</td>
<td>71</td>
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<td>30</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. guilliermondi</em></td>
<td>AmB</td>
<td>NR</td>
<td>Expired</td>
<td>1972</td>
<td>72</td>
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<td>39</td>
<td>IVDA</td>
<td>(N) Aortic, mitral, tricuspid</td>
<td><em>C. krusei</em></td>
<td>Clotrimazole</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
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<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>33 days</td>
<td>Expired</td>
<td>1972</td>
<td>73</td>
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<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. parakruzei</em></td>
<td>AmB</td>
<td>NR</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
</tr>
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<td>28</td>
<td>IVDA</td>
<td>(N) Tricuspid</td>
<td><em>C. tropicalis</em></td>
<td>AmB</td>
<td>1 month</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
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<td>IVDA Corticosteroid</td>
<td>(N) Pulmonary</td>
<td><em>C. stellatoidea</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1972</td>
<td>73</td>
</tr>
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<td>Abdominal surgery</td>
<td>(N) Tricuspid</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>2 months</td>
<td>Expired</td>
<td>1972</td>
<td>74</td>
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<td>(N) Aortic</td>
<td><em>C. parakruzei</em></td>
<td>AmB</td>
<td>1 week</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>38</td>
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<td>(N) Mitral</td>
<td><em>C. krusei</em></td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1975</td>
<td>78</td>
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<td>26</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. glabrata</em></td>
<td>AmB</td>
<td>10 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td><em>C. guilliermondi</em></td>
<td>AmB</td>
<td>1 week</td>
<td>Alive</td>
<td>1975</td>
<td>78</td>
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<td>AmB</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1975</td>
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<td><em>C. guilliermondi</em></td>
<td>AmB</td>
<td>5 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>C. albicans</em></td>
<td>AmB</td>
<td>10 weeks</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>63</td>
<td>IVDA</td>
<td>(N) Aortic</td>
<td><em>Candida spp.</em></td>
<td>AmB, then flucytosine (10d)</td>
<td>19 days</td>
<td>Expired</td>
<td>1975</td>
<td>78</td>
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<td>Peritonitis</td>
<td>(N) Mitral</td>
<td><em>C. glabrata</em></td>
<td>AmB</td>
<td>1.5 g</td>
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<td>1975</td>
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<td>Rheumatic heart disease</td>
<td>(N) Aortic and mitral</td>
<td><em>C. stellatoidea</em></td>
<td>AmB</td>
<td>2 weeks</td>
<td>Expired</td>
<td>1975</td>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
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<td>AmB</td>
<td>1 g</td>
<td>Expired</td>
<td>1975</td>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
<td>3 g</td>
<td>Alive</td>
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<td>19</td>
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<td>Bacterial endocarditis</td>
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<td>AmB</td>
<td>1 g</td>
<td>Expired</td>
<td>1975</td>
<td>19</td>
</tr>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
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<td>Alive</td>
<td>1976</td>
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<td>AmB</td>
<td>14 days</td>
<td>Expired</td>
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<td>Abdominal surgery</td>
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<td>AmB</td>
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<td><em>C. parapsilosis</em></td>
<td>AmB</td>
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<td>Alive</td>
<td>1976</td>
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<td>Affected valve (native or prosthetic)</td>
<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
<td>Reference</td>
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<td>(N) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>2 months</td>
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<td>1979</td>
<td>83</td>
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<td>(P) Aortic</td>
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<td>AmB</td>
<td>5 weeks</td>
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<td>1968</td>
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<td>AmB</td>
<td>5 months</td>
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<td>1970</td>
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<td>Candida spp.</td>
<td>AmB + flucytosine</td>
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<td>Expired</td>
<td>1971</td>
<td>87</td>
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<td>62</td>
<td>Mitral valve replacement</td>
<td>(P) Mitral</td>
<td>C. albicans</td>
<td>AmB + flucytosine</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1971</td>
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<td>AmB + flucytosine</td>
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<td>1975</td>
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<td>Mitral valve replacement</td>
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<td>AmB</td>
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<td>Alive</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. glabrata</td>
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<td>8 weeks</td>
<td>Expired</td>
<td>1975</td>
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<td>(P) Aortic</td>
<td>C. albicans</td>
<td>AmB</td>
<td>25 days</td>
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<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>Candida spp.</td>
<td>Flucytosine</td>
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<td>C. parapsilosis</td>
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<td>3 months</td>
<td>Expired</td>
<td>1977</td>
<td>93</td>
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<td>36</td>
<td>Aortic valve replacement</td>
<td>(P) Aortic</td>
<td>C. parapsilosis</td>
<td>AmB, then flucytosine, then miconazole</td>
<td>4 months</td>
<td>Expired</td>
<td>1977</td>
<td>93</td>
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<td>C. albicans</td>
<td>Miconazole</td>
<td>10 weeks</td>
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<td>1977</td>
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<td>IDDM, abdominal surgery</td>
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<td>C. glabrata</td>
<td>AmB + flucytosine</td>
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<td>C. parapsilosis</td>
<td>AmB</td>
<td>4 g</td>
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<td>1984</td>
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<td>52</td>
<td>IHSS</td>
<td>(N) Mitral</td>
<td>C. tropicalis</td>
<td>AmB</td>
<td>3.0 g</td>
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<td>Renal and pancreatic tail transplantation</td>
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<td>4 weeks</td>
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<td>1986</td>
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<td>36</td>
<td>Corticosteroid therapy for SLE</td>
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<td>8 weeks</td>
<td>32 week gestation</td>
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<td>Ventricular septal defect</td>
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<td>C. albicans</td>
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<td>Pancreaticoduodenectomy</td>
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<td>C. tropicalis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
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<td>8 days</td>
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<td>Age (yrs)</td>
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<td>Organism</td>
<td>Medical treatment</td>
<td>Duration</td>
<td>Outcome</td>
<td>Year</td>
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<td>Candidemia with central catheter</td>
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<td>C. albicans</td>
<td>AmB + flucytosine</td>
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<td>Expired</td>
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<td>Mitral commissurotomy</td>
<td>Mitral</td>
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<td>1994</td>
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<td>C. parapsilosis</td>
<td>AmB</td>
<td>6 weeks</td>
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<td>1994</td>
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<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
<td>Expired</td>
<td>1994</td>
<td>109</td>
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<td>C. parapsilosis</td>
<td>AmB</td>
<td>3 weeks</td>
<td>Expired</td>
<td>1994</td>
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<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>5 weeks</td>
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<td>Allogeneic BMT</td>
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<td>AmB, then fluconazole</td>
<td>4 months</td>
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<td>1994</td>
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<td>Aortic</td>
<td>C. parapsilosis</td>
<td>AmB + flucytosine</td>
<td>6 weeks</td>
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<td>66</td>
<td>None</td>
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<td>L-AmB + flucytosine, then fluconazole</td>
<td>8 weeks</td>
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<td>1996</td>
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<td>Aortic regurgitation</td>
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<td>C. albicans</td>
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<td>30 days</td>
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<td>68</td>
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<td>C. albicans</td>
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<td>4 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>113</td>
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<td>57</td>
<td>Alcoholic cirrhosis, candidemia untreated</td>
<td>Tricuspid</td>
<td>C. glabrata</td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>114</td>
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<td>Sickle cell disease</td>
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<td>C. tropicalis</td>
<td>AmB</td>
<td>6 weeks</td>
<td>Alive</td>
<td>1997</td>
<td>115</td>
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<td>42</td>
<td>Percutaneous transluminal coronary angioplasty</td>
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<td>C. parapsilosis and C. albicans</td>
<td>AmB, then fluconazole</td>
<td>1 g</td>
<td>Alive</td>
<td>1998</td>
<td>116</td>
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<td>57</td>
<td>Prolonged hyper-alimentation</td>
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<td>C. parapsilosis</td>
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AmB, amphotericin B; L-AmB, liposomal amphotericin B; flucytosine, flucytosine; IVDA, intravenous drug abuser; IDDM, insulin dependent diabetes mellitus; SLE, systemic lupus erythematosus; PDA, patent ductus arteriosus; VSD, ventricular septal defect; HIV, human immunodeficiency virus; NR, not recorded.

References


Candida endocarditis therapy


2015 ESC Guidelines for the management of infective endocarditis

The Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC)

Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM)

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ESC Working Groups: Cardiovascular Pharmacotherapy, Cardiovascular Surgery, Grown-up Congenital Heart Disease, Myocardial and Pericardial Diseases, Pulmonary Circulation and Right Ventricular Function, Thrombosis, Valvular Heart Disease.

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The disclosure forms of all experts involved in the development of these guidelines are available on the ESC website http://www.escardio.org/guidelines.

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Abbreviations and acronyms

3D three-dimensional
AIDS acquired immune deficiency syndrome
b.i.d. bis in die (twice daily)
BCNIE blood culture-negative infective endocarditis
CDRIE cardiac device-related infective endocarditis
CHD congenital heart disease
CIED cardiac implantable electronic device
CoNS coagulase-negative staphylococci
CPG Committee for Practice Guidelines
CRP C-reactive protein
CT computed tomography
E. Enterococcus
ESC European Society of Cardiology
ESR erythrocyte sedimentation rate
EuroSCORE European System for Cardiac Operative Risk Evaluation
FDG fluorodeoxyglucose
HF heart failure
HIV human immunodeficiency virus
HLAR high-level aminoglycoside resistance
i.m. intramuscular
i.v. intravenous
ICE International Collaboration on Endocarditis
ICU intensive care unit
ID infectious disease
IE infective endocarditis
Ig immunoglobulin
IVDA intravenous drug abuser
MIC minimum inhibitory concentration
MR magnetic resonance
MRI magnetic resonance imaging
MRSA methicillin-resistant Staphylococcus aureus
MSCT multislice computed tomography
MSSA methicillin-susceptible Staphylococcus aureus
NBTE non-bacterial thrombotic endocarditis
NICE National Institute for Health and Care Excellence
NVE native valve endocarditis
OPAT outpatient parenteral antibiotic therapy
PBP penicillin binding protein
PCR polymerase chain reaction
PET positron emission tomography
PVE prosthetic valve endocarditis
SOFA Sequential Organ Failure Assessment
SPECT single-photon emission computed tomography
TOE transoesophageal echocardiography
TTE transthoracic echocardiography
WBC white blood cell

by guest on January 14, 2016
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EuroSCORE European System for Cardiac Operative Risk Evaluation
1. Preamble

Guidelines summarize and evaluate all available evidence on a particular issue at the time of the writing process, with the aim of assisting health professionals in selecting the best management strategies for an individual patient with a given condition, taking into account the impact on outcome, as well as the risk–benefit ratio of particular diagnostic or therapeutic means. Guidelines and recommendations should help health professionals to make decisions in their daily practice. However, the final decisions concerning an individual patient must be made by the responsible health professional(s) in consultation with the patient and caregiver as appropriate.

A great number of Guidelines have been issued in recent years by the European Society of Cardiology (ESC) as well as by other societies and organisations. Because of the impact on clinical practice, quality criteria for the development of guidelines have been established in order to make all decisions transparent to the user. The recommendations for formulating and issuing ESC Guidelines can be found on the ESC website (http://www.escardio.org/Guidelines-&-Education/Clinical-Practice-Guidelines/Guidelines-development/Writing-ESC-Guidelines). ESC Guidelines represent the official position of the ESC on a given topic and are regularly updated.

Members of this Task Force were selected by the ESC to represent professionals involved with the medical care of patients with this pathology. Selected experts in the field undertook a comprehensive review of the published evidence for management (including diagnosis, treatment, prevention and rehabilitation) of a given condition according to ESC Committee for Practice Guidelines (CPG) policy. A critical evaluation of diagnostic and therapeutic procedures was performed, including assessment of the risk–benefit ratio. Estimates of expected health outcomes for larger populations were included, where data exist. The level of evidence and the strength of the recommendation of particular management options were weighed and graded according to predefined scales, as outlined in Tables 1 and 2.

The experts of the writing and reviewing panels provided declarations of interest forms for all relationships that might be perceived as real or potential sources of conflicts of interest. These forms were compiled into one file and can be found on the ESC website (http://www.escardio.org/guidelines). Any changes in declarations of interest that arise during the writing period must be notified to the ESC and updated. The Task Force received its entire financial support from the ESC without any involvement from the healthcare industry.

The ESC CPG supervises and coordinates the preparation of new Guidelines produced by task forces, expert groups or consensus panels. The Committee is also responsible for the endorsement process of these Guidelines. The ESC Guidelines undergo extensive review by the CPG and external experts. After appropriate revisions the Guidelines are approved by all the experts involved in the Task Force. The finalized document is approved by the CPG for publication in the European Heart Journal. The Guidelines were developed after careful consideration of the scientific and medical knowledge and the evidence available at the time of their dating.

The task of developing ESC Guidelines covers not only integration of the most recent research, but also the creation of educational tools and implementation programmes for the recommendations. To implement the guidelines, condensed pocket guidelines versions, summary slides, booklets with essential messages, summary cards for non-specialists, and an electronic version for digital applications (smartphones, etc.) are produced. These versions are abridged and thus, if needed, one should always refer to the full text version, which is freely available on the ESC website. The National Societies of the ESC are encouraged to endorse, translate and implement all ESC Guidelines. Implementation programmes are needed because it

### Table 1  Classes of recommendations

<table>
<thead>
<tr>
<th>Classes of recommendations</th>
<th>Definition</th>
<th>Suggested wording to use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Evidence and/or general agreement that a given treatment or procedure is beneficial, useful, effective.</td>
<td>Is recommended/is indicated</td>
</tr>
<tr>
<td>Class II</td>
<td>Conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of the given treatment or procedure.</td>
<td>Should be considered</td>
</tr>
<tr>
<td>Class IIa</td>
<td>Weight of evidence/opinion is in favour of usefulness/efficacy.</td>
<td>Should be considered</td>
</tr>
<tr>
<td>Class IIb</td>
<td>Usefulness/efficacy is less well established by evidence/opinion.</td>
<td>May be considered</td>
</tr>
<tr>
<td>Class III</td>
<td>Evidence or general agreement that the given treatment or procedure is not useful/effective, and in some cases may be harmful.</td>
<td>Is not recommended</td>
</tr>
</tbody>
</table>
has been shown that the outcome of disease may be favourably influenced by the thorough application of clinical recommendations.

Surveys and registries are needed to verify that real-life daily practice is in keeping with what is recommended in the guidelines, thus completing the loop between clinical research, writing of guidelines, disseminating them and implementing them into clinical practice.

Health professionals are encouraged to take the ESC Guidelines fully into account when exercising their clinical judgment, as well as in the determination and the implementation of preventive, diagnostic or therapeutic medical strategies. However, the ESC Guidelines do not override in any way whatsoever the individual responsibility of health professionals to make appropriate and accurate decisions in consideration of each patient’s health condition and in consultation with that patient and the patient’s caregiver where appropriate and/or necessary. It is also the health professional’s responsibility to verify the rules and regulations applicable to drugs and devices at the time of prescription.

### 2. Justification/scope of the problem

Infective endocarditis (IE) is a deadly disease.\(^1\)\(^2\) Despite improvements in its management, IE remains associated with high mortality and severe complications. Until recently, guidelines on IE were mostly based on expert opinion because of the low incidence of the disease, the absence of randomized trials and the limited number of meta-analyses.\(^3\)\(^–\)\(^7\)

The 2009 ESC Guidelines on the prevention, diagnosis and treatment of IE\(^8\) introduced several innovative concepts, including limitation of antibiotic prophylaxis to the highest-risk patients, a focus on healthcare-associated IE and identification of the optimal timing for surgery. However, several reasons justify the decision of the ESC to update the previous guidelines: the publication of new large series of IE, including the first randomized study regarding surgical therapy;\(^9\) important improvements in imaging procedures,\(^10\) particularly in the field of nuclear imaging; and discrepancies between previous guidelines.\(^5\)\(^–\)\(^8\) In addition, the need for a collaborative approach involving primary care physicians, cardiologists, surgeons, microbiologists, infectious disease (ID) specialists and frequently other specialists—namely the ‘Endocarditis Team’—has been underlined recently\(^11\)\(^,\)\(^12\) and will be developed in these new guidelines.

The main objective of the current Task Force was to provide clear and simple recommendations, assisting healthcare providers in their clinical decision making. These recommendations were obtained by expert consensus after thorough review of the available literature. An evidence-based scoring system was used, based on a classification of the strength of recommendations and the levels of evidence.

### 3. Prevention

#### 3.1 Rationale

The principle of antibiotic prophylaxis for IE was developed on the basis of observational studies and animal models and aimed at preventing the attachment of bacteria onto the endocardium after transient bacteraemia following invasive procedures. This concept led to the recommendation for antibiotic prophylaxis in a large number of patients with predisposing cardiac conditions undergoing a wide range of procedures.\(^13\)

The restriction of indications for antibiotic prophylaxis was initiated in 2002 because of changes in pathophysiological conceptions and risk–benefit analyses as follows:\(^14\)

- Low-grade but repeated bacteraemia occurs more frequently during daily routine activities such as toothbrushing, flossing or chewing, and even more frequently in patients with poor dental health.\(^15\)
- The accountability of low-grade bacteraemia was demonstrated in an animal model.\(^16\) The risk of IE may therefore be related more to cumulative low-grade bacteraemia during daily life rather than sporadic high-grade bacteraemia after dental procedures.
- Most case–control studies did not report an association between invasive dental procedures and the occurrence of IE.\(^17\)\(^–\)\(^19\)
- The estimated risk of IE following dental procedures is very low. Antibiotic prophylaxis may therefore avoid only a small number of IE cases, as shown by estimations of 1 case of IE per 150 000 dental procedures with antibiotics and 1 per 46 000 for procedures unprotected by antibiotics.\(^20\)
- Antibiotic administration carries a small risk of anaphylaxis, which may become significant in the event of widespread use. However, the lethal risk of anaphylaxis seems very low when using oral amoxicillin.\(^21\)
- Widespread use of antibiotics may result in the emergence of resistant microorganisms.\(^13\)
- The efficacy of antibiotic prophylaxis on bacteraemia and the occurrence of IE has only been proven in animal models. The effect on bacteraemia in humans is controversial.\(^15\)
- No prospective randomized controlled trial has investigated the efficacy of antibiotic prophylaxis on the occurrence of IE and it is unlikely that such a trial will be conducted given the number of subjects needed.\(^22\)

These points have been progressively taken into account in most guidelines, including the 2009 ESC guidelines,\(^5\)\(^,\)\(^8\)\(^,\)\(^23\)\(^–\)\(^26\) and led to the restriction of antibiotic prophylaxis to the highest-risk patients (patients with the highest incidence of IE and/or highest risk of adverse outcome from IE).

In 2008 the National Institute for Health and Care Excellence (NICE) guidelines went a step further and advised against any antibiotic prophylaxis for dental and non-dental procedures whatever
the patient’s risk. The authors concluded there was an absence of benefit of antibiotic prophylaxis, which was also highly cost-ineffective. These conclusions have been challenged since estimations of the risks of IE are based on low levels of evidence due to multiple extrapolations.

Four epidemiological studies have analysed the incidence of IE following restricted indications for antibiotic prophylaxis. The analysis of 2000–2010 national hospital discharge codes in the UK did not show an increase in the incidence of streptococcal IE after the release of NICE guidelines in 2008. The restriction of antibiotic prophylaxis was seen in a 78% decrease in antibiotic prescriptions before dental care. However, residual prescriptions raised concerns regarding a persisting use of antibiotic prophylaxis. A survey performed in 2012 in the UK showed that the majority of cardiologists and cardiac surgeons felt that antibiotic prophylaxis was necessary in patients with valve prosthesis or prior IE. Recently an analysis of UK data collected from 2000 to 2013 showed a significant increase in the incidence of IE in both high-risk and lower-risk patients in the UK starting in 2008. However, this temporal relationship should not be interpreted as a direct consequence of the NICE guidelines. These findings may be influenced by confounding factors, in particular changes in the number of patients at risk of hospitalizations and healthcare-associated IE. Moreover, microbiological data were not available. Thus we cannot know whether that increase is due to the microbiological species covered by antibiotic prophylaxis.

A repeated prospective 1-year population-based French survey did not show an increase in the incidence of IE, in particular streptococcal IE, between 1999 and 2008, whereas antibiotic prophylaxis had been restricted for native valve disease since 2002.

Two studies from the USA did not find a negative impact of the abandonment of antibiotic prophylaxis in native valve disease in the 2007 American Heart Association guidelines. A more recent analysis on an administrative database found an increase in the incidence of IE hospitalizations between 2000 and 2011, with no significant change after the change of American guidelines in 2007. The increase in IE incidence was observed for all types of microorganisms, but was significant for streptococci after 2007. It was not stated whether this was due to oral streptococci and if intermediate- or high-risk patients were involved.

The present guidelines maintain the principle of antibiotic prophylaxis in high-risk patients for the following reasons:

- The remaining uncertainties regarding estimations of the risk of IE, which play an important role in the rationale of NICE guidelines.
- The worse prognosis of IE in high-risk patients, in particular those with prosthetic IE.
- The fact that high-risk patients account for a much smaller number than patients at intermediate risk, thereby reducing potential harm due to adverse events of antibiotic prophylaxis.

### 3.2 Population at risk

Patients with the highest risk of IE can be placed in three categories (Table 3):

(1) Patients with a prosthetic valve or with prosthetic material used for cardiac valve repair: these patients have a higher risk of IE, a higher mortality from IE and more often develop complications of the disease than patients with native valves and an identical pathogen. This also applies to transcatheter-implanted prostheses and homografts.

(2) Patients with previous IE: they also have a greater risk of new IE, higher mortality and higher incidence of complications than patients with a first episode of IE.

(3) Patients with untreated cyanotic congenital heart disease (CHD) and those with CHD who have postoperative palliative shunts, conduits or other prostheses. After surgical repair with no residual defects, the Task Force recommends prophylaxis for the first 6 months after the procedure until endothelialisation of the prosthetic material has occurred.

### Table 3  Cardiac conditions at highest risk of infective endocarditis for which prophylaxis should be considered when a high-risk procedure is performed

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic prophylaxis should be considered for patients at highest risk for IE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Patients with any prosthetic valve, including a transcatheter valve, or those in whom any prosthetic material was used for cardiac valve repair.</td>
<td>IIa C</td>
<td></td>
</tr>
<tr>
<td>(2) Patients with a previous episode of IE.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) Patients with CHD:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Any type of cyanotic CHD.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Any type of CHD repaired with a prosthetic material, whether placed surgically or by percutaneous techniques, up to 6 months after the procedure or lifelong if residual shunt or valvular regurgitation remains.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic prophylaxis is not recommended in other forms of valvular or CHD.</td>
<td>III C</td>
<td></td>
</tr>
</tbody>
</table>

CHD = congenital heart disease; IE = infective endocarditis.

- **Class of recommendation.**
- **Level of evidence.**
- **Reference(s) supporting recommendations.**

Although American Heart Association/American College of Cardiology guidelines recommend prophylaxis in cardiac transplant recipients who develop cardiac valvulopathy, this is not supported by strong evidence and is not recommended by the ESC Task Force.

Antibiotic prophylaxis is not recommended for patients at intermediate risk of IE, i.e. any other form of native valve disease (including the most commonly identified conditions: bicuspid aortic valve, mitral valve prolapse and calcific aortic stenosis). Nevertheless, both intermediate- and high-risk patients should be advised of the importance of dental and cutaneous hygiene. These measures of general hygiene apply to patients and healthcare workers and should ideally be applied to the general population, as IE frequently occurs without known cardiac disease.
3.3 Situations and procedures at risk

3.3.1 Dental procedures

At-risk procedures involve manipulation of the gingival or periapical region of the teeth or perforation of the oral mucosa (including scaling and root canal procedures) (Table 5). The use of dental implants raises concerns with regard to potential risk due to foreign material at the interface between the buccal cavity and blood. Very few data are available. The opinion of the Task Force is that there is no evidence to contraindicate implants in all patients at risk. The indication should be discussed on a case-by-case basis. The patient should be informed of the uncertainties and the need for close follow-up.

3.3.2 Other at-risk procedures

There is no compelling evidence that bacteraemia resulting from respiratory tract procedures, gastrointestinal or genitourinary procedures, including vaginal and caesarean delivery, or dermatological or musculoskeletal procedures causes IE (Table 5).

3.4 Prophylaxis for dental procedures

Antibiotic prophylaxis should only be considered for patients at highest risk for endocarditis, as described in Table 3, undergoing at-risk dental procedures listed in Table 5, and is not recommended in other situations. The main targets for antibiotic prophylaxis in these patients are oral streptococci. Table 6 summarizes the main regimens of antibiotic prophylaxis recommended before dental procedures. Fluoroquinolones and glycopeptides are not recommended due to their unclear efficacy and the potential induction of resistance.

Table 4 Non-specific prevention measures to be followed in high-risk and intermediate-risk patients

<table>
<thead>
<tr>
<th>These measures should ideally be applied to the general population and particularly reinforced in high-risk patients:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Strict dental and cutaneous hygiene. Dental follow-up should be performed twice a year in high-risk patients and yearly in the others.</td>
</tr>
<tr>
<td>• Disinfection of wounds.</td>
</tr>
<tr>
<td>• Eradication or decrease of chronic bacterial carriage: skin, urine.</td>
</tr>
<tr>
<td>• Curative antibiotics for any focus of bacterial infection.</td>
</tr>
<tr>
<td>• No self-medication with antibiotics.</td>
</tr>
<tr>
<td>• Strict infection control measures for any at-risk procedure.</td>
</tr>
<tr>
<td>• Discourage piercing and tattooing.</td>
</tr>
<tr>
<td>• Limit the use of infusion catheters and invasive procedure when possible. Favour peripheral over central catheters, and systematic replacement of the peripheral catheter every 3–4 days. Strict adherence to care bundles for central and peripheral cannulae should be performed.</td>
</tr>
</tbody>
</table>

Table 5 Recommendations for prophylaxis of infective endocarditis in the highest-risk patients according to the type of at-risk procedure

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class$^a$</th>
<th>Level$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Dental procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis should only be considered for dental procedures requiring manipulation of the gingival or periapical region of the teeth or perforation of the oral mucosa</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for local anaesthetic injections in non-infected tissues, treatment of superficial caries, removal of sutures, dental X-rays, placement or adjustment of removable prosthodontic or orthodontic appliances or braces or following the shedding of deciduous teeth or trauma to the lips and oral mucosa</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>B. Respiratory tract procedures$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for respiratory tract procedures, including bronchoscopy or laryngoscopy, or transnasal or endotracheal intubation</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>C. Gastrointestinal or urogenital procedures or TOE$^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for gastroscopy, colonoscopy, cystoscopy, vaginal or caesarean delivery or TOE</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>D. Skin and soft tissue procedures$^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for any procedure</td>
<td>III</td>
<td>C</td>
</tr>
</tbody>
</table>

TOE = transoesophageal echocardiography.

$^a$Class of recommendation.

$^b$Level of evidence.

$^c$For management when infections are present, please refer to Section 3.5.3.

3.3.2 Other at-risk procedures

There is no compelling evidence that bacteraemia resulting from respiratory tract procedures, gastrointestinal or genitourinary procedures, including vaginal and caesarean delivery, or dermatological or musculoskeletal procedures causes IE (Table 5).

3.4 Prophylaxis for dental procedures

Antibiotic prophylaxis should only be considered for patients at highest risk for endocarditis, as described in Table 3, undergoing at-risk dental procedures listed in Table 5, and is not recommended in other situations. The main targets for antibiotic prophylaxis in these patients are oral streptococci. Table 6 summarizes the main regimens of antibiotic prophylaxis recommended before dental procedures. Fluoroquinolones and glycopeptides are not recommended due to their unclear efficacy and the potential induction of resistance.

Table 5 Continued

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class$^a$</th>
<th>Level$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. Respiratory tract procedures$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for respiratory tract procedures, including bronchoscopy or laryngoscopy, or transnasal or endotracheal intubation</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>C. Gastrointestinal or urogenital procedures or TOE$^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for gastroscopy, colonoscopy, cystoscopy, vaginal or caesarean delivery or TOE</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>D. Skin and soft tissue procedures$^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Antibiotic prophylaxis is not recommended for any procedure</td>
<td>III</td>
<td>C</td>
</tr>
</tbody>
</table>

$^a$Class of recommendation.

$^b$Level of evidence.

$^c$For management when infections are present, please refer to Section 3.5.3.

Table 6 Recommended prophylaxis for high-risk dental procedures in high-risk patients

<table>
<thead>
<tr>
<th>Situation</th>
<th>Antibiotic</th>
<th>Single-dose 30–60 minutes before procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>No allergy to penicillin or ampicillin</td>
<td>Amoxicillin or ampicillin$^a$</td>
<td>2 g orally or i.v.</td>
</tr>
<tr>
<td>Allergy to penicillin or ampicillin</td>
<td>Clindamycin</td>
<td>600 mg orally or i.v.</td>
</tr>
</tbody>
</table>

$^a$Alternatively, cefalexin 2 g i.v. for adults or 50 mg/kg i.v. for children, cefazolin or ceftriaxone 1 g i.v. for adults or 50 mg/kg i.v. for children. Cephalosporins should not be used in patients with anaphylaxis, angio-oedema, or urticaria after intake of penicillin or ampicillin due to cross-sensitivity.
Cephalosporins should not be used in patients with anaphylaxis, angio-oedema or urticaria after intake of penicillin or ampicillin due to cross-sensitivity.

### 3.5 Prophylaxis for non-dental procedures

Systematic antibiotic prophylaxis is not recommended for non-dental procedures. Antibiotic therapy is only needed when invasive procedures are performed in the context of infection.

#### 3.5.1 Respiratory tract procedures

Patients listed in Table 3 who undergo an invasive respiratory tract procedure to treat an established infection (i.e. drainage of an abscess) should receive an antibiotic regimen that contains an anti-staphylococcal drug.

#### 3.5.2 Gastrointestinal or genitourinary procedures

In the case of an established infection or if antibiotic therapy is indicated to prevent wound infection or sepsis associated with a gastrointestinal or genitourinary tract procedure in patients described in Table 3, it is reasonable that the antibiotic regimen includes an agent active against enterococci (i.e. ampicillin, amoxicillin or vancomycin; only in patients unable to tolerate beta-lactams). The use of intrauterine devices was regarded as contraindicated, but this was based on low levels of evidence. Use of an intrauterine device is now considered acceptable, in particular when other contraceptive methods are not possible and in women at low risk of genital infections.43

#### 3.5.3 Dermatological or musculoskeletal procedures

For patients described in Table 3 undergoing surgical procedures involving infected skin (including oral abscesses), skin structure or musculoskeletal tissue, it is reasonable that the therapeutic regimen contains an agent active against staphylococci and beta-haemolytic streptococci.

#### 3.5.4 Body piercing and tattooing

These growing societal trends are a cause for concern, particularly for individuals with CHD who are at increased susceptibility for the acquisition of IE. Case reports of IE after piercing and tattooing are increasing, particularly when piercing involves the tongue,44 although publication bias may over- or underestimate the problem. Currently no data are available on the incidence of IE after such procedures and the efficacy of antibiotics for prevention. Education of procedures and the efficacy of antibiotics for prevention should be discouraged not only in high-risk patients, but also in those with native valve disease. If undertaken, procedures should be performed under strictly sterile conditions, though antibiotic prophylaxis is not recommended.

#### 3.5.5 Cardiac or vascular interventions

In patients undergoing implantation of a prosthetic valve, any type of prosthetic graft or pacemakers, perioperative antibiotic prophylaxis should be considered due to the increased risk and adverse outcome of an infection45–49 (Table 7). The most frequent microorganisms underlying early (1 year after surgery) prosthetic valve infections are coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus*. Prophylaxis should be started immediately before the procedure, repeated if the procedure is prolonged and terminated 48 h afterwards. A randomized trial has shown the efficacy of 1 g intravenous (i.v.) cefazolin on the prevention of local and systemic infections before pacemaker implantation.45 Preoperative screening of nasal carriage of *S. aureus* is recommended before elective cardiac surgery in order to treat carriers using local mupirocin and chlorhexidine.46,47 Rapid identification techniques using gene amplification are useful to avoid delaying urgent surgery. Systematic local treatment without screening is not recommended. It is strongly recommended that potential sources of dental sepsis should be eliminated at least 2 weeks before implantation of a prosthetic valve or other intracardiac or intravascular foreign material, unless the latter procedure is urgent.48

#### Table 7 Recommendations for antibiotic prophylaxis for the prevention of local and systemic infections before cardiac or vascular interventions

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative screening of nasal carriage of <em>S. aureus</em> is recommended before elective cardiac surgery in order to treat carriers</td>
<td>I</td>
<td>A</td>
<td>46,47</td>
</tr>
<tr>
<td>Perioperative prophylaxis is recommended before placement of a pacemaker or implantable cardioverter defibrillator</td>
<td>I</td>
<td>B</td>
<td>45</td>
</tr>
<tr>
<td>Potential sources of sepsis should be eliminated ≥2 weeks before implantation of a prosthetic valve or other intracardiac or intravascular foreign material, except in urgent procedures</td>
<td>IIA</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Perioperative antibiotic prophylaxis should be considered in patients undergoing surgical or transcatheter implantation of a prosthetic valve, intravascular prosthetic or other foreign material</td>
<td>IIA</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Systematic local treatment without screening of <em>S. aureus</em> is not recommended</td>
<td>III</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

*Class of recommendation.*  
*Level of evidence.*  
*Reference(s) supporting recommendations.*

#### 3.5.6 Healthcare-associated infective endocarditis

Healthcare-associated IE represents up to 30% of all cases of IE and is characterized by an increasing incidence and a severe prognosis, thus presenting an important health problem.50,51 Although routine antimicrobial prophylaxis administered before most invasive
procedures is not recommended, aseptic measures during the insertion and manipulation of venous catheters and during any invasive procedures, including in outpatients, are mandatory to reduce the rate of this healthcare-associated IE.52

In summary, these guidelines propose continuing to limit antibiotic prophylaxis to patients at high risk of IE undergoing the highest-risk dental procedures. They highlight the importance of hygiene measures, in particular oral and cutaneous hygiene. Epidemiological changes are marked by an increase in IE due to staphylococcus and of healthcare-associated IE, thereby highlighting the importance of non-specific infection control measures.31,53 This should concern not only high-risk patients, but should also be part of routine care in all patients since IE occurring in patients without previously known heart disease now accounts for a substantial and increasing incidence. This means that although antibiotic prophylaxis should be restricted to the highest-risk patients, preventive measures should be maintained or extended to all patients with cardiac disease.

Although this section of the guidelines on IE prophylaxis is based on weak evidence, they have been strengthened recently by epidemiological surveys, most of which did not show an increased incidence of IE due to oral streptococci.31–35 Their application by patients should follow a shared decision-making process. Future challenges are to gain a better understanding of the mechanisms associated with valve infection, the adaptation of prophylaxis to the ongoing epidemiological changes and the performance of specific prospective surveys on the incidence and characteristics of IE.

4. The ‘Endocarditis Team’

IE is a disease that needs a collaborative approach for the following reasons:

- First, IE is not a single disease, but rather may present with very different aspects depending on the first organ involved, the underlying cardiac disease (if any), the microorganism involved, the presence or absence of complications and the patient’s characteristics.36 No single practitioner will be able to manage and treat a patient in whom the main clinical symptoms might be cardiac, rheumatological, infectious, neurological or other.
- Second, a very high level of expertise is needed from practitioners from several specialties, including cardiologists, cardiac surgeons, ID specialists, microbiologists, neurologists, neurosurgeons, experts in CHD and others. Echocardiography is known to have a major importance in the diagnosis and management of IE. However, other imaging techniques, including magnetic resonance imaging (MRI), multislice computed tomography (MSCT), and nuclear imaging, have also been shown to be useful for diagnosis, follow-up and decision making in patients with IE.10 Including all of these specialists in the team is becoming increasingly important.
- Finally, about half of the patients with IE undergo surgery during the hospital course.36 Early discussion with the surgical team is important and is considered mandatory in all cases of complicated IE [i.e. endocarditis with heart failure (HF), abscess or embolic or neurological complications].

Therefore the presence of an Endocarditis Team is crucial. This multidisciplinary approach has already been shown to be useful in the management of valve disease11 (the ‘Heart Valve Clinic’), particularly in the selection of patients for transcatheter aortic valve implantation procedures (‘Heart Team’ approach).56 In the field of IE, the team approach adopted in France, including standardized medical therapy, surgical indications following guideline recommendations and 1 year of close follow-up, has been shown to significantly reduce the 1-year mortality, from 18.5% to 8.2%.52 Other authors have recently reported similar results.56 Taking these reports together, such a team approach has been recommended recently as class IB in the 2014 American Heart Association/American College of Cardiology guideline for the management of patients with valvular heart disease.35

The present Task Force on the management of IE of the ESC strongly supports the management of patients with IE in reference centres by a specialized team (the ‘Endocarditis Team’). The main characteristics of the Endocarditis Team and the referring indications are summarized in Tables 8 and 9.

Table 8 Characteristics of the ‘Endocarditis Team’

<table>
<thead>
<tr>
<th>When to refer a patient with IE to an ‘Endocarditis Team’ in a reference centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patients with complicated IE (i.e. endocarditis with HF, abscess, or embolic or neurological complication or CHD), should be referred early and managed in a reference centre with immediate surgical facilities.</td>
</tr>
<tr>
<td>2. Patients with non-complicated IE can be initially managed in a non-reference centre, but with regular communication with the reference centre, consultations with the multidisciplinary ‘Endocarditis Team’, and, when needed, with external visit to the reference centre.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristics of the reference centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Immediate access to diagnostic procedures should be possible, including TTE, TOE, multislice CT, MRI, and nuclear imaging.</td>
</tr>
<tr>
<td>2. Immediate access to cardiac surgery should be possible during the early stage of the disease, particularly in case of complicated IE (HF, abscess, large vegetation, neurological, and embolic complications).</td>
</tr>
<tr>
<td>3. Several specialists should be present on site (the ‘Endocarditis Team’), including at least cardiac surgeons, cardiologists, anaesthesiologists, ID specialists, microbiologists and, when available, specialists in valve diseases, CHD, pacemaker extraction, echocardiography and other cardiac imaging techniques, neurologists, and facilities for neurosurgery and interventional neuroradiology.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Role of the ‘Endocarditis Team’</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The ‘Endocarditis Team’ should have meetings on a regular basis in order to discuss cases, take surgical decisions, and define the type of follow-up.</td>
</tr>
<tr>
<td>2. The ‘Endocarditis Team’ chooses the type, duration, and mode of follow-up of antibiotic therapy, according to a standardized protocol, following the current guidelines.</td>
</tr>
<tr>
<td>3. The ‘Endocarditis Team’ should participate in national or international registries, publicly report the mortality and morbidity of their centre, and be involved in a quality improvement programme, as well as in a patient education programme.</td>
</tr>
<tr>
<td>4. The follow-up should be organized on an outpatient visit basis at a frequency depending on the patient’s clinical status (ideally at 1, 3, 6 and 12 months after hospital discharge, since the majority of events occur during this period&quot;).</td>
</tr>
</tbody>
</table>

CHD = Congenital heart disease; CT = computed tomography; HF = heart failure; ID = Infectious disease; IE = infective endocarditis; MRI = magnetic resonance imaging; TOE = transoesophageal echocardiography; TTE = transthoracic echocardiography.
5. Diagnosis

5.1 Clinical features

The diverse nature and evolving epidemiological profile of IE ensure that it remains a diagnostic challenge. The clinical history of IE is highly variable according to the causative microorganism, the presence or absence of pre-existing cardiac disease, the presence or absence of prosthetic valves or cardiac devices and the mode of presentation. Thus IE should be suspected in a variety of very different clinical situations. It may present as an acute, rapidly progressive infection, but also as a subacute or chronic disease with low-grade fever and non-specific symptoms that may mislead or confuse initial assessment. Patients may therefore present to a variety of specialists who may consider a range of alternative diagnoses, including chronic infection; rheumatological, neurological and autoimmune diseases; or malignancy. The early involvement of a cardiologist and an ID specialist to guide management is highly recommended.

Up to 90% of patients present with fever, often associated with systemic symptoms of chills, poor appetite and weight loss. Heart murmurs are found in up to 85% of patients. Up to 25% of patients have embolic complications at the time of diagnosis. Therefore IE has to be suspected in any patient presenting with fever and embolic phenomena. Classic signs may still be seen in the developing world in subacute forms of IE, although peripheral stigmata of IE are increasingly uncommon elsewhere, as patients generally present at an early stage of the disease. However, vascular and immunological phenomena such as splinter haemorrhages, Roth spots and glomerulonephritis remain common. Emboli to the brain, lung or spleen occur in 30% of patients and are often the presenting feature. In a febrile patient, diagnostic suspicion may be strengthened by laboratory signs of infection, such as elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), leucocytosis, anaemia and microscopic haematuria. However, these signs lack specificity and have not been integrated into current diagnostic criteria. Atypical presentation is common in elderly or immunocompromised patients, in whom fever is less common than in younger individuals. A high index of suspicion and low threshold for investigation are therefore essential in these and other high-risk groups, such as those with CHD or prosthetic valves, to exclude IE or avoid delays in diagnosis.

5.2 Laboratory findings

In addition to specialized microbiological and imaging investigations, a number of laboratory investigations and biomarkers have been evaluated in sepsis/sepsis syndromes and endocarditis. The large number of proposed potential biomarkers reflects the complex pathophysiology of the disease process, involving pro- and anti-inflammatory processes, humoral and cellular reactions and both circulatory and end-organ abnormalities. However, owing to their poor positive predictive value for the diagnosis of sepsis and lack of specificity for endocarditis, these biomarkers have been excluded from being major diagnostic criteria and are only used to facilitate risk stratification.

Sepsis severity may be indicated by the demonstration of a number of laboratory investigations, including the degree of leucocytosis/leucopenia, the number of immature white cell forms, concentrations of CRP and procalcitonin, ESR and markers of end-organ dysfunction (lactataemia, elevated bilirubin, thrombocytopenia and changes in serum creatinine concentration); however, none are diagnostic for IE. Further, certain laboratory investigations are used in surgical scoring systems relevant to risk stratification in patients with IE, including bilirubin, creatinine and platelet count [Sequential Organ Failure Assessment (SOFA) score] and creatinine clearance [European System for Cardiac Operative Risk Evaluation (EuroSCORE) II]. Finally, the pattern of increase in inflammatory mediators or immune complexes may support, but not prove, the diagnosis of IE, including the finding of hypocomplementaemia in the presence of elevated antineutrophil cytoplasmic antibody in endocarditis-associated vasculitis or, where lead infection is suspected clinically, the laboratory finding of a normal procalcitonin and white cell count in the presence of significantly elevated CRP and/or ESR.

5.3 Imaging techniques

Imaging, particularly echocardiography, plays a key role in both the diagnosis and management of IE. Echocardiography is also useful for the prognostic assessment of patients with IE, for its follow-up under therapy and during and after surgery. Echocardiography is particularly useful for initial assessment of the embolic risk and in decision making in IE. Transoesophageal echocardiography (TOE) plays a major role both before and during surgery (intraoperative echocardiography). However, the evaluation of patients with IE is no longer limited to conventional echocardiography, but should include several other imaging techniques such as MSCT, MRI, 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET)/computed tomography (CT) or other functional imaging modalities.

5.3.1 Echocardiography

Echocardiography, either transthoracic echocardiography (TTE) or TOE, is the technique of choice for the diagnosis of IE, and plays a
key role in the management and monitoring of these patients. Echocardiography must be performed as soon as IE is suspected. TOE must be performed in case of negative TTE when there is a high index of suspicion for IE, particularly when TTE is of suboptimal quality. TOE should also be performed in patients with positive TTE to rule out local complications. The indications of echocardiographic examination for diagnosis and follow-up of patients with suspected IE are summarized in Table 10 and Figure 1. In patients with S. aureus bacteraemia, echocardiography is justified in view of the frequency of IE in this setting, the virulence of this organism and its devastating effects once intracardiac infection is established. In these patients, TTE or TOE should be considered according to individual patient risk factors and the mode of acquisition of S. aureus bacteraemia.

### Table 10 Role of echocardiography in infective endocarditis

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class**</th>
<th>Level*</th>
<th>Ref.†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommendations</strong></td>
<td><strong>Class</strong></td>
<td><strong>Level</strong></td>
<td><strong>Ref.</strong></td>
</tr>
<tr>
<td><strong>A. Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• TTE is recommended as the first-line imaging modality in suspected IE.</td>
<td>I</td>
<td>B</td>
<td>64,65</td>
</tr>
<tr>
<td>• TOE is recommended in all patients with clinical suspicion of IE and a negative or non-diagnostic TTE.</td>
<td>I</td>
<td>B</td>
<td>64, 68–71</td>
</tr>
<tr>
<td>• TOE is recommended in patients with clinical suspicion of IE, when a prosthetic heart valve or an intracardiac device is present.</td>
<td>I</td>
<td>B</td>
<td>64,71</td>
</tr>
<tr>
<td>• Repeat TTE and/or TOE within 5–7 days is recommended in case of initially negative examination when clinical suspicion of IE remains high.</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>• Echocardiography should be considered in Staphylococcus aureus bacteraemia.</td>
<td>Ila</td>
<td>B</td>
<td>66,67</td>
</tr>
<tr>
<td>• TOE should be considered in patients with suspected IE, even in cases with positive TTE, except in isolated right-sided native valve IE with good quality TTE examination and unequivocal echocardiographic findings.</td>
<td>Ila</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>B. Follow-up under medical therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Repeat TTE and/or TOE are recommended as soon as a new complication of IE is suspected (new murmur, embolism, persisting fever, HF, abscess, atrioventricular block).</td>
<td>I</td>
<td>B</td>
<td>64,72</td>
</tr>
</tbody>
</table>

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**HF** = heart failure; **IE** = infective endocarditis; **TOE** = transoesophageal echocardiography; **TTE** = transthoracic echocardiography. **Class of recommendation.** **Level of evidence.** **Reference(s) supporting recommendations.**

### Figure 1 Indications for echocardiography in suspected infective endocarditis.
Three echocardiographic findings are major criteria in the diagnosis of IE: vegetation, abscess or pseudoaneurysm and new dehiscence of a prosthetic valve (see Table 11 for anatomical and echocardiographic definitions). Nowadays, the sensitivity for the diagnosis of vegetations in native and prosthetic valves is 70% and 50%, respectively, for TTE and 96% and 92%, respectively, for TOE. Specificity has been reported to be around 90% for both TTE and TOE. Identification of vegetations may be difficult in the presence of pre-existing valvular lesions (mitral valve prolapse, degenerative calcified lesions), prosthetic valves, small vegetations (< 2–3 mm), recent embolization and in non-vegetant IE. Diagnosis may be particularly challenging in IE affecting intracardiac devices, even with the use of TOE. False diagnosis of IE may occur, and in some instances it may be difficult to differentiate vegetations from thrombi, Lambi’s excrescences, cusp prolapse, chordal rupture, valve fibroelastoma, degenerative or myxomatous valve disease, strands, systemic lupus (Libman–Sacks) lesions, primary antiphospholipid syndrome, rheumatoid lesions or marantic vegetations. Therefore the results of the echocardiographic study must be interpreted with caution, taking into account the patient’s clinical presentation and the likelihood of IE.

The sensitivity of TTE for the diagnosis of abscesses is about 50%, compared with 90% for TOE. Specificity higher than 90% has been reported for both TTE and TOE. Small abscesses may be difficult to identify, particularly in the earliest stage of the disease, in the postoperative period and in the presence of a prosthetic valve. IE must always be suspected in patients with new periprosthetic regurgitation, even in the absence of other echocardiographic findings of IE.

In cases with an initially negative examination, repeat TTE/TOE must be performed 5–7 days later if the clinical level of suspicion is still high, or even earlier in the case of S. aureus infection. Other imaging techniques should also be used in this situation (see section 5.5). Finally, follow-up echocardiography to monitor complications and response to treatment is mandatory (Figure 1).

Real-time three-dimensional (3D) TOE allows the analysis of 3D volumes of cardiac structures in any possible plane. A recent study has shown that conventional TOE underestimates vegetation size and that 3D TOE is a feasible technique for the analysis of vegetation morphology and size that may overcome the shortcomings of conventional TOE, leading to a better prediction of the embolic risk in IE. 3D TOE is particularly useful in the assessment of perivalvular extension of the infection, prosthetic valve dehiscence and valve perforation. Although in clinical practice 3D TOE is increasingly performed along with conventional TOE in many centres, at present 3D TOE should still be regarded as a supplement to standard echocardiography in most cases.

### 5.3.2 Multislice computed tomography

The potential risks of vegetation embolization and/or haemodynamic decompensation during coronary angiography (when indicated) have led to proposals to consider MSCT coronary angiography as an alternative technique for some patients with endocarditis.

MSCT can be used to detect abscesses/pseudoaneurysms with a diagnostic accuracy similar to TOE, and is possibly superior in the provision of information regarding the extent and consequences of any perivalvular extension, including the anatomy of pseudoaneurysms, abscesses and fistulae. In aortic IE, CT may additionally be useful to define the size, anatomy and calcification of the aortic valve, root and ascending aorta, which may be used to inform surgical planning. In pulmonary/right-sided endocarditis, CT may reveal concomitant pulmonary disease, including abscesses and infarcts.

In the evaluation of prosthetic valve dysfunction, one recent study has suggested that MSCT may be equivalent or superior to echocardiography for the demonstration of prostheses-related vegetations, abscesses, pseudoaneurysms and dehiscence. However, large comparative studies between the two techniques are missing, and echocardiography should always be performed first.

The higher sensitivity of MRI compared with CT for the detection of cerebral lesions is well known and has been confirmed in the context of endocarditis. However, in the critically ill patient, CT may be more feasible and practical and is an acceptable alternative when MRI is not available. MSCT angiography allows complete

### Table 11 Anatomical and echocardiographic definitions

<table>
<thead>
<tr>
<th>Surgery/necropsy</th>
<th>Echocardiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetation</td>
<td>Infected mass attached to an endocardial structure or on implanted intracardiac material.</td>
</tr>
<tr>
<td></td>
<td>Oscillating or non-ossilating intracardiac mass on valve or other endocardial structures, or on implanted intracardiac material.</td>
</tr>
<tr>
<td>Abscess</td>
<td>Perivalvular cavity with necrosis and purulent material not communicating with the cardiovascular lumen.</td>
</tr>
<tr>
<td></td>
<td>Thickened, non-homogeneous perivalvular area with echodense or echoluent appearance.</td>
</tr>
<tr>
<td>Pseudoaneurysm</td>
<td>Perivalvular cavity communicating with the cardiovascular lumen.</td>
</tr>
<tr>
<td></td>
<td>Pulsatile perivalvular echo-free space, with colour-Doppler flow detected.</td>
</tr>
<tr>
<td>Perforation</td>
<td>Interruption of endocardial tissue continuity.</td>
</tr>
<tr>
<td></td>
<td>Interruption of endocardial tissue continuity traversed by colour-Doppler flow.</td>
</tr>
<tr>
<td>Fistula</td>
<td>Communication between two neighbouring cavities through a perforation.</td>
</tr>
<tr>
<td></td>
<td>Colour-Doppler communication between two neighbouring cavities through a perforation.</td>
</tr>
<tr>
<td>Valve aneurysm</td>
<td>Saccular outpouching of valvular tissue.</td>
</tr>
<tr>
<td></td>
<td>Saccular bulging of valvular tissue.</td>
</tr>
<tr>
<td>Dehiscence of a prosthetic valve</td>
<td>Dehiscence of the prosthesis.</td>
</tr>
<tr>
<td></td>
<td>Paravalvular regurgitation identified by TTE/TOE, with or without rocking motion of the prosthesis.</td>
</tr>
</tbody>
</table>

TOE = transeosophageal echocardiography; TTE = transthoracic echocardiography.
visualization of the intracranial vascular tree and carries a lower contrast burden and risk of permanent neurological damage than conventional digital subtraction angiography, with a sensitivity of 90% and specificity of 86%. Where subarachnoid and/or intraparenchymal haemorrhage is detected, other vascular imaging (i.e. angiography) is required to diagnose or exclude a mycotic aneurysm if not detected on CT.

Contrast-enhanced MSCT has a high sensitivity and specificity for the diagnosis of splenic and other abscesses; however, the differentiation with infarction can be challenging. MSCT angiography provides a rapid and comprehensive exploration of the systemic arterial bed. Detailed multiplanar and 3D contrast-enhanced angiographic reconstructions allow vascular mapping with identification and characterization of peripheral vascular complications of IE and their follow-up.

5.3.3 Magnetic resonance imaging
Given its higher sensitivity than CT, MRI increases the likelihood of detecting cerebral consequences of IE. Different studies including systematic cerebral MRI during acute IE have consistently reported frequent lesions, in 60–80% of patients. Regardless of neurological symptoms, most abnormalities are ischaemic lesions (in 50–80% of patients), with more frequent small ischaemic lesions than larger territorial infarcts. Other lesions are found in <10% of patients and are parenchymal or subarachnoidal haemorrhages, abscesses or mycotic aneurysms.

Systematic cerebral MRI has an impact on the diagnosis of IE since it adds one minor Duke criterion in patients who have cerebral lesions and no neurological symptoms. In one study, findings of cerebral MRI upgraded the diagnosis of IE in 25% of patients presenting initially with non-definite IE, thereby leading to earlier detection of peripheral vascular complications of IE. MRI increases the likelihood of counting the findings of cerebral MRI.

To summarize, cerebral MRI allows for a better lesion characterization in patients with IE and neurological symptoms, whereas its impact on IE diagnosis is marked in patients with non-definite IE and without neurological symptoms.

5.3.4 Nuclear imaging
With the introduction of hybrid equipment for both conventional nuclear medicine [e.g. single-photon emission CT (SPECT)/CT] and PET (i.e. PET/CT), nuclear molecular techniques are evolving as an important supplementary method for patients with suspected IE and diagnostic difficulties. SPECT/CT imaging relies on the use of autologous radiolabelled leucocytes (\(^{111}\)In-oxine or \(^{99m}\)Tc-hexamethylpropyleneamine oxime) that accumulate in a time-dependent fashion in late images versus earlier images, whereas PET/CT is generally performed using a single acquisition time point (generally at 1 h) after administration of \(^{18}\)F-FDG, which is actively incorporated in vivo by activated leucocytes, monocyte-macrophages and CD4\(^+\) T-lymphocytes accumulating at the sites of infection.

Several reports have shown promising results for radiolabelled white blood cell (WBC) SPECT/CT and \(^{18}\)F-FDG PET/CT imaging in IE. The main added value of using these techniques is the reduction in the rate of misdiagnosed IE, classified in the ‘Possible IE’ category using the Duke criteria, and the detection of peripheral embolic and metastatic infectious events. Limitations to the use of \(^{18}\)F-FDG PET/CT are represented by localization of septic emboli in the brain, due to the high physiological uptake of this tracer in the brain cortex, and to the fact that at this site, metastatic infections are generally <5 mm, the spatial resolution threshold of current PET/CT scanners.

Caution must be exercised when interpreting \(^{18}\)F-FDG PET/CT results in patients who have recently undergone cardiac surgery, as a postoperative inflammatory response may result in non-specific \(^{18}\)F-FDG uptake in the immediate postoperative period. Furthermore, a number of pathological conditions can mimic the pattern of focally increased \(^{18}\)F-FDG uptake that is typically observed in IE, such as active thrombi, soft atherosclerotic plaques, vasculitis, primary cardiac tumours, cardiac metastasis from a non-cardiac tumour, post-surgical inflammation and foreign body reactions.

Radiolabelled WBC SPECT/CT is more specific for the detection of IE and infectious foci than \(^{18}\)F-FDG PET/CT and should be preferred in all situations that require enhanced specificity. Disadvantages of scintigraphy with radiolabelled WBC are the requirement of blood handling for radiopharmaceutical preparation, the duration of the procedure, which is more time consuming than PET/CT, and a slightly lower spatial resolution and photon detection efficiency compared with PET/CT.

An additional promising role of \(^{18}\)F-FDG PET/CT may be seen in patients with established IE, in whom it could be employed to monitor response to antimicrobial treatment. However, sufficient data are not available at this time to make a general recommendation.

5.4 Microbiological diagnosis
5.4.1 Blood culture–positive infective endocarditis
Positive blood cultures remain the cornerstone of diagnosis and provide live bacteria for both identification and susceptibility testing. At
at least three sets are taken at 30-min intervals, each containing 10 mL of blood, and should be incubated in both aerobic and anaerobic atmospheres. Sampling should be obtained from a peripheral vein rather than from a central venous catheter (because of the risk of contamination and misleading interpretation), using a meticulous sterile technique. This is virtually always sufficient to identify the usual causative microorganisms. The need for culture before antibiotic administration is self-evident. In IE, bacteraemia is almost constant and has two implications: (i) there is no rationale for delaying blood sampling with peaks of fever and (ii) virtually all blood cultures are positive. As a result, a single positive blood culture should be regarded cautiously for establishing the diagnosis of IE. The microbiology laboratory should be aware of the clinical suspicion of IE at the time of blood culture sampling. When a microorganism has been identified, blood cultures should be repeated after 48–72 h to check the effectiveness of treatment. Automated machines perform continuous monitoring of bacterial growth, which ensures quick provision of reports to physicians. When a positive blood culture bottle is identified, presumptive identification is based on Gram staining. This information is immediately given to clinicians in order to adapt presumptive antibiotic therapy. Complete identification is routinely achieved within 2 days, but may require longer for fastidious or atypical organisms. Since the delay between blood culture sampling and definitive identification of the organism responsible for the bacteraemia and antibiotic susceptibility testing is long, many improvements have been proposed to speed up the process of detection and identification. One of the most recent procedures for rapid bacterial identification is based on peptide spectra obtained by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. This technique has recently demonstrated its usefulness in clinical microbiology; it also has the potential for direct identification of bacterial colonies in the blood culture bottle supernatant.96

5.4.2 Blood culture-negative infective endocarditis

Blood culture-negative IE (BCNIE) refers to IE in which no causative microorganism can be grown using the usual blood culture methods. BCNIE can occur in up to 31% of all cases of IE and often poses considerable diagnostic and therapeutic dilemmas. BCNIE most commonly arises as a consequence of previous antibiotic administration, underlying the need for withdrawing antibiotics and repeating blood cultures in this situation. BCNIE can be caused by fungi or fastidious bacteria, notably obligatory intracellular bacteria. Isolation of these microorganisms requires culturing them on specialized media, and their growth is relatively slow. According to local epidemiology, systematic serological testing for Coxiella burnetii, Bartonella spp., Aspergillus spp., Mycoplasma pneumoniae, Brucella spp. and Legionella pneumophila should be proposed, followed by specific polymerase chain reaction (PCR) assays for Tropheryma whippelii, Bartonella spp. and fungi (Candida spp., Aspergillus spp.) from the blood97 (Table 12). Most studies using blood PCR for the diagnosis of BCNIE have highlighted the importance of Streptococcus gallolyticus and Streptococcus mitis, enterococci, S. aureus, Escherichia coli and fastidious bacteria, the respective prevalence of which varies according to the status and condition of the patient.98

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Diagnostic procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella spp.</td>
<td>Blood cultures, serology, culture, immunohistology, and PCR of surgical material.</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>Serology (IgG phase 1 &gt;1:800), tissue culture, immunohistology, and PCR of surgical material.</td>
</tr>
<tr>
<td>Bartonella spp.</td>
<td>Blood cultures, serology, culture, immunohistology, and PCR of surgical material.</td>
</tr>
<tr>
<td>Tropheryma whippelii</td>
<td>Histology and PCR of surgical material.</td>
</tr>
<tr>
<td>Mycoplasma spp.</td>
<td>Serology, culture, immunohistology, and PCR of surgical material.</td>
</tr>
<tr>
<td>Legionella spp.</td>
<td>Blood cultures, serology, culture, immunohistology, and PCR of surgical material.</td>
</tr>
<tr>
<td>Fungi</td>
<td>Blood cultures, serology, PCR of surgical material.</td>
</tr>
</tbody>
</table>

Ig = immunoglobulin; PCR = polymerase chain reaction.

When all microbiological assays are negative, the diagnosis of non-infectious endocarditis should be considered and assays for antinuclear antibodies as well as antiphospholipid syndrome [anticardiolipin antibodies [immunoglobulin (Ig)G and anti-β2-glycoprotein 1 antibodies [IgG and IgM]] should be performed. When all other tests are negative and the patient has a porcine bioprosthesis together with markers of allergic response, anti-pork antibodies should be sought.99

5.4.3 Histological diagnosis of infective endocarditis

Pathological examination of resected valvular tissue or embolic fragments remains the gold standard for the diagnosis of IE. All tissue samples that are excised during the course of the surgical removal of cardiac valves must be collected in a sterile container without fixative or culture medium. The entire sample should be taken to the diagnostic microbiology laboratory for optimal recovery and identification of microorganisms.

5.4.4 Proposed strategy for a microbiological diagnostic algorithm in suspected IE

A proposed diagnostic scheme is provided in Figure 2. When there is clinical suspicion of IE and blood cultures remain negative at 48 h, liaison with the microbiologist is necessary. A suggested strategy is the use of a diagnostic kit including blood cultures and systematic serological testing for C. burnetii, Bartonella spp., Aspergillus spp., L. pneumophila, Brucella spp., M. pneumoniae, as well as rheumatoid factor, the serological tests for antiphospholipid syndrome [anticardiolipin (IgG) and anti-β2-glycoprotein 1 (IgG and IgM)], antinuclear antibodies and anti-pork antibodies. In addition, cardiac valvular materials obtained at surgery have to be subjected to systematic culture, histological examination and PCR aimed at documenting the presence of fastidious organisms.
5.5 Diagnostic criteria

Besides the pathological aspect obtained after valve surgery, in clinical practice the diagnosis of IE usually relies on the association between an infective syndrome and recent endocardial involvement. This is the cornerstone of the various criteria proposed to facilitate the difficult diagnosis of this disease. Thus, in 2000, the modified Duke criteria were recommended for diagnostic classification (Table 13). These criteria are based on clinical, echocardiographic and biological findings, as well as the results of blood cultures and serologies.\(^87\) This classification has a sensitivity of approximately 80% overall when the criteria are evaluated at the end of patient follow-up in epidemiological studies.\(^100\) However, the modified Duke criteria show a lower diagnostic accuracy for early diagnosis in clinical practice, especially in the case of prosthetic valve endocarditis (PVE) and pacemaker or defibrillator lead IE, for which echocardiography is normal or inconclusive in up to 30% of cases.\(^101,102\) Recent advances in imaging techniques have resulted in an improvement in identification of endocardial involvements and extracardiac complications of IE.\(^10,103\) Thus recent works have demonstrated that cardiac/whole-body CT scan, cerebral MRI, \(^18\)F-FDG PET/CT and radiolabelled leucocyte SPECT/CT might improve the detection of silent vascular phenomena (embolic events or infectious aneurysms) as well as endocardial lesions.\(^79,80,83–85,93,94,104–108\) The addition of the results of these imaging modalities may improve the sensitivity of the modified Duke criteria in difficult cases.

### Table 13  Definition of infective endocarditis according to the modified Duke criteria (adapted from Li et al.\(^87\))

<table>
<thead>
<tr>
<th>Definite IE</th>
<th>Pathological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microorganisms demonstrated by culture or on histological examination of a vegetation, or a vegetation that has embolised, or an intracardiac abscess specimen; or</td>
</tr>
<tr>
<td></td>
<td>Pathological lesions; vegetation or intracardiac abscess confirmed by histological examination showing active endocarditis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Definite IE</th>
<th>Clinical criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 major criteria; or</td>
</tr>
<tr>
<td></td>
<td>1 major criterion and 3 minor criteria; or</td>
</tr>
<tr>
<td></td>
<td>5 minor criteria</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Possible IE</th>
<th>Pathological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 major criterion and 1 minor criterion; or</td>
</tr>
<tr>
<td></td>
<td>3 minor criteria</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rejected IE</th>
<th>Pathological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Firm alternate diagnosis; or</td>
</tr>
<tr>
<td></td>
<td>Resolution of symptoms suggesting IE with antibiotic therapy for ≤4 days; or</td>
</tr>
<tr>
<td></td>
<td>No pathological evidence of IE at surgery or autopsy, with antibiotic therapy for ≤4 days; or</td>
</tr>
<tr>
<td></td>
<td>Does not meet criteria for possible IE, as above</td>
</tr>
</tbody>
</table>

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**Figure 2** Microbiological diagnostic algorithm in culture-positive and culture-negative IE.
Given the recent published data, the Task Force proposes the addition of three further points in the diagnostic criteria (Table 14):

1. The identification of paravalvular lesions by cardiac CT should be considered a major criterion.

2. In the setting of the suspicion of endocarditis on a prosthetic valve, abnormal activity around the site of implantation detected by $^{18}$F-FDG PET/CT (only if the prosthesis was implanted for $>$3 months) or radiolabelled leucocyte SPECT/CT should be considered a major criterion.

3. The identification of recent embolic events or infectious aneurysms by imaging only (silent events) should be considered a minor criterion.

Table 14: Definitions of the terms used in the European Society of Cardiology 2015 modified criteria for the diagnosis of infective endocarditis

<table>
<thead>
<tr>
<th>Major criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Blood cultures positive for IE</td>
</tr>
<tr>
<td>a. Typical microorganisms consistent with IE from 2 separate blood cultures:</td>
</tr>
<tr>
<td>- Viridans streptococci, Streptococcus gordonii (Streptococcus bovis), HACEK group, Staphylococcus aureus or</td>
</tr>
<tr>
<td>- Community-acquired enterococci, in the absence of a primary focus; or</td>
</tr>
<tr>
<td>b. Microorganisms consistent with IE from persistently positive blood cultures:</td>
</tr>
<tr>
<td>- ≥2 positive blood cultures of blood samples drawn $&gt;$12 h apart; or</td>
</tr>
<tr>
<td>- All of 3 or a majority of ≥4 separate cultures of blood (with first and last samples drawn ≥1 h apart); or</td>
</tr>
<tr>
<td>c. Single positive blood culture for Coxiella burnetii or phase 1 IgG antibody titre $&gt;$1.800</td>
</tr>
</tbody>
</table>

2. Imaging positive for IE

a. Echocardiogram positive for IE:
   - Vegetation; |
   - Abscess, pseudoaneurysm, intracardiac fistula; |
   - Valvular perforation or aneurysm; |
   - New partial dehiscence of prosthetic valve. |

b. Abnormal activity around the site of prosthetic valve implantation detected by $^{18}$F-FDG PET/CT (only if the prosthesis was implanted for $>$3 months) or radiolabelled leucocyte SPECT/CT. |

c. Definite paravalvular lesions by cardiac CT.

Minor criteria

1. Predisposition such as predisposing heart condition, or injection drug use. |
2. Fever defined as temperature $>$38°C. |
3. Vascular phenomena (including those detected by imaging only): |
   - Major arterial emboli, septic pulmonary infarcts, infectious (mycotic) aneurysm, intracranial haemorrhage, conjunctival haemorrhages, and Janeway’s lesions. |
4. Immunological phenomena: glomerulonephritis, Osler’s nodes, Roth’s spots, and rheumatoid factor. |
5. Microbiological evidence: positive blood culture but does not meet a major criterion as noted above or serological evidence of active infection with organism consistent with IE.

Finally, $^{18}$F-FDG PET/CT and radiolabelled leucocyte SPECT/CT have proven their role in the diagnosis of cardiovascular electronic implanted devices, but the data are not sufficient for them to be included in the diagnostic criteria of the specific topic of IE on pacemaker or defibrillator leads.

In summary, echocardiography (TTE and TOE), positive blood cultures and clinical features remain the cornerstone of IE diagnosis. When blood cultures are negative, further microbiological studies are needed. The sensitivity of the Duke criteria can be improved by new imaging modalities (MRI, CT, PET/CT) that allow the diagnosis of embolic events and cardiac involvement when TTE/TOE findings are negative or doubtful. These criteria are useful, but they do not replace the clinical judgement of the Endocarditis Team.

6. Prognostic assessment at admission

The in-hospital mortality rate of patients with IE varies from 15% to 30%. Rapid identification of patients at highest risk of death...
may offer the opportunity to change the course of the disease (i.e. emergency or urgent surgery) and improve prognosis. Prognosis in IE is influenced by four main factors: patient characteristics, the presence or absence of cardiac and non-cardiac complications, the infecting organism and the echocardiographic findings (Table 15). The risk of patients with left-sided IE has been formally assessed according to these variables. Patients with HF, periannular complications and/or S. aureus infection are at highest risk of death and need for surgery in the active phase of the disease. When three of these factors are present, the risk reaches 79%. Therefore these patients with complicated IE should be referred early and managed in a reference centre with surgical facilities and preferably by an Endocarditis Team. A high degree of co-morbidity, diabetes, septic shock, moderate-to-severe ischaemic stroke, brain haemorrhage or the need for haemodialysis are also predictors of poor in-hospital outcome. Persistence of positive blood cultures 48–72 h after initiation of antibiotic treatment indicates a lack of infection control and is an independent risk factor for in-hospital mortality.

### Table 15: Predictors of poor outcome in patients with infective endocarditis

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Clinical complications of IE</th>
<th>Microorganism</th>
<th>Echocardiographic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Older age</td>
<td>Heart failure</td>
<td>Staphylococcus aureus</td>
<td>Periannular complications</td>
</tr>
<tr>
<td>Prosthetic valve IE</td>
<td>Renal failure</td>
<td>Fungi</td>
<td>Severe left-sided valve regurgitation</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>&gt;Moderate area of ischaemic stroke</td>
<td>Non-HACEK Gram-negative bacilli</td>
<td>Low left ventricular ejection fraction</td>
</tr>
<tr>
<td>Comorbidity (e.g., frailty, immunosuppression, renal or pulmonary disease)</td>
<td>Brain haemorrhage</td>
<td></td>
<td>Pulmonary hypertension</td>
</tr>
<tr>
<td></td>
<td>Septic shock</td>
<td></td>
<td>Large vegetations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Severe prosthetic valve dysfunction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Premature mitral valve closure and other signs of elevated diastolic pressures</td>
</tr>
</tbody>
</table>


Nowadays, 40–50% of patients undergo cardiac surgery during hospitalization. Surgical mortality in IE strongly depends on its indication. Among patients who need emergency or urgent surgery, septic shock, persistent signs of infection and renal failure are predictors of mortality. Predictably, patients with an indication for surgery who cannot proceed due to prohibitive surgical risk have the worst prognosis.

### 7. Antimicrobial therapy: principles and methods

#### 7.1 General principles

Successful treatment of IE relies on microbial eradication by antimicrobial drugs. Surgery contributes by removing infected material and draining abscesses. Host defences are of little help. This explains why bactericidal regimes are more effective than bacteriostatic therapy, both in animal experiments and in humans. Aminoglycosides synergize with cell-wall inhibitors (i.e. beta-lactams and glycopeptidases) for bactericidal activity and are useful for shortening the duration of therapy (e.g. oral streptococci) and eradicating problematic organisms (e.g. Enterococcus spp.).

One major hindrance to drug-induced killing is bacterial antibiotic tolerance. Tolerant microbes are not resistant (i.e. they are still susceptible to growth inhibition by the drug) but escape drug-induced killing and may resume growth after treatment discontinuation. Slow-growing and dormant microbes display phenotypic tolerance towards most antimicrobials (except rifampin to some extent). They are present in vegetations and biofilms (e.g. in PVE) and justify the need for prolonged therapy (6 weeks) to fully sterilize infected heart valves. Some bacteria carry mutations rendering them tolerant during both active growth and stationary (dormant) phases. Bactericidal drug combinations are preferred to monotherapy against tolerant organisms.

Drug treatment of PVE should last longer (at least 6 weeks) than that of native valve endocarditis (NVE) (2–6 weeks), but is otherwise similar, except for staphylococcal PVE, where the regimen should include rifampin whenever the strain is susceptible.

In NVE needing valve replacement by a prosthesis during antibiotic therapy, the postoperative antibiotic regimen should be that recommended for NVE, not for PVE. In both NVE and PVE, the duration of treatment is based on the first day of effective antibiotic therapy (negative blood culture in the case of initial positive blood culture), not on the day of surgery. A new full course of treatment should only start if valve cultures are positive, with the choice of antibiotic being based on the susceptibility of the latest recovered bacterial isolate.

Finally, there are six important considerations in the current recommendations:

1. The indications and pattern of use of aminoglycosides have changed. They are no longer recommended in staphylococcal NVE because their clinical benefits have not been demonstrated, but they can increase renal toxicity, when they are indicated in other conditions, aminoglycosides should be given in a single daily dose to reduce nephrotoxicity.
susceptible streptococci and normal renal function. Ceftriaxone and netilmicin can be given once daily in patients with IE due to beta-haemolytic streptococci137–139 if desensitization cannot be performed, patients allergic to beta-lactams should receive vancomycin. Teicoplanin has been proposed as an alternative,8 but requires loading doses (6 mg/kg/12 h for 3 days) followed by 6–10 mg/kg/day. Loading is critical because the drug is highly bound (>98%) to serum proteins and penetrates slowly into vegetables.140 However, only limited retrospective studies have assessed its efficacy in streptococcal IE.

Daptomycin and fosfomycin have been recommended for treating staphylococcal endocarditis and netilmicin for treating penicillin-susceptible oral and digestive streptococci, but they are considered alternative therapies in these guidelines because they are not available in all European countries. When daptomycin is indicated, it must be given at high doses (≥10 mg/kg once daily132) and combined with a second antibiotic to increase activity and avoid the development of resistance.133,134

Only published antibiotic efficacy data from clinical trials and cohort studies in patients with endocarditis (or bacteraemia if there are no endocarditis data) have been considered in these guidelines. Data from experimental endocarditis models have not been taken into account in most cases.

We are still using the Clinical and Laboratory Standards Institute minimum inhibitory concentration (MIC) breakpoints instead of the European Committee on Antimicrobial Susceptibility Testing ones because most endocarditis data are derived from studies using the former breakpoints.

Although a consensus was obtained for the majority of antibiotic treatments, the optimal treatment of staphylococcal IE and the empirical treatment are still debated.

7.2 Penicillin-susceptible oral streptococci and Streptococcus bovis group

Recommended regimens against susceptible streptococci (penicillin MIC ≤0.125 mg/L) are summarized in Table 16.6,8,135,136 The cure rate is expected to be >95%. In uncomplicated cases, short-term 2-week therapy can be administered by combining penicillin or ceftriaxone with gentamicin or netilmicin.137,138 Gentamicin and netilmicin can be given once daily in patients with IE due to susceptible streptococci and normal renal function. Ceftriaxone alone or combined with gentamicin or netilmicin given once a day is particularly convenient for outpatient therapy.137–139 If desensitization cannot be performed, patients allergic to beta-lactams should receive vancomycin. Teicoplanin has been proposed as an alternative,8 but requires loading doses (6 mg/kg/12 h for 3 days) followed by 6–10 mg/kg/day. Loading is critical because the drug is highly bound (>98%) to serum proteins and penetrates slowly into vegetables.140 However, only limited retrospective studies have assessed its efficacy in streptococcal141 and enterococcal142 IE.

7.3 Penicillin-resistant oral streptococci and Streptococcus bovis group

Penicillin-resistant oral streptococci are classified as intermediate resistant (MIC 0.25–2 mg/L) and fully resistant (MIC ≥4 mg/L). However, some guidelines consider an MIC >0.5 mg/L as fully resistant.6,8,135 Such resistant streptococci are increasing in number. Large strain collections have reported >30% of intermediate- and fully resistant Streptococcus mitis and Streptococcus oralis.142,143 Conversely, >99% of digestive streptococci remain penicillin susceptible.

Treatment guidelines for penicillin-resistant streptococcal IE rely on retrospective series. Compiling four of them, 47 of 60 patients (78%) were treated with penicillin or ceftriaxone, mostly combined with aminoglycosides, and some with either clindamycin or aminoglycosides alone.144–147 Most penicillin MICs were ≥1 mg/L. Fifty patients (83%) were cured and 10 (17%) died. Death was not related to resistance, but to the patients’ underlying conditions.146 Treatment outcomes were similar in PVE and NVE.145 Hence antibiotic therapy for penicillin-resistant and penicillin-susceptible oral streptococci is qualitatively similar (Table 16). However, in penicillin-resistant cases, aminoglycoside treatment must be given for at least 2 weeks and short-term therapy regimens are not recommended. Little experience exists with highly resistant isolates (MIC ≥4 mg/L), but vancomycin might be preferred in such circumstances (combined with aminoglycosides). There is very limited experience with daptomycin.

7.4 Streptococcus pneumoniae, beta-haemolytic streptococci (groups A, B, C, and G)

IE due to S. pneumoniae has become rare since the introduction of antibiotics. It is associated with meningoitis in up to 30% of cases,149 which requires special consideration in cases with penicillin resistance. Treatment of penicillin-susceptible strains (MIC ≤0.06 mg/L) is similar to that of oral streptococci (Table 16), except for the use of short-term 2-week therapy, which has not been formally investigated. The same holds true for penicillin intermediate (MIC 0.125–2 mg/L) or resistant strains (MIC ≥4 mg/L) without meningoitis, although for resistant strains some authors recommend high doses of cephalosporins (e.g. cefotaxime or ceftriaxone) or vancomycin. In cases with meningoitis, penicillin must be avoided because of its poor penetration of the cerebrospinal fluid, and should be replaced with ceftriaxone or cefotaxime alone or in association with vancomycin according to the antibiotic susceptibility pattern.

IE due to group A, B, C, or G streptococci—including Streptococcus anginosus group (S. constellatus, S. anginosus, and S. intermedius)—is relatively rare.151 Group A streptococci are uniformly susceptible to beta-lactams (MIC ≤0.12 mg/L), whereas other serogroups may display some degree of resistance. IE due to group B streptococci was once associated with the peripartum period, but it now occurs in other adults, especially the elderly. Group B, C, and G streptococci and S. anginosus produce abscesses and thus may require adjunctive surgery.151 Mortality from group B PVE is very high and cardiac surgery is recommended.152 Antibiotic treatment is similar to that of oral streptococci (Table 16), except that short-term therapy is not recommended. Gentamicin should be given for 2 weeks.
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage and route</th>
<th>Duration (weeks)</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains penicillin-susceptible (MIC ≤ 0.125 mg/L) oral and digestive streptococci</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Standard treatment: 4-week duration</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G <strong>or</strong> Amoxicillin or <strong>Ceftriaxone</strong></td>
<td>12–18 million U/day i.v. either in 4–6 doses or continuously</td>
<td>4</td>
<td>I</td>
<td>B</td>
<td>6,8, 135–139</td>
<td>Preferred in patients &gt; 65 years or with impaired renal or VIII (vestibulocochlear) cranial nerve functions. 6-week therapy recommended for patients with PVE</td>
</tr>
<tr>
<td><strong>Paediatric doses</strong>:</td>
<td>Penicillin G 200,000 U/kg/day i.v. in 4–6 divided doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amoxicillin 300 mg/kg/day i.v. in 4–6 equally divided doses</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone 100 mg/kg/day i.v. or i.m. in 1 dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Standard treatment: 2-week duration</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G <strong>or</strong> Amoxicillin <strong>or</strong> Ceftriaxone</td>
<td>12–18 million U/day i.v. either in 4–6 doses or continuously</td>
<td>2</td>
<td>I</td>
<td>B</td>
<td>6,8, 127, 135–138</td>
<td>Only recommended in patients with non-complicated NVE with normal renal function.</td>
</tr>
<tr>
<td><strong>Paediatric doses</strong>:</td>
<td>Penicillin G, amoxicillin, and ceftriaxone as above</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gentamicin 3 mg/kg/day i.v. or i.m. in 1 dose</td>
<td></td>
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<tr>
<td></td>
<td>Netilmicin 4–5 mg/kg/day i.v. in 1 dose</td>
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<tr>
<td><strong>In beta-lactam allergic patients</strong>:</td>
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<tr>
<td>Vancomycin</td>
<td>30 mg/kg/day i.v. in 2 doses</td>
<td>4</td>
<td>I</td>
<td>C</td>
<td></td>
<td>6-week therapy recommended for patients with PVE</td>
</tr>
<tr>
<td><strong>Paediatric doses</strong>:</td>
<td>Vancomycin 40 mg/kg/day i.v. in 2 or 3 equally divided doses</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Strains relatively resistant to penicillin (MIC 0.250–2 mg/L)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Standard treatment</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin G <strong>or</strong> Amoxicillin <strong>or</strong> Ceftriaxone <strong>combined with</strong> Gentamicin</td>
<td>24 million U/day i.v. either in 4–6 doses or continuously</td>
<td>4</td>
<td>I</td>
<td>B</td>
<td>6,8, 135, 136</td>
<td>6-week therapy recommended for patients with PVE</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg/day i.v. in 4–6 doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 g/day i.v. or i.m. in 1 dose</td>
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<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In beta-lactam allergic patients</strong>:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin <strong>with</strong> Gentamicin</td>
<td>30 mg/kg/day i.v. in 2 doses</td>
<td>4</td>
<td>I</td>
<td>C</td>
<td></td>
<td>6-week therapy recommended for patients with PVE</td>
</tr>
<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 dose</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

C<sub>min</sub> = minimum concentration; IE = infective endocarditis; i.m. = intramuscular; i.v. = intravenous; MIC = minimum inhibitory concentration; NVE = native valve endocarditis; PVE = prosthetic valve endocarditis; U = units.

*Refer to text for other streptococcal species; †Class of recommendation; ‡Level of evidence; †Reference(s) supporting recommendations; *Or ampicillin, same dosages as amoxicillin; †Preferred for outpatient therapy; ‡Paediatric doses should not exceed adult doses; §Renal function and serum gentamicin concentrations should be monitored once a week. When given in a single daily dose, pre-dose (trough) concentrations should be < 1 mg/L and post-dose (peak; 1 hours after injection) serum concentrations should be ~10–12 mg/L. 148. ‡Penicillin desensitization can be attempted in stable patients; †Serum vancomycin concentrations should achieve 10–15 mg/L at pre-dose (trough) level, although some experts recommend to increase the dose of vancomycin to 45–60 mg/kg/day i.v. in 2 or 3 divided doses to reach serum trough vancomycin levels (C<sub>min</sub>) of 15–20 mg/L, as in staphylococcal endocarditis. However, vancomycin dose should not exceed 2 g/d unless serum levels are monitored and can be adjusted to obtain a peak plasma concentration of 30–45 μg/mL 1 hour after completion of the i.v. infusion of the antibiotic; †Patients with penicillin-resistant strains (MIC > 2 mg/L) should be treated as enterococcal endocarditis (see Table 18).
7.5 *Granulicatella* and *Abiotrophia* (formerly nutritionally variant streptococci)

*Granulicatella* and *Abiotrophia* produce IE with a protracted course, which is associated with large vegetations (≥10 mm), higher rates of complications and valve replacement (around 50%), possibly due to delayed diagnosis and treatment. Antibiotic recommendations include penicillin G, ceftriaxone or vancomycin for 6 weeks, combined with an aminoglycoside for at least the first 2 weeks.153,154

7.6 *Staphylococcus aureus* and coagulase-negative staphylococci

*Staphylococcus aureus* is usually responsible for acute and destructive IE, whereas CoNS produce more protracted valve infections (except *S. lugdunensis*156 and some cases of *S. capitis*).156,157 Table 17 summarizes treatment recommendations for methicillin-susceptible and methicillin-resistant *S. aureus* and CoNS in both native and prosthetic valve IE. Of note, the addition of an aminoglycoside in staphylococcal native valve IE is no longer recommended because it increases renal toxicity.128,158 Short-term (2-week) and oral treatments have been proposed for uncomplicated right-sided native valve methicillin-susceptible *S. aureus* (MSSA) IE (see also section 12.4.2), but these regimens cannot be applied to left-sided IE. For penicillin-allergic patients with MSSA IE, penicillin desensitization might be attempted in stable patients since vancomycin is inferior to beta-lactams159 and should not be given. If beta-lactams cannot be given, where available, daptomycin should be chosen and given in combination with another effective antistaphylococcal drug to increase activity and avoid the development of resistance. Some experts have recommended a combination of high doses of cotrimoxazole plus clindamycin as an alternative for *S. aureus* IE.160 *S. lugdunensis* is always methicillin susceptible and can be treated with colistin.155

*Staphylococcus aureus* PVE carries a very high risk of mortality (>45%)161 and often requires early valve replacement. Other differences in comparison with NVE include the overall duration of therapy, the use of aminoglycosides and the addition of rifampin after 3–5 days of effective antibiotic therapy once the bacteremia has been cleared. The rationale supporting this recommendation is based on the antagonistic effect of the antibiotic combinations with rifampin against planktonic/replicating bacteria and the synergy seen against dormant bacteria within the biofilm, as it has been demonstrated in foreign body infection models and clinically in prosthetic orthopaedic and vascular infections. Although the level of evidence is poor, adding rifampin to the treatment of staphylococcal PVE is standard practice, although treatment may be associated with microbial resistance, hepatotoxicity and drug interactions.164

7.7 Methicillin-resistant and vancomycin-resistant staphylococci

Methicillin-resistant *S. aureus* (MRSA) produces low-affinity penicillin binding protein 2a (PBP2a), which confers cross-resistance to most beta-lactams. MRSA are usually resistant to multiple antibiotics, leaving only vancomycin and daptomycin to treat severe infections. However, vancomycin-intermediate *S. aureus* (MIC 4–8 mg/L) and hetero-vancomycin-intermediate *S. aureus* (MIC ≤2 mg/L, but with subpopulations growing at higher concentrations) have emerged worldwide and are associated with IE treatment failures.165,166 Moreover, some highly vancomycin-resistant *S. aureus* strains have been isolated from infected patients in recent years, requiring new approaches to treatment. In addition, a systematic review and meta-analysis of studies published between 1996 and 2011 in patients with MRSA bacteremia with vancomycin-susceptible strains (MIC ≤2 mg/L)167 showed that a high vancomycin MIC (≥1.5 mg/L) was associated with higher mortality. Daptomycin is a lipopeptide antibiotic approved for *S. aureus* bacteremia and right-sided IE.168 Cohort studies of *S. aureus* and CoNS IE128,168–170 have shown that daptomycin is at least as effective as vancomycin, and in two cohort studies of MRSA bacteremia with high vancomycin MICs (>1 mg/L),171,172 daptomycin was associated with better outcomes (including survival) compared with vancomycin. Importantly, daptomycin needs to be administered in appropriate doses and combined with other antibiotics to avoid further resistance in patients with IE.168,173 For this reason, daptomycin should be given at high doses (≥10 mg/kg), and most experts recommend it be combined with beta-lactams133 or fosfomycin134 (beta-lactams and (probably fosfomycin) increase membrane daptomycin binding by decreasing the positive surface charge) for NVE and with gentamicin and rifampin for PVE.168,173,174

Other alternatives include fosfomycin plus imipenem,175 newer beta-lactams with relatively good PBP2a affinity such as ceftaroline,176 quinupristin–dalfopristin with or without beta-lactams,177,178 beta-lactams plus oxazolidinones (linezolid),179 beta-lactams plus vancomycin180 and high doses of trimethoprim/sulfamethoxazole and clindamycin.160 Such cases warrant collaborative management with an ID specialist.

7.8 *Enterococcus* spp.

Enterococcal IE is primarily caused by *Enterococcus faecalis* (90% of cases) and, more rarely, by *Enterococcus faecium* (5% of cases or other species).151 They pose two major problems. First, enterococci are highly resistant to antibiotic-induced killing, and eradication requires prolonged administration (up to 6 weeks) of synergistic bactericidal combinations of two cell wall inhibitors (ampicillin plus ceftriaxone, which synergize by inhibiting complementary PBPs) or one cell wall inhibitor with aminoglycosides (Table 18). Second, they may be resistant to multiple drugs, including aminoglycosides [high-level aminoglycoside resistance (HLAR)], beta-lactams (via PBPs modification and sometimes beta-lactamases) and vancomycin.182

Fully penicillin-susceptible strains (penicillin MIC ≤8 mg/L) are treated with penicillin G or ampicillin (or amoxicillin) combined with gentamicin. Ampicillin (or amoxicillin) might be preferred since MICs are two to four times lower. Gentamicin resistance is frequent in both *E. faecalis* and *E. faecium*.182 An aminoglycoside MIC >500 mg/L (HLAR) is associated with the loss of bactericidal synergism with cell wall inhibitors, and aminoglycosides should not be used in such conditions. Streptomycin may remain active in such cases and is a useful alternative.
**Table 17** Antibiotic treatment of infective endocarditis due to Staphylococcus spp.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage and route</th>
<th>Duration (weeks)</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Native valves</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Methicillin-susceptible staphylococci</td>
<td>(flu)cloxacillin or oxacillin</td>
<td>12 g/d i.v. in 4–6 doses</td>
<td>4–6</td>
<td>I</td>
<td>B</td>
<td>6.8, 128, 133, 136, 138</td>
</tr>
<tr>
<td></td>
<td>Paediatric doses:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gentamicin addition is not recommended because clinical benefit has not been demonstrated and there is increased renal toxicity</td>
</tr>
<tr>
<td></td>
<td>200–300 mg/kg/day i.v. in 4–6 equally divided doses</td>
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<tr>
<td>Alternative therapy*</td>
<td>Cotrimoxazole*</td>
<td>1800 mg/day i.v. in 3 doses</td>
<td>1</td>
<td>IIb</td>
<td>C</td>
<td>for Staphylococcus aureus</td>
</tr>
<tr>
<td>with Clindamycin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Paediatric doses:</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Sulfamethoxazole 4800 mg/day and Trimethoprim 960 mg/day (i.v. in 4–6 doses)</td>
<td>1 iv. + 5 oral intake</td>
<td>IIb</td>
<td>C</td>
<td>for Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1800 mg/day i.v. in 3 doses</td>
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</tr>
<tr>
<td><em><em>Penicillin-allergic patients</em> or methicillin-resistant staphylococci</em>*</td>
<td>Vancomycinb *</td>
<td>30–60 mg/kg/day i.v. in 2–3 doses</td>
<td>4–6</td>
<td>I</td>
<td>B</td>
<td>Cephalosporins (cefazolin 6 g/day or cefotaxime 6 g/day i.v. in 3 doses) are recommended for penicillin-allergic patients with non-anaphylactic reactions with methicillin-susceptible endocarditis</td>
</tr>
<tr>
<td>with Clindamycin</td>
<td></td>
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<td></td>
<td>Paediatric doses:</td>
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<tr>
<td></td>
<td>40 mg/kg/day i.v. in 2–3 equally divided doses</td>
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</tr>
<tr>
<td>Alternative therapy*</td>
<td>Daptomycinc,d</td>
<td>10 mg/kg/day i.v. once daily</td>
<td>4–6</td>
<td>I</td>
<td>A</td>
<td>Daptomycin is superior to vancomycin for MSSA and MRSA bacteraemia with vancomycin MIC &gt; 1 mg/L</td>
</tr>
<tr>
<td>with Clindamycin</td>
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<td></td>
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<td></td>
<td>Paediatric doses:</td>
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</tr>
<tr>
<td></td>
<td>10 mg/kg/day i.v. once daily</td>
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<tr>
<td>Alternative therapy*</td>
<td>Cotrimoxazole*</td>
<td>1800 mg/day i.v. in 3 doses</td>
<td>1</td>
<td>IIb</td>
<td>C</td>
<td>for Staphylococcus aureus</td>
</tr>
<tr>
<td>with Clindamycin</td>
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<td></td>
<td>Paediatric doses:</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole 4800 mg/day and Trimethoprim 960 mg/day (i.v. in 4–6 doses)</td>
<td>1 iv. + 5 oral intake</td>
<td>IIb</td>
<td>C</td>
<td>for Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1800 mg/day i.v. in 3 doses</td>
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<tr>
<td><strong>Prosthetic valves</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Methicillin-susceptible staphylococci</td>
<td>(flu)cloxacillin or oxacillin with Rifampin* and Gentamicin</td>
<td>12 g/d i.v. in 4–6 doses</td>
<td>≥ 6</td>
<td>I</td>
<td>B</td>
<td>6.8, 128, 133, 136</td>
</tr>
<tr>
<td></td>
<td>Paediatric doses:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Starting rifampin 3–5 days later than vancomycin and gentamicin has been suggested by some experts. Gentamicin can be given in a single daily dose in order to reduce renal toxicity</td>
</tr>
<tr>
<td></td>
<td>900–1200 mg i.v. or orally in 2 or 3 divided doses</td>
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<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 or 2 doses</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Paediatric doses:</td>
<td>Oxaclillin and (flu)cloxacillin as above</td>
<td>3 mg/kg/day i.v. or orally in 3 equally divided doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin-allergic patients* and methicillin-resistant staphylococci</td>
<td>Vancomycinb with Rifampin* and Gentamicin</td>
<td>30–60 mg/kg/day i.v. in 2–3 doses</td>
<td>≥ 6</td>
<td>I</td>
<td>B</td>
<td>6.8, 128, 133, 136</td>
</tr>
<tr>
<td></td>
<td>Paediatric doses:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Starting rifampin 3–5 days later than vancomycin and gentamicin has been suggested by some experts. Gentamicin can be given in a single daily dose in order to reduce renal toxicity</td>
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<tr>
<td></td>
<td>900–1200 mg i.v. or orally in 2 or 3 divided doses</td>
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</tr>
<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 or 2 doses</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

AUC = area under the curve; Cmin = minimum concentration; IE = infective endocarditis; MIC = minimum inhibitory concentration; MRSA = methicillin-resistant Staphylococcus aureus; MSSA = methicillin-susceptible S. aureus; PVE = prosthetic valve endocarditis.

*Renal function, serum Cotrimoxazole concentrations should be monitored once/week (twice/week in patients with renal failure); Serum trough vancomycin levels (Cmin) should be ≥20 mg/L. A vancomycin AUC/MIC > 400 is recommended for MRSA infections; Monitor plasma CPAK levels at least once a week. Some experts recommend adding cloxacillin (2 g/4 h i.v.) or fosfomycin (2 g/6 h i.v.) to daptomycin in order to increase activity and avoid the development of daptomycin resistance; Daptomycin and fosfomycin are not available in some European countries; Rifampin is believed to play a special role in prosthetic device infection because it helps eradicate bacteria attached to foreign material. Starting rifampin is associated with a high frequency of microbial resistance and is not recommended. Rifampin increases the hepatic metabolism of warfarin and other drugs; Renal function and serum gentamicin concentrations should be monitored once/week (twice/week in patients with renal failure); Paediatric doses should not exceed adult doses; Penicillin desensitization can be attempted in stable patients; Class of recommendation; Level of evidence; Reference(s) supporting recommendations. No clinical benefit of adding rifampicin or gentamicin.
There have been two important advances in recent years. First is the demonstration, in several cohort studies of Enterococcus faecalis IE including hundreds of cases, that ampicillin plus ceftriaxone is as effective as ampicillin plus gentamicin for non-HLAR Enterococcus faecalis IE. It is also safer, without any nephrotoxicity.183–185 In addition, this is the combination of choice for treating HLAR Enterococcus faecalis IE. Second, the total daily dose of gentamicin can be given in a single daily dose instead of the two or three divided doses recommended up to now, and the length of the treatment for non-HLAR Enterococcus faecalis IE may be safely shortened from 4–6 weeks to 2 weeks, reducing the rates of nephrotoxicity to very low levels.129,136,186

Beta-lactam and vancomycin resistance are mainly observed in Enterococcus faecium. Since dual resistance is rare, beta-lactam might be used against vancomycin-resistant strains and vice versa. Varying results have been reported with quinupristin–dalfopristin (not active against Enterococcus faecalis), linezolid, daptomycin (combined with ampicillin, ertapenem or ceftaroline) and tigecycline. Again, these situations require the expertise of an ID specialist.

### Table 18: Antibiotic treatment of infective endocarditis due to Enterococcus spp.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage and route</th>
<th>Duration, weeks</th>
<th>Class*</th>
<th>Level*</th>
<th>Ref.†</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>**Beta-lactam and gentamicin-susceptible strains (for resistant isolates see <strong>a,b,c)</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin* with Gentamicin§</td>
<td>200 mg/kg/day i.v. in 4–6 doses</td>
<td>4–6</td>
<td>I</td>
<td>B</td>
<td>6.8, 129, 135, 136, 186</td>
<td>6-week therapy recommended for patients with &gt;3 months symptoms or PVE</td>
</tr>
<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 dose</td>
<td>2–6**</td>
<td>I</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paediatric doses:*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Ampicillin 300 mg/kg/day i.v. in 4–6 equally divided doses Gentamicin 3 mg/kg/day i.v. or i.m. in 3 equally divided doses</td>
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<td></td>
</tr>
<tr>
<td>Amoxicillin as above Ceftriaxone 100 mg/kg/12 h i.v. or i.m.</td>
<td></td>
<td>6</td>
<td>I</td>
<td>B</td>
<td>183–185</td>
<td>This combination is active against Enterococcus faecalis strains with and without HLAR, being the combination of choice in patients with HLAR E. faecalis endocarditis. This combination is not active against E. faecium</td>
</tr>
<tr>
<td>Amoxicillin* with Ceftriaxone</td>
<td>200 mg/kg/day i.v. in 4–6 doses</td>
<td>6</td>
<td>I</td>
<td>B</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4 g/day i.v. or i.m. in 2 doses</td>
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<tr>
<td>Paediatric doses:*</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Amoxicillin as above Ceftriaxone 100 mg/kg/12 h i.v. or i.m.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin* with Gentamicin§</td>
<td>30 mg/kg/day i.v. in 2 doses</td>
<td>6</td>
<td>I</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mg/kg/day i.v. or i.m. in 1 dose</td>
<td>6</td>
<td>I</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paediatric doses:*</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Vancomycin 40 mg/kg/day i.v. in 2–3 equally divided doses. Gentamicin as above</td>
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</tbody>
</table>

HLAR: high-level aminoglycoside resistance; IE: infective endocarditis; MIC: minimum inhibitory concentration; PBP: penicillin binding protein; PVE: prosthetic valve endocarditis.

*aHigh-level resistance to gentamicin (MIC > 500 mg/L): if susceptible to streptomycin, replace gentamicin with streptomycin 15 mg/kg/day in two equally divided doses.

*bBeta-lactam resistance: (i) if due to beta-lactamase production, replace ampicillin with amoxicillin–sulbactam or amoxicillin with amoxicillin–clavulanate; (ii) if due to PBP alteration, use vancomycin-based regimens.

*cMultiresistance to aminoglycosides, beta-lactams and vancomycin: suggested alternatives are (i) daptomycin 10 mg/kg/day plus ampicillin 200 mg/kg/day i.v. in four to six doses; (ii) linezolid 2 × 600 mg/day i.v. or orally for ≥8 weeks (IIa, C) (monitor haematological toxicity); (iii) quinupristin–dalfopristin 3 × 7.5 mg/kg/day for ≥8 weeks. Quinupristin–dalfopristin is not active against Enterococcus faecalis; (iv) for other combinations (daptomycin plus ertapenem or ceftaroline), consult infectious diseases specialists.

*dMonitor serum levels of aminoglycosides and renal function as indicated in Table 16.

*ePaediatric doses should not exceed adult doses.

*fMonitor serum vancomycin concentrations as stated in Table 16.

*gClass of recommendation.

*hLevel of evidence.

†Reference(s) supporting recommendations.

§Or ampicillin, same dosages as amoxicillin.

**Some experts recommend giving gentamicin for only 2 weeks (IIa, B).
weeks in PVE. If they do not produce beta-lactamase, ampicillin (12 g/day i.v. in four or six doses) plus gentamicin (3 mg/kg/day divided into two or three doses) for 4–6 weeks is an option. Ciprofloxacin (400 mg/8–12 h i.v. or 750 mg/12 h orally) is a less well-validated alternative.\textsuperscript{180,189}

### 7.9.2 Non-HACEK species

The International Collaboration on Endocarditis (ICE) reported non-HACEK Gram-negative bacteria in 49 of 2761 (1.8%) IE cases.\textsuperscript{190} Recommended treatment is early surgery plus long-term therapy with bactericidal combinations of beta-lactams and aminoglycosides, sometimes with additional quinolones or cotrimoxazole. In vitro bactericidal tests and monitoring of serum antibiotic concentrations may be helpful. Because of their rarity and severity, these conditions should be discussed by the Endocarditis Team or with an ID specialist.

### 7.10 Blood culture–negative infective endocarditis

The main causes of BCNIE are summarized in section 5.4.2.\textsuperscript{191,192} Treatment options are summarized in Table 19.\textsuperscript{192,193} Consultation with an ID specialist from the Endocarditis Team is recommended.

### 7.11 Fungi

Fungi are most frequently observed in PVE and in IE affecting i.v. drug abusers (IVDAs) and immunocompromised patients.\textsuperscript{190} Candida and Aspergillus spp. predominate, the latter resulting in BCNIE.\textsuperscript{199,200} Mortality is very high (>50%), and treatment necessitates combined antifungal administration and surgical valve replacement.\textsuperscript{135,198–200} Antifungal therapy for Candida IE includes liposomal amphotericin B (or other lipid formulations) with or without flucytosine or an echinocandin at high doses; and for Aspergillus IE, voriconazole is the drug of choice and some experts recommend the addition of an echinocandin or amphotericin B.\textsuperscript{135,198,200,201}Suppressive long-term treatment with oral azoles (fluconazole for Candida and voriconazole for Aspergillus) is recommended, sometimes for life.\textsuperscript{135,198,201} Consultation with an ID specialist from the Endocarditis Team is recommended.

### 7.12 Empirical therapy

Treatment of IE should be started promptly. Three sets of blood cultures should be drawn at 30-min intervals before initiation of antibiotics.\textsuperscript{192} The initial choice of empirical treatment depends on several considerations:

1. Whether the patient has received previous antibiotic therapy,
2. Whether the infection affects a native valve or a prosthesis [and if so, when surgery was performed (early vs. late PVE)],
3. The place of the infection (community, nosocomial, or non-nosocomial healthcare-associated IE) and knowledge of the local epidemiology, especially for antibiotic resistance and specific genuine culture-negative pathogens (Table 19).
4. Cloxacillin/cefazolin administration is associated with lower mortality rates than other beta-lactams, including...

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**Table 19**  
Antibiotic treatment of blood culture-negative infective endocarditis (adapted from Brouqui et al.\textsuperscript{193})

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Proposed therapy*</th>
<th>Treatment outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brucella</em> spp.</td>
<td>Doxycycline (200 mg/24 h) plus cotrimoxazole (960 mg/12 h) plus rifampin (300–600/24 h) for ≥3–6 months* orally</td>
<td>Treatment success defined as an antibody titre &lt;1:60. Some authors recommend adding gentamicin for the first 3 weeks.</td>
</tr>
<tr>
<td><em>C. burnetii</em> (agent of Q fever)</td>
<td>Doxycycline (200 mg/24 h) plus hydroxychloroquine (200–600 mg/24 h)* orally (≥18 months of treatment)</td>
<td>Treatment success defined as anti-phase 1 IgG titre &lt;1:200, and IgA and IgM titres &lt;1:50.</td>
</tr>
<tr>
<td><em>Bartonella</em> spp.*</td>
<td>Doxycycline 100 mg/12 h orally for 4 weeks plus gentamicin (3 mg/24 h) i.v. for 2 weeks</td>
<td>Treatment success expected in ≥90%.</td>
</tr>
<tr>
<td><em>Legionella</em> spp.</td>
<td>Levofoxacin (500 mg/12 h) i.v. or orally for ≥6 weeks or clarithromycin (500 mg/12 h) i.v. for 2 weeks, then orally for 4 weeks plus rifampin (300–1200 mg/24 h)</td>
<td>Optimal treatment unknown.</td>
</tr>
<tr>
<td><em>Mycoplasma</em> spp.</td>
<td>Levofoxacin (500 mg/12 h) i.v. or orally for ≥6 months*</td>
<td>Optimal treatment unknown.</td>
</tr>
<tr>
<td><em>T. whipplei</em> (agent of Whipple’s disease)*</td>
<td>Doxycycline (200 mg/24 h) plus hydroxychloroquine (200–600 mg/24 h)* orally for ≥18 months</td>
<td>Long-term treatment, optimal duration unknown.</td>
</tr>
</tbody>
</table>

*Owing to the lack of large series, the optimal duration of treatment of IE due to these pathogens is unknown. The presented durations are based on selected case reports.

Consultation with an ID specialist is recommended.

Addition of streptomycin (15 mg/kg/24 h in 2 doses) for the first few weeks is optional.

Doxycycline plus hydroxychloroquine (with monitoring of serum hydroxychloroquine levels) is significantly superior to doxycycline.\textsuperscript{194}

Several therapeutic regimens have been reported, including aminopenicillins (ampicillin or amoxicillin, 12 g/24 h i.v.) or cephalosporins (ceftriaxone, 2 g/24 h i.v.) combined with aminoglycosides (gentamicin or netilmicin).\textsuperscript{195} Doses are as for streptomycoccal and enterococcal IE (Tables 16 and 18).\textsuperscript{196,197}

Newer fluoroquinolones (levofloxacin, moxifloxacin) are more potent than ciprofloxacin against intracellular pathogens such as *Mycoplasma* spp., *Legionella* spp., and *Chlamydia* spp.

Treatment of Whipple’s IE remains highly empirical. In the case of central nervous system involvement, sulfadiazine 1.5 g/6 h orally must be added to doxycycline. An alternative therapy is ceftriaxone (2 g/24 h i.v.) for 2–4 weeks or penicillin G (2 million U/h i.v.) and streptomycin (1 g/24 h) i.v. for 2–4 weeks followed by cotrimoxazole (800 mg/12 h) orally.

Trimethoprim is not active against *T. whipplei*. Successes have been reported with long-term therapy (>1 year).
Suggested regimens for empirical treatment in acute patients are summarized in Table 20. NVE and late PVE regimens should cover staphylococci, streptococci and enterococci. Early PVE or healthcare-associated IE regimens should cover methicillin-resistant staphylococci, enterococci and, ideally, non-HACEK Gram-negative pathogens. Once the pathogen is identified (usually in <48 h), the antibiotic treatment must be adapted to its antimicrobial susceptibility pattern.

### 7.13 Outpatient parenteral antibiotic therapy for infective endocarditis

Outpatient parenteral antibiotic therapy (OPAT) is used to consolidate antimicrobial therapy once critical infection-related complications are under control (e.g., perivalvular abscesses, acute HF, septic emboli and stroke). Two different phases may be identified during the course of antibiotic therapy: (i) a first critical phase (the first 2 weeks of therapy), during which OPAT has a restricted indication; and (ii) a second, continuation phase (beyond 2 weeks of therapy), where OPAT may be feasible. Table 21 summarizes the salient questions to address when considering OPAT for IE.

### Table 20 Proposed antibiotic regimens for initial empirical treatment of infective endocarditis in acute severely ill patients (before pathogen identification)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage and route</th>
<th>Class</th>
<th>Level</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Community-acquired native valves or late prosthetic valves (&gt;12 months post surgery) endocarditis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin with (Fl)cloxacillin or oxacillin with Gentamicin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12 g/day i.v. in 4–6 doses</td>
<td>IIa</td>
<td>C</td>
<td>Patients with BCNIE should be treated in consultation with an ID specialist.</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;d&lt;/sup&gt; with Gentamicin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30–60 mg/kg/day i.v. in 2–3 doses</td>
<td>IIb</td>
<td>C</td>
<td>For penicillin-allergic patients</td>
</tr>
<tr>
<td><strong>Early PVE (&lt;12 months post surgery) or nosocomial and non-nosocomial healthcare associated endocarditis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;d&lt;/sup&gt; with Gentamicin&lt;sup&gt;d&lt;/sup&gt; with Rifampin</td>
<td>900–1200 mg i.v. or orally in 2 or 3 divided doses</td>
<td>IIb</td>
<td>C</td>
<td>Rifampin is only recommended for PVE and it should be started 3–5 days later than vancomycin and gentamicin has been suggested by some experts. In healthcare associated native valve endocarditis, some experts recommend in settings with a prevalence of MRSA infections &gt;5% the combination of cloxacillin plus vancomycin until they have the final S. aureus identification</td>
</tr>
</tbody>
</table>

BCNIE = blood culture-negative infective endocarditis; ID = infectious disease; i.m. = intramuscular; i.v. = intravenous; PVE = prosthetic valve endocarditis.

<sup>a</sup>Initial blood cultures are negative and there is no clinical response, consider BCNIE aetiology (see Section 7.10) and maybe surgery for molecular diagnosis and treatment, and extension of the antibiotic spectrum to blood culture-negative pathogens (doxycycline, quinolones) must be considered.

<sup>b</sup>Class of recommendation.

<sup>c</sup>Level of evidence.

<sup>d</sup>Monitoring of gentamicin or vancomycin dosages is as described in Tables 16 and 17.

---

**Table 21 Criteria that determine suitability of outpatient parenteral antibiotic therapy for infective endocarditis (adapted from Andrews et al.**

<table>
<thead>
<tr>
<th>Phase of treatment</th>
<th>Guidelines for use</th>
</tr>
</thead>
</table>
| **Critical phase (weeks 0–2)** | • Complications occur during this phase  
• Preferred inpatient treatment during this phase  
• Consider OPAT if oral streptococci or Streptococcus bovis<sup>2</sup> native valve<sup>3</sup> patient stable, no complications |
| **Continuation phase (beyond week 2)** | • Consider OPAT if medically stable  
• Do not consider OPAT if HF; concerning echocardiographic features, neurological signs, or renal impairment |
| **Essential for OPAT** | • Educate patient and staff  
• Regular post-discharge evaluation (nurses 1/day, physician in charge 1 or 2/week)<sup>4</sup>  
• Prefer physician-directed programme, not home-infusion model |

HF = heart failure; ID = infectious disease; IE = infective endocarditis; OPAT = outpatient parenteral antibiotic therapy; PVE = prosthetic valve endocarditis.

<sup>2</sup>For other pathogens, consultation with an ID specialist is recommended.

<sup>3</sup>For patients with late PVE; consultation with an ID specialist is recommended.

<sup>4</sup>General physician can see the patient once a week, if needed.
8. Main complications of left-sided valve infective endocarditis and their management

Surgical treatment is required in approximately half of the patients with IE because of severe complications. Reasons to consider early surgery in the active phase (i.e. while the patient is still receiving antibiotic treatment) are to avoid progressive HF and irreversible structural damage caused by severe infection and to prevent systemic embolism. On the other hand, surgical therapy during the active phase of the disease is associated with significant risk. Surgery is justified in patients with high-risk features that make the possibility of cure with antibiotic treatment unlikely and who do not have co-morbid conditions or complications that make the prospect of recovery remote. Age per se is not a contraindication to surgery.

Early consultation with a cardiac surgeon is recommended in order to determine the best therapeutic approach. Identification of patients requiring early surgery is frequently difficult and is an important objective of the 'Heart Team'. Each case must be individualized and all factors associated with increased risk identified at the time of diagnosis. Frequently the need for surgery will be determined by a combination of several high-risk features.

In some cases, surgery needs to be performed on an emergency (within 24 h) or urgent (within a few days, <7 days) basis, irrespective of the duration of antibiotic treatment. In other cases, surgery can be postponed to allow 1 or 2 weeks of antibiotic treatment under careful clinical and echocardiographic observation before an elective surgical procedure is performed. The three main indications for early surgery in IE are HF, uncontrolled infection and prevention of embolic events (Table 22).

Table 22  Indications and timing of surgery in left-sided valve infective endocarditis (native valve endocarditis and prosthetic valve endocarditis)

<table>
<thead>
<tr>
<th>Indications for surgery</th>
<th>Timinga</th>
<th>Classb</th>
<th>Levelc</th>
<th>Ref.d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic or mitral NVE or PVE with severe acute regurgitation, obstruction or fistula causing refractory pulmonary oedema or cardiogenic shock</td>
<td>Emergency</td>
<td>I</td>
<td>B</td>
<td>111,115, 213,216</td>
</tr>
<tr>
<td>Aortic or mitral NVE or PVE with severe regurgitation or obstruction causing symptoms of HF or echocardiographic signs of poor haemodynamic tolerance</td>
<td>Urgent</td>
<td>I</td>
<td>B</td>
<td>37,115, 209,216, 220,221</td>
</tr>
<tr>
<td>2. Uncontrolled infection (abscess, false aneurysm, fistula, enlarging vegetation)</td>
<td>Urgent</td>
<td>I</td>
<td>B</td>
<td>37,209, 216</td>
</tr>
<tr>
<td>Infection caused by fungi or multiresistant organisms</td>
<td>Urgent/ elective</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Persisting positive blood cultures despite appropriate antibiotic therapy and adequate control of septic metastatic foci</td>
<td>Urgent</td>
<td>IIa</td>
<td>B</td>
<td>123</td>
</tr>
<tr>
<td>PVE caused by staphylococci or non-HACEK gram-negative bacteria</td>
<td>Urgent/ elective</td>
<td>IIa</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3. Prevention of embolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic or mitral NVE or PVE with persistent vegetations &gt;10 mm after one or more embolic episode despite appropriate antibiotic therapy</td>
<td>Urgent</td>
<td>I</td>
<td>B</td>
<td>9,58,72, 113,222</td>
</tr>
<tr>
<td>Aortic or mitral NVE with vegetations &gt;10 mm, associated with severe valve stenosis or regurgitation, and low operative risk</td>
<td>Urgent</td>
<td>IIa</td>
<td>B</td>
<td>9</td>
</tr>
<tr>
<td>Aortic or mitral NVE or PVE with isolated very large vegetations (&gt;30 mm)</td>
<td>Urgent</td>
<td>IIa</td>
<td>B</td>
<td>113</td>
</tr>
<tr>
<td>Aortic or mitral NVE or PVE with isolated large vegetations (&gt;15 mm) and no other indication for surgery*</td>
<td>Urgent</td>
<td>IIb</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

HACEK = Haemophilus parainfluenzae, Haemophilus aphrophilus, Haemophilus paraphrophilus, Haemophilus influenzae, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae and Kingella denitrificans; HF = heart failure; IE = infective endocarditis; NVE = native valve endocarditis; PVE = prosthetic valve endocarditis.

*Emergency surgery: surgery performed within 24 h; urgent surgery: within a few days; elective surgery: after at least 1–2 weeks of antibiotic therapy.

1Class of recommendation.
2Level of evidence.
3Reference(s) supporting recommendations.
4Surgery may be preferred if a procedure preserving the native valve is feasible.
or worsening severe aortic or mitral regurgitation, although intracardiac fistulae and, more rarely, valve obstruction may also lead to HF.

Valvar regurgitation in native IE may occur as a result of mitral chordal rupture, leaflet rupture (flail leaflet), leaflet perforation or interference of the vegetation mass with leaflet closure. A particular situation is infection of the anterior mitral leaflet secondary to an infected regurgitant jet of a primary aortic IE. Resultant aneurysm formation on the atrial side of the mitral leaflet may later lead to mitral perforation.

Clinical presentation of HF may include dyspnoea, pulmonary oedema and cardiogenic shock. Among the large ICE Prospective Cohort Study patients with HF and IE, 66% were in New York Heart Association class III or IV. In addition to clinical findings, TTE is of crucial importance for initial evaluation and follow-up.

Valve perforation, secondary mitral lesions and aneurysms are best assessed using TOE. Echocardiography is also useful to evaluate the haemodynamic consequences of valvar dysfunction, measurement of pulmonary artery pressure, detection of pericardial effusion and assessment and monitoring of left ventricular systolic function and left and right heart filling pressures. B-type natriuretic peptide has potential use in the diagnosis and monitoring of HF in IE. Both elevated levels of cardiac troponins and B-type natriuretic peptide are associated with adverse outcomes in IE. Moderate to severe HF is the most important predictor of in-hospital, 6-month and 1-year mortality.

8.2.1 Persisting infection

The definition of persisting infection is arbitrary and consists of fever and persisting positive cultures after 7–10 days of antibiotic treatment. Persisting fever is a frequent problem observed during treatment of IE. Usually, temperature normalizes within 7–10 days under specific antibiotic therapy. Persisting fever may be related to several factors, including inadequate antibiotic therapy, resistant organisms, persisting lines, locally uncontrolled infection, embolic complications or extracardiac site of infection and adverse reaction to antibiotics. Management of persisting fever includes replacement of i.v. lines, repeat laboratory measurements, blood cultures, echocardiography, and the search for an intracardiac or extracardiac focus of infection.

8.2.2 Perivalvular extension in infective endocarditis

Perivalvular extension of IE is the most frequent cause of uncontrolled infection and is associated with a poor prognosis and high likelihood of the need for surgery. Perivalvular complications include abscess formation, pseudoaneurysms and fistulae (defined in Table 11).

Perivalvular abscess is more common in aortic IE (10–40% in NVE and is frequent in PVE (56–100%)). In mitral IE, perivalvular abscesses are usually located posteriorly or laterally. In aortic IE, perivalvular extension occurs most frequently in the mitral-aortic intervalvular fibrosa. Serial echocardiographic studies have shown that abscess formation is a dynamic process, starting with aortic root wall thickening and extending to the development of fistulae. In one study, the most important risk factors for perivalvular complications were prosthetic valve, aortic location and infection with CoNS.

Pseudoaneurysms and fistulae are severe complications of IE and are frequently associated with very severe valvular and perivalvular damage. The frequency of fistula formation in IE has been reported to be 1.6%, with S. aureus being the most commonly associated organism (46%).

Despite high rates of surgery in this population (87%), hospital mortality remains high (41%). Other complications due to major extension of infection are less frequent and may include ventricular septal defect, third-degree atrio-ventricular block and acute coronary syndrome. Perivalvular extension should be suspected in cases with persistent unexplained fever or new atrio-ventricular block. Therefore an electrocardiogram should be performed frequently during continuing treatment, particularly in aortic IE. TOE, MSCT and PET/CT are particularly useful for the diagnosis of perivalvular complications.
while the sensitivity of TTE is $<50\%$ (see section 5). Indeed, perivalvular extension is frequently discovered on a systematic TOE. However, small abscesses can be missed, even using TOE, particularly those in a mitral location when there is co-existent annular calcification.101

8.2.3 Indications and timing of surgery in the presence of uncontrolled infection in infective endocarditis (Table 22)

The results of surgery when the reason for the procedure is uncontrolled infection are worse than when surgery is performed for other reasons.124,235

8.2.3.1 Persistent infection

In some cases of IE, antibiotics alone are insufficient to eradicate the infection. Surgery has been indicated when fever and positive blood cultures persist for several days (7–10 days) despite an appropriate antibiotic regimen and when extracardiac abscesses (spleen, vertebral, cerebral or renal) and other causes of fever have been excluded. However, the best timing for surgery in this difficult situation is unclear. Recently it has been demonstrated that persistent blood cultures 48–72 h after initiation of antibiotics are an independent risk factor for hospital mortality.122 These results suggest that surgery should be considered when blood cultures remain positive after 3 days of antibiotic therapy, after the exclusion of other causes of persistent positive blood cultures (adapted antibiotic regimen).

8.2.3.2 Signs of locally uncontrolled infection

Signs of locally uncontrolled infection include increasing vegetation size, abscess formation, false aneurysms, and the creation of fistulae.213,236,237 Persistent fever is also usually present and surgery is recommended as soon as possible. Rarely when there are no other reasons for surgery and fever is easily controlled with antibiotics, small abscesses or false aneurysms can be treated conservatively under close clinical and echocardiographic follow-up.

8.2.3.3 Infection by microorganisms at low likelihood of being controlled by antimicrobial therapy

Surgery is indicated in fungal IE238,239 in cases of multiresistant organisms (e.g. MRSA or vancomycin-resistant enterococci) or in the rare infections caused by Gram-negative bacteria. Surgery should also be considered in PVE caused by staphylococci or non-HACEK Gram-negative bacteria. In NVE caused by S. aureus, surgery is indicated if a favourable early response to antibiotics is not achieved161,240,241 (Table 22). Finally, surgery should be performed in patients with PVE and S. aureus infection.

In summary, uncontrolled infection is most frequently related to perivalvular extension or ‘difficult-to-treat’ organisms. Unless severe co-morbidity exists, the presence of locally uncontrolled infection is an indication for early surgery in patients with IE.

8.3 Prevention of systemic embolism

8.3.1 Embolic events in infective endocarditis

Embolic events are a frequent and life-threatening complication of IE related to the migration of cardiac vegetations. The brain and spleen are the most frequent sites of embolism in left-sided IE, while pulmonary embolism is frequent in native right-sided and pacemaker lead IE. Stroke is a severe complication and is associated with increased morbidity and mortality.105 Conversely, embolic events may be totally silent in 20–50% of patients with IE, especially those affecting the splenic or cerebral circulation, and can be diagnosed by non-invasive imaging.83,85,242 Thus systematic abdominal and cerebral CT scanning may be helpful. However, contrast media should be used with caution in patients with renal impairment or haemodynamic instability because of the risk of worsening renal impairment in combination with antibiotic nephrotoxicity.

Overall, embolic risk is very high in IE, with embolic events occurring in 20–50% of patients.72,242–249 However, the risk of new events (occurring after initiation of antibiotic therapy) is only 6–21%.72,115,243 A study from the ICE group250 demonstrated that the incidence of stroke in patients receiving appropriate antimicrobial therapy was 4.8/1000 patient-days in the first week of therapy, falling to 1.7/1000 patient-days in the second week, and further thereafter.

8.3.2 Predicting the risk of embolism

Echocardiography plays a key role in predicting embolic events.72,115,246–252 although prediction remains difficult in the individual patient. Several factors are associated with increased risk of embolism, including the size and mobility of vegetations,72,242,246–253 the location of the vegetation on the mitral valve,72,246–249 the increasing or decreasing size of the vegetation under antibiotic therapy,72,254 particular microorganisms (S. aureus,72 S. bovis,255 Candida spp.), previous embolism,72 multivalvular IE246 and biological markers.255 Among these, the size and mobility of the vegetations are the most potent independent predictors of a new embolic event.253

Patients with vegetations $>10\ mm$ in length are at higher risk of embolism,72,255 and this risk is even higher in patients with larger ($>15\ mm$) and mobile vegetations, especially in staphylococcal IE affecting the mitral valve.256 A recent study113 found that the risk of neurological complications was particularly high in patients with very large ($>30\ mm$) vegetations.

Several factors should be taken into account when assessing embolic risk. In a recent study of 847 patients with IE, the 6-month incidence of new embolism was 8.5%.222 Six factors (age, diabetes, atrial fibrillation, previous embolism, vegetation length and S. aureus infection) were associated with an increased embolic risk and were used to create an ‘embolic risk calculator’.

whatever the risk factors observed in an individual patient, it must be re-emphasized that the risk of new embolism is highest during the first 2 weeks following initiation of antibiotic therapy and rapidly decreases thereafter, particularly beyond 2 weeks,72,242,250 although some risk persists indefinitely while vegetations remain present, particularly for very large vegetations.113 For this reason, the benefits of surgery to prevent embolism are greatest during the first 2 weeks of antibiotic therapy, when embolic risk peaks.

8.3.3 Indications and timing of surgery to prevent embolism in infective endocarditis (Table 22)

Avoiding embolic events is difficult since the majority occur before admission.222 The best means to reduce the risk of an embolic event is the prompt institution of appropriate antibiotic therapy.83 While promising,256,257 the addition of antiplatelet therapy did not reduce the risk of embolism in the only published randomized study.258
The exact role of early surgery in preventing embolic events remains controversial. In the Euro Heart Survey, vegetation size was one of the reasons for surgery in 54% of patients with NVE and in 25% of those with PVE, but was rarely the only reason. The value of early surgery in an isolated large vegetation is controversial. A recent randomized trial demonstrated that early surgery in patients with large vegetations significantly reduced the risk of death and embolic events compared with conventional therapy. However, the patients studied were at low risk and there was no significant difference in all-cause mortality at 6 months in the early surgery and conventional-treatment groups.

Finally, the decision to operate early for prevention of embolism must take into account the presence of previous embolic events, other complications of IE, the size and mobility of the vegetation, the likelihood of conservative surgery and the duration of antibiotic therapy. The overall benefits of surgery should be weighed against the operative risk and must consider the clinical status and co-morbidity of the patient.

The main indications and timing of surgery to prevent embolism are given in Table 22. Surgery is indicated in patients with persisting vegetations >10 mm after one or more clinical or silent embolic events despite appropriate antibiotic treatment. Surgery may be considered in patients with large (>15 mm) isolated vegetations on the aortic or mitral valve, although this decision is more difficult and must be very carefully individualized according to the probability of conservative surgery.

Surgery undertaken for the prevention of embolism must be performed very early, during the first few days following initiation of antibiotic therapy (urgent surgery), as the risk of embolism is highest at this time. In summary, embolism is very frequent in IE, complicating 20–50% of cases of IE, but falling to 6–21% after initiation of antibiotic therapy. The risk of embolism is highest during the first 2 weeks of antibiotic therapy and is clearly related to the size and mobility of the vegetation, although other risk factors exist. The decision to operate early to prevent embolism is always difficult and specific for the individual patient. Governing factors include the size and mobility of the vegetation, previous embolism, type of microorganism and duration of antibiotic therapy.

9. Other complications of infective endocarditis

9.1 Neurological complications

Symptomatic neurological complications occur in 15–30% of patients with IE and are mainly the consequence of embolism from vegetations. Neurological manifestations occur before or at IE diagnosis in a majority of cases, but new or recurrent events can also take place later in the course of IE. Clinical presentation is variable and may include multiple symptoms or signs in the same patient, but focal signs predominate and ischaemic strokes are most commonly diagnosed. Transient ischaemic attack, intracerebral or subarachnoid haemorrhage, brain abscess, meningo- and toxic encephalopathy are also seen, and firm evidence supports that additional clinically silent cerebral embolisms occur in 35–60% of IE patients. S. aureus IE is more frequently associated with neurological complications compared with IE caused by other bacteria. Vegetation length and mobility also correlate with embolic tendency. Neurological complications are associated with an excess mortality, as well as sequelae, particularly in the case of stroke. Rapid diagnosis and initiation of appropriate antibiotics are of major importance to prevent a first or recurrent neurological complication. Early surgery in high-risk patients is the second mainstay of embolism prevention, while antithrombotic drugs have no role (see section 12.7).

Successful management of IE requires a combined medical and surgical approach in a substantial proportion of patients. Following a neurological event, the indication for cardiac surgery often remains or is strengthened, but must be balanced with perioperative risk and postoperative prognosis. Randomized studies are not possible and cohort studies suffer from bias that can only be partly compensated for by statistical methods. However, the risk of postoperative neurological deterioration is low after a silent cerebral emboli or transient ischaemic attack, and surgery is recommended without delay if an indication remains. After an ischaemic stroke, cardiac surgery is not contraindicated unless the neurological prognosis is judged too poor. Evidence regarding the optimal time interval between stroke and cardiac surgery is conflicting, but recent data favour early surgery. If cerebral haemorrhage has been excluded by cranial CT and neurological damage is not severe (i.e. coma), surgery indicated for HF, uncontrolled infection, abscess or persistent high embolic risk should not be delayed and can be performed with a low neurological risk (3–6%) and good probability of complete neurological recovery. Conversely, in cases with intracranial haemorrhage, neurological prognosis is worse and surgery should generally be postponed for at least 1 month, although one recent study has reported a relatively low risk of neurological deterioration in IE patients undergoing surgery within 2 weeks after an intracranial haemorrhage. The Task Force has thus decided to adapt the level of evidence to a class IIa. If urgent cardiac surgery is needed, close cooperation with the neurosurgical team and the Endocarditis Team is mandatory. Table 23 and Figure 4 summarize the recommended management of neurological complications in IE.

Cerebral imaging is mandatory for any suspicion of neurological complication of IE. CT scanning, with or without contrast agent, is most often performed. The higher sensitivity of MRI, with or without contrast gadolinium enhancement, allows for better detection and analysis of cerebral lesions in patients with neurological symptoms, and this may have an impact on the timing of surgery (see section 5). In patients without neurological symptoms, cerebral MRI often detects lesions that may change the therapeutic strategy; in particular, the indications and timing of surgery. Cerebral MRI often detects microbleeds (round T2* hypointensities with a diameter ≤10 mm) in patients with IE. The lack of association with parenchymal haemorrhage and the absence of postoperative neurological complications in patients with microbleeds suggest that microbleeds should not be interpreted as active bleeding and should not lead to postponed surgery when this is indicated.

In summary, symptomatic neurological events develop in 15–30% of all patients with IE and additional silent events are frequent. Stroke (ischaemic and haemorrhagic) is associated with excess mortality. Rapid diagnosis and initiation of appropriate antibiotics are of major importance to prevent a first or recurrent neurological complication. After a first neurological event, cardiac surgery, if
9.2 Infectious aneurysms

Infectious (mycotic) aneurysms result from septic arterial embolism to the intraluminal space or vasa vasonum or from subsequent spread of infection through the intimal vessels. Infectious aneurysms are typically thin walled and friable and, as such, exhibit a high tendency to rupture and haemorrhage. No predictor of rupture has been identified and, in contrast to non-infectious aneurysms, size does not appear to be a reliable predictor of potential rupture.268,269

An intracranial location is most common and the reported frequency of 2–4% is probably an underestimation since some infectious aneurysms are clinically silent.267,270 Early detection and treatment of infectious aneurysms is essential given the high morbidity and mortality rate secondary to rupture. Clinical presentation is highly variable (i.e. focal neurological deficit, headache, confusion, seizures), so imaging should be systematically performed to detect intracranial infectious aneurysms in any case of IE with neurological symptoms.268

Cerebral CT and MRI both reliably diagnose infectious aneurysms with good sensitivity and specificity.271 However, conventional angiography remains the gold standard and should be performed when non-invasive techniques are negative and suspicion remains.267

Owing to the lack of randomized trials, there is no widely accepted standard management for infectious aneurysms. Thus management should be provided by an Endocarditis Team and tailored to the individual patient. Some infectious aneurysms may resolve during antibiotic treatment, while others require surgical or endovascular intervention depending on the occurrence of rupture and the location in the artery bed, as well as the clinical status of the patient.268,269

Regarding intracranial infectious aneurysms, ruptured aneurysms should be treated immediately by surgical or endovascular procedures. Unruptured infectious aneurysms should be followed by serial cerebral imaging under antibiotic therapy. If the size of the aneurysm increases or resolves completely, surgical or endovascular intervention might be considered before the procedure, depending on associated cerebral lesions, the haemodynamic status of the patient and the risk of the procedure.

9.3 Splenic complications

Splenic infarcts are common and very often asymptomatic. Persistent or recurrent fever, abdominal pain and bacteraemia suggest the presence of complications (splenic abscess or rupture). Although

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### Table 23 Management of neurological complications of infective endocarditis

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Classa</th>
<th>Levelb</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>After a silent embolism or transient ischaemic attack, cardiac surgery, if indicated, is recommended without delay</td>
<td>I</td>
<td>B</td>
<td>105, 263</td>
</tr>
<tr>
<td>Neurosurgery or endovascular therapy is recommended for very large, enlarging or ruptured intracranial infectious aneurysms</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Following intracranial haemorrhage, surgery should generally be postponed for ≥ 1 month</td>
<td>Ila</td>
<td>B</td>
<td>264–266</td>
</tr>
<tr>
<td>After a stroke, surgery indicated for HF, uncontrolled infection, abscess, or persistent high embolic risk should be considered without any delay as long as coma is absent and the presence of cerebral haemorrhage has been excluded by cranial CT or MRI</td>
<td>Ila</td>
<td>B</td>
<td>267, 268</td>
</tr>
<tr>
<td>Intracranial infectious aneurysms should be looked for in patients with IE and neurological symptoms. CT or MR angiography should be considered for diagnosis. If non-invasive techniques are negative and the suspicion of intracranial aneurysm remains, conventional angiography should be considered</td>
<td>Ila</td>
<td>B</td>
<td>267, 268</td>
</tr>
</tbody>
</table>

CT = computed tomography; HF = heart failure; IE = infective endocarditis; MR = magnetic resonance; MRI = magnetic resonance imaging.

*a Class of recommendation.

*b Level of evidence.

*c Reference(s) supporting recommendations.
spleen emboli are common, spleenic abscesses are rare. Persistent or recurrent fever and bacteremia suggest the diagnosis. These patients should be evaluated by abdominal CT, MRI or ultrasound. Recently PET has proved useful for the diagnosis of splenic metastatic infection in patients with IE. Treatment consists of appropriate antibiotic regimens. Splenectomy may be considered for splenic rupture or large abscesses, which respond poorly to antibiotics alone, and should be performed before valvular surgery unless the latter is urgent. Rarely, splenectomy and valvular surgery are performed during the same operative time. Percutaneous drainage is an alternative for high-risk surgical candidates.

9.4 Myocarditis and pericarditis

Cardiac failure may be due to myocarditis, which is frequently associated with abscess formation or immune reaction. Ventricular arrhythmias may indicate myocardial involvement and imply a poor prognosis. Myocardial involvement is best assessed using TTE and cardiac MRI.

The inflammatory response, HF, perianular complications or infection itself can cause pericardial effusion, which could be a sign of more severe IE. Rarely, ruptured pseudoaneurysms or fistulae may communicate with the pericardium, with dramatic and often fatal consequences. Purulent pericarditis is rare and may necessitate surgical drainage.

9.5 Heart rhythm and conduction disturbances

Conduction disorders are uncommon complications of IE. According to data from patient registries, their frequency is between 1% and 15% of cases and their presence is associated with worse prognosis and higher mortality. Conduction abnormalities (mainly first-, second-, and third-degree atrio-ventricular blocks, rarely bundle branch blocks) are due to spread of the infection beyond the endocardium, from valves to the conduction pathways, and are generally associated with peri-valvular complications. Complete atrio-ventricular block is most often associated with involvement of the left-sided valves (aortic, 36%; mitral, 33%). This is because of the anatomical relationship with the atrio-ventricular node, which is close to the non-coronary aortic cusp and the anterior mitral leaflet. In a study of patients with IE and complete atrio-ventricular block, pathology workup revealed the presence of an infection, frequently accompanied by abscesses and fistulae, affecting the conduction pathways; in cases of paroxysmal atrio-ventricular block, inflammation was observed at this level, which would explain the reversibility of the event.

The occurrence of conduction abnormalities during electrocardiographic monitoring in patients with endocarditis can therefore alert physicians to the appearance of peri-valvular complications.

In the case of embolization of vegetation fragments into a coronary artery, the resulting myocardial ischaemia can be the substrate for the onset of tachyarrhythmias. Atrial fibrillation can be observed in patients with IE and may be present before IE or occur as a complication of IE. Atrial fibrillation has been reported to be more frequent in the elderly and to be associated with a poor prognosis. More recently, in a large prospective series of IE, atrial fibrillation was found to be associated with an increased embolic risk, as were other factors (age, diabetes, previous embolism, vegetation length and S. aureus infection). Consequently, atrial fibrillation has the potential to increase the risk of both congestive HF and embolism in IE. However, there is no specific study on this situation and no international consensus for the care of these patients. The management of anticoagulation therapy in these patients should be taken on an individual basis by the Endocarditis Team.

9.6 Musculoskeletal manifestations

Musculoskeletal symptoms (arthralgia, myalgia, back pain) are frequent during IE. Rheumatological manifestations may be the first manifestations of IE and can delay its diagnosis, especially when classic manifestations are less evident and a variety of antibodies (i.e. positive antineutrophil cytoplasmic antibody test) induced by infections are present. Arthralgia occurs in about 10% of patients, while myalgia is present in 12–15%. Back pain is observed in about 13% of cases, and lumbar pain is the most common symptom in patients with IE and vertebral osteomyelitis. Periarticular arthritis occurs in about 14% of cases. The prevalence of spondylodiscitis in patients with IE is about 1.8–15%. Pyogenic vertebral osteomyelitis occurs in 4.6–19% of IE patients with a high incidence of streptococcal and staphylococcal bacteria. IE can complicate or be complicated by pyogenic osteomyelitis. The prevalence of IE in vertebral osteomyelitis is higher in patients with Streptococcus viridans IE, but preferably MRI, of the spine or whole-body PET/CT should be performed in IE patients with back or bone pain. Conversely, echocardiography should be performed in patients with a definite diagnosis of pyogenic spondylodiscitis/osteomyelitis and underlying cardiac conditions predisposing to IE.

In definite spondylodiscitis and osteomyelitis, prolonged antibiotic therapy is generally required until no signs of inflammatory activity are detected by 18F-FDG PET/CT. Other musculoskeletal manifestations are less common in IE and include sacroiliitis in about 1% of cases, a condition mimicking polymyalgia rheumatica with pain and morning stiffness of the shoulders and hips, proximal muscle weakness in about 0.9% of cases and cutaneous leucocytoclastic vasculitis (purpuric skin lesions) in 3.6% of cases.

9.7 Acute renal failure

Acute renal failure is a common complication of IE and may worsen the prognosis of IE. The onset of renal dysfunction is independently associated with increased risk of in-hospital death and post-operative events.

Acute renal dysfunction occurs in about 6–30% of patients. Causes are often multifactorial:

(i) immune complex and vasculitic glomerulonephritis; (ii) renal infarction, mostly due to septic emboli, occurring at any time during the course of the disease; (iii) haemodynamic impairment in cases with HF or severe sepsis or after cardiac surgery; (iv) antibiotic toxicity (acute interstitial nephritis), notably related to aminoglycosides, vancomycin (synergistic toxicity with aminoglycosides) and even high-dose penicillin; and (v) nephrotoxicity of contrast agents used for imaging purposes.

Haemodialysis may be required in some patients with advanced renal failure and is associated with high mortality.
failure of a milder degree is often reversible. To mitigate this complication, antibiotic doses should be adjusted for creatinine clearance with careful monitoring of serum levels (aminoglycosides and vancomycin). Imaging with nephrotoxic contrast agents should be avoided when possible in patients with haemodynamic impairment or previous renal insufficiency.

10. Surgical therapy: principles and methods

10.1 Operative risk assessment

Few studies have evaluated the utility of operative risk scores in the setting of IE. Although EuroSCORE II is frequently used, it was developed and validated predominantly for coronary artery bypass grafting and valve surgery. Risk scores specific to IE surgery have been developed: (i) from the Society of Thoracic Surgeons database using 13,617 patients and (ii) an additional NVE risk score from a single centre using 440 patients by De Feo et al. A study compared the prognostic utility of these contemporary risk scores for mortality and morbidity after IE surgery in 146 patients. Here, although EuroSCORE II discriminated mortality and postoperative morbidity (in particular, stroke), the Society of Thoracic Surgeons endocarditis score and the De Feo et al. score performed better at predicting operative mortality after surgery for active IE. However, the relevance of these findings is limited by the small number of patients involved. Similar to previous studies, preoperative use of inotropes or an intra-aortic balloon pump, prior coronary artery bypass surgery and renal failure requiring dialysis were independent predictors of operative and long-term mortality.

Finally, although no single operative risk score is perfect, preoperative assessment of operative risk is of utmost importance. Although the theoretical indications for surgery in IE are clear (Table 22), their practical application relies largely on the clinical status of the patient, the patient’s co-morbidities and the patient’s operative risk.

10.2 Preoperative and perioperative management

10.2.1 Coronary angiography

Coronary angiography is recommended according to the ESC Guidelines on the management of valvular heart disease in men >40 years, in post-menopausal women and in patients with at least one cardiovascular risk factor or a history of coronary artery disease. Exceptions arise when there are aortic vegetations that may be dislodged during catheterization or when emergency surgery is necessary. In these situations, high-resolution CT may be used to rule out significant coronary artery disease in haemodynamically stable patients.

10.2.2 Extracardiac infection

If a primary focus of infection likely to be responsible for IE has been identified, it must be eradicated before cardiac surgical intervention unless valve surgery is urgent. In any case, it should be eradicated before the end of antibiotic therapy.

10.2.3 Intraoperative echocardiography

Intraoperative TOE is most useful to determine the exact location and extent of infection, guide surgery, assess the result and help in early postoperative follow-up.

10.3 Surgical approach and techniques

The two primary objectives of surgery are total removal of infected tissues and reconstruction of cardiac morphology, including repair or replacement of the affected valve(s).

Where infection is confined to the valve cusps or leaflets, any method to repair or replace the valve may be used. However, valve repair is favoured whenever possible, particularly when IE affects the mitral or tricuspid valve without significant destruction. Perforations in a single valve cusp or leaflet may be repaired with an untreated or glutaraldehyde-treated autologous or bovine pericardial patch. Isolated or multiple ruptured chordae may be replaced by polytetrafluoroethylene neo-chordae.

More extensive destruction of a single leaflet or the presence of an abscess is not necessarily a contraindication for valve repair. Rather, intraoperative assessment of the valve after debridement is of paramount importance in order to evaluate whether the remaining tissue is of sufficient quality to achieve a durable repair. The need for a patch to achieve a competent valve, whether pericardial, tricuspid autograft or a flipped-over mitral patch, has not been associated with worse results in terms of recurrence of IE or mitral regurgitation when performed by experienced surgeons.

To avoid paravalvular leaks in complex cases with locally uncontrolled infection, total excision of infected and devitalized tissue should be followed by valve replacement and repair of associated defects to secure valve fixation.

Mechanical and biological prostheses have similar operative mortality. Therefore the Task Force does not favour any specific valve substitute but recommends a tailored approach for each individual patient and clinical situation. The use of foreign material should be kept to a minimum. Small abscesses can be closed directly, but larger cavities should be allowed to drain into the pericardium or circulation.

In mitral valve IE, successful valve repair can be achieved by experienced teams in up to 80% of patients, but such results may not be matched in non-specialist centres. Moreover, although surgery may be deferred if control of the infection by antibiotic therapy appears evident in the absence of cardiac failure, early operation has been associated in recent reports with a repair rate of 61–80% and improved in-hospital and long-term survival. Residual mitral regurgitation should be assessed using intraoperative TOE. Mitral subannular, annular or supraannular tissue defects are preferably repaired with autologous or bovine pericardium, a prosthetic valve then being secured to the reconstructed/reinforced annulus, if necessary. The choice of technique depends on the vertical extension of the lesion/tissue defect. The use of mitral valve homografts and pulmonary autografts (Ross II procedure) has been suggested, but their application is limited by poor availability and difficulty of the surgical technique, and the results have not been consistent.

In aortic IE, replacement of the aortic valve using a mechanical or biological prosthesis is the technique of choice. Nevertheless, in
centres with great expertise, aortic valve repair in IE can be achieved in up to 33% of patients. However, experience with aortic valve repair in this setting is still very limited and there is no evidence that repair is associated with improved outcomes compared with replacement.313,314 Owing to their natural biocompatibility, the use of cryopreserved or sterilized homografts has been suggested to reduce the risk of persistent or recurrent infection, especially in the presence of annular abscesses.315,316 It is expert opinion and standard strategy in many institutions that the use of a homograft is to be favoured over valve prostheses, particularly in the presence of root abscess.316,317 However, mechanical prostheses and xenografts have led to similar results in terms of persistent or recurrent infection and survival if associated with complete debridement of annular abscesses.313,318 Homografts or stentless xenografts may be preferred in PVE or in cases where there is extensive aortic root destruction with aorto-ventricular discontinuity.315,319 The anterior mitral leaflet of the aortic homograft can be effectively used for reconstruction of the outflow tract. A monoblock aorto-mitral homograft has been suggested as a surgical option for extensive bivalvular IE.320 In experienced hands, the Ross procedure may be used in children or adolescents to facilitate growth and in young adults for extended durability.321,322

Cardiac transplantation may be considered in extreme cases where repeated operative procedures have failed to eradicate persistent or recurrent PVE.323

10.4 Postoperative complications

Postoperative patient management should follow the usual recommendations after valvular surgery324 but should also take into account the specifics of IE. Postoperative follow-up should be particularly cautious given the in-hospital mortality of patients operated on for acute IE on an emergency or urgent basis, which ranges from 10% to 20% in most series,1 and the increased risk of postoperative complications.

Among the most frequent complications are severe coagulopathy requiring treatment with clotting factors, re-exploration of the chest for bleeding or tamponade, acute renal failure requiring haemodialysis, stroke, low cardiac output syndrome, pneumonia and atriointerventricular block following radical resection of an aortic root abscess with the need for pacemaker implantation.325 A preoperative electrocardiogram demonstrating left bundle branch block predicts the need for a postoperative permanent pacemaker.23 When a patient does not survive surgery, the cause of death is often multifactorial.325

11. Outcome after discharge: follow-up and long-term prognosis

Following in-hospital treatment, the main complications include recurrence of infection, HF, need for valve surgery and death.57,326,327

11.1 Recurrences: relapses and reinfections

The actual risk of recurrence among survivors of IE varies between 2% and 6%.313,328–332 Two main types of recurrence are distinguishable: relapse and reinfection. Although not systematically differentiated in the literature, the term ‘relapse’ refers to a repeat episode of IE caused by the same microorganism, while ‘reinfection’ describes an infection caused by a different microorganism.38 When the same species is isolated during a subsequent episode of IE, there is often uncertainty as to whether the repeat infection is a relapse of the initial infection or a new infection (reinfection). In these cases, molecular methods including strain-typing techniques should be employed.8,38 When these techniques or the identity of both isolates is unavailable, the timing of the second episode of IE may be used to distinguish relapse from reinfection. Thus, although variable, the time between episodes is usually shorter for relapse than for reinfection. Generally speaking, a recurrence caused by the same species within 6 months following the initial infection represents relapse, whereas later events suggest reinfection.38 For these purposes, storage of IE isolates for at least 1 year is recommended.8,38

Factors associated with an increased rate of relapse are listed in Table 24. Relapses are most often due to insufficient duration of original treatment, suboptimal choice of initial antibiotics or a persistent focus of infection. When the duration of therapy has been insufficient or the choice of antibiotic incorrect, relapse should be treated for a further 4–6 weeks depending on the causative microorganism and its antibiotic susceptibility (remembering that resistance may develop in the meantime).

Patients with previous IE are at risk of reinfection332 and prophylactic measures should be very strict. Reinfection is more frequent in IVDA332 (especially in the year after the initial episode),332,333 in PVE,334 in patients undergoing chronic dialysis326,332 and in those with multiple risk factors for IE.8 Patients with reinfection are at higher risk of death and need for valve replacement.325,332 Paravalvular destruction is associated with a higher rate of recurrence and a higher operative mortality.331 In a large series of surgically managed NVE (358 cases), 21% had paravalvular destruction, and freedom from recurrent PVE at 15 years was 78.9%.331

The type of valve implanted has no effect on the risk of recurrent IE.325,331 Aortic valve and root replacement with a prosthetic

![Table 24 Factors associated with an increased rate of relapse](image_url)
11.2 Short-term follow-up
A first episode of IE should not be seen as an ending once the patient has been discharged. Residual severe valve regurgitation may decompensate left ventricular function, or valve deterioration may progress despite bacteriological cure, usually presenting with acute HF. After completion of treatment, recommendations for surgery follow conventional guidelines.\textsuperscript{35,36} A consequence of increasing rates of surgery during the active phase of infection, the need for late valve surgery is low, ranging from 3% to 8% in recent series.\textsuperscript{326–328}

Patients should be educated about the signs and symptoms of IE after discharge. They should be aware that recurrence could occur in IE and that new onset of fever, chills or other signs of infection mandate immediate evaluation, including procurement of blood cultures before empirical use of antibiotics. To monitor the development of secondary HF, an initial clinical evaluation and baseline TTE should be performed at the completion of antimicrobial therapy and repeated serially, particularly during the first year of follow-up.

Clinical follow-up should be done by the Endocarditis Team or by a Heart Valve Clinic specialist.\textsuperscript{11,337} Regular clinical and echocardiographic follow-up should be performed during the first year following completion of treatment.\textsuperscript{8,12} This Task Force also recommends to take blood samples (i.e. white cell count, CRP, etc.), and blood cultures systematically at the initial visit, and otherwise if there is clinical suspicion.

Good oral health maintenance, preventive dentistry and advice about skin hygiene, including tattoos and skin piercing, are mandatory. Deficiencies in dental surveillance contribute to the continuous gradual increase in the incidence of IE.\textsuperscript{30,337} This increase underlines the need for repeating the principles of IE prevention at each follow-up visit.

11.3 Long-term prognosis
In recent series, the crude long-term survival rates after the completion of treatment were estimated to be 80–90% at 1 year, 70–80% at 2 years and 60–70% at 5 years.\textsuperscript{37,336–332} The main predictors of long-term mortality are older age, co-morbidities, recurrences and HF, especially when cardiac surgery cannot be performed.\textsuperscript{57,327,330}

Compared with an age- and sex-matched general population, patients surviving a first episode of IE have a significantly worse survival.\textsuperscript{57} This excess mortality is especially high within the first few years after hospital discharge and can be explained by late complications such as HF, higher risk of recurrences and higher patient vulnerability.\textsuperscript{57,339} In fact, most recurrences and late cardiac surgeries occurred during this period of time.\textsuperscript{57,328,329}

In summary, recurrences are rare following IE and may be associated with inadequate initial antibiotic therapy, resistant microorganisms, persistent focus of infection, i.e. drug abuse and chronic dialysis. Patients with IE must be informed of the risk of recurrence and educated about how to diagnose and prevent a new episode of IE. The need for late valve surgery is low.

12. Management of specific situations
12.1 Prosthetic valve endocarditis
PVE is the most severe form of IE and occurs in 1–6% of patients with valve prostheses,\textsuperscript{338} with an incidence of 0.3–1.2% per patient-year.\textsuperscript{316,233,339,340} PVE accounts for 10–30% of all cases of IE\textsuperscript{341} and affects mechanical and bioprosthetic valves equally. PVE was observed in 16% of cases of IE in a French survey,\textsuperscript{322} in 26% of cases in the Euro Heart Survey\textsuperscript{54} and in 20% of 2670 patients with definite IE in the ICE Prospective Cohort Study.\textsuperscript{340} PVE is still associated with difficulties in diagnosis, determination of the optimal therapeutic strategy and poor prognosis.

12.1.1 Definition and pathophysiology
Early PVE is defined as IE occurring within 1 year of surgery and late PVE as IE occurring beyond 1 year, because of significant differences between the microbiological profiles observed before and after this time point.\textsuperscript{3,342} However, this is an artificial distinction. What is important is not the time from the valve replacement procedure to the onset of IE, but whether IE is acquired perioperatively and which microorganism is involved. A recent large, prospective, multicentre, international registry reported that 37% of PVE cases were associated with nosocomial infection or non-nosocomial healthcare-associated infections in outpatients with extensive healthcare contact.\textsuperscript{340}

The pathogenesis of PVE differs according to both the type of contamination and the type of prosthetic valve. In cases with perioperative contamination, the infection usually involves the junction between the sewing ring and the annulus, leading to perivalvular abscess, dehiscence, pseudo-aneurysms and fistulae.\textsuperscript{339,343,344} In late PVE, additional mechanisms may exist. For example, in late bioprosthetic PVE, infection is frequently located on the leaflets of the prosthesis, leading to vegetations, cusp rupture and perforation. PVE has recently been reported after transcatheter aortic bioprosthetic valve implantation, which should be managed in the same manner as other prosthetic valves.\textsuperscript{345,346} The risk of prosthetic valve implantation endocarditis increases with the use of orotracheal intubation and a self-expandable valve system.

The consequence of PVE is usually new prosthetic regurgitation. Less frequently, large vegetations may cause prosthetic valve obstruction, which can be diagnosed by TOE and sometimes by TTE or fluoroscopy.

12.1.2 Diagnosis
Diagnosis is more difficult in PVE than in NVE. Clinical presentation is frequently atypical, particularly in the early postoperative period, in which fever and inflammatory syndromes are common in the absence of IE. However, persistent fever should trigger the suspicion of PVE. As in NVE, diagnosis of PVE is based mainly on the results of echocardiography and blood cultures. However, both are more frequently negative in PVE.\textsuperscript{340} Although TOE is mandatory in suspected PVE (Figure 3), its diagnostic value is lower than in NVE. A negative echocardiogram is frequently observed in PVE\textsuperscript{5} and does not rule out the diagnosis, but identification of a new periprosthetic leak is a major criterion, in which case an additional imaging modality could be considered (such as CT or nuclear imaging).
In PVE, staphylococcal and fungal infections are more frequent and streptococcal infection less frequent than in NVE. Staphylococci, fungi and Gram-negative bacilli are the main causes of early PVE, while the microbiology of late PVE mirrors that of NVE, with staphylococci, oral streptococci, S. bovis and enterococci being the most frequent organisms, more likely due to community-acquired infections. Staphylococci and enterococci are the most common agents in prosthetic valve implantation endocarditis.345,346

The Duke criteria have been shown to be helpful for the diagnosis of NVE, with a sensitivity of 70–80%, but are less useful in PVE because of their lower sensitivity in this setting.348,349 Recently, nuclear techniques, particularly 18F-FDG PET/CT, have been shown to be useful for the diagnosis of PVE.93 The addition of abnormal FDG uptake as a novel major criterion for PVE has thus been pointed out. An algorithm for evaluation of patients with suspected PVE, including echocardiography and PET/CT has been suggested (see Figure 3).93

12.1.3 Prognosis and treatment

A very high in-hospital mortality rate of 20–40% has been reported in PVE.338,341 As in NVE, prognostic assessment is of crucial importance in PVE, as it allows identification of high-risk subgroups of patients in whom an aggressive strategy may be necessary. Several factors have been associated with poor prognosis in PVE, including older age, diabetes mellitus, healthcare-associated infections, staphylococcal or fungal infection, early PVE, HF, stroke and intracardiac abscess. Among these, complicated PVE and staphylococcal infection are the most powerful markers. These patients need aggressive management, consisting of antibiotic therapy and early radical surgery.

Antimicrobial therapy for PVE is similar to that for NVE. An exception is S. aureus PVE, which requires a more prolonged (≥6 weeks) antibiotic regimen (particularly in association with aminoglycosides) and frequent use of rifampin.

Surgery for PVE follows the general principles outlined for NVE. Radical debridement in these cases means removal of all infected foreign material, including the original prosthesis, and any calcium remaining from previous surgery. Homografts, stentless xenografts or autografts may be considered in aortic PVE, and homograft or xenograft root replacement is indicated for any abnormality of the aortic root that distorts the aortic sinuses. Alternatively, a valved Dacron conduit can be used.

The best therapeutic option in PVE is still debated.221,354–359 Although surgery is generally considered the best option when PVE causes severe prosthetic dysfunction or HF,220 it was performed in only 50% of patients with PVE in the Euro Heart Survey,54 a similar rate as for patients with NVE. Other groups have reported similar data.221,340 Early surgery was associated with lower in-hospital and 1-year mortality in a large cohort of 4166 patients including both native and prosthetic valve IE complicated by HF.216 Conversely, after adjustment for differences in clinical characteristics and survival bias, early valve replacement was not associated with lower mortality compared with medical therapy in a large international cohort.37 However, in these series, surgery was beneficial in the subgroup of patients with the greatest need for surgery, including valve regurgitation, vegetation and dehiscence or paravalvular abscess/fistula.37

Therefore a surgical strategy is recommended for PVE in high-risk subgroups identified by prognostic assessment, i.e. PVE complicated by HF, severe prosthetic dysfunction, abscess or persistent fever (Table 22). Emergency surgery is indicated only in cases with refractory congestive HF leading to pulmonary oedema or shock, as in NVE. Conversely, patients with uncomplicated non-staphylococcal and non-fungal late PVE can be managed conservatively.350,357,358 However, patients who are initially treated medically require close follow-up because of the risk of late events.

In summary, PVE represents 20% of all cases of IE, with an increasing incidence. The diagnosis of PVE is more difficult than for NVE. Complicated PVE and staphylococcal PVE are associated with a worse prognosis if treated without surgery. These forms of PVE must be managed aggressively. Patients with uncomplicated, non-staphylococcal late PVE can be managed conservatively with close follow-up.

12.2 Infective endocarditis affecting cardiac implantable electronic devices

12.2.1 Introduction

Infection of cardiac implantable electronic devices (CIEDs) is a severe disease associated with high mortality.360 The increased rates of CIED implantation coupled with increased implantation in older patients with more co-morbidities have set the stage for higher rates of CIED infection and the increasing frequency of IE in these patients.361 The reported incidence of permanent pacemaker infection varies widely among studies.362,363 A population-based study found an incidence of CIED infection of 1.9 per 1000 device-years and a higher probability of infection after implantable cardioverter defibrillators compared with permanent pacemakers.362 Both diagnosis and therapeutic strategy are particularly difficult in these patients.365

12.2.2 Definitions of cardiac device infections

A distinction should be made between local device infection and cardiac device-related IE (CDRIE). Local device infection is defined as an infection limited to the pocket of the cardiac device and is clinically suspected in the presence of local signs of inflammation at the generator pocket, including erythema, warmth, fluctuance, wound dehiscence, erosion, tenderness or purulent drainage.366 CDRIE is defined as an infection extending to the electrode leads, cardiac valve leaflets or endocardial surface. However, differentiating local device infection and CDRIE is frequently difficult. In one study,367 a culture of intravascular lead segments was positive in 72% of 50 patients with manifestations strictly limited to the implantation site. However, the possibility of intraoperative contamination of the lead tip cannot be excluded in these patients.

12.2.3 Pathophysiology

The pocket may become infected at the time of implantation, during subsequent surgical manipulation of the pocket or if the generator or subcutaneous electrodes erode through the skin. Pocket infection may track along the intravascular portion of the electrode to involve the intracardiac portion of the pacemaker or implantable cardioverter defibrillator. Alternatively, the pocket or intracardiac portion of the electrode may become infected as a result of haematogenous seeding during a bacteraemia secondary to a distant infected focus. The consequence may be formation of vegetations, which can be found anywhere from the insertion vein to the superior vena cava, on the lead or on the tricuspid valve, as well as on the
right atrial and ventricular endocardium. Septic pulmonary embolism is a very frequent complication of CDRIE.

12.2.4 Risk factors
Several factors have been associated with CIED infections. Patient factors include renal failure, corticosteroid use, congestive HF, haematoma formation, diabetes mellitus and anticoagulation use. In addition, procedural characteristics may also play an important role in the development of CIED infection. The factors associated with an increased risk of infection include the type of intervention, device revisions, the site of intervention, the amount of indwelling hardware, the use of pre-procedural temporary pacing, failure to administer perioperative antimicrobial prophylaxis, fever within the 24 h before implantation and operator experience.

12.2.5 Microbiology
Staphylococci, and especially CoNS, account for 60–80% of cases in most reported series. A variety of CoNS species have been described. Methicillin resistance among staphylococci varies among studies, but a low frequency of methicillin-resistant CoNS has been reported among individuals with no healthcare contact, whereas a high rate of methicillin resistance in CoNS is associated with a healthcare environment source. Polymicrobial infection sometimes involves more than one species of CoNS. Corynebacterium spp., Propionibacterium acnes, Gram-negative bacilli and Candida spp. are rarely identified as pathogens in CIED infection.

12.2.6 Diagnosis
Clinical presentation is frequently misleading, with predominant respiratory and rheumatological symptoms as well as local signs of infection. CIED must be suspected in the presence of unexplained fever in a patient with a CIED. Fever is frequently blunted, particularly in elderly patients. As in other forms of IE, echocardiography and blood cultures are the cornerstones of diagnosis. Staphylococcus aureus bacteraemia might be the sole manifestation of device infection.

Echocardiography plays a key role in CDRIE and is helpful for the diagnosis of both lead vegetations and tricuspid involvement, quantification of tricuspid regurgitation, sizing of vegetations and follow-up after lead extraction. Several prognostic features may be better defined on TTE than on TOE, such as pericardial effusion, ventricular dysfunction and pulmonary vascular pressure estimations. TOE has superior sensitivity and specificity to TTE for diagnosis of lead-related endocarditis.

TOE allows visualization of the lead in atypical locations, such as the proximal superior vena cava, and of regions that are difficult to visualize by TTE. In addition, the sensitivity of TOE for left-sided involvement and for perivalvular extension of infection is superior to that of TTE. Considering their complementary role, it is recommended to perform both investigations in suspected CDRIE.

In the presence of infective material along the lead course not providing typical vegetations of measurable size, both TTE and TOE may be falsely negative in CDRIE. Intracardiac echocardiography was recently found to be feasible and effective in cardiac device patients and to have a superior sensitivity for the detection of vegetations in cardiac devices.

A normal echographic examination does not rule out CDRIE. In difficult cases, other modalities such as radiolabelled leucocyte scintigraphy and 18F-FDG PET/CT scanning have been described as additive tools in the diagnosis of CDRIE and related complications, including pulmonary septic embolism. The Duke criteria are difficult to apply in these patients because of lower sensitivity. Modifications of the Duke criteria have been proposed including local signs of infection and pulmonary embolism as major criteria.

12.2.7 Treatment
CDRIE must be treated by prolonged antibiotic therapy associated with complete hardware removal.

12.2.8 Antimicrobial therapy
Antimicrobial therapy for CDRIE should be individualized and based on culture and susceptibility results if possible. Because most CDRIE infections are secondary to staphylococcal species and, of those, up to 50% are methicillin-resistant vancomycin should be administered initially as empirical antibiotic coverage until microbiological results are known. Daptomycin, approved for right-sided IE and bacteraemia attributable to S. aureus, is a promising molecule to treat CIED infection. Before hardware removal, but after blood cultures, i.v. antibiotics should be initiated. There are no clinical trial data to define the optimal duration of antimicrobial therapy. The duration of therapy should be 4–6 weeks in most cases. At least 2 weeks of parenteral therapy is recommended after extraction of an infected device for patients with bloodstream infection.

At least 2 weeks of parenteral therapy for at least 4 weeks. Collection of vegetations should be performed only in centres committed to a procedural volume allowing the maintenance of skills of adequately trained teams and able to provide immediate cardiothoracic surgery backup in the event of emergency thoracotomy or sternotomy.

Pulmonary embolism as a result of vegetation displacement during extraction occurs frequently, particularly when vegetations are
However, these episodes are frequently asymptomatic, and percutaneous extraction remains the recommended method even in cases of large vegetations, as overall risks are even higher with surgical extraction.

Some authors recommend surgery in patients with very large vegetations. Until additional data are available, decisions regarding percutaneous versus surgical removal of leads with vegetations should be individualized.

Other indications for a surgical approach to lead removal include patients who need a contemporary valve replacement or repair for IE or patients who have significant retained hardware after attempts at percutaneous removal. However, mortality associated with surgical removal is high in these frequently elderly patients with associated co-morbidities.

### 12.2.10 Reimplantation

The first step before reimplantation is a re-evaluation of the indication for CIED implantation. In a significant number of cases, reimplantation is not necessary. The device should be reimplanted on the contralateral side. There is no clear recommendation concerning the optimal timing of reimplantation. Factors such as persistent bacteremia, persistent vegetation and pacemaker and implantable cardioverter defibrillator dependency should be considered and the decision adapted to the individual patient. Immediate reimplantation should be avoided, owing to the risk of new infection.

Blood cultures should be negative for at least 72 h before placement of a new device. In cases of evidence of remnant valvular infection, implantation should be delayed for at least 14 days.

Temporary pacing is a risk factor for subsequent cardiac device infection and should be avoided if possible. In pacing-dependent patients, temporary use of active fixation leads connected to external devices is described as a “bridge,” permitting earlier mobilization with a reduced risk of pacing-related adverse events.

### 12.2.11 Prophylaxis

Although there are no large controlled studies on this topic, antibiotic prophylaxis is recommended before implantation. A first-generation cephalosporin, such as cefazolin (6 g/day for 24–36 h after the intervention), is usually used as prophylaxis and should be parenterally administered 1 h before the procedure.

Vancomycin, teicoplanin and daptomycin may be considered instead of cefazolin in centres where oxacillin resistance among staphylococci is high, in high-risk patients or in patients with contraindications to cephalosporins. They should always be started before the procedure according to the drug pharmacokinetics.

**In summary, CDRIE is one of the most difficult forms of IE to diagnose and must be suspected in the presence of frequently misleading symptoms, particularly in elderly patients. Prognosis is poor, probably because of its frequent occurrence in elderly patients with associated co-morbidities. In the majority of patients, CDRIE must be treated by prolonged antibiotic therapy and device removal. Table 25 summarizes the main features concerning diagnosis, treatment and prevention of CDRIE.**

### Table 25 Cardiac device-related infective endocarditis: diagnosis, treatment and prevention

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Three or more sets of blood cultures are recommended before prompt initiation of antimicrobial therapy for CIED infection</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>2. Lead-tip culture is indicated when the CIED is explanted</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3. TOE is recommended in patients with suspected CDRIE with positive or negative blood cultures, independent of the results of TTE, to evaluate lead-related endocarditis and heart valve infection</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4. Intracardiac echocardiography may be considered in patients with suspected CDRIE, positive blood cultures and negative TTE and TOE results</td>
<td>IIb</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5. Radionuclide leucocyte scintigraphy and 18F-FDG PET/CT scanning may be considered additive tools in patients with suspected CDRIE, positive blood cultures and negative echocardiography</td>
<td>IIb</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>B. Principles of treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Prolonged (i.e. before and after extraction) antibiotic therapy and complete hardware (device and leads) removal are recommended in definite CDRIE, as well as in presumably isolated pocket infection</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>2. Complete hardware removal should be considered on the basis of occult infection without another apparent source of infection</td>
<td>IIa</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3. In patients with NVE or PVE and an intracardiac device with no evidence of associated device infection, complete hardware extraction may be considered</td>
<td>IIb</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td><strong>C. Mode of device removal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Percutaneous extraction is recommended in most patients with CDRIE, even those with vegetations &gt;10 mm</td>
<td>I</td>
<td>B</td>
<td>382, 391, 405</td>
</tr>
</tbody>
</table>
Table 25  Continued

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Surgical extraction should be considered if percutaneous extraction is incomplete or impossible or when there is associated severe destructive tricuspid IE</td>
<td>Ila</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3. Surgical extraction may be considered in patients with large vegetations (&gt;20 mm)</td>
<td>Iib</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

D. Reimplantation

1. After device extraction, reassessment of the need for reimplantation is recommended | I | C | |
2. When indicated, definite reimplantation should be postponed if possible, to allow a few days or weeks of antibiotic therapy | Ila | C | |
3. A ‘temporary’ ipsilateral active fixation strategy may be considered in pacemaker-dependent patients requiring appropriate antibiotic treatment before reimplantation | Iib | C | |
4. Temporary pacing is not routinely recommended | III | C | |

E. Prophylaxis

1. Routine antibiotic prophylaxis is recommended before device implantation | I | B | 367, 368, 371 |
2. Potential sources of sepsis should be eliminated ≥2 weeks before implantation of an intravascular cardiac foreign material, except in urgent procedures | Ila | C | |

CDRIE = cardiac device-related infective endocarditis; CIED = cardiac implantable electronic device; FDG = fluorodeoxyglucose; IE = infective endocarditis; NVE = native valve endocarditis; PET = positron emission tomography; PVE = prosthetic valve endocarditis; TOE = transoesophageal echocardiography; TTE = transthoracic echocardiography.

*aClass of recommendation.

*bLevel of evidence.

*cReference(s) supporting recommendations.

12.3 Infective endocarditis in the intensive care unit

Admission to the intensive care unit (ICU) is frequently a part of the normal patient pathway following surgery for IE. In addition, patients with IE may be admitted to the ICU due to haemodynamic instability related to severe sepsis, overt HF and/or severe valvular pathology or organ failure from IE-related complications.

The incidence of nosocomial infection is increasing and patients may develop IE as a result of healthcare-associated infection acquired during hospital or intensive care admission. Finally, the diagnosis of IE can be challenging, being made only post-mortem in a number of patients. Despite advances in diagnosis and treatment, mortality remains particularly high in critically ill patients, ranging from 29% to 84%.411,414,415

Estimation of the number of patients requiring ICU admission for IE is challenging. In a retrospective, multicentre, observational study of 4106 patients admitted to four medical ICUs, IE was identified in 0.8% of admissions.416 Reasons for admission to the ICU were congestive cardiac failure (64%), septic shock (21%), neurological deterioration (15%) and cardiopulmonary resuscitation (9%).416 Critical care morbidity is high, with up to 79% of patients requiring mechanical ventilation, 73% inotropic support and 39% developing renal failure.

12.3.1 Organisms

Limited data are available regarding causative organisms for IE in the ICU. Case series have revealed *Staphylococcus* spp. to be the most common causative agent, accounting for 74% of all nosocomial IE cases. Streptococci are the second most common causative organisms. Fungal IE is an increasing problem in the ICU, with *Candida* occurring significantly more often in ICU than non-ICU hospitalized patients.417 There should be a high index of suspicion for fungal IE in the ICU setting, in particular where there is failure to respond to empirical antifungal therapy.

12.3.2 Diagnosis

The diagnostic criteria for IE in the ICU are identical to those for the non-ICU patient population. However, clinical manifestations may be atypical and the classic features may be masked by concomitant pathology and critical care interventions. Thus pyrexia may be attributed to co-existing hospital-acquired infections, neurological manifestations masked by the confounding factors of sedation, ICU-related delirium, concomitant multiple pathologies and acute kidney injury ascribed to co-existing pathologies. Echocardiography can be challenging in the intensive care setting, with a reduced sensitivity of TTE for the diagnosis of IE. There should be a relatively low threshold for TOE in critically ill patients with *S. aureus* catheter-related bloodstream infection because of its high propensity to cause IE, and also, if negative, this may allow short antibiotic treatment.

12.3.3 Management

Patients with severe sepsis or septic shock should be managed according to protocolised international guidelines.418 Antimicrobial management and indications for surgery in patients with IE are described in sections 7 and 10, respectively. However, emergency/salvage status accounts for the highest mortality rates in registry data for patients operated on for IE.299 and patients with SOFA scores >15 on the day of surgery have extremely poor outcomes.125 Decision-making in this most critically ill patient population where indications and contraindications for cardiac surgery co-exist is challenging and should be undertaken in the context of the multi-professional, multidisciplinary Endocarditis Team environment.

12.4 Right-sided infective endocarditis

Right-sided IE accounts for 5–10% of IE cases.419,420 Although it may occur in patients with a pacemaker, ICD, central venous catheter or CHD, this situation is most frequently observed in IVDAs, especially in patients with concomitant human immunodeficiency virus (HIV) seropositivity or in immunosuppressed patients.420–422 *S. aureus* is the predominant organism (60–90% of cases)419,423 with methicillin-resistant strains becoming more prevalent.414 The frequency of polymicrobial infections is also rising.424 The tricuspid valve is most
frequently affected, but other valves—including left-sided—may also become infected. In-hospital mortality is approximately 7%.

12.4.1 Diagnosis and complications

The usual manifestations of right-sided IE are persistent fever, bacteremia and multiple septic pulmonary emboli, which may manifest as chest pain, cough or haemoptysis. When systemic emboli occur, paradoxical embolism or associated left-sided IE should be considered. Isolated right HF is rare, but can be caused by pulmonary hypertension or severe right-sided valvular regurgitation or obstruction. Pulmonary hypertension can be secondary to left-sided IE.

TTE usually allows assessment of tricuspid involvement because of the anterior location of this valve and usual large vegetations. Eustachian and pulmonary valves should always be assessed. TOE is more sensitive in the detection of pulmonary vegetations and associated left-sided involvement.

12.4.2 Prognosis and treatment

Vegetation length >20 mm and fungal aetiology were the main predictors of death in a large retrospective cohort of right-sided IE in IVDA.

Consistent data show that 2-week treatment may be sufficient and that the addition of an aminoglycoside may be unnecessary. In HIV-infected patients, a CD4 count <200 cells/µL has a high prognostic value.

12.4.2.1 Antimicrobial therapy

The choice of empiric antimicrobial therapy depends on the suspected microorganism, type of drug and solvent used by the addict and the infection location. In any case, S. aureus must always be covered. Initial treatment includes penicillinase-resistant penicillins, vancomycin or daptomycin, depending on the local prevalence of MRSA, in combination with gentamicin. If the patient is a pentazocine addict, an antipseudomonas agent should be added. If an IVDA uses brown heroin dissolved in lemon juice, Candida spp. (not Candida albicans) should be considered and antifungal treatment added. Once the causative organisms have been isolated, therapy has to be adjusted.

Consistent data show that 2-week treatment may be sufficient and that the addition of an aminoglycoside may be unnecessary. Two-week treatment with oxacillin (or cloxacillin) without gentamicin or without aminoglycosides is effective for most patients with isolated tricuspid IE if all the following criteria are fulfilled:

- MSSA,
- Good response to treatment,
- Absence of metastatic sites of infection or empyema,
- Absence of cardiac and extracardiac complications,
- Absence of associated prosthetic valve or left-sided valve infection,
- <20 mm vegetation, and
- Absence of severe immunosuppression (<200 CD4 cells/µL) with or without acquired immune deficiency syndrome (AIDS).

Alternatively, when conventional i.v. route therapy is not possible, right-sided S. aureus IE in IVDA may also be treated with oral ciprofloxacin [750 mg bis in die (b.i.d.)] plus rifampicin (300 mg b.i.d.) provided that the strain is fully susceptible to both drugs, the case is uncomplicated and patient adherence is monitored carefully. One randomized controlled study has demonstrated the non-inferiority of daptomycin compared with standard therapy in the treatment of S. aureus infections, including right-sided IE. When using daptomycin, most authors recommend using high doses (10 mg/kg/24 h) and combining it with ciprofloxacin or fosfomycin to avoid the development of resistance to this drug. Glycopeptides (e.g. vancomycin) or daptomycin are the agents of choice for MRSA infections. Vancomycin may have a lower efficacy in infections caused by MRSA strains with a vancomycin MIC >1 µg/mL. In these cases, daptomycin would be the drug of choice. For organisms other than S. aureus, therapy in IVDA does not differ from that in non-IVDA.

12.4.2.2 Surgery

Given the high recurrence rate of IE due to continued drug abuse, surgery should generally be avoided in IVDA with right-

### Table 26

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Classa</th>
<th>Levelb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical treatment should be considered in the following scenarios:</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>- Microorganisms difficult to eradicate (e.g. persistent fungi) or bacteremia for &gt;7 days (e.g. S. aureus, P. aeruginosa) despite adequate antimicrobial therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Persistent tricuspid valve vegetations &gt;20 mm after recurrent pulmonary emboli with or without concomitant right heart failure or right HF secondary to severe tricuspid regurgitation with poor response to diuretic therapy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aClass of recommendation.
bLevel of evidence.
sided native IE, but it has to be considered in the following situations (Table 26):

- Right HF secondary to severe tricuspid regurgitation with poor response to diuretic therapy;
- IE caused by organisms that are difficult to eradicate (e.g. persistent fungi) or bacteremia for at least 7 days (e.g. S. aureus, Pseudomonas aeruginosa) despite adequate antimicrobial therapy; and
- Tricuspid valve vegetations > 20 mm that persist after recurrent pulmonary embolii with or without concomitant right HF.426-433

Cardiac surgery in HIV-infected IVDAs with IE does not worsen the prognosis of either the IE or the HIV.

Recent nationwide data have shown that the three most frequent surgical strategies for tricuspid valve IE are valvectomy, valve repair and valve replacement.429 Tricuspid valve replacement accounted for the majority of cases, with most receiving a bioprosthetic valve. Some authors prefer valve repair (avoiding artificial material whenever possible) over valve replacement, but the former did not improve outcomes over valve replacement or valvectomy.429 Valvectomy without prosthetic replacement can be done in extreme cases, but may be associated with severe postoperative right HF, particularly in patients with pulmonary hypertension. In these cases, the valve can be subsequently replaced once infection has been cured and drug use discontinued. Pulmonary valve replacement should be avoided, but if judged necessary, use of a pulmonary homograft (or, if unavailable, a xenograft valve) is preferred.

In summary, right-sided IE is primarily a disease that affects IVDAs and patients with CHD. Diagnostic features include respiratory symptoms and fever. S. aureus is responsible for most cases. TTE is of major value in these patients. Despite relatively low in-hospital mortality, right-sided IE has a high risk of recurrence in IVDAs and surgery is recommended only for intractable symptoms, failure of medical therapy, recurrent septic emboli to the lungs or paradoxical emboli.

12.5 Infective endocarditis in congenital heart disease

The population of children and adults with CHD is expanding, and this is the major substrate for IE in younger patients. However, our knowledge of IE in this setting is limited since systematic studies are few and often retrospective and selection bias associated with studies from highly specialized centres hampers universal application.

The reported incidence of IE in CHD is 15–140 times higher than that in the general population (the highest estimate originating from a highly specialized unit).442,443 The incidence is lower in children (0.04% per year) than in adults with CHD (0.1% per year).444,445 The reported proportion of CHD in patients with IE varies (probably due to selection bias) by between 2% and 60%,446–450 with a consistent minor male dominance.443,451,452

Some simple lesions, such as secundum atrial septal defect and pulmonary valve disease, carry a low risk of IE, while others, such as bicuspid aortic valve, carry higher risk. However, CHD often consists of multiple cardiac lesions, each contributing to the total risk of IE. For example, the incidence of IE is considerably higher in patients with a ventricular septal defect when there is associated aortic regurgitation.453

The distribution of causative organisms does not differ from the pattern found in acquired heart disease, with streptococci and staphylococci being the most common strains.443,451,452

As in other groups, the diagnosis of IE is often made too late, highlighting the need to consider the diagnosis of IE in any patient with CHD presenting with ongoing fever or other signs of ongoing infection. Blood cultures should be taken before starting antibiotic treatment. The principal symptoms, complications and basis for diagnosis do not differ from IE in general. However, right-sided IE is more frequent in CHD than in acquired cardiac disease. The superiority of TOE over TTE has not been systematically studied in this setting. Nevertheless, complex anatomy and the presence of artificial material may reduce the rate of detection of vegetations and other features of IE, thus favouring the addition of TOE, particularly in the adult group.443 However, a negative study does not exclude the diagnosis.

Care of CHD patients with IE, from diagnosis to treatment, is best provided by specialized CHD centres with expertise in imaging, surgery and intensive care. Cardiac surgery is appropriate when medical therapy fails, when serious haemodynamic complications arise and when there is a high risk of devastating septic embolism.

IE in CHD carries a mortality rate of 4–10%.443,451,452,454 This better prognosis compared with acquired heart disease may reflect the higher proportion of right-heart IE or the better care in CHD centres. Primary prevention is vital.455 The importance of good oral, dental and skin hygiene has already been emphasized, and antibiotic prophylaxis is indicated in high-risk groups as defined in section 3. However, there is also an educational problem, especially in patients not followed in specialist CHD centres, and awareness of the risk of IE and the need for preventive measures are not satisfactorily highlighted in the population with CHD.456 Cosmetic tattooing and piercing, at least involving the tongue and mucous membranes, should be discouraged in this group.

Surgical repair of CHD often reduces the risk of IE, provided there is no residual lesion.417,457 However, in other cases when artificial valve substitutes are implanted, the procedure may increase the overall risk of IE. There are no scientific data justifying cardiac surgery or percutaneous interventions (e.g. closure of a patent ductus arteriosis) with the sole purpose of eliminating the risk of IE.458 Cardiac repair as a secondary preventive measure to reduce the risk of recurrent IE has been described but not systematically studied.

In summary, IE in CHD is rare and more frequently affects the right heart. Care of CHD patients with IE, from diagnosis to treatment, is best provided by specialist CHD centres with expertise in imaging, surgery and intensive care. This applies to most patients with CHD. Complex anatomy makes echocardiographic assessment difficult. However, the diagnosis should be considered in all CHD patients with ongoing infection or fever. Prognosis is better than in other forms of IE, with a mortality rate of <10%. Preventive measures and patient education are of particular importance in this population.

12.6 Infective endocarditis during pregnancy

A challenge for the physician during pregnancy in the cardiac patient is the changing cardiovascular physiology, which can mimic cardiac disease and confuse the clinical picture.459,460 The incidence of IE during pregnancy has been reported to be 0.006%.196 The incidence of IE in patients with cardiac disease is 0–1.2% and is higher in women with a mechanical prosthetic valve.461–464 Therefore IE in pregnancy is extremely rare and is either a complication of a pre-existing cardiac lesion or the result of i.v. drug abuse. Maternal mortality approaches 33%, with most deaths relating to HF or an embolic event, while foetal...
mortality is reported to be about 29%. Close attention should be paid to any pregnant woman with unexplained fever and a cardiac murmur.

Rapid detection of IE and appropriate treatment is important in reducing the risk of both maternal and foetal mortality. Despite the high foetal mortality, urgent surgery should be performed during pregnancy in women who present with HF due to acute regurgitation.

### 12.7 Antithrombotic therapy in infective endocarditis

Indications for anticoagulant and antiplatelet therapy are the same in IE patients as in other patients, and evidence does not support the initiation of medications interfering with the coagulation system as adjunctive therapy for IE. Thrombolytic therapy is generally contraindicated and has sometimes resulted in severe intracranial haemorrhage, but thrombectomy could be an alternative in selected patients with ischaemic stroke related to IE (see section 9.1). The risk of intracranial haemorrhage may be increased in patients already on oral anticoagulants when IE is diagnosed, especially in patients with S. aureus PVE. On the other hand, ongoing oral anticoagulants during IE development may diminish early embolic tendencies.

The recommendations for management of anticoagulant therapy in IE patients are based on a low level of evidence, and decisions should be made on an individual basis by the Endocarditis Team. The role of bridging therapy with unfractionated or low molecular weight heparin has not been studied in patients with IE, but may have reasonable advantages in special situations (i.e. in unstable patients) before surgical decisions are made or to avoid drug interactions.

Evidence does not support initiation of antiplatelet therapy in patients diagnosed with IE, despite promising results in experimental studies. Some cohort studies indicate a possible reduction in the rate of embolic complications or IE development in subgroups of patients already on antiplatelet therapy, but the data are contradictory.

### 12.8 Non-bacterial thrombotic endocarditis and endocarditis associated with cancers

#### 12.8.1 Non-bacterial thrombotic endocarditis

Non-bacterial thrombotic endocarditis (NBTE) (i.e. marantic endocarditis, Libman–Sacks endocarditis or verrucous endocarditis) is characterized by the presence of sterile vegetations consisting of fibrin and platelet aggregates on cardiac valves. These vegetations are associated with neither bacteremia nor with destructive changes of the underlying valve. It is also quite relevant to differentiate true NBTE versus patients with negative blood cultures due to previous antibiotic therapy.

NBTE is a condition associated with numerous diseases such as cancer, connective tissue disorders (i.e. systemic lupus erythematosus patients possessing antiphospholipid antibodies, called Libman–Sacks endocarditis), autoimmune disorders, hypercoagulable states, sepsis, severe burns or chronic diseases such as tuberculosis, uraemia or AIDS. It is a potentially life-threatening source of thromboembolism, its main clinical manifestation.

It is essential to differentiate NBTE from IE. The same initial diagnostic workup used for IE is recommended. The diagnosis of NBTE is difficult and relies on strong clinical suspicion in the context of a disease process known to be associated with NBTE, the presence of a heart murmur, the presence of vegetations not responding to antibiotic treatment and evidence of multiple systemic emboli.

The presence of a new murmur or a change in a pre-existing murmur, although infrequent, in the setting of a predisposing disease should alert the clinician to consider NBTE. Valvular vegetations in NBTE are usually small, broad based and irregularly shaped. They have little inflammatory reaction at the site of attachment, which make them more friable and detachable. Following embolization, small remnants on affected valves (≤3 mm) may result in false-negative echocardiography results. TOE should be ordered when there is a high suspicion of NBTE. Left-sided (mitral more than aortic) and bilateral vegetations are more consistent with NTBE than with IE. When an early TOE examination is performed, the diagnosis of NTBE is improved.

Comprehensive haematological and coagulation studies should be performed to search for a potential cause. Multiple blood cultures should be undertaken to rule out IE, although negative blood cultures

### Table 27 Recommendations for the use of antithrombotic therapy

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interruption of antiplatelet therapy is recommended in the presence of major bleeding</td>
<td>I</td>
<td>B</td>
<td>257</td>
</tr>
<tr>
<td>In intracranial haemorrhage, interruption of all anticoagulation is recommended</td>
<td>I</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>In ischaemic stroke without haemorrhage, replacement of oral anticoagulant (anti-vitamin K) therapy by unfractionated or low molecular weight heparin for 1–2 weeks should be considered under close monitoring</td>
<td>IIa</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>In patients with intracranial haemorrhage and a mechanical valve, unfractionated or low molecular weight heparin should be reintiated as soon as possible following multidisciplinary discussion</td>
<td>IIa</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>In the absence of stroke, replacement of oral anticoagulant therapy by unfractionated or low molecular weight heparin for 1–2 weeks should be considered in the case of Staphylococcus aureus IE under close monitoring</td>
<td>IIa</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Thrombolytic therapy is not recommended in patients with IE</td>
<td>III</td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

IE = infective endocarditis.
Class of recommendation.
Level of evidence.
Reference(s) supporting recommendations.
There is very limited experience with new oral anticoagulant treatment in the field of IE.
can be observed in IE (i.e. previous antibiotic therapy, HACEK group, fungi, etc.). Immunological assays for antiphospholipid syndrome (i.e. lupus anticoagulant, anticardiolipin antibodies, and anti-β2-glycoprotein 1 antibodies; at least one must be positive for the diagnosis of antiphospholipid syndrome on at least two occasions 12 weeks apart) should be undertaken in patients presenting with recurrent systemic emboli or known systemic lupus erythematosus.477

NTBE is first managed by treating the underlying pathology. If there is no contraindication, these patients should be anticoagulated with unfractioned or low molecular weight heparin or warfarin, although there is little evidence to support this strategy. In NTBE, the use of direct thrombin or factor Xa inhibitors has not been evaluated. In antiphospholipid syndrome, lifelong anticoagulation is indicated. A trial comparing rivaroxaban (a factor Xa inhibitor) and warfarin in patients with thrombotic antiphospholipid syndrome is currently in progress.478 However, anticoagulation is associated with a risk of haemorrhagic conversion of embolic events. CT of the brain should be performed in patients with NBTE and cerebral attack before anticoagulation to rule out intracranial haemorrhage.

Surgical intervention, valve debridement and/or reconstruction are often not recommended unless the patient presents with recurrent thromboembolism despite well-controlled anticoagulation. Other indications for valve surgery are the same as for IE. In the context of cancer, a multidisciplinary approach is recommended (Endocarditis Team).

12.8.2 Infective endocarditis associated with cancer

IE may be a potential marker of occult cancers. In a large, Danish, nationwide, population-based cohort study, 997 cancers were identified among 8445 IE patients with a median follow-up of 3.5 years. The risk of abdominal and haematological cancers was high soon after IE diagnosis (within the first 3 months) and remained higher than expected in the long-term follow-up (>12 months) for abdominal cancer.479

Several bacteria have been reported in association with colonic cancer, with the strongest and best-documented relationship with S. bovis infection, specifically the S. gallolyticus subspecies; S. bovis infection has been related to the presence of gastrointestinal neoplasia, which in most cases is colonic adenoma or carcinoma.480 However, it is still a source of debate whether the association of S. bovis/S. gallolyticus IE with colorectal tumours is merely a consequence of the gastrointestinal lesion or could trigger or promote colorectal cancer.481

In the setting of S. bovis IE, there is a need for proper microbiological classification. In case of S. bovis/S. gallolyticus IE, it is recommended to rule out occult colon cancer during hospitalization. In the absence of any tumour, scheduling an annual colonoscopy is highly suggested.482

As for other tests (i.e. faecal occult blood), the serology-based detection of colorectal cancer—serum IgG concentrations against S. bovis antigens—is neither sensitive (not all colorectal tumours are colonized by S. bovis) nor specific.483

FDG PET/CT is increasingly used in the diagnostic workup of IE. It may play an interesting role in detecting gastrointestinal pathological activity and guide colonoscopy. However, negative PET/CT does not rule out significant colonic pathology. No study has examined its clinical value for the detection of occult colorectal cancer in patients with S. bovis/S. gallolyticus IE.

13. To do and not to do messages from the guidelines

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Class</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prophylaxis/prevention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic prophylaxis should be considered for patients at highest risk for IE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Patients with any prosthetic valve, including transcatheter valve, or those in whom any prosthetic material was used for cardiac valve repair</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>b. Patients with a previous episode of IE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Patients with congenital heart disease (i.e. any type of cyanotic congenital heart disease or any type of congenital heart disease repaired with a prosthetic material)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic prophylaxis is not recommended in other forms of valvular or congenital heart disease</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>Dental procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic prophylaxis should only be considered for dental procedures requiring manipulation of the gingival or periapical region of the teeth or perforation of the oral mucosa</td>
<td>IIa</td>
<td>C</td>
</tr>
<tr>
<td>Antibiotic prophylaxis is not recommended for local anaesthetic injections in non-infected tissues, treatment of superficial caries, removal of sutures, dental X-rays, placement or adjustment of removable prosthodontic or orthodontic appliances or braces, or following the shedding of deciduous teeth or trauma to the lips and oral mucosa</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>Other procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic prophylaxis is not recommended for respiratory tract procedures, including bronchoscopy or laryngoscopy, transnasal or endotracheal intubation, gastroscopy, colonoscopy, cystoscopy, vaginal or caesarean delivery, TOE or skin and soft tissue procedures</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>2. Recommendations for referring patients to the Reference Centre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with complicated IE should be evaluated and managed at an early stage in a reference centre with immediate surgical facilities and the presence of a multidisciplinary Endocarditis Team, including an ID specialist, a microbiologist, a cardiologist, imaging specialists, a cardiac surgeon and, if needed, a specialist in CHD</td>
<td>IIa</td>
<td>B</td>
</tr>
<tr>
<td>For patients with non-complicated IE managed in a non-reference centre, there should be early and regular communication with the reference centre and, when needed, visits to the reference centre, should be made</td>
<td>IIa</td>
<td>B</td>
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<tr>
<td>3. Diagnosis</td>
<td></td>
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<tr>
<td>TTE is recommended as the first-line imaging modality in suspected IE</td>
<td>I</td>
<td>B</td>
</tr>
<tr>
<td>TOE is recommended in all patients with clinical suspicion of IE and a negative or non-diagnostic TTE</td>
<td>I</td>
<td>B</td>
</tr>
</tbody>
</table>
Routine antibiotic prophylaxis is recommended before device implantation. Temporary pacing is not routinely recommended for reimplantation. After device extraction, reassessment of the need for extraction is recommended in most cases. Percutaneous extraction is recommended in most infections, as well as in presumably isolated pocket remnants (new murmur, embolism, persisting fever, HF, abscess, atrioventricular block).

The recommendations for the use of antithrombotic therapy include interruption of antithrombotic therapy in the presence of major bleeding, interruption of all anticoagulation in intracranial hemorrhage, thrombolytic therapy not recommended in patients with IE.

### Recommendations for the use of antithrombotic therapy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Class</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interruption of antithrombotic therapy in the presence of major bleeding</td>
<td>I</td>
<td>B</td>
</tr>
<tr>
<td>Interruption of all anticoagulation in intracranial hemorrhage</td>
<td>I</td>
<td>C</td>
</tr>
<tr>
<td>Thrombolytic therapy not recommended in patients with IE</td>
<td>III</td>
<td>C</td>
</tr>
</tbody>
</table>

### 7. Recommendations for the use of antithrombotic therapy

#### Interruption of antithrombotic therapy

- **Class I**
  - **Level B**: Interruption of antithrombotic therapy is recommended in the presence of major bleeding.
  - **Level C**: Interruption of all anticoagulation is recommended in intracranial hemorrhage.
  - **Level III**: Thrombolytic therapy is not recommended in patients with IE.

### 6. Cardiac device-related infective endocarditis

#### Prolonged (i.e., before and after extraction) antibiotic therapy and complete hardware (device and leads) removal are recommended in definite CDRIE, as well as in presumably isolated pocket infection

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#### Percutaneous extraction is recommended in most patients with CDRIE, even those with vegetation ≥10 mm

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#### After device extraction, reassessment of the need for reimplantation is recommended

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#### Temporary pacing is not routinely recommended

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#### Routine antibiotic prophylaxis is recommended before device implantation

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### 5. Neurological complications

#### After a silent embolism or transient ischemic attack, cardiac surgery, if indicated, is recommended without delay

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#### Neurosurgery or endovascular therapy are indicated for very large, enlarging or ruptured intracranial infectious aneurysm

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#### Following intracranial hemorrhage, surgery should generally be postponed for ≥1 month

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<tr>
<td>IIa</td>
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### 4. Treatment

#### Aortic or mitral NVE or PVE with severe regurgitation or obstruction causing symptoms of HF or echocardiographic signs of poor haemodynamic tolerance must be treated by urgent surgery

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#### Locally uncontrolled infection (abscess, false aneurysm, fistula, enlarging vegetation) must be treated by urgent surgery

<table>
<thead>
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#### Infection caused by fungi or multiresistant organisms must be treated by urgent surgery

<table>
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<td>I</td>
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</table>

#### Aortic or mitral NVE or PVE with persistent vegetation >10 mm after ≥1 embolic episodes despite appropriate antibiotic therapy must be treated by urgent surgery

<table>
<thead>
<tr>
<th>Class</th>
<th>Level</th>
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<tbody>
<tr>
<td>I</td>
<td>B</td>
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</table>

### 14. Appendix

**ESC Committee for Practice Guidelines (CPG)**: Jose Luis Zamorano (Chairperson) (Spain), Victor Aboyans (France), Stephan Achenbach (Germany), Stefan Agewall (Norway), Lina Badimon (Spain), Gonzalo Barón-Escquiáis (Spain), Helmut Baumgartner (Germany), Jeroen J. Bax (The Netherlands), Héctor Bueno (Spain), Picciione Carerj (Italy), Veronica Dean (France), Çetin Erol (Turkey), Donna Fitzsimons (UK), Oliver Gaemperli (Switzerland), Paulus Kirchhof (UK/Germany), Philippe Kolb (Belgium), Patrizio Lancellotti (Belgium), Gregory Y.H. Lip (UK), Petros Nihoyannopoulos (UK), Massimo F. Piepoli (Italy), Piotr Ponikowski (Poland), Marco Roffi (Switzerland), Adam Torbicki (Poland), Antonio Vaz Carneiro (Portugal), Stephan Windecker (Switzerland).

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**Japan**: Japanese Circulation Society, K. Naber; **Korea**: Korean Society of Cardiology, K. Naber; **Latvia**: Latvian Society of Cardiology, Ilja Sars; **Lithuania**: Lithuanian Society of Cardiology, T. P. Virbalis; **Luxembourg**: Luxembourg Society of Cardiology, Herminio Koósko-Blanquet; **Macedonia**: Macedonian Society of Cardiology, Georgios C. Georgiou; **Netherlands**: Dutch Society of Cardiology, H. J. D. van Dijk; **Norway**: Norwegian Society of Cardiology, Øyvind Østby; **Poland**: Polish Society of Cardiology, Anna J. Pietraszewicz; **Portugal**: Portuguese Society of Cardiology, François Delahaye; **Romania**: Romanian Society of Cardiology, I. Sturjan; **Russia**: Russian Society of Cardiology, O. E. Vlasova; **Serbia**: Serbian Society of Cardiology, S. K. Kostic; **Slovakia**: Slovakian Society of Cardiology, M. Minarik; **Spain**: Spanish Society of Cardiology, J. J. Bax; **Sweden**: Swedish Society of Cardiology, S. O. Lindgren; **Switzerland**: Swiss Society of Cardiology, J. P. C. Güthner; **Turkey**: Turkish Society of Cardiology, C. Erol; **Ukraine**: Ukrainian Society of Cardiology, P. V. Lisk; **United Kingdom**: UK Core Practice Group, J. J. Bax; **United States**: American College of Cardiology, J. L. Bax; **USSR**: Union of Soviet Socialist Republics, S. K. Kostic; **Yugoslavia**: Yugoslav Society, M. Maksimovic.
15. References


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CME questions for this article are available at: European Heart Journal http://www.oxfordjournals.org/eurheartj and European Society of Cardiology http://www.escardio.org/ guidelines


Candida Infective Endocarditis: an Observational Cohort Study with a Focus on Therapy

Christopher J. Arnold, Melissa Johnson, Arnold S. Bayer, Suzanne Bradley, Efthymia Giannitsioti, José M. Miró, Pilar Tornos, Pierre Tattevin, Jacob Strahilevitz, Denis Spelman, Eugene Athan, Francisco Nacinovich, Claudio Q. Fortes, Cristiane Lamas, Bruno Barsic, Nuria Fernández-Hidalgo, Patricia Muñoz, Vivian H. Chu, for the ICE Investigators

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Candida infective endocarditis is a rare disease with a high mortality rate. Our understanding of this infection is derived from case series, case reports, and small prospective cohorts. The purpose of this study was to evaluate the clinical features and use of different antifungal treatment regimens for Candida infective endocarditis. This prospective cohort study was based on 70 cases of Candida infective endocarditis from the International Collaboration on Endocarditis (ICE)-Prospective Cohort Study and ICE-Plus databases collected between 2000 and 2010. The majority of infections were acquired nosocomially (67%). Congestive heart failure (24%), prosthetic heart valve (46%), and previous infective endocarditis (26%) were common comorbidities. Overall mortality was high, with 36% mortality in the hospital and 59% at 1 year. On univariate analysis, older age, heart failure at baseline, persistent candidemia, nosocomial acquisition, heart failure as a complication, and intracardiac abscesses were associated with higher mortality. Mortality was not affected by use of surgical therapy or choice of antifungal agent. A subgroup analysis was performed on 33 patients for whom specific antifungal therapy information was available. In this subgroup, 11 patients received amphotericin B-based therapy and 14 received echinocandin-based therapy. Despite a higher percentage of older patients and nosocomial infection in the echinocandin group, mortality rates were similar between the two groups. In conclusion, Candida infective endocarditis is associated with a high mortality rate that was not impacted by choice of antifungal therapy or by adjunctive surgical intervention. Additionally, echinocandin therapy was as effective as amphotericin B-based therapy in the small subgroup analysis.

Candida infective endocarditis (CIE) accounts for only 1 to 2% of all cases of infective endocarditis (IE) (1). This infection is important because it is associated with an exceptionally high mortality rate ranging from 30 to 80% (1–5). In addition, rates of fungemia have increased significantly in recent years, resulting in a growing number of patients at risk for this disease (2, 6).

Due to its rarity, our understanding of the clinical features, treatment, and mortality of CIE has been derived predominantly from retrospective reviews of case series, case reports, and several small prospective series (1, 2, 7). The standard-of-care treatment for CIE has historically been an amphotericin B-based regimen coupled with adjunctive surgical therapy. However, the options for treating invasive Candida infections changed with the development of the echinocandins. Echinocandins have fungicidal activity and exert their effect by inhibiting beta-glucan synthesis and disrupting the fungal cell wall. In 2003, caspofungin, the first echinocandin, was approved as therapy for invasive candidiasis, and since that time there has been a small but growing body of literature regarding echinocandin use in CIE (1, 2, 8–13). This has resulted in the addition of echinocandins to both the most recent Infectious Diseases Society of America (IDSA) and European Society for Microbiology and Clinical Infectious Diseases (ESCMID) guidelines for treatment of CIE, which now recommend either an amphotericin B-based regimen or an echinocandin-based regimen, both of these in combination with adjunctive surgical therapy if possible (14, 15). Nevertheless, these guidelines are based largely on case reports, case series, and clinical experience. To date, the largest prospective series have included 30 and 33 patients, respectively (1, 2). Additionally, there are no studies to date comparing amphotericin B- to echinocandin-based therapy for candidal infective endocarditis.

In this study, we used two large, contemporary, multinational, prospective cohorts of patients to better investigate the clinical features, treatment, and predictors of mortality in patients with CIE. Additionally, we compared amphotericin B- to echinocandin-based therapy in a subset of the cohort.
MATERIALS AND METHODS

Study design. Data for this observational cohort study were derived from the International Collaboration on Endocarditis Prospective Cohort Study (ICE-PCS) and ICE-Plus databases. ICE-PCS and ICE-Plus have each been previously described, including a detailed description of the ICE organization and methodologies for data collection and cataloging (3, 16, 17). Briefly, the ICE-PCS database contains prospective data on 4,794 patients with definite IE from 64 sites in 28 countries occurring between June 2000 and September 2006. The ICE-Plus database contains prospective data on 1,112 patients with definite IE from 29 sites in 16 countries occurring between September 2008 and December 2010. Data for each of these databases were gathered prospectively via a case report form (CRF) developed by ICE collaborators according to standard definitions (3, 18).

Additionally, for this study, a supplemental CRF was sent to enrolling sites from which cases of IE were identified. This supplemental CRF was designed to obtain detailed information regarding antifungal therapy and additional risk factors for CIE, as well as 42-day follow-up information. The ICE databases are maintained at the Duke Clinical Research Institute (DCRI), which serves as the coordinating center for the ICE studies, with institutional review board approvals from the Duke University School of Medicine and the participating ICE-PCS and ICE-Plus sites.

Study population. Patients were included in this study if they met both of the following criteria: (i) diagnosis of definite IE by the modified Duke criteria (19) and (ii) fungal IE caused by a Candida species only. Only patients for whom supplemental CRF information was obtained were included in the subgroup analysis specifically examining the association between antifungal therapy and outcomes.

Definitions. Infective endocarditis was defined according to the modified Duke criteria (19). A predisposing valvular condition was defined as having a native valve known to be affected at baseline by regurgitation or stenosis. Liver disease included a composite of mild, moderate, and severe disease as defined by a Child’s Pugh score of ≥5. Renal disease was defined as a composite of acute kidney injury (AKI), chronic kidney disease (CKD) at all stages, and end-stage renal disease (ESRD), including patients on hemodialysis (HD). Endocarditis device included the presence of either a pacemaker, an internal cardiac defibrillator (ICD), a left ventricular assist device (LVAD), or a right ventricular assist device (RVAD). The presence of any prosthetic material was defined to include patients who had any of the following: prosthetic valve, endocarditis device, intravenous graft material, prosthetic joint, orthopedic rod, and bone plates or screws.

Hospital-acquired IE was defined as IE developing in a patient hospitalized for more than 48 h prior to the onset of signs/symptoms consistent with IE. Health care–associated IE was defined as IE diagnosed within 48 h of admission in an outpatient with extensive health care contact as reflected by any of the following criteria: (i) receipt of intravenous therapy, wound care, or specialized nursing care at home within the 30 days prior to the onset of infection; (ii) attendance at a hospital or hemodialysis clinic or receipt of intravenous chemotherapy within the 30 days before the onset of infection; (iii) hospitalization in an acute care hospital for 2 or more days in the 90 days before the onset of infection; or (iv) residence in a nursing home or long-term care facility (20). Community-acquired IE was defined as IE diagnosed at the time of admission (or within 48 h of admission) in a patient not fulfilling the criteria for health care–associated IE.

Paravalvular complication was defined as the presence of any of the following in a patient with native valve IE: paravalvular abscess, paravalvular fistula, or valvular perforation. Prosthetic valve complication was defined as the presence of any of these same complications in a patient with prosthetic valve IE. Persistently positive blood culture was defined as having positive blood cultures >72 h following initiation of antifungal therapy.

For the subgroup analysis on antifungal therapy, patients were assigned to treatment groups based on the antifungal drug that they received for the majority of the first 30 days of therapy. These groups were termed majority regimen backbone groups. Patients receiving an echinocandin-based regimen for >15 days of the first 30 days of treatment were classified as being in the echinocandin backbone therapy group, and those receiving an amphotericin B-based regimen for >15 days of the first 30 days of treatment were placed in the amphotericin B backbone therapy group. An amphotericin B-based regimen was defined as a regimen that included any of the following: amphotericin B deoxycholate, amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC), or amphotericin B liposomal formulation (LAmB). An echinocandin-based regimen was defined as a regimen that contained caspofungin, micafungin, or anidulafungin. A treatment regimen was defined as a majority combination therapy regimen if the patient received at least two antifungal drugs concomitantly for >15 days of the first 30 days of therapy. A treatment regimen was defined as receiving any combination therapy if the patient received >1 day of two antifungal drugs concomitantly at any point during therapy. Suppressive antifungal therapy was defined as transition of antifungal therapy to azole-based therapy following initial treatment period for patients treated with either amphotericin B- or echinocandin-based therapy. For patients treated from onset of infection with azole-based therapy, suppressive therapy was defined as a duration of azole therapy of >120 days.

Outcomes. Clinical characteristics, complications (both clinical and echocardiographic), and mortality were compared between those receiving amphotericin B-based therapy and those receiving echinocandin-based therapy. These same variables were compared between the following groups: (i) those receiving adjunctive surgical therapy versus those receiving medical therapy alone and (ii) those infected with Candida albicans versus those infected with Candida parapsilosis. Additionally, univariate analysis was performed to look for predictors of in-hospital and 1-year mortality in the overall cohort.

Statistical analysis. All statistical analyses were performed using JMP Pro (version 11.0). Patients’ demographics and clinical variables were described as means and standard deviations for continuous data and proportions for categorical data. The χ² or Fisher exact test was used to compare categorical variables between groups, as appropriate. The Student t test or 1-way analysis of variance (ANOVA) was used to test significant differences of continuous variables between groups, as appropriate. A two-tailed P value of 0.05 or less was considered significant.

RESULTS

A total of 70 cases of definite Candida infective endocarditis (CIE) were identified, 52 cases from ICE-PCS and 18 cases from ICE-Plus. Forty-three patients (61%) were men. The mean age was 54.3 years. The majority of patients were over the age of 50 (63%), and nearly half were over the age of 60 (Table 1). Forty-six percent of patients had a prosthetic cardiac valve, and 20% had an endocarditis device. The most common other comorbidities were congestive heart failure (CHF), diabetes mellitus (DM), and renal disease. Twenty-six percent of patients had a history of a previous episode of infective endocarditis (IE) (Table 1).

Over half of the infections were hospital acquired, and only 27% were community acquired (Table 1). Among the 19 patients with community-acquired disease, 7 (37%) engaged in intravenous drug abuse (IVDA). Of the remaining 12 patients with community-acquired disease, 7 had prosthetic valves (2 with a concomitant endocarditis device), 2 had endocarditis devices alone, and 3 had only one of the following nonoverlapping comorbidities: HIV, renal disease, or liver disease.

The most common clinical complication was systemic embolization (34%), followed by CHF (31%) and intracardiac abscesses (24%) (Table 1). Echocardiographic evidence of complications was present in 19% of those with native valves and 34% of those with prosthetic valves (Table 1). Sixty-one patients (87%) had
evidence of at least one clinical or echocardiographic complication.

The most common organisms isolated were *C. albicans* (*n* = 31) and *C. parapsilosis* (*n* = 19), comprising over 70% of the cases (Table 2). One patient was infected with both *C. albicans* and *C. parapsilosis* and was excluded from the analysis comparing infections with these two organisms. Those infected with *C. parapsilosis* were more likely to be diabetic (42% versus 16%, *P* = 0.04) and were more likely to have community-acquired infection (44% versus 14%, *P* = 0.04) than were those with *C. albicans*. The majority of other patient characteristics and outcomes were similar between these two organisms (Table 1).

Thirty-two patients (46%) were treated with adjunctive surgical therapy. Patients receiving surgery were younger than those receiving medical therapy alone (Table 3). Those with intracardiac abscess were more likely to receive adjunctive surgical therapy...
TABLE 2 Microbiology of the overall cohort

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. (%) in overall cohort (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>31 (44)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>19 (27)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>7 (10)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>4 (6)</td>
</tr>
<tr>
<td>C. krusei</td>
<td>1 (1)</td>
</tr>
<tr>
<td>C. albicans plus C. parapsilosis</td>
<td>1 (1)</td>
</tr>
<tr>
<td>NOSa</td>
<td>7 (10)</td>
</tr>
</tbody>
</table>

a NOS, not otherwise specified.

(38% versus 13%, P = 0.02). All other characteristics evaluated were similar between those receiving adjunctive surgical therapy and those receiving medical therapy alone. There was no difference in in-hospital or 1-year mortality (Table 3).

The all-cause mortality of the overall CIE cohort was 36% in hospital and 59% at 1 year. This did not differ by therapy or by species. On univariate analysis, CHF at baseline was found to be a predictor of both in-hospital and 1-year mortality (Table 4). Other predictors of in-hospital mortality included older age and persistently positive blood cultures, while other predictors of 1-year mortality were nosocomial acquisition of infection, CHF as a complication, and intracardiac abscess (Table 4).

Detailed data regarding antifungal therapy were obtained for 33 patients using the supplemental CRF. The majority of patients received either an amphotericin B-based regimen (n = 11) or an echinocandin-based regimen (n = 14) (Table 5). Of the remaining patients, 6 received primarily azole-based therapy and 2 received a combination of amphotericin B and an echinocandin. The two patients receiving both amphotericin B and an echinocandin as their primary backbone regimen were excluded from the analysis when comparing the two therapies. Overall, 45% of patients received combination antifungal therapy at some point during treatment. The most common concomitantly prescribed antifungal was flucytosine, followed by fluconazole.

Treatment regimens were highly varied, with many patients undergoing sequential changes in therapy. The most common reason for a change in therapy was renal failure, followed by transition to suppressive therapy. Overall, 21/33 (64%) patients received amphotericin B at some point during their treatment course. Twelve of these patients (57%) developed acute kidney injury necessitating a change in therapy; amphotericin B therapy was discontinued altogether in 8 patients (38%) and changed to a lipid-based preparation of amphotericin B in the remaining 4 patients. Discontinuation of therapy due to adverse events was not observed in any patients receiving echinocandin-based therapy.

There was a higher percentage of older patients in the echinocandin group than in the amphotericin B group (Table 5). The majority of infections in the amphotericin B group were community acquired (82%), compared to less than half of the infections in the echinocandin group (42%) (P = 0.05). The rates of utilization of combination antifungal therapy, suppressive antifungal therapy, and adjunctive surgery did not differ between the two groups. Mortalities measured at all 3 time points (in hospital, 42 days, and 1 year) did not differ between the two groups (Table 5).

**DISCUSSION**

*Candida* IE (CIE) is a rare, but often deadly, disease. To date, our understanding of its clinical features and treatment practices has been based largely on case series and reports. Prospective studies have been small, with the two largest to date including 30 and 33 patients, respectively (1, 2). An earlier study by Baddley et al. included 33 patients from 2000 to 2005 and was the first examination of CIE cases from the ICE database (2). Our current study has an additional 37 patients from 2005 to 2010, making it the largest prospective study to date on this serious infection. It is also the first to compare relatively newer antifungal therapy (echinocandins) to historically standard therapy (amphotericin B).

Similar to prior studies, we found a high proportion of health care-associated infections (1, 2). Data previously reported from the ICE cohort showed a 51.5% incidence of health care-associated infection (2). In our current analysis, this has risen to 67%,
which is consistent with data indicating Candida as an emerging pathogen for nosocomial bloodstream infections (21). In conjunction with the high proportion of health care-associated infection was the overall advanced age of the CIE population, with nearly half of the patients being over the age of 60. Elderly patients with multiple comorbidities are more likely to have contact with the health care system and thus are more likely to acquire this predominantly nosocomial infection. With respect to community-acquired infection, intravenous drug abuse (IVDA) is classically associated with CIE; nevertheless, fewer than half of community-acquired CIE cases were associated with IVDA. Among those with non-IVDA community-acquired infection, most patients had a prosthetic valve or endocavity device as risk factors. Community-acquired infection outside IVDA, prosthetic valve, or an endocavity device was exceedingly rare, occurring in only 3 patients (4%). The mortality rate for CIE was exceptionally high in our cohort. The in-hospital mortality rate was over one-third, and the 1-year mortality rate approached two-thirds, similar to what has been reported in the literature (1, 2, 7). Despite advances in antifungal therapy and surgical technique, the mortality rate has remained this high throughout studies over time. Furthermore, in our study mortality did not appear to be impacted by either use of adjunctive surgical therapy or choice of antifungal therapy. This is likely reflective of the overall poor health of elderly hospitalized patients with multiple comorbidities who are predisposed to acquiring this infection. Indeed, baseline characteristics such as older age, preexisting heart failure, and nosocomial acquisition

<p>| TABLE 4 Predictors of in-hospital and 1-year mortality on univariate analysis for overall Candida infective endocarditis cohorta |
|-----------------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>In-hospital mortality, no. positive/total no. (%)</th>
<th>RR (95% CI)</th>
<th>1-yr mortality, no. positive/total no. (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>5/19 (26)</td>
<td>1 (ref)</td>
<td>11/18 (61)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>14/31 (45)</td>
<td>1.72 (0.74–4)</td>
<td>19/29 (66)</td>
<td>1.07 (0.68–1.69)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>5/26 (19)</td>
<td>1 (ref)</td>
<td>12/22 (55)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>≥50</td>
<td>20/44 (45)</td>
<td><strong>2.36 (1.01–5.54)</strong></td>
<td>28/41 (68)</td>
<td><strong>1.25 (0.81–1.93)</strong></td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native valve</td>
<td>14/38 (37)</td>
<td>1 (ref)</td>
<td>21/32 (66)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Prosthetic valve</td>
<td>11/32 (34)</td>
<td>0.93 (0.49–1.76)</td>
<td>19/31 (61)</td>
<td>0.93 (0.64–1.36)</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>19/55 (35)</td>
<td>1 (ref)</td>
<td>31/48 (65)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6/15 (40)</td>
<td>1.16 (0.56–2.38)</td>
<td>9/15 (60)</td>
<td>0.93 (0.58–1.48)</td>
</tr>
<tr>
<td>No CHF at baseline</td>
<td>14/53 (26)</td>
<td>1 (ref)</td>
<td>24/47 (51)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>CHF at baseline</td>
<td>11/17 (65)</td>
<td><strong>2.45 (1.38–4.33)</strong></td>
<td>16/16 (100)</td>
<td><strong>1.96 (1.48–2.59)</strong></td>
</tr>
<tr>
<td>First episode of IE</td>
<td>19/51 (37)</td>
<td>1 (ref)</td>
<td>28/44 (64)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>History of previous IE</td>
<td>6/18 (33)</td>
<td>0.89 (0.43–1.88)</td>
<td>11/18 (61)</td>
<td>0.96 (0.62–1.48)</td>
</tr>
<tr>
<td>Clinical complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No stroke</td>
<td>24/62 (39)</td>
<td>1 (ref)</td>
<td>36/55 (65)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1/8 (13)</td>
<td>0.32 (0.05–2.07)</td>
<td>4/8 (50)</td>
<td>0.76 (0.37–1.57)</td>
</tr>
<tr>
<td>No systemic embolization</td>
<td>17/46 (37)</td>
<td>1 (ref)</td>
<td>28/41 (68)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Systemic embolization</td>
<td>8/24 (33)</td>
<td>0.90 (0.46–1.78)</td>
<td>12/22 (55)</td>
<td>0.80 (0.52–1.23)</td>
</tr>
<tr>
<td>No CHF as complication</td>
<td>14/48 (29)</td>
<td>1 (ref)</td>
<td>20/43 (47)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>CHF as complication</td>
<td>11/22 (50)</td>
<td>1.71 (0.93–3.15)</td>
<td>20/20 (100)</td>
<td><strong>2.15 (1.56–2.96)</strong></td>
</tr>
<tr>
<td>No intracardiac abscess</td>
<td>17/53 (32)</td>
<td>1 (ref)</td>
<td>27/48 (56)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Intracardiac abscess</td>
<td>8/17 (47)</td>
<td>1.47 (0.77–2.78)</td>
<td>13/15 (87)</td>
<td><strong>1.54 (1.12–2.12)</strong></td>
</tr>
<tr>
<td>Bloodstream clearance ≤72 h</td>
<td>14/56 (25)</td>
<td>1 (ref)</td>
<td>29/50 (58)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Persistently positive blood cultures</td>
<td>9/19 (75)</td>
<td><strong>3 (1.72–5.25)</strong></td>
<td>9/11 (82)</td>
<td>1.41 (0.98–2.03)</td>
</tr>
<tr>
<td>Echocardiographic complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No paravalvarular complication</td>
<td>20/56 (36)</td>
<td>1 (ref)</td>
<td>31/51 (61)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Paravalvarular complication</td>
<td>4/13 (31)</td>
<td>0.86 (0.35–2.09)</td>
<td>8/11 (73)</td>
<td>1.20 (0.78–1.83)</td>
</tr>
<tr>
<td>Prosthetic valve complication</td>
<td>6/21 (29)</td>
<td>1 (ref)</td>
<td>12/21 (57)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Prosthetic valve complication</td>
<td>5/11 (45)</td>
<td>1.60 (0.62–4.06)</td>
<td>7/10 (70)</td>
<td>1.22 (0.71–2.12)</td>
</tr>
<tr>
<td>Mode of acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community acquired</td>
<td>3/19 (16)</td>
<td>1 (ref)</td>
<td>6/16 (38)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Hospital/health care associated</td>
<td>21/47 (45)</td>
<td>2.83 (0.96–8.38)</td>
<td>32/44 (73)</td>
<td><strong>1.93 (1.01–3.74)</strong></td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical therapy alone</td>
<td>13/38 (34)</td>
<td>1 (ref)</td>
<td>21/34 (62)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Adjunctive surgical therapy</td>
<td>12/32 (38)</td>
<td>1.10 (0.58–2.05)</td>
<td>19/29 (66)</td>
<td>1.06 (0.73–1.54)</td>
</tr>
</tbody>
</table>

Abbreviations: CHF, congestive heart failure; IE, infective endocarditis; RR, risk ratio; CI, confidence interval; ref, reference. Boldface indicates statistically significant values.
were all associated with higher mortality on univariate analysis. Higher mortality was also associated with clinical developments such as refractory candidemia and new CHF, features which may help identify candidates for early, aggressive interventions.

Adjunctive surgical therapy has long been considered to be the gold standard in treating CIE. The current IDSA and ESCMID guidelines recommend surgical therapy if possible (14, 15); however, this is based largely on case series and reports as well as expert

### TABLE 5 Antifungal therapy for Candida infective endocarditis subgroup analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall treatment subgroup (n = 33)</th>
<th>Amphotericin B group (n = 11)</th>
<th>Echinocandin group (n = 14)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>13 (39)</td>
<td>3 (27)</td>
<td>3 (21)</td>
<td>1.00</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>12 (36)</td>
<td>5 (45)</td>
<td>7 (50)</td>
<td>0.82</td>
</tr>
<tr>
<td>Other</td>
<td>8 (24)</td>
<td>2 (18)</td>
<td>4 (29)</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>61.0 (55.5–66.4)</td>
<td>52.4 (43.4–61.3)</td>
<td>62.5 (52.6–72.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>≥50</td>
<td>26 (79)</td>
<td>8 (73)</td>
<td>10 (71)</td>
<td>1.00</td>
</tr>
<tr>
<td>≥60</td>
<td>19 (58)</td>
<td>3 (27)</td>
<td>10 (71)</td>
<td>0.05</td>
</tr>
<tr>
<td>≥70</td>
<td>14 (42)</td>
<td>1 (9)</td>
<td>7 (50)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Community acquired</strong></td>
<td>10 (31)</td>
<td>9 (82)</td>
<td>6 (42)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosthetic valve</td>
<td>13 (39)</td>
<td>3 (27)</td>
<td>7 (50)</td>
<td>0.41</td>
</tr>
<tr>
<td>Predisposing valve condition</td>
<td>8 (24)</td>
<td>4 (36)</td>
<td>3 (21)</td>
<td>0.66</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>1 (3)</td>
<td>0</td>
<td>1 (7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Endocarditv device</td>
<td>8 (24)</td>
<td>1 (9)</td>
<td>5 (36)</td>
<td>0.18</td>
</tr>
<tr>
<td>Previous IE</td>
<td>8 (24)</td>
<td>4 (36)</td>
<td>2 (14)</td>
<td>0.35</td>
</tr>
<tr>
<td>CHF</td>
<td>12 (36)</td>
<td>4 (36)</td>
<td>6 (43)</td>
<td>1.00</td>
</tr>
<tr>
<td>Intravenous catheter</td>
<td>16 (52)</td>
<td>5 (50)</td>
<td>4 (29)</td>
<td>0.40</td>
</tr>
<tr>
<td>Any prothetic material</td>
<td>20 (61)</td>
<td>5 (45)</td>
<td>10 (71)</td>
<td>0.24</td>
</tr>
<tr>
<td>Renal disease</td>
<td>12 (36)</td>
<td>5 (45)</td>
<td>5 (36)</td>
<td>0.70</td>
</tr>
<tr>
<td>Liver disease</td>
<td>5 (16)</td>
<td>3 (33)</td>
<td>1 (7)</td>
<td>0.26</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>10 (30)</td>
<td>2 (18)</td>
<td>5 (36)</td>
<td>0.41</td>
</tr>
<tr>
<td>Cancer</td>
<td>5 (15)</td>
<td>0</td>
<td>2 (14)</td>
<td>0.49</td>
</tr>
<tr>
<td>IVDA</td>
<td>2 (6)</td>
<td>2 (18)</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>ICU in last 14 days</td>
<td>11 (33)</td>
<td>3 (27)</td>
<td>3 (21)</td>
<td>1.00</td>
</tr>
<tr>
<td>Surgery in last 30 days</td>
<td>11 (33)</td>
<td>3 (27)</td>
<td>3 (21)</td>
<td>1.00</td>
</tr>
<tr>
<td>TPN</td>
<td>7 (21)</td>
<td>2 (18)</td>
<td>2 (14)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Echocardiographic complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regurgitation</td>
<td>17 (52)</td>
<td>6 (55)</td>
<td>6 (43)</td>
<td>0.56</td>
</tr>
<tr>
<td>Paravalvular complication</td>
<td>8 (24)</td>
<td>2 (18)</td>
<td>3 (21)</td>
<td>1.00</td>
</tr>
<tr>
<td>Prosthetic valve complication</td>
<td>4 (31)</td>
<td>1 (33)</td>
<td>0</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Clinical complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>4 (13)</td>
<td>1 (10)</td>
<td>3 (21)</td>
<td>0.61</td>
</tr>
<tr>
<td>Embolization</td>
<td>11 (33)</td>
<td>3 (27)</td>
<td>4 (29)</td>
<td>1.00</td>
</tr>
<tr>
<td>CHF</td>
<td>13 (39)</td>
<td>3 (27)</td>
<td>7 (50)</td>
<td>0.41</td>
</tr>
<tr>
<td>Intracardiac abscess</td>
<td>11 (33)</td>
<td>5 (45)</td>
<td>3 (21)</td>
<td>0.39</td>
</tr>
<tr>
<td>Mycotic aneurysm</td>
<td>1 (3)</td>
<td>1 (10)</td>
<td>0</td>
<td>0.42</td>
</tr>
<tr>
<td>Persistently positive cultures</td>
<td>2 (6)</td>
<td>0</td>
<td>1 (7)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Majority regimen combination antifungal therapy</td>
<td>13 (39)</td>
<td>5 (45)</td>
<td>5 (36)</td>
<td>0.62</td>
</tr>
<tr>
<td>Any combination antifungal therapy</td>
<td>15 (45)</td>
<td>6 (55)</td>
<td>6 (43)</td>
<td>0.56</td>
</tr>
<tr>
<td>Suppressive antifungal therapy received</td>
<td>14 (42)</td>
<td>5 (45)</td>
<td>6 (43)</td>
<td>0.90</td>
</tr>
<tr>
<td>Adjunctive surgical therapy</td>
<td>13 (39)</td>
<td>6 (55)</td>
<td>5 (36)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In hospital</td>
<td>13 (39)</td>
<td>5 (45)</td>
<td>4 (29)</td>
<td>0.43</td>
</tr>
<tr>
<td>42 days</td>
<td>14 (42)</td>
<td>5 (45)</td>
<td>5 (36)</td>
<td>0.62</td>
</tr>
<tr>
<td>1 yr</td>
<td>21 (66)</td>
<td>7 (64)</td>
<td>9 (69)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Abbreviations: IE, infective endocarditis; CHF, congestive heart failure; IVDA, intravenous drug abuse; ICU, intensive care unit; TPN, total parenteral nutrition.

* p value for comparison of amphotericin B-based therapy to echinocandin-based therapy. Boldface indicates statistically significant values.
Candida Infective Endocarditis

opinion. A large meta-analysis published in 2005 reported a trend toward improved survival with surgical therapy, although this did not meet statistical significance (odds ratio [OR], 0.56; confidence interval [CI], 0.16 to 1.99) (7). Interestingly, in that analysis survival among those receiving combination antifungal therapies appeared similar to that of those receiving adjunctive surgical therapy. One conclusion suggested by the authors was that newer antifungal therapies potentially lent hope to those who could not undergo surgical therapy. In our study, mortality did not differ between those undergoing surgical therapy and those receiving medical therapy alone. The two groups appeared similar overall with respect to distribution of comorbidities. Additionally, the surgical group was comprised of younger patients, which should bias the results toward a better outcome with surgical therapy over medical therapy. Acknowledging the small numbers included in each study, similar to the study by Steinbach et al. (7), our study calls into question the dogma of recommending surgical therapy for all patients with CIE, based solely on the organism.

Amphotericin B-based therapy has long been considered the standard therapy for CIE, based largely on experience and case series. The echinocandins are in comparison relatively new agents, having been approved for candidemia only within the past decade. Like amphotericin B, the echinocandins are fungicidal, and similar to the lipid formulations of amphotericin B, they have good activity against candidal biofilms (12, 22, 23). Although the two therapies have been compared for treatment of invasive candidiasis, no studies have compared them for treatment of infective endocarditis (24).

Our study is the first to attempt to compare the amphotericin B- and echinocandin-based therapies for CIE. Despite the limited number of patients in the subgroup analysis comparing therapies, there are still some important findings. There was no difference in mortality between patients receiving the two therapies. The echinocandin group had several factors that should have biased toward a worse outcome, including a statistically significant higher percentage of older patients as well as a higher percentage of nosocomial infection, both of which were shown to be associated with higher mortality on univariate analysis. Additionally, although not statistically significant, there were a higher percentage of patients with prosthetic valves in the echinocandin group. Despite these differences, the mortalities did not differ between the two groups. Coupling this with the substantially lower rate of adverse events, specifically renal failure, associated with echinocandin therapy, echinocandin-based therapy appears to be an attractive option for this disease. Given the observational nature of this study and small sample size, no definitive recommendations can be made; however, our study provides additional supporting evidence for the use of echinocandins in CIE (14).

Our study, like all studies to date on CIE, is limited by small sample size. While the overall cohort represents the largest prospective cohort to date, the subgroup for antifungal therapy was small at only 33 patients. This may have limited our ability to demonstrate statistically significant differences between the therapy groups. Additionally, we were limited to analyzing antifungal therapy in only those patients whose enrolling sites completed the supplemental CRF, which could result in selection bias. Although the majority of the data were collected prospectively, the data on antifungal therapy were obtained retrospectively. Since this is an observational study, definitive conclusions about antifungal treatment regimen cannot be drawn; however, a randomized treatment trial for CIE would be logistically impossible to perform.

In conclusion, CIE is a rare but potentially devastating infection that affects older individuals with health care exposure. Although our study is small, it lends support to a growing body of evidence for the use of echinocandin-based therapy in the treatment of CIE based on a lower rate of renal dysfunction and similar mortality. Furthermore, similar to a previous study, our study calls into question the necessity of surgical therapy as a rule in all patients with CIE. Although the rarity of this disease makes it challenging to investigate, future studies are needed to validate these findings.

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Penicillium marneffei Infection and Recent Advances in the Epidemiology and Molecular Biology Aspects

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INTRODUCTION

Penicillium marneffei is an emerging pathogenic fungus that can cause a fatal systemic mycosis in patients infected with human immunodeficiency virus (HIV). P. marneffei infection is endemic in tropical Asia, especially Thailand, northeastern India, China, Hong Kong, Vietnam, and Taiwan (25, 33, 61, 125, 151, 185). The organism is a relatively recent addition to known southeast Asian mycoses, being discovered in 1956 as an infection of bamboo rats (16, 136). However, the importance of P. marneffei as a human disease was recognized only when the HIV pandemic arrived in Asia and prevalences of infection increased in local populations as well as in visitors from areas where the infection is not endemic (63, 119, 120, 147, 180). The disease is, after tuberculosis and cryptococcosis, the third most common opportunistic infection in patients with AIDS in northern Thailand (151). Common manifestations of disseminated P. marneffei infection in AIDS patients are fever, anemia, weight loss, lymphadenopathy, hepatosplenomegaly, respiratory signs, and skin lesions. Patients who do not receive the appropriate antifungal treatment have a poor prognosis; however, primary treatment with amphotericin B and secondary prophylaxis with itraconazole are effective (153). P. marneffei-infection patients who are HIV positive need prolonged suppressive therapy to prevent relapse. Laboratory diagnosis of P. marneffei infection requires microscopic demonstration of intracellular P. marneffei yeast cells in the infected tissue and the culture of the fungus from clinical specimens. Under the microscope, P. marneffei appears as a unicellular organism with round to oval cells. These cells may divide by cross wall formation within macrophages, or, alternatively, to form extracellular elongated cells. The unique feature of P. marneffei relative to other penicillia is its thermal dimorphism (136). This ability to grow as a mycelium at 25°C and as a yeast at 37°C is the organism’s principal virulence factor. General laboratory diagnosis is time-consuming despite the development of several tests for early diagnosis, including serodiagnostic and molecular assay-based diagnostic methods. There is a clear need for more rapid diagnostic tools to help the physician to make more rapid diagnoses and thus initiate an early treatment.

P. marneffei has been isolated from the internal organs of four species of rodents (Rhizomys sinensis, Rhizomys pruinosus, Rhizomys sumatrensis, and Campomys badius) and from soil.
samples that have been collected from bamboo rat burrows (55, 177). Studies on the molecular epidemiology of this fungus have been reported (46, 64, 71, 95, 163, 171), and recently developed typing systems coupled with modern population genetic analyses have shown that humans and bamboo rats share genetically identical isolates (46, 47, 55). Expanding such surveys will further characterize \textit{P. marneffei} and help in explaining the enigmatic natural history of this fungal infection. Recently, novel molecular tools have been developed to study the genetics of \textit{P. marneffei}. Several genes of this fungus that are involved in asexual development and/or fungal morphogenesis have been cloned and characterized (7–9, 12, 13, 30, 160, 193, 194). Other genes that are involved in the host immune response have been found (15, 123). The determination of the \textit{P. marneffei} genome, anticipated for 2005, will enable a study of the molecular networks underpinning these expressed genetic factors. Such analyses will clarify the molecular mechanisms of fungal morphogenesis, pathogenesis, and host-fungus interactions in future studies of this important and unique pathogen.

**HISTORY AND CLASSIFICATION**

**Discovery of \textit{P. marneffei} and \textit{P. marneffei} Infection**

\textit{Penicillium marneffei} was first isolated from the hepatic lesions of a bamboo rat (\textit{Rhizomys sinensis}) that had been maintained in captivity for experimental infections at the Pasteur Institute of Indochina, Dalat, South Vietnam, in 1956 (16). This bamboo rat died spontaneously from the reticuloendothelial mycosis. The fungus was named \textit{Penicillium marneffei}, in honor of Hubert Marneffe, director of the Pasteur Institute of Indochina (136). Human penicilliosis marneffei was first described as a laboratory-acquired infection when researcher G. Segretain accidentally pricked his own finger with a needle filled with \textit{P. marneffei} that was being used to inoculate hamsters (137). He developed a small nodule at the site of inoculation, followed by axillary lymphadenopathy; this accidental infection was cured by intensive treatment with oral nystatin for 30 days (40).

The mycology of \textit{P. marneffei} was first described by Segretain in 1959 (136). \textit{P. marneffei} was classified in the section Asymmetrica, subsection Divaricata, in the classification of Raper and Thom (126), which is equivalent to Pitt’s subgenus \textit{Furcatum}. Pitt (122) later assigned \textit{P. marneffei} to the subgenus \textit{Biverticillum}. This classification was confirmed by Frisvad and Filtenborg (51) on the basis of similar physiology and secondary metabolite profiles. A phylogenetic analysis of \textit{P. marneffei}, as assessed by the nucleotide sequences of nuclear and mitochondrial RNA gene regions, demonstrated that \textit{P. marneffei} is closely related to the species of the \textit{Penicillum} subgenus \textit{Biverticillum} and sexual \textit{Talaromyces} species with asexual bi- verticillate \textit{Penicillium} states (105).

The first naturally occurring human case of penicilliosis marneffei was reported in 1973 by Di Salvo and collaborators (39); the patient was an American minister with Hodgkin’s disease who had been living in southeast Asia. The second reported case, in 1984, was also in an American who had traveled in the Far East (118). Here, the patient had recurrent episodes of hemoptysis, which were thought to be caused by bronchiectasis. A pneumonectomy revealed granulomata, and tissue sections showed yeast-like cells of \textit{P. marneffei} that were confirmed by fungus culture. In the same year, five more cases were reported from Bangkok, Thailand (72). Eight cases of \textit{P. marneffei} infection were reported from China in 1985 (32); these were observed between 1964 and 1983. A further 20 cases were subsequently reported from the Guangxi region in southern China (33, 99, 182), and six more were reported from Hong Kong (18, 19, 146, 190).

The rarity of human penicilliosis marneffei changed when the global HIV-AIDS pandemic arrived in southeast Asia. From 1988, cases of \textit{P. marneffei} infection started being observed in patients with advanced HIV infection. The first cases were in foreign AIDS patients who had visited regions of endemicity (119, 120) and in HIV patients who were native to regions of endemicity within Thailand (135). The majority of infections by \textit{P. marneffei} were diagnosed in AIDS patients in Thailand; however, infections were also observed in Cambodia (4), China (101), Hong Kong (20, 85, 164, 166, 185), India (125), Malaysia (132), Taiwan (21, 25, 64, 66, 103), and Vietnam (61, 67). Cases from outside the region of endemicity were observed in HIV-infected patients from Australia (60, 75), Belgium (35), France (54, 62, 63, 169), Germany (129, 147), Japan (113, 167), Sweden (77), Switzerland (10, 52, 88), The Netherlands (65, 87), the United Kingdom (6, 111, 119, 178), and the United States (114). Currently, the prevalence of human penicilliosis marneffei infection is increasing in areas where HIV infection is on the increase, for instance, Vietnam (67). However, where transmission of HIV has been reduced by control methods, concomitant decreases in the numbers of cases of penicilliosis marneffei have been seen (Fig. 1).

**Mycology**

\textit{Penicillium marneffei} is the only known \textit{Penicillium} species that exhibits temperature-dependent dimorphic growth. At temperatures below 37°C, the fungus grows as mycelia, bearing conidiophores and conidia typical of the genus \textit{Penicillium}. At 37°C on artificial medium or in human tissue, the fungus grows in a yeast-like form with the formation of fission arthroconidium cells. The fission yeast cells represent the parasitic form of \textit{P. marneffei}. This form is seen in the intracellular infection of the macrophages. The mold-to-yeast conversion or phase transition, which is thermally regulated, is a diagnostic characteristic of \textit{P. marneffei}. In contrast to \textit{P. marneffei}, the other \textit{Penicillium} species are not dimorphic and are more like \textit{Aspergillus}, with hyphae in tissue.

Research on the biochemical properties of \textit{P. marneffei} has been focused on its enzymatic activities (189). The secreted enzymatic activities of 10 \textit{P. marneffei} isolates during the mid-log growth phase were analyzed. Both mycelia and yeast expressed alkaline phosphatase, acid phosphatase, and naphthol-AS-BI phosphatase in \textit{Coxiella burnetti} and phospholipase and esterase in \textit{Candida albicans} (3, 69, 109, 165). In order to determine
whether secreted enzymes are linked to virulence in *P. marneffei*, further work, focused on purifying such enzymes and characterizing their effects, is needed. A study by Wong et al. (186) on the biochemical properties of *P. marneffei* secreted enzymes and their possible use in strain biotyping showed that all 32 isolates of *P. marneffei* examined possessed the urease enzyme. All isolates assimilated glucose, maltose, and cellobiose. However, some heterogeneity between isolates was observed in their biochemical profiles. A total of 65, 84, and 72% of the isolates were able to assimilate trehalose, xylose, and nitrate, respectively, and when galactose (0.015 to 0.25%) was the sole carbon source in the medium, inhibition of growth occurred in all isolates. Several strains possessed the enzyme β-galactosidase. From these biochemical properties, 17 different biotypes were recognized.

**PATHOGENESIS AND CLINICAL FEATURES**

**Clinical Manifestations**

Between June 1990 and June 2004, 1,843 cases of disseminated *P. marneffei* infection were seen in HIV-infected patients at Chiang Mai University Hospital. Approximately 6,709 cases of *P. marneffei* infection were diagnosed in Thailand between September 1984 and October 2004 (HIV/AIDS epidemiology report, Division of Epidemiology, Ministry of Public Health, Thailand [http://epid.moph.go.th]). Patients infected with *P. marneffei* had clinical histories that exhibited various degrees of severity. The symptoms and signs of 74 HIV-infected patients with disseminated *P. marneffei* infection, observed between November 1993 and January 1996 at Chiang Mai University Hospital, have been summarized (177). Most cases presented with fever, weight loss, skin lesions, generalized lymphadenopathy, and hepatomegaly. Respiratory signs were also observed. Skin lesions were seen in 63 patients (85%). The observed lesions in 54 of these 63 patients were papules with central necrosis, and the rest were papules or maculopapules. All patients acquired lesions on the face and neck. Other body sites where skin lesions were found included the upper extremities (33 patients [52%]), trunk (25 patients [39%]), and lower extremities (19 patients [30%]). Six patients also had popular lesions on the palate. The average number of CD4+ T lymphocytes in these patients was 64 cells/mm³ (standard deviation, 47 cells/mm³). Fifty-six patients (76%) were anemic, with a measured hemoglobin level of 10 g/dl or less. With the possible exception of the skin lesions, it was not possible to unequivocally attribute these abnormal clinical findings to *P. marneffei* infection alone. This is because they may also be caused by other opportunistic infections associated with late-stage HIV infection, as well as the immunodeficiency virus itself.

Mucocutaneous, oral, and facial manifestations have been reported in patients with *P. marneffei* infection (26, 79, 84, 151, 161, 181). Entire facial skin and oral manifestations have been described as papular and ulcerated lesions. Soft palate was also involved. Some patients having hepatic penicilliosis without any skin lesions have been described (78). Osteoarticular lesions were seen in multiple sites of patients with disseminated *P. marneffei* infection; here the sites of bone infection were the ribs, long bones, skull, lumbar vertebrae, scapula, and temporomandibular region (72, 100, 107, 154). Arthritis involved both large peripheral joints and small joints of the fingers and multiple swollen joints. Multiple lytic bone lesions involving flat bones of the skull, long bones, and small bones of the fingers were also seen (19, 32, 107). The similarity of these lesions to those observed in other systemic infectious diseases, for example, cryptococcosis, blastomycosis, African histoplasmosis, and tuberculosis (40), makes differential diagnosis problematic.

**Pathology**

*P. marneffei* appears to be a primary pulmonary pathogen that disseminates to other internal organs by hematogenous...
spread. Severity of the disease depends upon the immunological status of the host. Clinical signs of *P. marneffei* infection in both HIV-positive and HIV-negative patients have been summarized (40). In the HIV-positive group, the rapid onset and severity of symptoms in the absence of early treatment were striking. Infected tissues can show different histopathological reactions. In immunocompromised patients, necrotizing reactions with macrophage and histiocyte infiltrations are seen (33, 40, 151). *P. marneffei* yeast-like cells are observed to be associated with both the intracellular and extracellular environments of both macrophages and histiocytes. The intracellular yeasts are oval or spherical cells of 2 to 3 μm in diameter, which multiply by binary fission. Elongated cells of up to 13 μm long can be observed extracellularly. In immunocompetent patients, granulomatous and supplicative reactions are frequently seen in the lung, skin, liver, and subcutaneous tissues (33, 72). The formation of central necrosis and multiple abscesses could be seen in the reactions. *P. marneffei* infection may be considered the homolog of histoplasmosis, since both *P. marneffei* and *Histoplasma capsulatum* exploit the macrophage as a host cell, and both organisms can cause acute or persistent pulmonary and disseminated infection and reactivation disease (31, 190).

**Immunology**

The mechanisms of host-fungus interaction and host immune response in *P. marneffei* infection are poorly understood. Infection is presumably via inhalation of conidia from the environment; however, aerosolization of infectious particles, and subsequent infection, has never been definitively shown. Phagocytic cells are likely to be the primary line of the host defense against this fungus. *P. marneffei* conidia are able to recognize fibronectin and bind to laminin via a sialic acid-specific lectin (56, 57). This reaction may play an important role in the attachment of conidia to bronchoalveolar epithelia before ingestion by host mononuclear phagocytes. A study of the interactions between human leukocytes and heat-killed yeast-phase *P. marneffei* revealed that monocyte-derived macrophages bind and phagocytose *P. marneffei*, even in the absence of opsonization. The entry of *P. marneffei* into macrophages is divalent cation independent, and the pathogen gains entry into the host cells by pinocytosis (86). The results of that study suggested that the major receptor(s) recognizing *P. marneffei* is a glycoprotein with exposed N-acetyl-β-glucosaminyl groups. In another intracellular fungus, *Histoplasma capsulatum*, the HSP60 protein has been identified as a specific ligand or adhesin on the surface of the fungus. This ligand is recognized by the CD11/CD18 receptors on the host macrophages (106). However, the surface ligand involved in the specific binding of *P. marneffei* to the host cell receptors has not thus far been identified.

In healthy hosts, *P. marneffei* can be cleared within 2 to 3 weeks, depending on the size of the inoculum, whereas in nude mice or in T-cell-depleted mice, *P. marneffei* infection is fatal (89, 90, 179). These results demonstrated that T cells, and in particular CD4 + T cells, are necessary for clearing this fungal infection in mice. For humans, it was also shown that the deficiency of a CD4 + T-cell-dependent immunity contributes to the development of fatal disseminated penicilliosis marneffei in AIDS patients (150). A commonality in the host immunological response to intracellular pathogens is that activation of macrophages by T-cell-derived cytokines is necessary for defense against such infections (28, 91–93, 96, 155); it appears that this is also the case regarding infection by *P. marneffei*.

Both human and mouse macrophages are able to control *P. marneffei* growth and to kill intracellular yeast cells when activated in vitro by T-cell-derived cytokines, such as gamma interferon (IFN-γ). It was demonstrated that intracellular *P. marneffei* was damaged via the l-arginine-dependent nitric oxide (NO) pathway in murine macrophages stimulated with IFN-γ. *P. marneffei* could also stimulate a respiratory burst regardless of whether opsonins are present, and serum factors are required for *P. marneffei* to stimulate tumor necrosis factor alpha (TNF-α) release (134). The ability of unopsonized *P. marneffei* to parasitize mononuclear phagocytes without stimulating the production of TNF-α may enhance the virulence of this intracellular organism.

As described above, several studies have proved that T cells and macrophages are important for protection against the *P. marneffei* infection. However, little is known about the role of T-cell cytokines in the immune response against this fungus. Recently, by the use of an in vitro analysis of a sublethal *P. marneffei* infection in BALB/c mice (3 × 10³ conidia/mouse), it was demonstrated that protective immunity follows a Th1 response, with high levels of interleukin-12 (IL-12). IFN-γ, and TNF-α being developed (144). This finding is consistent with the general knowledge that a Th1 response plays a crucial role in host resistance to intracellular pathogens (135), as in mycobacterial infections (45) and infections with other fungi, such as *Histoplasma capsulatum*, *Coccidioides immitis*, and *Candida albicans* (59). The role of the phosphoprotein osteopontin (OPN) in IL-12 production from peripheral blood mononuclear cells stimulated with *P. marneffei* was investigated (86). The results of that work demonstrated that OPN, secreted from monocytes, is involved in the production of IL-12 from peripheral blood mononuclear cells after stimulation with *P. marneffei* and that the OPN production is regulated by granulocyte-macrophage colony-stimulating factor. These results also indicated the possible involvement of the mannose receptor as a signal-transducing receptor for triggering the secretion of OPN by *P. marneffei*-stimulated peripheral blood mononuclear cells. These findings suggested that OPN may polarize the Th1 cytokine response and may also contribute to the host’s defense against *P. marneffei* infection.

The responses of rabbit pulmonary alveolar macrophages and circulating human mononuclear phagocytes to *P. marneffei* conidia have been reported (131). These cells manifested antifungal activity against *P. marneffei*; the circulating monocytes responded to conidia with an oxidative burst which was significantly enhanced by a macrophage colony-stimulating factor. In addition, studies utilizing electron microscopy demonstrated that intact conidia were enclosed in phagosomes at 7 h. Subsequently, the conidia then became intraphagosomal, and they exhibited damage, by 12 h. The role of human neutrophils in
the host defense against *P. marneffei* infection has been studied (93, 94). These results indicated that granulocyte-macrophage colony-stimulating factor enhanced the antifungal activity of human neutrophils against the yeast form of *P. marneffei* but not the conidia. This activity was mediated by exocytosis of the granular cytolytic molecules from neutrophils rather than by reactive oxygen-dependent mechanisms.

As for other intracellular pathogens, surviving within the phagocytes is the primary key to a successful invasion by *P. marneffei*. However, the mechanism of survival of *P. marneffei* under oxidative stress within the macrophage remains unknown. Many fungi have been shown to survive within the phagocytic environment (97). The mechanism of resistance for these organisms may function by inhibiting the production of reactive oxygen metabolites or by neutralizing inhibitory host metabolites. Youngchim et al. (189) found an expression of acid phosphatase activity by *P. marneffei*. Production of acid phosphatase is considered to be one of the virulence factors for intracellular pathogens, such as *Coxiella burnetii* (3), and *Francisella tularensis* (127). When a pathogen produces acid phosphatase, the concomitant decrease in intracellular pH may improve the survival of the organism by inhibiting the phagocyte respiratory burst. This hypothesis has found some support from studies of the antimicrobial activity of chloroquine against *P. marneffei* by using human THP1 and mouse J774 macrophages. These results revealed that the drug’s antifungal activity was due to an increase in the intravacuolar pH and a disruption of pH-dependent metabolic processes (156); the increase in pH within the phagocytic vacuole may directly reduce fungal growth, or it may inhibit pH-dependent yeast virulence factors, such as acid phosphatase activity. In addition, iron overload was found to significantly reduce the antifungal activity of gamma interferon-lipopolysaccharide-activated human THP1 and mouse J774 macrophages (155). That work suggested that iron availability critically affects immunity and the pathogenicity of *P. marneffei*. In *H. capsulatum*, the expression of at least three catalases which detoxify hydrogen peroxide has been identified (188). The expression of these enzymes could contribute to survival of *H. capsulatum* inside the host cell. In *P. marneffei*, an antigenic catalase-peroxidase protein-encoding gene (*cpeA*) was recently isolated by antibody screening of a cDNA yeast-phase library of this fungus (123). The high expression of this *cpeA* gene at 37°C may contribute to the survival of this fungus within the host cells.

**LABORATORY DIAGNOSIS**

**Diagnosis by Staining Methods and Cultures**

Diagnosis of infection by *Penicillium marneffei* is commonly made by identifying the fungus in clinical specimens by microscopy and culture. Clinical specimens that are commonly used include bone marrow aspirate, blood, lymph node biopsies, skin biopsies, skin scrapings, sputum, bronchoalveolar lavage pellet, pleural fluid, liver biopsies, cerebrospinal fluid, pharyngeal ulcer scrapings, palatal papule scrapings, urine, stool samples, and kidney, pericardium, stomach, or intestine specimens (40, 151). Rapid presumptive diagnosis can be made by microscopic examination of Wright-stained bone marrow aspirates and/or touch smear of skin biopsy or lymph node biopsy specimens (150). In patients with fulminant infection, *P. marneffei* could be observed in peripheral blood smears (112, 151). *P. marneffei* can be seen in histopathological sections stained with hematoxylin and eosin, Grocott methenamine silver, or periodic acid-Schiff stain. The organisms appear as fission arthroconidia or unicellular round to oval cells, which may divide by cross wall formation in macrophages or histiocytes (26, 63, 65, 112, 119, 120, 141, 142, 150, 164, 168, 180). The cross wall formation can differentiate yeast cells of *P. marneffei* from those of *Histoplasma capsulatum*, which also appear as intracellular yeasts (Fig. 2). Extracellular elongated or sausage-shaped cells of *P. marneffei*, with one or two septa, may also be seen (40, 151).

*P. marneffei* in paraffin-embedded, formalin-fixed tissue from infected guinea pigs could be identified by the use of a monoclonal antibody directed to *Aspergillus* galactomannan (44). Here, it seems that the monoclonal antibody used detected a specific galactomannan that appeared to have at least one identical epitope in *P. marneffei* and *Aspergillus* spp. (115). These two fungi have different morphologies as seen in tissue sections. *Aspergillus* appears as mycelial mats composed of radiating hyphae with regular septation and dichotomous branchings. Conversely, the tissue form of *P. marneffei* develops as a unique yeast-like structure of 2 to 3 μm in diameter.
which multiplies by binary fission or schizogony. Immunoperoxidase staining for the tissue form of *P. marneffei* was developed by using rabbit antisera raised against the 3-day-old yeast culture filtrate antigens of *P. marneffei* (175). *P. marneffei* in deparaffinized tissue sections of skin biopsies from patients with *P. marneffei* infection could be clearly demonstrated as intracellular yeasts.

A specific indirect fluorescent-antibody reagent for the rapid identification of *P. marneffei* in histologic sections was developed by using rabbit antiglobulins against yeast-like culture filtrate antigens of *P. marneffei* (80). The antiglobulins, adsorbed with *Histoplasma capsulatum*, specifically stained the yeast-like cells of *P. marneffei* in tissue sections from six patients with *P. marneffei* infection. None of the tissue sections from 10 humans with histoplasmosis stained with this specific antiglobulin. Murine immunoglobulin (IgM) monoclonal antibodies raised against *P. marneffei* mycelial culture filtrate antigens were also proved to react strongly in immunofluorescent staining with the yeast cells in the tissue biopsies of patients (162). Rapid diagnosis of *P. marneffei* infection, particularly in patients with lymphadenopathy, could be made by the use of fine-needle aspiration cytology (Fig. 2A and B) (17, 108). The use of this method of diagnosis is potentially beneficial to patients in whom lymphadenopathy is confined to deep intrabdominal nodes.

Definite diagnosis of disseminated *P. marneffei* infection is based on mycological culture, and studies have demonstrated high sensitivity from bone marrow (100%), blood (76%), and skin biopsy (90%) (151). The fungus grows in a mycelial phase at 25°C on Sabouraud glucose agar without cycloheximide. Mold-to-yeast conversion is achieved by subculturing onto brain heart infusion agar and incubating at 37°C (136). Identification of *P. marneffei* is based upon the morphology of the colony, its mold-to-yeast conversion, and the organism’s microscopic morphology.

A specific exoantigen test using the immunodiffusion (ID) technique was developed to identify *P. marneffei* culture (139). The concentrated culture filtrate from 6-week-old, 25°C shake cultures of a standard strain of *P. marneffei* was employed as a reference antigen. Rabbit antiglobulin raised against this antigen was used in an ID test to identify *P. marneffei* and proved especially useful for cultures that were difficult to convert or that had an atypical colony morphology (82). Two to four specific precipitin lines are seen in a positive reaction, and the exoantigen test can be used to differentiate *P. marneffei* from other *Penicillium* species, such as *P. citrinum* and *P. commune* (138). The anti-*P. marneffei* antiglobulin adsorbed with *P. primulinum* antigens can also be used in an ID test to differentiate between these two closely related species.

**Serologic Diagnosis**

Several methods have been developed for detecting specific antibodies against antigens of *P. marneffei* in clinical specimens. These tests have the potential to provide a rapid diagnostic method for identifying *P. marneffei* infection, thus enabling initial therapeutic management. The micro-ID test using *P. marneffei* (mycelial phase) exoantigens was applied to detect precipitin antibodies in a patient’s serum specimen. Thirteen serum specimens which were collected serially from the same HIV-positive patient infected with *P. marneffei* gave positive results 2 months after the initial treatment (180). However, another application of the ID test to detect *P. marneffei* antibodies in individual sera from 17 patients with *P. marneffei* infection revealed positive results for only 2 of the 17 serum specimens, showing low sensitivity of the test (81).

An indirect fluorescent-antibody test for detecting IgG antibodies in patients infected with *P. marneffei* was developed using germinating conidia and yeast forms as antigens (192). The test was evaluated with serum samples from 103 patients with persistent fever and 78 normal subjects. Eight cases with persistent fever and documented *P. marneffei* infection had an IgG titer of 160 or more. The remaining 95 patients without *P. marneffei* infection and 78 healthy controls had a titer of 40 or below. This test has the potential to provide rapid presumptive diagnosis and might supplement conventional culture.

Immunoblot assay using crude antigens of *P. marneffei* and serum specimens from patients were analyzed. The protein antigens produced during the growth phase of the yeast form were found to be more immunoreactive than the antigens obtained from the mycelial form (172). These findings corresponded to the infective stage of this organism, which was the intra- and extracellular yeast phase in the patient’s lesions. Four immunogenic yeast proteins of 200, 88, 54, and 50 kDa were produced in a large quantity during the deceleration and early stationary phases of growth. When these four proteins were tested with serum specimens from 33 *P. marneffei*-infected AIDS patients, reactivities to them were detected in 73, 94, 61, and 58%, respectively. The 200- and 88-kDa proteins may be common antigens that occur in other environmental fungi. These proteins elicited weak reactivity in high proportions of serum specimens from HIV-infected patients without *P. marneffei* infection and normal persons. However, the 54- and 50-kDa proteins proved more promising and were strongly reactive with serum specimens from *P. marneffei*-infected patients (172). Interestingly, in one serum specimen from an HIV-infected patient, strong reactivities to the 54- and 50-kDa proteins could be detected 2 months before the definite diagnosis was made by fungal culture. These results indicate that there are at least two yeast-phase immunoreactive proteins (54 and 50 kDa), which are relatively specific for *P. marneffei*. The use of these yeast-phase immunoreactive proteins for diagnosis should be studied further with more serum samples. Another diagnostic antigen of 38 kDa, which was prepared from acetone-precipitated culture filtrate of mycelial-phase cells, has also been reported (27).

A study of whole cytoplasmic antigens from yeast-form *P. marneffei* confirmed that *P. marneffei* infection could trigger a humoral response (73). Antigens of 88, 61, 54, and 50 kDa, identified by Western blotting, were purified by a combination of liquid isoelectric focusing (Rotofor system) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Prep-cell system). These antigens were screened separately with pooled sera from patients infected with *P. marneffei*, pooled normal sera, and pooled sera from patients with candidiasis, aspergillosis, histoplasmosis, and cryptococcosis. The 61-, 54-, and 50-kDa antigens were specific for *P. marneffei*, with no cross-reactivity with sera from patients with other mycoses. The 88-kDa antigen was slightly cross-reactive with sera from patients with cryptococcosis. The 61-kDa antigen was an addi-
tional candidate for the development of specific antibody tests for disseminated \textit{P. marneffei} infection.

An enzyme-linked immunosorbent assay (ELISA)-based antibody test was developed with a recombinant \textit{P. marneffei} mannoprotein (Mp1p) for the serodiagnosis of \textit{P. marneffei} infection (15). Evaluation of the test revealed high specificity (100\%) and approximately 80\% sensitivity (14 of 17) in HIV-seropositive patients infected with \textit{P. marneffei}. The anti-Mp1p antibody was also used in an ELISA-based method for the detection of the mannoprotein Mp1p in the sera of patients (14). The antigen test had an overall sensitivity of 65\% (17 of 26). The combined antibody and antigen tests for the diagnosis of \textit{P. marneffei} infection had a sensitivity of 88\% (23 of 26), with a positive predictive value of 100\% and a negative predictive value of 96\%.

Several additional methods for detecting circulating \textit{P. marneffei} antigens have been developed. Pastorex \textit{Aspergillus} is a latex agglutination test kit using a monoclonal antibody to detect \textit{Aspergillus fumigatus} galactomannan in serum specimens from patients with aspergillosis. This monoclonal antibody was found to cross-react with \textit{P. marneffei} antigen (121, 170). The reagent was used to detect galactomannan in an experimental infection with \textit{P. marneffei}. However, the titer of antigen detected was lower than that in infection with \textit{Aspergillus} (170). A specific latex agglutination (LA) for the detection of \textit{P. marneffei} antigens was developed (81). The LA test detected antigens in 13 of 17 serum samples (76\%) and in 2 urine specimens. The LA titers ranged from 1:64 to 1:4,096. The use of ID antibody and LA antigen tests concurrently increased the sensitivity to 82\%. These tests appeared to be highly specific (100\%), since none were positive with sera from 15 Thai control patients, 6 serum samples containing cryptococcal antigen, or 6 urine specimens positive for \textit{Histoplasma} polysaccharide antigens.

An enzyme immunoassay for the quantitation of \textit{P. marneffei} urinary antigen was developed by using fluorescein isothiocyanate-labeled purified rabbit hyperimmune IgG (37). The hyperimmune serum was prepared by injecting New Zealand White rabbits subcutaneously with killed whole yeast-like cells of \textit{P. marneffei}. The urine antigen detection assay had a diagnostic sensitivity of 97\% and specificity of 98\% at a cutoff titer of 1:40. The same polyclonal anti-\textit{P. marneffei} antibody was used further in a dot blot ELISA and a latex agglutination test for the detection of \textit{P. marneffei} urinary antigen (36). The overall sensitivities of the tests were as follows: dot blot ELISA, 94\%; ELISA, 97\%; and LA test, 100\%. The specificities were 97, 98, and 99\%, respectively. All tests were highly sensitive and specific. These tests have the potential to be used to assess responses to antifungal therapy in serial urinary antigen testing during treatment and subsequent follow-up.

A monoclonal antibody-based sandwich ELISA was developed for the detection of \textit{P. marneffei} antigen in clinical specimens from patients with \textit{P. marneffei} infection (116, 162). When used at a 1:2 dilution, 13 of 18 culture-positive serum samples proved to be antigen positive. When samples were used undiluted, sensitivity was improved, with three additional specimens proving positive. The test was also found to be useful for the detection of secreted antigen in urine samples. However, to determine the actual sensitivity and specificity of the test, a larger number of specimens from patients with \textit{P. marneffei} and other diseases need to be tested.

**Molecular Diagnosis**

The PCR technique is extremely sensitive and has been used effectively for the specific detection of many fungi. Molecular diagnosis of \textit{P. marneffei} is based on specific oligonucleotide primers designed from the internally transcribed spacer and 5.8S rRNA gene (ITS1-5.8S-ITS2) of \textit{P. marneffei} (105). The specificity of these \textit{P. marneffei} primers was tested in a nested PCR; fungal DNA was first amplified with the primer pair ITS5 and ITS4 (184) and nested PCR subsequently performed with primer pair PM1 and PM4 or PM2 and PM4. The primer pair PM2 and PM4 was 100\% successful in amplifying \textit{P. marneffei} DNA, yielding a 347-bp PCR product, and this method was used successfully to identify \textit{P. marneffei} from a skin biopsy (167). To identify medically important yeast-like fungi, specific oligonucleotide probes for several pathogens, including \textit{P. marneffei}, have been developed and tested in a PCR-enzyme immunoassay method (102). In those studies, minor cross-reactivity has been observed for a \textit{Blastomyces dermatitidis} probe used against \textit{C. immitis} DNA and for an \textit{H. capsulatum} probe used against \textit{C. albicans}. However, no cross-reactivity to \textit{P. marneffei} was seen. The method was able to rapidly identify DNA obtained from fungi in a pure culture, and the application of this test in a clinical laboratory setting needs to be evaluated further.

An oligonucleotide probe based on the 18S rRNA gene of \textit{P. marneffei} has been designed and has proved specific for \textit{P. marneffei} in a PCR-hybridization reaction, regardless of whether the fungus was isolated from humans or from natural habitats. The sensitivity of the technique was about 0.1 pg/μl of DNA. In addition, this PCR-hybridization technique was used to detect \textit{P. marneffei} DNA in EDTA-blood samples collected from AIDS patients with \textit{P. marneffei} infection (174). Although the method was shown to be highly sensitive and specific, the hybridization technique as described is labor-intensive and requires a high level of competence in the laboratory. To address these concerns, single and nested PCR methods for the rapid identification of \textit{P. marneffei} were then developed using newly designed specific primers, also based on the 18S rRNA gene sequence of \textit{P. marneffei} (176). The sensitivities of single and nested PCR were 1.0 pg/μl and 1.8 fg/μl, respectively, and successful discrimination of a very young culture of \textit{P. marneffei} (2-day-old filamentous colony, 2 mm in diameter) could be performed by the use of this assay. Further diagnostic PCR methods have also been described; a one-tube seminested PCR assay was developed to identify \textit{P. marneffei} DNA based on the 18S rRNA sequences (124). This assay was sensitive and could identify \textit{P. marneffei} DNA both from pure cultures and two clinical samples. The utility of both of these PCR methods for the early diagnosis of the disease needs to be studied further.

**TREATMENT**

The mortality rate of patients with \textit{P. marneffei} infection has been very high, especially when physicians do not make an early diagnosis and commence treatment early. By using an in
viral susceptibility test, the fungus was demonstrated to be highly susceptible to miconazole, itraconazole, ketoconazole, and fluconazole (152). Amphotericin B showed intermediate antifungal activity, while fluconazole was the least active. Some strains of \textit{P. marneffei} were resistant to fluconazole. The clinical and microbiological responses correlated with the overall patterns of in vitro susceptibility to the azoles, whereas results with amphotericin B were more difficult to assess. In this study of 80 patients with disseminated \textit{P. marneffei} infection, no maintenance therapy was used. The study found that 12 out of 40 patients who initially responded to treatment relapsed within 6 months (152). These results suggested that a long-term secondary prophylaxis might be needed to prevent recurrence of this infection.

A double-blind trial was conducted to evaluate itraconazole as a secondary prophylaxis against \textit{P. marneffei} infection in 71 HIV-infected patients, who were enrolled in a maintenance study (153). The initial standard antifungal therapy comprised 2 weeks of parenteral amphotericin B at a dose of 0.6 mg/kg/ day, followed by 400 mg of itraconazole per day orally in two divided doses for the next 10 weeks. The patients were then randomly assigned to receive either oral itraconazole (200 mg daily) or a placebo as maintenance therapy. None of the 36 patients assigned to itraconazole had a relapse within 1 year, whereas 20 of the 35 patients assigned to a placebo had relapses. The results suggest that in patients infected with HIV who successfully complete primary treatment of \textit{P. marneffei} infection, a secondary prophylaxis with oral itraconazole (200 mg once daily) is well tolerated and prevents relapses of \textit{P. marneffei} infection.

**ECOLOGY AND EPIDEMIOLOGY**

Despite a decade of research on the epidemiology and ecology of \textit{Penicillium marneffei}, the basic ecology of this unique pathogen remains enigmatic. A core issue is whether the human disease, penicillosis marneffei, occurs as a consequence of zoonotic (animal) or sapronotic (environmental) transmission. In other words, the ecological reservoir(s) of human penicillosis marneffei remains unknown to this date.

\textit{Penicillium marneffei} was originally isolated from a bamboo rat, \textit{Rhizomys sinensis}, by Capponi et al. in 1956 (16). Since then, a number of studies have surveyed rodent species and firmly established four species of bamboo rats as enzootic reservoirs of infection: \textit{Rhizomys sinensis}, \textit{R. pruinosus}, \textit{R. sumatrensis}, and the reddish-brown subspecies of \textit{Cannomys badius} (1, 24, 34, 55, 98, 183). These studies have shown that, within these susceptible species, the prevalence of infection varies widely across southeast Asia. This finding suggests either that there are regional variations in the endemicity of infection or that there are geographical variations in the predisposition to infection within different species of bamboo rats. A recent study by Gugnani et al. (55) comprehensively surveyed six species of sympatric rodents (\textit{Bandicota bengalensis}, \textit{Rattus norvegicus}, \textit{Rattus rattus}, \textit{Rattus nigripes}, \textit{Mus musculus}, and indigenous reddish-brown \textit{C. badius} \(n = 182\)) from bamboo plantations in Manipur State, northeastern India, and found that only \textit{C. badius} harbored infection. As all the rodents in this area presumably have approximately similar exposure rates, this study provides strong evidence that there exist host-specific factors that govern infection. Surveillance of the rodent reservoirs of the ascomycete pathogens \textit{Coccidioides immitis} and \textit{Coccidioides posadasii} in the United States have shown similar interspecific variation in the prevalence of infection, suggesting that this may be a general feature of pathogenic fungi that infect rodents (42, 43). Intraspecific variation in the prevalence of infection has also been found between subspecies of bamboo rat, where a study by Chariyalertksak et al. (24) in the Chiang Mai region, Thailand, showed that all 51 animals of the greyish-black subspecies of \textit{C. badius} were negative for \textit{P. marneffei} while 3 of the 10 rats (30\%) in the reddish-brown group were positive. Taken together, these studies indicate that the distribution of prevalences of \textit{P. marneffei} in rodents has (i) a host component and (ii) a geographical component.

A correct description of the factors governing the distribution of \textit{P. marneffei} infection in bamboo rats is necessary to describe the epidemiology of this disease in human populations. The reporting of autochthonous infections in humans has so far provided the best method of describing the geographic range of \textit{P. marneffei}, and such reports have defined the endemic range to include Thailand, southern China, Taiwan, Hong Kong, Laos, Cambodia, Malaysia, Vietnam, Myanmar (Burma), and northeastern India (4, 33, 41, 125, 140, 143). While a single case of the disease has also been observed in an African from Ghana who had no history of travel to southeast Asia (104), no further cases have been reported, suggesting that \textit{P. marneffei} is in fact endemic to southeast Asia. The geographic ranges of the lesser and greater bamboo rats (\textit{Cannomys} spp. and \textit{Rhizomys} spp.) broadly follow the known distribution of \textit{P. marneffei}. While this may be considered circumstantial evidence that bamboo rats are an obligate stage in the life cycle of the fungus, it may also be that the ecotype favored by these rodents is simply shared with \textit{P. marneffei} and that infections within these rodents are examples of sylvatic sapronotic infections. Early attempts to epidemiologically link bamboo rats and human infection showed no association; a case-control study of HIV-positive patients, performed in northern Thailand by Chariyalertksak et al. (23), did not implicate bamboo rats as a reservoir of infection for humans. Rather, age (16 to 30 years) and an agricultural occupation were found as factors that were independently associated with an increased risk of infection. Temporal analyses of the incidence within northern Thailand over a 4-year period (22) showed that there was extensive seasonal variation in infection rates and that increased disease was associated with the rainy season. These studies suggest that soil exposure, especially during the rainy season, is a critical risk factor associated with infection by \textit{P. marneffei}.

If these studies are correct and \textit{P. marneffei} infection is in fact a sapronosis (rather than a zoonosis), then the fungus must have a reservoir in the environment. Many species of penicillia are soil saprophytes. However, despite extensive efforts, attempts to recover soil isolates of \textit{P. marneffei} have met with only limited success. Vanittanakom et al. (173) demonstrated 80 to 85\% recovery of CFU after 3 days of incubation from sterilized soil seeded with \textit{P. marneffei}; however, the recovery from nonsterile soil seeded with the fungus was only 6\%. A recent laboratory study has demonstrated that \textit{P. marneffei} can survive in sterile soil for several weeks but can survive for only...
a few days in nonsterile soil (76); these studies suggest that survivability in the soil when faced with natural fungal competitors may be limited. Deng et al. isolated \textit{P. marneffei} from three soil samples collected from the burrows of \textit{Rhizomys pruinosus} (33) and Chariyalertsak et al. (24) were able to recover \textit{P. marneffei} from one out of 28 soil samples collected from the burrows of \textit{Rhizomys sumatrensis}. However, to date no attempts to recover \textit{P. marneffei} from environments other than those that are intimately associated with bamboo rats have been successful, and definitive proof of an environmental reservoir for \textit{P. marneffei} within the soil, or other substrates, is still lacking.

**Molecular Epidemiology**

Modern molecular methods provide the best opportunity for dissecting the epidemiology of \textit{P. marneffei} and revealing its fundamental life history traits (158). This is because multilocus genotypes can be generated that (i) identify isolates of a similar or identical genetic background that are derived from a common infective population, (ii) when applied to systematically sampled isolates can describe the hierarchical organization of population structure (i.e., whether there is a single large population or a number of genetically isolated subpopulations), (iii) can identify the reproductive mode (i.e., whether the fungus is asexual and clonal, sexual and recombining, or a combination of both), and (iv) can provide information on the deeper phylogenetic and evolutionary history of the pathogen.

**Randomly sampling genetic variation within the \textit{P. marneffei} genome.** Until recently, molecular approaches to typing \textit{P. marneffei} have relied on surveying the genome by using methods that randomly sample for genetic variation. Vanittanakom et al. (171) used HaeIII digests of genomic DNA to search for restriction fragment length polymorphisms (RFLPs) in order to differentiate \textit{P. marneffei} isolates from the Chiang Mai region, northern Thailand. The 22 human isolates in their study were classified in two DNA types, with type I representing 16 (73%) and type II representing 6 (27%) isolates; of the 23 bamboo rat isolates, all 20 from \textit{R. sumatrensis} were type I and all 3 from \textit{C. badius} were type II. In a separate study of 20 \textit{P. marneffei} isolates from Taiwanese patients, Hsueh et al. (64) used the same restriction digestion assay of genomic DNA to uncover the same two HaeIII RFLP patterns that had been found in Thailand and at approximately the same frequencies (type I, 75%; type II, 25%). However, the use of randomly amplified polymorphic DNA (RAPD) assays yielded eight different RAPD patterns, suggested that there was greater genetic diversity in the Taiwanese population than had been uncovered by the RFLP assay (64). Imwinhaya et al. (71) developed a fingerprinting assay for \textit{P. marneffei} by using the tetranucleotide repeat primer \((GACA)_3\) and the phage M13 core sequence. This assay identified four genotypes that varied in frequency between northern and southern Thailand. A separate study used pulsed-field gel electrophoresis of 69 \textit{P. marneffei} isolates from several regions of Thailand, using the restriction enzyme \textit{NotI} (163). This study revealed two macrorestriction patterns (MPI and MPII) that could be grouped into nine subprofiles (MPIa to MPIf and MPIIa to MPIIc) by using HaeIII digestion, yielding 54 genotypes in total. However, no correlation between the restriction patterns from various \textit{P. marneffei} isolates and geographic region or specimen source was observed in these studies.

Substantial drawbacks of all these typing systems are that (i) discriminatory power is low due to the small numbers of alleles, (ii) the reproducibility of RAPD and macrorestriction profiles between laboratories can be low (5), and (iii) alleles may not be homologous despite being of similar sizes (128, 158). In order to address these concerns, recent efforts have focused on developing typing schemes that focus on specific, sequence-characterized regions of the genome that have been isolated by data mining of the DNA sequence that has been generated by \textit{P. marneffei} genomics programs (191).

**Sequence-specific assays of genetic variation in the \textit{P. marneffei} genome.** There are two main technologies that target specific regions of the fungal genome in order to discriminate between isolates: multilocus sequence typing (MLST) (46, 110) and multilocus microsatellite typing (MLMT) (46, 49, 50). MLST is becoming the technique of choice for bacterial species and the fungus \textit{Candida albicans} (11, 130) and works by characterizing isolates by sequencing housekeeping genes (usually seven). The alleles present at each locus are combined into a multilocus sequence type, which is then deposited in a species-specific online database held at http://www.mlst.net/. However, the use of MLST is inappropriate when the species being typed contain insufficient genetic variation to differentiate isolates, as is the case for a number of bacterial and eukaryotic species (48, 148). At this stage, it is unclear whether levels of genetic diversity found in \textit{P. marneffei} housekeeping loci are high enough to allow MLST to be used to discriminate between isolates.

To circumvent the problem of low levels of genetic variation, MLMT targets loci that contain di-, tri-, or tetranucleotide repeats. These repeats ("microsatellites") (157) are more highly variable than housekeeping loci due to the accumulation of length polymorphisms as a consequence of slippage by DNA polymerase during genome replication. The alleles at each locus are scored by electrophoresing PCR-amplified loci through an automated sequencer, typing the length polymorphisms, and then combining the alleles from each locus into a multilocus microsatellite type (MT). These MTs can then be used to query online databases held at http://www.mlst.net/. The resulting outputs from these queries can be used to analyze the population genetic structure of the organism or to test epidemiological hypotheses.

Fisher et al. (47) screened 1.7 Mb of \textit{P. marneffei} genome sequence for microsatellite motifs, using all possible permutations of di-, tri-, and tetranucleotide motifs with a minimum repeat number of six; this search resulted in 30 dinucleotide, 14 trinucleotide, and 5 tetranucleotide repeats being discovered. A similar study on the same genome sequence by Lasker and Ran (95) uncovered only three microsatellites; why this discrepancy between the two studies exists is unclear, although the software used in the latter study excluded tri- and tetranucleotide repeats. Of the 49 loci identified by Fisher et al. (47), 24 were chosen and amplified as multiplex PCRs in four groups of six loci and used to type a panel of 29 clinical and bamboo rat isolates chosen from across the endemic range of \textit{P. marneffei} (46, 47, 55). Of the 24 loci, 23 were amplifiable and 21 were polymorphic with between 2 and 14 alleles present at
each locus, comprising 19 unique MTs in total. Clustering of isolates based on the microsatellite genetic distance $D_1$ (53) showed that isolates occur within one of two geographically separated clades that account for 26% of the total observed genetic diversity (46). The “eastern” clade contained isolates from mainland China, Hong Kong, Indonesia, and Vietnam, while the “western” clade contained isolates from Thailand and India, showing that *P. marneffei* has an extensive geographic component to its population genetic structure. Within the eastern and western clades, extensive linkage disequilibria existed between loci (measured by the index of association) (145), suggesting that there were either genetically differentiated subpopulations or extensive clonal reproduction occurring within the existing populations. A study over a smaller geographical scale in Manipur, India, showed that while the MTs of isolates were identical within bamboo plantations, they were dissimilar between plantations (55). This finding suggests that the population genetic structure of *P. marneffei* may in fact be partitioned over local, as well as large, geographical scales, although further studies are necessary to confirm the generality of this finding.

Fisher et al. (46) genotyped the type isolate *P. marneffei* CBS 388.87 (136) and showed that it was characterized by a unique MT and was therefore dissimilar to any human isolates (55); a similar result was found by comparing 2 bamboo rat isolates with 32 clinical isolates in another study (95). However, a comparison of 10 bamboo rat isolates collected in Manipur, India, showed that a single bamboo rat isolate was identical at all 21 microsatellite loci to the human isolate CBS 101038, isolated in 1998 (125). This is the first direct molecular epidemiological evidence that humans and bamboo rats share identical strains of *P. marneffei* and that host-to-host transmission may occur. However, it is equally possible that, in this case, coinfection had occurred from a common environmental source, and this possibility needs to be investigated. To address these issues, ongoing studies in northern Thailand are using highly sensitive real-time PCR assays to screen rigorously samples of (i) bamboo rat populations, (ii) environmental isolate samples, and (iii) human clinical populations. The application of highly discriminatory MLMT techniques to characterize these isolates will then provide a unique means to definitively identify the natural cycles of infection by *P. marneffei* in nature.

**MOLECULAR GENETICS**

Morphogenesis in *P. marneffei*

The generalized life cycle of *Penicillium marneffei* can be divided into three distinct phases: (i) a multicellular, filamentous (mold) vegetative form; (ii) an asexual reproductive stage (conidiogenesis); and (iii) a unicellular yeast-like/arthroconidial phenotype. Temperature and nutrition play a significant role in determining which particular phase is exhibited. Current evidence suggests that these two environmental conditions actually influence the expression, or the absence of expression, of particular genes that regulate cellular differentiation in *P. marneffei* (2, 30, 31).

**Mold phase of *P. marneffei***. Laboratory cultures of *P. marneffei* incubated at 25°C to 30°C resemble all other species of *Penicillium* in that growth occurs as a mold bearing the asexual reproductive structures (conidia) typical of the genus (Fig. 3A). In particular, young colonies grown on Sabouraud glucose agar are relatively thin and powdery to velvet in texture. Cultures may appear bluish gray-green initially, but as the *P. marneffei* colony matures, it becomes reddish yellow in color and produces a pink or red-rose pigment that readily diffuses into the surrounding medium (31). However, not all penicilli producing a red pigment are *P. marneffei*.

Microscopically, the conidiophores of *P. marneffei* (70 to 150 by 2.5 to 3.0 μm) usually possess bivarcilated penicilli, but monoverticillate structures may also be present (Fig. 3A). Each penicillus is composed of three to five metulae (7 to 11 μm long) bearing four to seven phialides (6 to 10 by 2.5 to 3.0 μm), ampulliform to acerose in shape. Attached to the phialides in short chains are single-celled, smooth-walled to echinulated conidia (3 to 4 μm in diameter). Conspicuous connectors between each conidium in the chain are readily observed. Spiral hyphae are occasionally present (39). This unusual structure of hyphae has also been observed in several isolates of *P. marneffei* which were isolated from HIV-AIDS patients (175).

Typically, the conidia are uninucleate, and when incubated at 25°C under the appropriate conditions, they swell isotropically for up to 6 to 12 h before undergoing polarized growth to produce a germ tube (2, 30, 31). Continued incubation at 25°C
results in the formation of true hyphae by apical extension of the germ tubes. The establishment of cross walls subapically produces individual cells. The subapical cells may undergo a repolarization to form branches of filaments that also grow by apical extension to form a mycelial network (Fig. 3B). Within this thallus, older cells tend to be uninucleate, whereas actively growing cellular compartments are usually multinucleate. The latter observation suggests that nuclear and cellular division become uncoupled during periods of rapid cellular growth.

Molecular studies have identified a gene intimately involved in germination (194). This gene, designated gasC, encodes a G protein subunit belonging to the G-protein signaling pathway that regulates normal germination. Deletion of this gene results in a significant delay of the germination process. However, gasC apparently has no role in vegetative growth, yeast development, or the dimorphic switch. Curiously, it does seem to be involved in a secondary metabolic pathway related to the red pigment production by *P. marneffei*.

Other studies have identified a transcription factor encoded by the gene *tupA* (160). This gene represses yeast development at 25°C while maintaining hyphal growth. However, *tupA* is not responsible for the initiation of hyphal formation. Instead, it appears to be required for the correct polarity of growth by yeast cells (see below). Interestingly, the *P. marneffei* gene stlA also has no apparent role in the initiation of vegetative hyphal development, although it is a homolog of the *STE12* gene in *Saccharomyces cerevisiae* and *Candida albicans*, which regulates the yeast-hyphal transition (8).

Hyphal development is also affected by the normal deposition of actin. The *P. marneffei* gene *actB*, which encodes a RAC homolog of a family of GTPase proteins, plays a crucial role in activity (13). The absence of this gene affects polarized growth and cell division in both hyphal development and conidiogenesis.

**Conidiogenesis in *P. marneffei***. The formation of conidia by *P. marneffei* results from a well-programmed series of cellular changes that produce the complex, multicellular conidiophores described above. At 25°C, conidiogenesis begins following the establishment of aerial hyphae on a solid medium. Liquid shake cultures of *P. marneffei* do not usually permit the formation of conidia (2, 30, 31). Hence, an air interface appears to be essential for conidiogenesis.

Conidiophore development begins as multicellular stalk cells arise from specialized “foot cells” of the hyphae lying at the culture/air interface. Secondary stalks, termed rama, may be formed by septation of the primary stalk cells. Eventually, the tips of the stalk cells differentiate into the uninucleate cells comprising the sterigmata (metulae and phialides). The latter divide by budding rather than apical extension and septation. The metulae are formed first and then gives rise to the various phialides. In turn, the phialides generate uninucleate conidia in a similar budding fashion. Interestingly, the conidia are produced in a basipetal mode; i.e., older conidia are displaced by newer ones. Hence, the first conidium in a chain represents the oldest cell, whereas the youngest is closest to the phialide from which it was born. Such cellular production is opposite of the acropetal mode of development exhibited by the remaining cells of the fungal thallus; i.e., the younger cell is the first one in the hypha or conidiophore stalk.

The molecular signals that induce conidiogenesis are regulated by a transcription factor encoded by the *abaA* gene (7). This gene is expressed only during conidiogenesis, and its transcript is not found during normal vegetative hyphal development. Deletion of *abaA* results in defective phialides and the absence of conidia. Other evidence suggests that this transcription factor also helps control cell cycle events during conidiogenesis.

Other genes involved in the formation of conidia include the transcription factor gene *stuA* and the *gasA* gene, encoding the G protein subunit (9, 193). Like *abaA*, *stuA* is expressed only during conidiogenesis. Loss of *stuA* results in the absence of metulae and phialides. Curiously, conidia are still formed, but they are located at the tips of conidiophores. *gasA* plays a role in the normal timing of conidiogenesis and appears to be involved in the molecular decision between vegetative growth or formation of conidia. Interestingly, like *gasC*, this gene appears to also have a role in the secondary metabolic pathway leading to red pigment synthesis by the hyphal form of *P. marneffei*.

**Dimorphism in *P. marneffei***: **arthroconidiogenesis and the yeast phase**. Among the penicillia, few species exhibit a sexual means of reproduction. Most species of *Penicillium* grow only as molds, reproducing solely via the asexual production of conidia. None are known to be dimorphic except *P. marneffei* (29, 30). The means by which *P. marneffei* undergoes dimorphism, a process termed phase transition, has been well studied at the morphological level and has triggered great interest in the molecular mechanisms governing this unique type of cellular differentiation.

Dimorphism in *P. marneffei* is thermally controlled. At 37°C, germinated conidia produce hyphae that are generally shorter in length and wider in diameter than those produced at 25°C (2, 30, 31). Moreover, the 37°C-derived hyphae are much more highly branched than their 25°C-derived counterparts. Within 48 h upon a suitable growth substrate, each compartment within a hyphal strand produced at 37°C becomes uninucleate as cell and nuclear division are coupled. These individual cells, containing a single nucleus, are divided from each other by a double-layered septum. These cells, termed prearthroconidia (Fig. 3C), are shorter (approximately 15 to 20 µm) along their axis than vegetative hyphal cells formed at 25°C. Left undisturbed, the prearthroconidia tend to remain attached to one another, yet even slight disturbances cause the prearthroconidial cells to break apart from one another along the midline of the double septum separating them. By definition, these cells are now termed arthroconidia, and they function as independent asexual propagules. In liquid cultures incubated at 37°C, the action of aeration by shaking leads to a population consisting mostly of single, unattached arthroconidia (Fig. 3D).

Arthroconidial formation, however, is merely a transitory stage to production of a yeast phase. Continued incubation of arthroconidia at 37°C initiates polarized growth and subsequent elongation of the cell shape. Eventually, following nuclear division, the cell divides by fission to form two uninucleate cells, much in the same way that cell division occurs in *Schizosaccharomyces pombe*. These newly formed cells can then again propagate by fission. Ultimately, the culture will consist entirely of yeast cells. In a liquid culture, the cells appear elliptical and generally detached from one another. On many rich solid media, a smooth cream-to-beige colony that
consists almost entirely of yeast cells. Notably, cultures of \textit{P. marneffei} grown at 37°C do not produce the diffusible red pigment that is so readily secreted by the mold phase.

A number of genetic studies have focused upon genes involved in the dimorphic switch in \textit{P. marneffei}. Several of those previously mentioned, i.e., \textit{stuA}, \textit{stfA}, \textit{gasA}, \textit{gasC}, and \textit{cflB}, have no role in yeast cell development or the dimorphic switch. However, others do have some role to play in the regulation of dimorphism or yeast growth. For example, the \textit{abaA} gene appears to control the coupling of nuclear and cellular division in prearthroconidial cells (7). However, once arthroconidia begin to develop as yeasts, a second series of genes appears to take over the coupling of cell cycle events.

With regard to yeast growth, two genes seem to be intimately involved in cell polarity. One gene in \textit{P. marneffei}, designated \textit{cflA}, is a homolog of \textit{CDC42} of \textit{S. cerevisiae} (12). \textit{CDC42} encodes a Rho-related GTPase that functions in cell signaling and polarity. In \textit{P. marneffei}, \textit{cflA} controls the correct morphogenesis of yeast cells. Mutants with mutations in the \textit{cflA} gene form aberrant yeast cells, although they are still capable of switching to filamentous growth when the incubation temperature is shifted to 25°C. However, the hyphae formed by these mutants exhibit defects in the hyphal tips where apical growth occurs. Curiously, the \textit{cflA} mutations have no effect upon conidiogenesis. Similarly, \textit{tupA} regulates the correct polarity in yeast cells that form at 37°C (160).

Two additional points regarding dimorphism in \textit{P. marneffei} are significant to note. First, arthroconidial development and subsequent yeast cell formation can also occur by shifting mold cultures from 25°C to 37°C. Initially, nuclear and cellular division must become coupled in the apical cells of the hyphal filaments. Once this event is established, then arthroconidio genesis occurs, followed by yeast cell formation. Conversely, yeast cells and arthroconidia formed at 37°C will cease their unique mode of development when shifted to 25°C and initiate apical growth with concomitant uncoupling of nuclear and cellular division typical of the mold phase. These observations clearly indicate that phase transition in \textit{P. marneffei} is a readily controllable and reversible process that is regulated solely by temperature. While nutritional factors may influence the extent to which phase transition may proceed, the event of the dimorphic switch rests entirely upon a temperature-induced signal that presently remains unknown.

A final important observation regarding the dimorphic nature of \textit{P. marneffei} pertains to its in vivo state. Conidia are regarded as the infectious propagule of \textit{P. marneffei}. Upon inhalation, conidia are believed to be phagocytized by alveolar macrophages, where they reside as intracellular parasites growing in the yeast phase. From there, the fungus can spread to other parts of the body. Interestingly, the yeast phase appears to develop directly from the phagocytized conidia without first forming hyphae, as is typical of conidia incubated at 37°C in vitro. Hyphae have never been reported in patients infected with \textit{P. marneffei}. Until now, no studies have reported data on the yeast-hyphal transition in animal models. It is therefore not known whether mycelia occur in the initial stages of infection. This is in contrast to the case for non-\textit{P. marneffei} species, which grow filamentously in vivo on those rare occasions when they cause disease. Why such a distinct type of phase transition occurs remains unclear and will require further investigation.

The focus of such investigations must be twofold and include the response of the invading fungus to the host as well as that of the host to the engulfed fungus.

**GENOMICS OF \textit{P. marneffei}**

To understand the fundamental cellular machinery that underpins thermal dimorphism, disease pathogenesis, and the interaction between the fungus and the immune system, a comprehensive knowledge of the \textit{P. marneffei} genome is required. The field of human fungal pathogen genomics is currently progressing at great speed; complete genome sequences have currently been completed for \textit{Candida albicans}, \textit{Candida glabrata}, \textit{Aspergillus fumigatus}, and \textit{Coccidioides immitis}, and four genomes are currently available for \textit{Cryptococcus neoformans}. However, there is currently no publicly available genome sequence for \textit{P. marneffei}.

Pulsed-field gel electrophoresis of genomic DNA of \textit{P. marneffei} has revealed three chromosomes (5.0, 4.0, and 2.2 Mb), although this is likely an underestimate of the true number (149), as genomes in the genus \textit{Aspergillus} tend to have eight or more chromosomes (http://www.broad.mit.edu). Prospective genome sequencing of \textit{P. marneffei} has been performed by Yuen and collaborators (191). The genome size of \textit{P. marneffei} has been estimated to be in the range of 17.8 to 26.2 Mb (191), compared to an estimated as 22 to 35 Mb in \textit{H. capsulatum} (170) and 31 Mb in \textit{Aspergillus nidulans} (http://www.broad.mit.edu). Sequencing a shotgun library of the \textit{P. marneffei} genome yielded 2,303 random sequence tags (RSTs), corresponding to a 9% genome coverage, with 11.7, 6.3, and 17.4% of the RSTs having sequence similarity to yeast-specific sequences, nonyeast fungus sequences, and both (common sequences), respectively (191). Analysis of the RSTs revealed genes for information transfer (ribosomal protein genes, tRNA synthetase subunit genes, translation initiation genes, and elongation factor genes), metabolism, and compartmentalization, including several multidrug resistance protein genes and homologs of fluconazole resistance genes. In addition, the RSTs uncovered the presence of genes encoding pheromone homologs and ankyrin repeat-containing proteins of other fungi and algae. These findings indicate that \textit{P. marneffei} may have a sexual component to its life cycle; however, further work is necessary to determine whether these genes are still functionally intact and capable of undergoing transcription to form functional gene products.

From this \textit{P. marneffei} sequencing project, a contig that contains the complete sequence (35 kb) of the mitochondrial genome was assembled (191). The gene contents, gene orders, and gene sequences were analyzed and compared to those of yeasts and molds (187). Interestingly, the results of comparative analyses revealed that the mitochondrial genome of \textit{P. marneffei} is more closely related to those of molds, especially to that of \textit{Aspergillus nidulans}, than to those of yeasts.

Many investigators have focused on phase-specific genes in the pathogenic dimorphic fungi, for example, in \textit{H. capsulatum}. Several genes that are expressed predominantly in the yeast phase of \textit{H. capsulatum} have been identified: \textit{yps-3} (83), \textit{cdc2} (38), and \textit{CBP1} (117). Some genes specific to the mold phase have been identified: alpha- and beta-tubulin genes (58), \textit{MS8} (159), and \textit{CATA} (74). In addition, several genes that were
highly expressed in the mold or yeast phase of \textit{H. capsulatum} have been identified using a genomic shotgun microarray (68). In \textit{P. marneffei}, the phase-specific genes have been studied by utilizing differential display techniques (30). This work has shown that many of the genes whose expression is upregulated during the mold-to-yeast transition are related to those genes involved in energy metabolism. The malate synthase-encoding gene was induced when the temperature was shifted to 37°C, whereby the pathogenic yeast phase of \textit{P. marneffei} is formed. The role of \textit{cpeA} in the pathogenesis of \textit{P. marneffei} will require further investigations.

**ACKNOWLEDGMENTS**

This work was supported by the Thailand Research Fund, the European Commission, and Medical Research Grants, Faculty of Medicine, Chiang Mai University (for research by Thira Sirisantha and Nonmuang Vanittanakom). Portions of Chester R. Cooper’s research were supported by a grant from the National Science Foundation (DBI-0330863). Matthew C. Fisher was supported by a Wellcome Trust Biodiversity Research Fellowship.

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**Penicillium marneffei** infection in HIV
Andrew P. Ustianowski\(^a\), Tran P.M. Sieu\(^b\) and Jeremy N. Day\(^a,c\)

**Introduction**
Penicilliosis – infection with *Penicillium marneffei* – is an endemic disease in areas of South East Asia that causes fever, lymphadenopathy, hepatosplenomegaly and cutaneous lesions. Cases were rare and sporadic before the HIV pandemic, but penicilliosis is now the third most common AIDS-defining illness (after tuberculosis and cryptococcosis) in South East Asia, and it has been reported from Northeast India across Burma (Myanmar), Thailand, Cambodia, Vietnam, Taiwan and Southern China, to Indonesia (Fig. 1) [1]. Increasingly, patients are diagnosed in other parts of the world having been exposed in Asia, and therefore it is increasingly important for all those working in the field of HIV to recognize this emerging disease [2,3].

**Epidemiology**
The only known natural hosts are bamboo rats (*Rhizomys* and *Cannomys* spp.) and humans [4–7]. *P. marneffei* can be isolated from the soil around the burrows, although only rarely from other environmental sources. The exact route of acquisition in humans is unknown but it is thought unlikely to be from direct contact with the rodents and is presumed to be via inhalation and, rarely, inoculation. In Thailand human infection is seasonal, particularly coinciding with rainy seasons, and it has been associated with soil exposure [8,9]. There is no evidence of person-to-person spread.

Infections have been described solely in those exposed in Asia, except for one case in an HIV-infected African male with no such travel history [10]. It has become the third most common HIV-related opportunistic infection in South East Asia, accounting for 15% of all HIV-related illness in Northern Thailand, affecting 10% of AIDS patients in Hong Kong, and becoming the second most common pathogen isolated from blood cultures in the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam after *Cryptococcus neoformans* [11,12]. Those with CD4+ lymphocyte counts below 100/μl are principally affected [2,9,13].

**Microbiology**
*P. marneffei* has the unique feature among the *Penicillium* spp. of being thermally dimorphic; at 25°C it grows as a mycelium (similar to *Aspergillus* spp.) but at 37°C it grows as a yeast. This thermal switching is demonstrable in the laboratory on artificial media and, *in vivo*, it is thought to...
be key to virulence. In humans and rats the infectious propagule is presumed to be the conidia derived from the mycelial form. Once in mammalian tissue the yeast form is assumed. Thus, unlike in aspergillosis, hyphae are not demonstrable in human tissue.

There is little description of the biochemical properties of the organism published in the literature. Wong et al. described 32 isolates and identified 17 different biotypes according to sugar assimilation and fermentation tests [14]. All isolates were urease positive and growth was inhibited by high concentrations of galactose. Youngchim et al. [15] described the enzymatic activities of 10 isolates. They found that yeast and mycelial phases exhibited alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, whereas trypsin, chymotrypsin and $\alpha$-fucosidase activities were absent. Some isolates had esterase, lipase and galactosidase activities. Some of these enzymes have previously been implicated in the virulence of fungal and nonfungal pathogens [16,17].

Multilocus microsatellite typing and multilocus sequence typing schemes are in development for *P. marneffei* (http://pmarneffei.multilocus.net/). Analyses resulting from these typing schemes show that there are two phylologically distinct groups of *P. marneffei* [1]: an Eastern and a Western clade. The clinical significance, if any, of these two clades is yet to be determined.

**Clinical features**

*P. marneffei* was initially isolated in 1956 from the liver of a bamboo rat, and human infection was first demonstrated in 1959 when the researcher himself, Segretain, accidentally self-inoculated the original rat isolate into his finger while working in his laboratory [18,19]. He developed a localized ulcer and lymphadenitis, and was treated with oral nystatin. His subsequent recovery was therefore probably spontaneous, because nystatin has negligible oral bioavailability. Confirmation as a natural pathogen of humans came in 1973 with the description of disseminated disease in a missionary with Hodgkin’s disease [20].

There is now extensive experience of this systemic mycosis in endemic countries, and most of the case series have originated from these areas [11,12,21–31]. Presentation can be myriad, with fever, lymphadenopathy, hepatosplenomegaly and cutaneous lesions commonly being reported. Table 1 illustrates the frequencies of clinical signs and symptoms from the larger series [11,21]. There have also been case reports of patients presenting in nonendemic areas who have exhibited similar clinical features [2]. It is important to note that, other than in one case, all such patients had a clear history of exposure in endemic regions before their subsequent diagnosis [10].

The skin lesions commonly appear as papules (characteristically with central umbilication, resembling molluscum contagiosum), nodules, or necrotic lesions, and they are predominantly located over the face and upper trunk (Figs 2 and 3). The skin lesions are very similar to those observed in disseminated cryptococcosis, and concomitant cryptococcosis and other opportunistic infections are common in patients with penicilliosis [9]. In Thailand a
cryptococcal co-infection rate at diagnosis of 5% has been reported. In another series [11] the investigators identified a 10% risk for developing cryptococcosis within the first 4 months after diagnosis of penicilliosis.

Laboratory diagnosis

Laboratory diagnosis continues to depend on microscopic identification of the fungus with confirmation by culture, although there has been increasing recent interest in the use of serodiagnostics and nucleic acid assays.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Duong [23]</th>
<th>Supparatpinyo et al. [11]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>98</td>
<td>93</td>
</tr>
<tr>
<td>Anaemia</td>
<td>74.8</td>
<td>78</td>
</tr>
<tr>
<td>Weight loss</td>
<td>71.6</td>
<td>76</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>69.7</td>
<td>71</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>52.3</td>
<td>58</td>
</tr>
<tr>
<td>Cough</td>
<td>49.7</td>
<td>–</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>43.9</td>
<td>51</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>23.2</td>
<td>31</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>13.5</td>
<td>16</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>4.5</td>
<td>–</td>
</tr>
<tr>
<td>Osteolytic lesions</td>
<td>3.9</td>
<td>–</td>
</tr>
<tr>
<td>Arthritis</td>
<td>3.9</td>
<td>–</td>
</tr>
<tr>
<td>Ulcer</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Jaundice</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>Positive blood culture</td>
<td>54.2</td>
<td>76</td>
</tr>
</tbody>
</table>

Values are expressed as percentage affected.

Microscopy and culture

Microscopically, *P. marneffei* appears as oval or round intracellular yeasts, most commonly in biopsies of bone marrow, lymph node, liver and cutaneous lesions. More rarely the infection has been diagnosed directly in sputum, pleural fluid, cerebrospinal fluid, pericardium, stool, urine and fine-needle aspirates of lymph nodes [11,32]. The differential diagnosis of such intracellular yeasts includes histoplasmosis (which also has similar clinical presentations) and cryptococcosis (which is associated with more neurological symptoms and less respiratory involvement, lymphadenopathy and hepatosplenomegaly) [33,34]. *P. marneffei* has characteristic cross-wall formations (Fig. 4), and confirmatory immunohistochemistry can

The classical cross-wall formation is demonstrated.
be performed with antibodies raised to *Aspergillus* galactomannan or other more specific *Penicillium* antigens [35,36].

The classical culture characteristics of thermal dimorphism and the production of red pigment are easily demonstrated. Bone marrow, blood and biopsies of skin lesions all have high culture yield (100%, 76% and 90%, respectively) [11].

**Serodiagnosis**

For serodiagnosis various methods have been developed to assess host antibody production (such as immunoblot, immunofluorescence antibody test and enzyme-linked immunosorbent assay), but they have thus far been trialled on only small numbers of patients or there have been issues with sensitivity and specificity [37**]. These have not yet entered routine clinical practice.

There has been recent interest in detecting circulating galactomannan. The *Penicillium* galactomannan has considerable homology to that of *Aspergillus*, and commercial assays for the detection of the latter were recently investigated in *P. marneffei* infection [38*]. Sera from 11 out of 15 culture confirmed penicilliosis cases were positive (optimal density index >0.5), although 9% of HIV-positive control individuals were apparent false positives.

**Nucleic acid assays**

Polymerase chain reaction assays, detecting fungal DNA, have been developed. High sensitivity and specificity have been reported, but the protocols remain labour (and equipment) intensive and have yet to enter routine clinical practice [37**].

**Treatment**

Disseminated disease is thought to be universally fatal if untreated.

*In vitro*, *P. marneffei* is highly sensitive to itraconazole, voriconazole, ketoconazole, miconazole, terbinafine and 5-fluorocytosine; intermediate sensitive to amphotericin B; but largely resistant to fluconazole [39–42]. No clear data are presently available for the echinocandins, although they may work poorly against the pathogenic yeast phase [43].

**Acute infection**

There have been no randomized controlled trials on the acute treatment of penicilliosis, and thus treatment choices must be based upon data from case series and in-vitro data on antifungal sensitivities.

In a case series of 80 consecutive HIV-positive Thai patients with penicilliosis [44], the authors described responses to treatment with amphotericin B, itraconazole, or fluconazole. In addition, 30 isolates underwent antifungal sensitivity testing. The failure rates (defined as persistent fungaemia, lack of clinical improvement, or clinical deterioration) were 22.8% for amphotericin B, 25% for itraconazole, 63.6% for fluconazole and 100% for no treatment. Treatment choice was at the discretion of the attending physician without knowledge of the minimum inhibitory concentration of antifungal drugs for the isolates. Consistent with the poorer response to fluconazole, there were consistently higher in-vitro minimum inhibitory concentrations for this drug (73% of isolates were classified as borderline susceptible or resistant). Of isolates tested for amphotericin B susceptibility, 41% were classified as only moderately sensitive or resistant, but despite this the *Penicillium*-attributable death rate was lowest in patients receiving this drug. All isolates were sensitive to 5-fluorocytosine.

A subsequent series included 74 HIV patients with disseminated penicilliosis treated with amphotericin B 0.6 mg/kg per day for 2 weeks followed by itraconazole 400 mg/day for 10 weeks [45]. All patients received co-trimoxazole as primary prophylaxis for *Pneumocystis jirovecii* but not antiretroviral therapy. Remarkably, there were no deaths in the study. The reason for the lack of deaths is not clear from the report, but it could conceivably be due to exclusion of more seriously ill patients who would have been unable to consent to enter the study.

Unfortunately, amphotericin B is a prohibitively expensive drug for most patients at risk of penicilliosis. A small uncontrolled study utilizing itraconazole alone demonstrated a disappointingly slow clearance of fungaemia [46]. A more recent study from Chang Mai, Thailand, has examined the in-vivo efficacy of voriconazole [47*]. Two patients received intravenous voriconazole (6 mg/kg twice daily on day 1, followed by 4 mg/kg twice daily) stepping down to oral voriconazole (200 mg twice daily), and a further nine were treated with oral voriconazole alone (400 mg twice daily on day 1, followed by 200 mg twice daily). There were favourable responses in eight of the nine evaluable patients, and the therapy was well tolerated. Further research into this newerazole is warranted.

**Secondary prophylaxis**

Before the widespread introduction of highly active antiretroviral therapy (HAART), it was recognized that there was a very high relapse rate after initial therapy (of the order of 11.5 cases per 100 person-months) [11,44,46]. A subsequent randomized, double-blind, placebo-controlled study of itraconazole secondary prophylaxis (200 mg once daily) [48] was discontinued early because all relapses were within the placebo arm. Such long-term prophylaxis has therefore been adopted.
Discontinuation of secondary prophylaxis
Several reports have investigated the discontinuation of itraconazole secondary prophylaxis after immune restoration due to HAART; however, all have been retrospective observational studies.

There were no relapses after itraconazole discontinuation in 33 patients with a CD4<sup>+</sup> lymphocyte count above 100/µl for more than 6 months who were followed for a median of 18 months, or in another study conducted in patients stabilized on HAART, which unfortunately did not specify CD4<sup>+</sup> lymphocyte counts [49<sup>**</sup>, 50]. One relapse was described in a series of 19 patients who discontinued prophylaxis at a median CD4<sup>+</sup> lymphocyte count of 95/µl (18 patients had a CD4<sup>+</sup> count <200/µl and 10 <100/µl), equating to a relapse rate of 1.72/100 patient-years [34].

It therefore appears reasonable to discontinue secondary prophylaxis after significant immune restoration from antiretrovirals, although exact criteria need to be established in larger, prospective, randomized studies.

Primary prophylaxis
The potential for primary prophylaxis against penicilliosis has been explored in a randomized placebo-controlled double-blinded study of itraconazole (200 mg/day) in those with CD4<sup>+</sup> lymphocyte counts below 150/µl [51]. Only eight out of 129 patients were receiving antiretroviral therapy. Although there was a numerical decrease in the incidence of *P. marneffei* infection (principally in those with CD4<sup>+</sup> count <100/µl) there was no survival advantage of being on itraconazole, and this intervention cannot presently be endorsed. The trial should be repeated in patients also receiving antiretrovirals.

Summary of treatment
At present the standard therapy remains 14 days of intravenous amphotericin B, followed by 10 weeks of oral itraconazole (400 mg/day) and subsequent itraconazole secondary prophylaxis (at 200 mg/day). It is generally recognized, although there is no directly confirmatory evidence, that institution of HAART is also important. Discontinuation of secondary prophylaxis may be considered after immune restoration from antiretrovirals (potentially if the CD4<sup>+</sup> lymphocyte count increases to >100/µl for at least 6 months).

Future research
The genome of *P. marneffei* is being sequenced in a collaboration between Melbourne University and the J. Craig Venter Institute (formerly The Institute for Genome Research). The sequence will become freely available. There are active research groups in Thailand, China, Australia, Vietnam, UK and the USA, and current research includes investigation into dimorphic switching and its relationship with pathogenicity, fungal and disease epidemiology, and the interaction between the environment and human disease.

Further, well constructed studies are required to establish the optimal treatment for acute infection (including the roles of the newer azoles and oral-only regimens) and the criteria for discontinuation of secondary prophylaxis.

Conclusion
Previously a very rare diagnosis, the advent of the HIV pandemic has greatly increased the frequency of penicilliosis. It is now endemic throughout South East Asia, usually presenting with fever, lymphadenopathy, hepatosplenomegaly and cutaneous lesions. Routine diagnosis still requires identification of the organism, although there is increasing interest in serodiagnosis, and antigen and nucleic acid assays. The mainstay of therapy is amphotericin B followed by itraconazole, although the newer azoles appear promising. Secondary itraconazole prophylaxis is required until there has been significant immune restoration. Further research is ongoing or required into pathogenicity, epidemiology and optimal management, in order to achieve better understanding and control of this emerging infectious disease.

Acknowledgements
With thanks to Dr M Fisher (Imperial College, UK) for assistance with Fig. 1.

Dr Ustianowski has received lecturing fees from the HIV division of Gilead Sciences. Gilead also market AmBisome (liposomal amphotericin).

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 103).

HIV infection and AIDS


27 This paper provides an excellent review of the epidemiology and present state of diagnostics for P. marneffei.


39 This paper investigates the important area of establishing criteria for discontinuation of secondary intracranial prophylaxis in those with immune restoration after HAART, implying that CD4 lymphocyte count above 100/µl for more than 6 months may be adequate.


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Combination Antifungal Therapy for Mold Infections: Much Ado about Nothing?

Jose A. Vazquez
Division of Infectious Diseases, Henry Ford Hospital, Wayne State University School of Medicine, Detroit, Michigan

In general, mortality rates associated with systemic fungal infections have not improved much in more than a decade, although the number of antifungal agents available for the treatment of serious fungal infections has increased in the past few years. A possible approach to decreasing mortality rates associated with fungal infections may be to treat patients with combinations of different classes of antifungals. Recently, in vitro and animal studies evaluating different combinations of antifungal agents have demonstrated important synergistic and/or additive activity against many genera of fungi. However, prudence is required, because some antifungal combinations have demonstrated antagonistic activity. Well-controlled clinical trials are still necessary to define the most efficacious antifungal combination. In addition, these clinical trials should evaluate the adverse event profile of the combination regimens, as well as their pharmacoeconomic impact.

Recent epidemiologic studies have demonstrated an increase in mortality rates associated with infection due to invasive molds, such as *Aspergillus* species, *Zygomycetes*, *Fusarium* species, and *Scedosporium* species [1, 2]. The development and approval of potent new antifungal agents with broader spectrums of activity and different mechanisms of action have changed the way that we manage many of these infections. The unique properties of these antifungals afford us the opportunity to evaluate newer antifungal combinations that eventually may be used to treat serious fungal infections.

There are several possible reasons for using 2 or more antifungals simultaneously, instead of using a single agent. One reason is to achieve fungicidal activity that may not be possible with only 1 agent. Second, combinations may enable us to diminish drug dosages, and thus, diminish the adverse event profile while increasing efficacy. Another reason may be to delay or possibly prevent the emergence of drug-resistant mutants. In addition, combination therapy may provide broader spectrum coverage for seriously ill patients.

There are also disadvantages to using antifungal combinations. In general, because at least 2 drugs are being administered, the cost of therapy will be greater. Second, there is a greater chance of drug interactions and adverse effects. Finally, the use of drug combinations may produce a false sense of security, because the physician may feel that all possible pathogens are being covered.

Currently available antifungal agents include fluconazole, miconazole, itraconazole, voriconazole, posaconazole, and amphotericin B, all of which act by altering the cellular membrane [3, 4]. Flucytosine acts intracellularly by inhibiting the protein synthesis of the fungal cell. New additions include the echinocandins caspofungin, micafungin, and anidulafungin [5]. The echinocandins act by inhibition of 1,3-β-glucan synthase required for the formation of the cell wall [6]. These new antifungals imply that new combinations are possible [7–9].

IN VITRO ANTIFUNGAL COMBINATIONS AGAINST *ASPERGILLUS* SPECIES

To date, >60 in vitro studies evaluating the activity of different antifungal combinations against *Aspergillus* species have been published. An extensive assessment of the current literature can be reviewed in table 1. Several of the studies are described below.

**Polyene-flucytosine azole combinations.** Te Dorsthorst et al. [10] evaluated the interactions of amphotericin B, itraconazole, and terbinaine against *Aspergillus fumigatus*. The combinations of amphotericin B–itraconazole and amphotericin B–terbinaine were found to be antagonistic, whereas the combination of itraconazole–terbinaine was synergistic. In one of the earlier studies evaluating the activity of sequential an-
Table 1. In vitro susceptibility assays evaluating antifungal combinations against *Aspergillus* species.

<table>
<thead>
<tr>
<th>Study, organism (s)</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Results $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Te Dorsthorst et al. [10]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>AmB</td>
<td>Itz</td>
<td>Antagonism</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Tbf</td>
<td>Antagonism</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Itz</td>
<td>Tbf</td>
<td>Synergy</td>
</tr>
<tr>
<td>Te Dorsthorst et al. [11]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Itz</td>
<td>Antagonistic</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Itz</td>
<td>5-FC</td>
<td>Antagonistic</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em></td>
<td>AmB</td>
<td>5-FC</td>
<td>Synergy</td>
</tr>
<tr>
<td>Kontoyiannis et al. [12]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Itz</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Maesaki et al. [13]</td>
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<tr>
<td><em>A. fumigatus</em></td>
<td>AmB (preincubation)</td>
<td>Mcz or Flz</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Flz (preincubation)</td>
<td>AmB</td>
<td>Antagonism</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Mcz (preincubation)</td>
<td>AmB</td>
<td>Synergy</td>
</tr>
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<td><em>A. fumigatus</em></td>
<td>AmB (simultaneous)</td>
<td>Mcz or Flz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Ghannoum et al. [14]</td>
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<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Cfgn</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Vcz</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Meletiadis et al. [15]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>AmB</td>
<td>Itz</td>
<td>Antagonism, synergy</td>
</tr>
<tr>
<td>Perkhofer et al. [16]</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>A. fumigatus, A. flavus, Aspergillus niger, and A. terreus</em></td>
<td>AmB</td>
<td>Pcz</td>
<td>Conidia, 12% synergy, hyphae, 75% synergy</td>
</tr>
<tr>
<td><em>A. fumigatus, A. flavus, A. niger, and A. terreus</em></td>
<td>AmB</td>
<td>Vcz</td>
<td>Conidia, 43% synergy, hyphae, 37% synergy</td>
</tr>
<tr>
<td>Ryder et al. [17]</td>
<td></td>
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<tr>
<td><em>A. fumigatus and A. niger</em></td>
<td>Tbf</td>
<td>AmB</td>
<td>Synergy, cidal</td>
</tr>
<tr>
<td><em>A. fumigatus and A. niger</em></td>
<td>Tbf</td>
<td>Itz</td>
<td>Synergy, cidal</td>
</tr>
<tr>
<td><em>A. fumigatus and A. niger</em></td>
<td>Tbf</td>
<td>Vcz</td>
<td>Synergy, cidal</td>
</tr>
<tr>
<td><em>A. fumigatus and A. niger</em></td>
<td>Tbf</td>
<td>Flz</td>
<td>Synergy, cidal</td>
</tr>
<tr>
<td>Mosquera et al. [18]</td>
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<tr>
<td><em>A. fumigatus</em></td>
<td>Tbf</td>
<td>Itz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td><em>A. fumigatus (Itz resistant)</em></td>
<td>Tbf</td>
<td>Flz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Tbf</td>
<td>AmB</td>
<td>Indifference, antagonism</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Tbf</td>
<td>5-FC</td>
<td>Indifference, antagonism</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>Tbf</td>
<td>5-FC</td>
<td>Indifference, antagonism</td>
</tr>
<tr>
<td>Manavathu et al. [19]</td>
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<tr>
<td><em>A. fumigatus</em></td>
<td>Mfgn</td>
<td>AmB</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Synergy</td>
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<td><em>A. fumigatus</em></td>
<td>Mfgn</td>
<td>Vcz</td>
<td>Synergy</td>
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<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Synergy</td>
</tr>
<tr>
<td>Arikan et al. [20]</td>
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<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Additivity, indifference</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>Perea et al. [21]</td>
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</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Cfgn</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Cfgn</td>
<td>Cfgn</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>Cfgn</td>
<td>Cfgn</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>Shalit et al. [22]</td>
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<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Itz</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Cfgn</td>
<td>Itz</td>
<td>Additivity, indifference</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Itz</td>
<td>Synergy, indifference</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>Cfgn</td>
<td>Itz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>Manavathu et al. [23]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Itz</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Pcz</td>
<td>Synergy</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Rcz</td>
<td>Additivity</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Kontoyiannis et al. [24]</td>
<td></td>
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</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Cfgn (pre-exposure)</td>
<td>Itz</td>
<td>Dose-dependent activity enhancement</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Itz (pre-exposure)</td>
<td>Cfgn</td>
<td>Dose-dependent activity enhancement</td>
</tr>
</tbody>
</table>

(continued)
Table 1. (Continued.)

<table>
<thead>
<tr>
<th>Study, organism(s)</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Resultsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabatelli [25]</td>
<td>Cfgn</td>
<td>Pcz</td>
<td>Synergy, 23%; additivity, 70%</td>
</tr>
<tr>
<td>O’Shaughnessy et al. [26]</td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Additivity, synergy</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Mfgn</td>
<td>Vcz</td>
<td>Additivity, synergy</td>
</tr>
<tr>
<td>Dannaoui et al. [27]</td>
<td>Cfgn</td>
<td>AmB</td>
<td>Additivity</td>
</tr>
<tr>
<td>A. fumigatus and A. terreus</td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Additivity, synergy</td>
</tr>
<tr>
<td>A. fumigatus and A. terreus</td>
<td>Cfgn</td>
<td>5-FC</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>A. fumigatus and A. terreus</td>
<td>Cfgn</td>
<td>AmB plus 5-FC</td>
<td>Synergistic, additivity</td>
</tr>
<tr>
<td>Cuenca-Estrella et al. [28]</td>
<td>AmB</td>
<td>Itz</td>
<td>Indifference</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>AmB</td>
<td>Vcz</td>
<td>Synergy, 43%</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>AmB</td>
<td>Cfgn</td>
<td>Synergy, 7%–35%</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Itz</td>
<td>Cfgn</td>
<td>Indifference</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Vcz</td>
<td>Cfgn</td>
<td>Synergy</td>
</tr>
<tr>
<td>Lewis and Kontoyiannis [29]</td>
<td>Mfgn</td>
<td>Vcz</td>
<td>Synergy</td>
</tr>
<tr>
<td>A. terreus</td>
<td>Mfgn</td>
<td>Vcz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Philip et al. [30]</td>
<td>Afgn</td>
<td>Itz</td>
<td>Synergy</td>
</tr>
<tr>
<td>A. fumigatus, A. flavus, A. niger and A. terreus</td>
<td>Afgn</td>
<td>Vcz</td>
<td>Synergy</td>
</tr>
<tr>
<td>A. fumigatus, A. flavus, A. niger and A. terreus</td>
<td>Afgn</td>
<td>AmB</td>
<td>Indifference</td>
</tr>
<tr>
<td>Chiu et al. [31]</td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>Synergy</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>Synergy</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>AmB</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>ABLC</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>LAmB</td>
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<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>Itz</td>
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<td>NikZ</td>
<td>Vcz</td>
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<td>NikZ</td>
<td>Pcz</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>Rcz</td>
</tr>
<tr>
<td></td>
<td>A. fumigatus</td>
<td>NikZ</td>
<td>Cfgn</td>
</tr>
<tr>
<td>Nguyen et al. [32]</td>
<td>A. fumigatus</td>
<td>Azithromycin</td>
<td>AmB</td>
</tr>
<tr>
<td>Var’t Hof et al. [33]</td>
<td>A. fumigatus</td>
<td>AmB</td>
<td>Histatin 5, dhvar 4, magainin 2, PGLa</td>
</tr>
<tr>
<td>Afetra et al. [34]</td>
<td>A. fumigatus</td>
<td>Itz</td>
<td>Amiodarone, lansoprazole, risedipine</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Itz</td>
<td>Amiloride, lidocaine, verapamil, fluphenazone</td>
<td>Indifference</td>
</tr>
<tr>
<td>Steinbach et al. [35]</td>
<td>A. fumigatus</td>
<td>Cfgn</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Cfgn</td>
<td>Cyclosporine</td>
<td>Synergy</td>
</tr>
</tbody>
</table>

**NOTE.** ABL, amphotericin B lipid complex; Afgn, anidulafungin; AmB, amphotericin B; Cfgn, caspofungin; 5-FC, 5-fluorouracil; Flz, fluconazole; Itz, itraconazole; LAmB, liposomal amphotericin B; Mfgn, micafungin; NikZ, nikkomycin Z; Pcz, posaconazole; Rcz, ravuconazole; Tbf, terbinafine; Vcz, voriconazole...

Percentages are percentage of isolates.

Tifungals against *A. fumigatus*, Maesaki et al. [13] evaluated simultaneous versus sequential combinations of amphotericin B–miconazole and amphotericin B–fluconazole. Pretreatment with amphotericin B and subsequent treatment with either miconazole or fluconazole resulted in improved efficacy, compared with simultaneous exposure. In addition, pretreatment with azoles and subsequent exposure to amphotericin B had efficacy that was no different than that of monotherapy.

Perkhofer et al. [16] evaluated the efficacy of posaconazole and voriconazole administered in combination with amphotericin B against *Aspergillus* species. They found that the combinations were more active against hyphae than they were against conidia. Synergy was noted in 75% of isolates exposed to posaconazole–amphotericin B and in only 37% of isolates exposed to voriconazole–amphotericin B.

In a unique study, Liu et al. [36] described the attenuated efficacy of itraconazole against *A. fumigatus* after exposure to fluconazole. The authors observed that, after serial passages on fluconazole-containing medium, the minimum fungicidal concentrations of itraconazole increased. This may be clinically relevant for patients receiving fluconazole prophylaxis who experience a breakthrough mold infection and whose treatment...
Table 2. Animal models evaluating antifungal combinations against *Aspergillus* species.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Drug combination</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Árroyo et al. [37]</td>
<td>Murine</td>
<td>AmB, rifampin AmB, 5-FC</td>
<td>Indifference</td>
</tr>
<tr>
<td>Schmitt et al. [38]</td>
<td>Murine IPA</td>
<td>AmB, Ktz</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Lewis et al. [39]</td>
<td>Murine IPA</td>
<td>AmB, Itz</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Najvar et al. [40]</td>
<td>Murine IPA</td>
<td>AmB, Pcz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Meletiadis et al. [41]</td>
<td>IPA in neutropenic rabbits</td>
<td>LAmB, Rcz</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Gavardá et al. [42]</td>
<td>IPA in rats</td>
<td>Vcz, Tbf</td>
<td>No improvement in survival</td>
</tr>
<tr>
<td>Kirkpatrick et al. [43]</td>
<td>Intravenous conidia in immunosuppressed rabbits</td>
<td>AmB, Tbf</td>
<td>No improvement in survival</td>
</tr>
<tr>
<td>Kohno et al. [44]</td>
<td>Murine IPA</td>
<td>Mfgn, Mfgn, Itz, Mfgn, 5-FC</td>
<td>Synergy, 65% of isolates; synergy, 45% of isolates; synergy, 55% of isolates</td>
</tr>
<tr>
<td>Kirkpatrick et al. [45]</td>
<td>Intravenous conidia in immunosuppressed rabbits</td>
<td>AmB, Vcz, Cfgn, Vcz</td>
<td>Improved survival and reduction in CFUs for both combinations</td>
</tr>
<tr>
<td>Petraitis et al. [46]</td>
<td>IPA in neutropenic rabbits</td>
<td>Mfgn, Rcz</td>
<td>Improved survival and reduction in CFUs</td>
</tr>
<tr>
<td>Graybill et al. [47]</td>
<td>Immunosuppressed mice</td>
<td>Mfgn, AmB</td>
<td>Indifference</td>
</tr>
<tr>
<td>Capilla Luque et al. [48]</td>
<td>Murine systemic IA</td>
<td>Mfgn, AmB, Mfgn, Itz, Mfgn, NikZ</td>
<td>Indifference for all 3 combinations</td>
</tr>
<tr>
<td>Chandrasekar et al. [49]</td>
<td>Immunosuppressed mice</td>
<td>ABLC, Cfgn</td>
<td>Indifference</td>
</tr>
<tr>
<td>Chandrasekar et al. [50]</td>
<td>IPA in guinea pigs</td>
<td>Vcz, AmB; Vcz, Mfgn</td>
<td>Indifference for both combinations</td>
</tr>
<tr>
<td>Clemens et al. [51]</td>
<td>Murine CNS aspergillosis</td>
<td>Vcz, AmB; AmB, Mfgn, Cfgn, AmB, Cfgn</td>
<td>Enhanced activity; prolonged survival; prolonged survival</td>
</tr>
<tr>
<td>Clemens et al. [52]</td>
<td>Murine CNS aspergillosis</td>
<td>ABLC, Mfgn; ABLC, Cfgn; ABLC, Itz; ABLC, Vcz</td>
<td>Indifference; indifference; indifference; improved survival</td>
</tr>
<tr>
<td>MacCallum et al. [53]</td>
<td>IA in neutropenic guinea pigs</td>
<td>Cfgn, Vcz</td>
<td>Additivity</td>
</tr>
<tr>
<td>Dennis et al. [54]</td>
<td>p47phox −/− murine model of chronic granulomatous disease</td>
<td>Mfgn, AmB</td>
<td>Prolonged survival</td>
</tr>
<tr>
<td>Olsen et al. [55]</td>
<td>Murine systemic IA</td>
<td>Mfgn, LAmB</td>
<td>Indifference</td>
</tr>
<tr>
<td>Petraitis et al. [56]</td>
<td>IPA in neutropenic rabbits</td>
<td>Afgn, AmB</td>
<td>Improved survival, reduction in CFUs</td>
</tr>
</tbody>
</table>

**NOTE.** ABLC, amphotericin B lipid complex; Afgn, anidulafungin; AmB, amphotericin B; Cfgn, caspofungin; CFU, colony-forming unit; 5-FC, 5-fluorouracil; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis; Itz, itraconazole; Ktz, ketoconazole; LAmB, liposomal amphotericin B; Mfgn, micafungin; NikZ, nikkomycin Z; Pcz, posaconazole; Rcz, ravuconazole; Tbf, terbinafine; Vcz, voriconazole.
is changed to a broader spectrum azole, such as voriconazole or posaconazole. This effect has not yet been described in patients, and therefore, the clinical relevance is unclear.

**Terbinafine combinations.** Ryder et al. [17] evaluated the combination of terbinafine with several azoles. They noted that terbinafine–amphotericin B displayed an additive to synergistic interaction, whereas terbinafine–itraconazole and terbinafine–voriconazole showed potent synergy and fungicidal activity. The combination terbinafine–fluconazole also displayed an additive to synergistic effect against many *Aspergillus* species isolates.

**Echinocandin combinations.** Although the echinocandins are relatively new antifungals, a significant amount of data regarding their use has been rapidly generated. The interaction of caspofungin–voriconazole was evaluated in a group of *Aspergillus* isolates [21]. Synergy was demonstrated for 88% of the strains, whereas additive was seen in 12% of strains. Manavathu et al. [23] evaluated the activity of several azoles in combination with caspofungin against *A. fumigatus*. The results demonstrated synergy with caspofungin–itraconazole and caspofungin–posaconazole, whereas caspofungin–ravuconazole and caspofungin–voriconazole were no different than monotherapy.

Kontoyiannis et al. [24] evaluated the sequential exposure of *A. fumigatus* to the combination of caspofungin–itraconazole. The investigators exposed *Aspergillus* strains to itraconazole and subsequently exposed the same strains to caspofungin. The sequential addition of the second antifungal resulted in a dose-dependent increase in activity. Similarly, exposure to caspofungin with subsequent exposure to itraconazole also led to an increased activity. Although sequential therapy is not routine, it does occur when patients are receiving antifungals as part of a prophylaxis regimen and experience a breakthrough mold infection.

Dannaoui et al. [27] evaluated the efficacy of triple-drug combinations against *A. fumigatus* and *Aspergillus terreus*. The investigators evaluated caspofungin–amphotericin B, caspofungin–voriconazole, and caspofungin–fluconazole, as well as the triple-drug combination caspofungin–amphotericin B—fluconazole. The results revealed that the most potent 2-drug combination was caspofungin–fluconazole, which demonstrated synergy in 62% of isolates and additivity in 38%. The triple-drug regimen, caspofungin–amphotericin B—fluconazole, demonstrated 100% additivity but no synergy. However, caspofungin–voriconazole—fluconazole demonstrated 67% synergy and 33% additivity. The results are important for those clinicians who use 2-drug and 3-drug combinations without any evidence-based data to confirm the efficacy or safety of these complex drug-drug interactions.

Cuenca-Estrella et al. [28] evaluated caspofungin in combination with either amphotericin B, itraconazole, or voriconazole and compared these combinations with the combinations amphotericin B—itraconazole and amphotericin B—voriconazole. The assays revealed that therapy with amphotericin B—itraconazole and amphotericin B—voriconazole were no different than monotherapy. In contrast, amphotericin B—voriconazole demonstrated synergy in 43% of the isolates, and caspofungin—voriconazole demonstrated synergy in 50% of the isolates. Lewis and Kontoyiannis [29] evaluated the activity of micafungin—voriconazole against *A. fumigatus*, *Aspergillus flavus*, and *A. terreus*. The investigators showed enhanced activity against *A. fumigatus* and *A. terreus* but not against *A. flavus*.

Philip et al. [30] evaluated anidulafungin in combination with either itraconazole or voriconazole against *A. fumigatus*, *A. flavus*, *Aspergillus niger*, and *A. terreus*. Both anidulafungin—itraconazole and anidulafungin—voriconazole demonstrated synergy against *A. fumigatus* and *A. flavus*. However, no difference was seen with respect to activity against *A. niger* and *A. terreus*.

### Experimental Animal Models Evaluating Antifungal Combinations Against *Aspergillus* Species

There are >50 reports describing experimental animal models that evaluate the activity of antifungal combinations against *Aspergillus* species. Table 2 contains a more extensive review and additional details regarding many of these studies.

**Polyene-azole—fluconazole combinations.** Arroyo et al. [37] published one of the earliest animal models evaluating the efficacy of combination antifungal therapy in treating aspergillosis. With use of a murine model of disseminated aspergillosis, the investigators evaluated suboptimal doses of amphotericin B administered in combination with either rifampin or fluconazole. They noted that survival rates improved when either combination was used. However, none of the regimens produced complete mycological cures. Lewis et al. [39] used a murine model of pulmonary aspergillosis to compare the efficacy of amphotericin B monotherapy with that of amphotericin B administered after a 3-day exposure to itraconazole. In these experiments, the group that was pretreated with itraconazole was associated with poorer mycological efficacy and lower survival rates. Moreover, increased dosages of amphotericin B were not able to overcome this antagonistic effect.

Najvar et al. [40] evaluated posaconazole—amphotericin B in treating infections due to *A. flavus* in immunosuppressed mice. The antifungals were given either concurrently or with a 1-day period of exposure to posaconazole, followed by administration of amphotericin B. In contrast with the antagonism noted by Lewis et al. [39], these investigators did not detect any antagonism.

**Terbinafine combinations.** Kirkpatrick et al. [43] evaluated terbinafine—amphotericin B in a rabbit model of disseminated aspergillosis. Their results revealed that terbinafine alone had
Table 3. Clinical results of various antifungal combinations against various mold infections.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Setting</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codish et al. [57]</td>
<td>Aspergillus fumigatus</td>
<td>AmB</td>
<td>FC</td>
<td>Pulmonary aspergillosis</td>
<td>Clinical response and resolution</td>
</tr>
<tr>
<td>Schaffner and Baker [58]</td>
<td>A. fumigatus</td>
<td>Itz (pre-exposure)</td>
<td>AmB</td>
<td>Refractory aspergillosis</td>
<td>Clinical failure</td>
</tr>
<tr>
<td>Bajjoka et al. [59]</td>
<td>A. fumigatus</td>
<td>LAmB</td>
<td>Itz</td>
<td>Patient 1: liver transplant recipient with IPA; patient 2: liver transplant recipient with epidural abscess</td>
<td>Death in both patients</td>
</tr>
<tr>
<td>Kontoyiannis et al. [60]</td>
<td>Aspergillus species</td>
<td>Cfgn</td>
<td>LAmB</td>
<td>Retrospective review of patients with HM and IA (50 patients)</td>
<td>Combination therapy had success rate of 41% as primary therapy and 6% as salvage therapy</td>
</tr>
<tr>
<td>Marr et al. [61]</td>
<td>Aspergillus species</td>
<td>Vcz</td>
<td>Cfgn</td>
<td>Prospective comparison of salvage therapy after failure of AmB therapy; patients switched to Vcz alone (n = 31) or Vcz plus Cfgn (n = 16)</td>
<td>Combination therapy associated with decreased mortality and improved survival at 3 months</td>
</tr>
<tr>
<td>Kontoyiannis et al. [62]</td>
<td>Aspergillus species</td>
<td>Itz</td>
<td>LAmB</td>
<td>Retrospective review of HM and IA in 146 patients who received LAmB and 33 who received LAmB plus Itz</td>
<td>Survival rate in monotherapy group, 10%; no survivors in combination therapy group</td>
</tr>
<tr>
<td>Maertens et al. [63]</td>
<td>Aspergillus species</td>
<td>Cfgn</td>
<td>AmB, Itz, Vcz</td>
<td>Multicenter study involving 53 patients with IA refractory to or intolerant of antifungal therapy</td>
<td>Success rate was 55% at end of therapy and 49% at 3 months; survival at 3 months was 55%</td>
</tr>
<tr>
<td>Singh et al. [64]</td>
<td>Aspergillus species</td>
<td>Cfgn</td>
<td>Vcz</td>
<td>IA, SOT, MCT, prospective, comparative study of primary therapy vs. historic control group receiving LAmB</td>
<td>Survival at 90 days was 68% in the combination therapy group (n = 40) and 51% in the control group (n = 47)</td>
</tr>
<tr>
<td>Raad et al. [65]</td>
<td>Aspergillus species</td>
<td>Cfgn</td>
<td>Vcz</td>
<td>Retrospective, IA, HM, comparative of Cfgn vs. Vcz vs. Cfgn plus Vcz as primary therapy (n = 65) and salvage therapy (n = 69)</td>
<td>Primary therapy response rate was 19%, 30%, and 50% for Vcz vs. Cfgn vs. combination therapy; salvage therapy mortality rate at 6 months was 82% for Cfgn vs. 52% for Vcz plus Cfgn</td>
</tr>
<tr>
<td>Denning et al. [66]</td>
<td>Aspergillus species</td>
<td>Mfgn</td>
<td>OLAT</td>
<td>MCT, prospective, IPA in HM or HSCT comparing Mfgn alone (n = 34) vs. OLAT (n = 191)</td>
<td>Response rates for Mfgn: 50% as primary therapy vs. 41% as salvage therapy; response rates for OLAT: 29% as primary therapy vs. 34% as salvage therapy</td>
</tr>
<tr>
<td>Durand-Joly et al. [67]</td>
<td>Fusarium oxysporum</td>
<td>ABLC</td>
<td>Vcz</td>
<td>Case report of disseminated infection in a patient with HM</td>
<td>Clinical cure with no relapse after 8 months</td>
</tr>
<tr>
<td>Rothe et al. [68]</td>
<td>F. oxysporum</td>
<td>AmB</td>
<td>Tbf</td>
<td>Case report of disseminated infection after chemotherapy in a patient with HM</td>
<td>Clinical cure without relapse</td>
</tr>
<tr>
<td>Makowsky et al. [69]</td>
<td>Fusarium verticilliodes</td>
<td>AmB followed by ABLC</td>
<td>Cfgn</td>
<td>Case report of disseminated infection in a patient with HM</td>
<td>Patient initially responded to AmB plus Cfgn, switched to Vcz with recurrence of infection and ALL, died of disseminated disease</td>
</tr>
<tr>
<td>Voitl et al. [70]</td>
<td>Rhizopus species</td>
<td>LAmB</td>
<td>Cfgn</td>
<td>Peritoneal infection in a non-immunocompromised patient</td>
<td>Death</td>
</tr>
<tr>
<td>Verma et al. [71]</td>
<td>Rhizopus and Aspergillus species</td>
<td>ABLC</td>
<td>Cfgn plus Vcz</td>
<td>Pulmonary infection in HSCT with GVHD</td>
<td>Clinical cure and survival</td>
</tr>
<tr>
<td>Vazquez et al. [72]</td>
<td>Zygomycetes</td>
<td>Cfgn</td>
<td>LAmB</td>
<td>HM with rhinocerebral infection</td>
<td>Clinical response and survival</td>
</tr>
<tr>
<td>Rickerts et al. [73]</td>
<td>Zygomycetes</td>
<td>Cfgn</td>
<td>Pcz</td>
<td>HM with disseminated infection</td>
<td>Clinical cure without surgical intervention</td>
</tr>
<tr>
<td>Steinbach et al. [74]</td>
<td>Scedosporium prolificans</td>
<td>Vcz</td>
<td>Cfgn</td>
<td>Osteomyelitis in immunocompetent child</td>
<td>Clinical cure</td>
</tr>
<tr>
<td>Howden et al. [75]</td>
<td>S. prolificans</td>
<td>Vcz</td>
<td>Tbf</td>
<td>Disseminated infection after HSCT</td>
<td>Successfully treated with therapy and debridement</td>
</tr>
<tr>
<td>Gosbell et al. [76]</td>
<td>S. prolificans</td>
<td>Vcz</td>
<td>Tbf</td>
<td>Osteomyelitis in a corneal host</td>
<td>Successful treatment</td>
</tr>
<tr>
<td>Bhat et al. [77]</td>
<td>S. prolificans</td>
<td>Vcz</td>
<td>Tbf</td>
<td>Brain abscess in HM</td>
<td>Clinical cure</td>
</tr>
</tbody>
</table>

(continued)
no antifungal activity, whereas the combination of terbinafine–amphotericin B demonstrated indifference.

Echinocandin combinations. The activity of caspofungin–voriconazole against *A. fumigatus* was evaluated in a neutropenic model of invasive aspergillosis [45]. The combination was no more effective than voriconazole monotherapy. However, compared with monotherapy, the combination therapy reduced the fungal burden in different organs and sterilized a greater percentage of cultures. A study by Chandrasekar et al. [49] evaluated voriconazole administered with either amphotericin B or micafungin in a guinea pig model of pulmonary aspergillosis. These investigators were also unable to demonstrate any difference between monotherapy and combination therapy.

Clemons et al. [51] evaluated micafungin, caspofungin, amphotericin B, liposomal amphotericin B, amphotericin B lipid complex, and voriconazole alone and in combination in a model of murine CNS aspergillosis. The investigators concluded that liposomal amphotericin B in combination with either micafungin, caspofungin, or voriconazole prolonged the survival of the animals. Increased efficacy was seen with liposomal amphotericin B–voriconazole. Petraitis et al. [56] evaluated anidulafungin–voriconazole in a model of invasive aspergillosis in neutropenic rabbits. The results showed a reduction in fungal burden with voriconazole–anidulafungin therapy, compared with monotherapy. In addition, the use of combination therapy was associated with a significant decrease in organism-mediated injury.

**CLINICAL TRIALS EVALUATING ANTIFUNGAL COMBINATIONS AGAINST ASPERGILLUS SPECIES**

Because of the inherent difficulties encountered in performing clinical trials, most of the current literature that describes the use of antifungal combinations in invasive aspergillosis are derived from case reports or small single-center studies (table 3). Codish et al. [57] described the use of amphotericin B–flucytosine in a patient with pulmonary aspergillosis who demonstrated a prompt clinical response and resolution of infection. Another early report described a patient who experienced failure of treatment with amphotericin B after previously being treated with itraconazole [58]. The investigators were also able to demonstrate the loss of amphotericin B activity against *A. fumigatus* strains that had been previously exposed to subinhibitory concentrations of itraconazole.

In a small, single-center study, Marr et al. [61] evaluated the efficacy of voriconazole therapy, compared with voriconazole–caspofungin therapy, in patients who had experienced failure of initial antifungal therapy. In this study, patients received voriconazole monotherapy or combination salvage therapy. Overall, the patients who received combination therapy had a decrease in mortality and an improved 3-month survival rate.

Nikkomycin Z is a nucleoside-peptide and a competitive inhibitor of chitin synthase, a participant in fungal cell wall biogenesis [79, 31]. Ganesan et al. [80] evaluated the activity of nikkomycin Z in combination with either amphotericin B, liposomal amphotericin B, amphotericin B lipid complex, itraconazole, voriconazole, ravuconazole, posaconazole, caspofungin, or micafungin. The only combinations that demonstrated synergy were nikkomycin Z administered with either caspofungin or micafungin.

Nguyen et al. [32] evaluated the efficacy of azithromycin–

**Table 3.** (Continued.)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Setting</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shenep et al. [78]</td>
<td><em>Pythium insidiosum</em></td>
<td>Itz</td>
<td>Tbf</td>
<td>Cellulitis in an otherwise healthy 2-year-old child</td>
<td>Successful response and cure</td>
</tr>
</tbody>
</table>

**NOTE.** ALL, acute lymphocytic leukemia; AmB, amphotericin B; Cfgn, caspofungin; FC, flucytosine; GVHD, graft-versus-host disease; HM, hematologic malignancy; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis; Itz, itraconazole; LAmB, liposomal amphotericin B; MCT, multicenter study; Mfgn, micafungin; OLAT, other licensed antifungal; Pcz, posaconazole; SOT, solid-organ transplantation; tbf, terbinafine; Vcz, voriconazole.

**Table 4.** In vitro susceptibility combination assays against *Fusarium* species.

<table>
<thead>
<tr>
<th>Study, organism(s)</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghannoum et al. [14]</td>
<td><em>Fusarium</em> species</td>
<td>AmB</td>
<td>Vcz</td>
</tr>
<tr>
<td><strong>Cordoba et al. [81]</strong></td>
<td><em>Fusarium</em> species</td>
<td>Vcz</td>
<td>Tbf</td>
</tr>
<tr>
<td><strong>Fusarium species</strong></td>
<td><em>Fusarium species</em></td>
<td>Vcz</td>
<td>AmB</td>
</tr>
<tr>
<td><strong>Fusarium species</strong></td>
<td>Itz</td>
<td>Tbf</td>
<td>Synergy, indifference</td>
</tr>
<tr>
<td><strong>Heyn et al. [82]</strong></td>
<td><em>Fusarium solani</em></td>
<td>Mfgn</td>
<td>Vcz</td>
</tr>
<tr>
<td><strong>Arikan et al. [20]</strong></td>
<td><em>F. solani</em></td>
<td>Cfgn</td>
<td>AmB</td>
</tr>
<tr>
<td><strong>Fusarium oxysporum</strong></td>
<td>Cfgn</td>
<td>AmB</td>
<td>Synergy</td>
</tr>
<tr>
<td><strong>Philip et al. [30]</strong></td>
<td><em>Fusarium</em> species</td>
<td>Afgn</td>
<td>Itz</td>
</tr>
<tr>
<td><strong>Fusarium species</strong></td>
<td>Afgn</td>
<td>Vcz</td>
<td>Indifference</td>
</tr>
<tr>
<td><strong>Fusarium species</strong></td>
<td>Afgn</td>
<td>AmB</td>
<td>Indifference</td>
</tr>
<tr>
<td><strong>Li and Rinaldi [83]</strong></td>
<td><em>Fusarium species</em></td>
<td>NiKz</td>
<td>Flz</td>
</tr>
<tr>
<td><strong>Fusarium species</strong></td>
<td>NiKz</td>
<td>Itz</td>
<td>Indifference</td>
</tr>
</tbody>
</table>

**NOTE.** Afgn, anidulafungin; AmB, amphotericin B; Cfgn, caspofungin; Itz, itraconazole; Mfgn, micafungin; NiKz, nikkomycin Z; Tbf, terbinafine; Vcz, voriconazole.
amphotericin B against *Aspergillus* species. The investigators demonstrated synergy against all 25 isolates tested. They also showed that the effect was attributable to a 70% inhibition of protein synthesis caused by azithromycin. In another unique study, investigators evaluated histatin 5 and its analogues, administered in combination with either amphotericin B, fluconazole, or flucytosine, against *Aspergillus* species [33]. These peptides bind to the lipid bilayer of cell membranes, leading to loss of integrity and cell death. The results demonstrated some synergy when combined with amphotericin B. The synergy may be attributable to the disruption of cellular membranes by the peptides and an increased amphotericin B uptake.

In an effort to develop a new approach to treating aspergillosis, Afeltra et al. [34] evaluated the interaction of itraconazole with 7 different membrane-active compounds (amiodarone, amiloride, lidocaine, lansoprazole, nifedipine, verapamil, and fluphenazine) against *A. fumigatus*. Synergy was noted with several of the combinations (table 2).

Calcineurin inhibitors, such as cyclosporine, tacrolimus, and sirolimus, have exhibited some in vitro activity against *Aspergillus* species. Steinbach et al. [35] demonstrated that cyclosporine or tacrolimus administered in combination with caspofungin had limited synergy against *A. fumigatus*.

### FUSARIA M SPECIES

Disseminated fusarial infections are increasingly recognized in immunocompromised hosts (table 4) [84]. In addition, we also recognize that the different species of *Fusarium* have different antifungal susceptibility profiles.

**In vitro studies.** In vitro studies are shown in table 4. Ghan-noum et al. [14] showed that amphotericin B–voriconazole was synergistic against one strain and antagonistic against another strain of *Fusarium* species. Arikan et al. [20] evaluated the efficacy of caspofungin–amphotericin B against *Fusarium* species and reported synergy in 50% of the isolates, additivity in 17%, and indifference in 33%.

**Clinical trials and case reports.** Clinical trials and case reports involving *Fusarium* species are shown in table 3. There are no evidence-based guidelines recommending combination therapy. Most of the recommendations are derived from non-comparative trials or case reports [84].

### ZYGOMYCETES

Data for Zygomyces are shown in table 5. Zygomycosis is an increasingly recognized fungal infection in immunocompro-mised hosts, especially in hematopoietic stem cell transplant and solid-organ transplant recipients [88, 89]. In addition, we
Table 6. In vitro susceptibility assays evaluating antifungal combinations against various molds.

<table>
<thead>
<tr>
<th>Study, organism(s)</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walsh et al. [95]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium apiospermum</td>
<td>AmB</td>
<td>Mcz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>S. apiospermum</td>
<td>AmB</td>
<td>Itz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>S. apiospermum</td>
<td>AmB</td>
<td>Flz</td>
<td>Synergy, additivity</td>
</tr>
<tr>
<td>Meletiades et al. [96]</td>
<td>Tbf</td>
<td>Itz</td>
<td>Synergy, 85%–95% of isolates</td>
</tr>
<tr>
<td>Scedosporium prolificans</td>
<td>AmB</td>
<td>Mfgn</td>
<td>Synergy, 82% of isolates</td>
</tr>
<tr>
<td>S. prolificans</td>
<td>Tbf</td>
<td>Vcz</td>
<td>Synergy</td>
</tr>
<tr>
<td>S. prolificans</td>
<td>Tbf</td>
<td>Itz</td>
<td>Synergy</td>
</tr>
<tr>
<td>Yustes and Guarro [98]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. apiospermum</td>
<td>AmB</td>
<td>Mfgn</td>
<td>Synergy, 32% of isolates</td>
</tr>
<tr>
<td>Heyn et al. [82]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium species</td>
<td>Mfgn</td>
<td>Vcz</td>
<td>Synergy, 75% of isolates</td>
</tr>
<tr>
<td>Yustes and Guarro [98]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. apiospermum</td>
<td>AmB</td>
<td>Mfgn</td>
<td>Synergy, 82% of isolates</td>
</tr>
<tr>
<td>Li and Rinaldi [83]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scedosporium species</td>
<td>NikZ</td>
<td>Flz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Scedosporium species</td>
<td>NikZ</td>
<td>Itz</td>
<td>Indifference</td>
</tr>
<tr>
<td>Ghannoum et al. [14]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudaallescheria boydii, S. prolificans, and Acremonium species</td>
<td>Vcz</td>
<td>AmB</td>
<td>Additivity, 20%; indifference, 80%</td>
</tr>
<tr>
<td>P. boydii, S. prolificans, and Acremonium species</td>
<td>Vcz</td>
<td>Mfgn</td>
<td>Additivity, 13%; indifference, 87%</td>
</tr>
<tr>
<td>P. boydii, S. prolificans, and Acremonium species</td>
<td>Vcz</td>
<td>Rcz</td>
<td>Additivity, 13%; indifference, 87%</td>
</tr>
<tr>
<td>P. boydii, S. prolificans, and Acremonium species</td>
<td>Vcz</td>
<td>Flz</td>
<td>Indifference, 100%</td>
</tr>
<tr>
<td>P. boydii, S. prolificans, and Acremonium species</td>
<td>Vcz</td>
<td>AmB</td>
<td>Indifference, 100%</td>
</tr>
</tbody>
</table>

**NOTE.** AmB, amphotericin B; Flz, fluconazole; Itz, itraconazole; Mcz, miconazole; Mfgn, micafungin; NikZ, nikkomycin Z; Rcz, ravuconazole; Tbf, terbinafine; Vcz, voriconazole.

also know that, within the Zygomycetes, the different genera have variable in vitro susceptibility patterns [90].

**In vitro activity.** Dannaoui et al. [85] evaluated the activity of antifungal combinations against different Zygomycetes. The investigators evaluated amphotericin B–rifampin, amphotericin B–flucytosine, amphotericin B–terbinafine, and terbinafine–voriconazole. The interaction of amphotericin B–rifampin revealed synergy in 69% of the strains and additivity in 31%. The combination of amphotericin B–terbinafine demonstrated synergy in 20% of the strains.

With use of a unique assay, Chamilos et al. [86] evaluated lovastatin–voriconazole against different Zygomycetes. Lovastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the rate-limiting enzyme in the mevalonate pathway. This study was able to show synergy. A study by Guembe et al. [87] described synergistic activity with caspofungin–posaconazole, although caspofungin administered alone has no known activity against the Zygomycetes.

**Experimental animal studies.** Spellberg et al. [91] evaluated the efficacy of amphotericin B lipid complex–caspofungin in murine zygomycosis. In this study, mice were infected with *Rhizopus oryzae*. The results showed that treatment with the combination decreased the death rate and reduced the residual fungal burden, compared with that found in control subjects. However, only amphotericin B lipid complex significantly reduced fungal burdens in the brain.

**Scedosporium species**

*Scedosporium* species are increasingly recognized as significant human pathogens, especially in immunosuppressed hosts [92]. *Scedosporium prolificans* and *Scedosporium apiospermum* (*Pseudallescheria boydii*) are the most frequently isolated species and are known to possess intrinsic resistance to many of the conventional antifungal agents [92–94].

**In vitro studies.** Data for in vitro studies are shown in table 6. Walsh et al. [95] evaluated the efficacy of amphotericin B and azoles against *S. apiospermum*. Their study showed that the combinations were either additive or synergistic in 67% of the isolates. In a recent study, Yustes and Guarro [98] evaluated the efficacy of amphotericin B–micafungin against *Scedospor-
ium species and were able to show synergy in 82% of S. prolificans isolates but in only 32% of S. apiospermum isolates.

In an interesting study, Afeltra et al. [99] evaluated the efficacy of amphotericin B–pentamidine against isolates of S. prolificans. Although pentamidine administered alone has no activity, the addition of amphotericin B showed synergy in 93% of the isolates. Bocanegra et al. [100] evaluated the combination of caspofungin–liposomal amphotericin B in a neutropenic murine model of invasive S. prolificans, and they found that only high-dose liposomal amphotericin B prolonged survival.

There have been only a few case reports evaluating the use of combination therapy in scedosporiosis (table 3). Bhat et al. [77] reported excellent results in a patient with chronic granulomatous disease who developed a brain abscess due to S. prolificans. The authors were able to demonstrate in vitro synergy with voriconazole-terbinafine, and because of this, the patient was treated with the combination, resulting in the resolution of the brain infection without surgical intervention.

CONCLUSIONS

The current Mycoses Study Group–Infectious Diseases Society of America guidelines do not recommend the use of combination therapy for the treatment of any form of fungal infection. However, because of the advent of new antifungal agents with broader spectrums and improved toxicity profiles, the use of combination therapy without any good, evidence-based data has become very prevalent in treating seriously ill immunocompromised patients.

Although appropriate clinical trials evaluating treatment with antifungal combinations have not been performed, there are several antifungal combinations that appear to have improved in vitro and in vivo efficacy and deserve careful evaluation. For treatment of infection due to Aspergillus species, the in vitro combinations of amphotericin B–caspofungin, amphotericin B–voriconazole, voriconazole-caspofungin, voriconazole-micafungin, and caspofungin-itraconazole show enough synergistic or additive activity to justify their evaluation in clinical trials and are worth pursuing. For the treatment of infection due to Fusarium species, the combinations of amphotericin B–caspofungin, amphotericin B–voriconazole, and voriconazole-terbinafine also show evidence of synergy.

On the other hand, we must also use caution in this approach, because several combinations have demonstrated antagonism. For example, against Aspergillus species, the combination of amphotericin B–itraconazole consistently demonstrated antagonism. Some antifungal combinations (e.g., amphotericin B–voriconazole) seem to demonstrate synergy in some studies and indifference or antagonism in other studies. Therefore, before selecting an antifungal combination, one must be aware of the number of inherent problems associated with in vitro combination assays, the different animal models that have been evaluated, and finally, the lack of human clinical trials.

Future studies will, no doubt, take advantage of the novel mechanisms of action and the broad-spectrum activity of the new antifungal agents in an attempt to reduce the high mortality rate associated with fungal infection. Because of the paucity of available evidence-based data, there is no single, magical combination of antifungal agents that can be considered superior to monotherapy in treating any form of invasive fungal infection.

In conclusion, some combinations of new antifungal agents and older antifungal agents have been shown to possess synergistic or additive activity against many fungi. However, caution is still required because of the lack of evidence-based clinical data and the occasional disparity found among the different studies that have evaluated the interactions in vitro or in vivo. Well-controlled, randomized, multicenter clinical trials are still required to adequately determine the most efficacious antifungal regimens for specific fungal infections. In addition to evaluating for efficacy, future studies should also evaluate the adverse event potentials of the combination regimens and the pharmacoeconomic impact that these regimens can have.

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References


Treatment of Endogenous Fungal Endophthalmitis: Focus on New Antifungal Agents

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Endogenous fungal endophthalmitis, involving only the chorioretinal structures or extending to involve the vitreous (vitritis), is a sight-threatening infection requiring early appropriate therapy. Endophthalmitis is a relatively frequent complication of candidemia and less commonly occurs in patients who have invasive aspergillosis. Because the eye is a protected compartment, penetration of systemically administered antifungal agents is highly variable. In the posterior segment of the eye, amphotericin B (AmB) achieves very poor concentrations, but fluconazole concentrations are high. Among newer antifungal agents, voriconazole shows the most promise, because therapeutic concentrations for most Candida and Aspergillus species are achieved in the vitreous, and its antifungal activity is broad. In contrast, neither posaconazole nor the 3 echinocandins achieve adequate therapeutic concentrations in the vitreous. For sight-threatening macular involvement and vitritis, intravitreal injection of either AmB or voriconazole is helpful to achieve high local antifungal activity as quickly as possible. We review the available evidence regarding the most appropriate use of antifungal agents for endogenous fungal endophthalmitis, with the emphasis on treatment of infections due to Candida species.

Intraocular fungal infections originate either exogenously, as occurs with penetrating trauma and postoperative infections, or endogenously from hematogenous spread. Endogenous infections range from isolated chorioretinitis to chorioretinitis with extension into the vitreous. For the purposes of this article, the term fungal endophthalmitis refers to chorioretinitis with or without associated vitritis.

Current Infectious Diseases Society of America (IDSA) guidelines for the management of endogenous Candida endophthalmitis recommend intravenous AmB deoxycholate (AmB-d) and oral flucytosine, possibly with vitrectomy and intravitreal AmB-d, as therapy for patients with sight-threatening infections, and fluconazole for less severe cases [1]. These recommendations are based on animal data and clinical experience reported almost entirely before the introduction of the extended spectrum triazoles, voriconazole and posaconazole, and the echinocandins. Based on a few case reports, most of which deal with keratitis and not endophthalmitis, the IDSA guidelines for the management of invasive eye infection caused by Aspergillus suggest voriconazole, given either systemically or by intravitreal injection, as an alternative treatment to intravenous and intravitreal AmB-d [2]. We sought to determine the role for the use of newer antifungal agents for the more common Candida endogenous endophthalmitis and for the less common Aspergillus endogenous endophthalmitis. Much of the data on the use of new agents for the treatment of endophthalmitis are reported in the ophthalmic literature that is not routinely perused by infectious diseases specialists. We confined our review to reports dealing with endogenous Candida or Aspergillus endophthalmitis and only...
included those reports of keratitis or exogenous endophthalmitis that included data on intraocular concentrations of antifungal agents.

Implicit in the approach to treatment of endogenous endophthalmitis is the assumption that achieving adequate concentrations of antifungal agents in the infected tissues is crucial to success. The choroid and retina are highly vascular compared with the vitreous, and the vascular compartments are separated from intraocular structures by the blood-ocular barrier. Thus, infection localized to the chorioretinal layers, which are not protected by this barrier, can be treated with systemic antifungal agents, but treatment of other intraocular infections requires penetration of the antifungal agent through this relatively impermeable barrier. Chorioretinal lesions outside of the macula are often treated solely with systemic antifungal agents. However, sight-threatening lesions in the macula and choroidal endophthalmitis with vitritis usually necessitate intravitreal injection of antifungal agents, with or without vitrectomy. The available clinical data support this approach but have not been rigorously tested; most of the data are derived from reports of small case series that generally focus on a single approach.

The second assumption in treating endogenous endophthalmitis is that an examination has been performed by an ophthalmologist familiar with fungal endophthalmitis soon after the diagnosis of candidemia or the occurrence of ocular symptoms. If endophthalmitis is found, follow-up examinations should be routinely performed to evaluate the response to therapy and the development of complications.

**OLDER ANTIFUNGAL AGENTS**

**Amphotericin B**

The greatest clinical experience has been accrued with AmB-d. Early studies noted minimal or no penetration of AmB-d into the vitreous in rabbits or humans [3–5]. More recent studies have confirmed the poor penetration of both AmB-d and lipid formulations of AmB into the vitreous in noninflamed rabbit eyes. However, multiple-dose studies over 7 days in rabbits with endotoxin-induced uveitis, which presumably compromises the blood-ocular barrier, showed that higher levels could be achieved with liposomal AmB (47 ± .21 μg/mL) than with AmB-d (.16 ± .04 μg/mL) [6]. Although patients have been successfully treated with systemic AmB-d, failures are common, and the toxicity of the drug is well known [7–9].

Because of the low intraocular levels attained with systemic administration, AmB-d has been injected directly into the vitreous for treatment of severe endophthalmitis. Early studies demonstrated retinal toxicity in rabbits with doses >10 μg [10]; however, others noted histopathologic evidence of focal retinal damage at doses as low as 1 μg [11]. Injection directly adjacent to the retina increased the risk of damage. Ganglion cell damage and retinal detachment were thought to be secondary to increased membrane permeability induced by AmB-d [10].

In rabbits, lipid formulations of AmB have been compared with AmB-d [12, 13]. Dose-related toxicity involving the retinal ganglion cells was found with all formulations but was less with liposomal AmB than with AmB lipid complex and AmB-d [12]. A study in primates suggested reduced toxicity when liposomal AmB was administered intravitreally and minimal toxicity when <30 μg of AmB-d was used [14]. After intravitreal injection in normal rabbit eyes, the intraocular half-life of AmB-d was 7–15 days, compared with only 1.8 days in vitrectomized eyes [15].

The reported clinical experience of intravitreal injection of AmB-d in humans is limited to case reports and small case series. Doses from 20 to 100 μg have been administered without toxicity [16–19]. However, the dose typically administered ranges from 5 to 10 μg. A total of 30 eyes were injected with AmB-d in this dosage range in 2 case series without the occurrence of significant toxicity [18, 20]. Intravitreal AmB-d has been used as sole treatment for endogenous *Candida* endophthalmitis to avoid systemic toxicity [21], but this approach cannot be recommended. Intravitreal AmB-d is used as an adjunctive therapy along with systemic antifungal agents in patients who have sight-threatening endophthalmitis caused by *Candida* species and in most cases of *Aspergillus* endophthalmitis.

**Flucytosine**

Flucytosine is an adjunctive agent that can be used in combination with AmB for the treatment of *Candida* endophthalmitis [1]. It is synergistic with AmB in killing *Candida* and achieves high levels in all intraocular compartments in rabbits and humans [3, 22, 23]. Minimal additional benefit was noted when flucytosine was added to fluconazole in a study in rabbits with endophthalmitis [23]. It is not known whether this combination might be helpful in humans.

**Fluconazole**

Clinical experience using fluconazole for *Candida* endophthalmitis has increased over the last 20 years. Experimental data in rabbits show that the levels achieved in the vitreous are approximately 50% of peak plasma levels and 150% of trough plasma levels [24]. In one study, higher concentrations were achieved in the vitreous than in the aqueous humor [25], and somewhat higher levels have been noted in inflamed eyes [26]. Fewer data are available in humans, but it appears that vitreous concentrations are approximately 70% of those in plasma [27, 28]. The drug is welltolerated when injected intravitreally [28]; however, few studies have examined intravitreal fluconazole use, most likely because vitreous levels are high with systemic administration.
The data in rabbits with experimental Candida endophthalmitis are conflicting. Some studies have shown success with fluconazole, even when treatment was delayed for 5 days [29]; others have shown success when treatment was started within 24 h of infection but failure when it was delayed for 7 days [26]. In a rabbit model of disseminated candidiasis, AmB-d performed better than fluconazole in eradicating organisms from the vitreous in one study [30], but opposite results were noted in another study [23].

Studies in humans have shown response rates ≥90% [20, 28, 31], but the data are somewhat obfuscated because some patients had vitrectomy and intravitreal AmB-d, in addition to systemic fluconazole. Failure of fluconazole also has been reported [32–34]. Because of its excellent intraocular concentrations and safety, fluconazole has become a preferred therapy that is used by many physicians for susceptible organisms. It is usually given as the sole agent for chorioretinitis and combined with intravitreal therapy and/or vitrectomy for more advanced disease with vitreal involvement.

**NEWER ANTIMICROBIAL AGENTS**

**Voriconazole**

After the large outbreak of Fusarium keratitis in contact lens wearers in 2005, interest in voriconazole to treat fungal eye infections increased among ophthalmologists, who realized the benefits of this broad-spectrum triazole agent in treating that difficult problem. Many studies on the distribution of voriconazole within ocular compartments were performed during treatment of complicated Fusarium keratitis, and, in contrast to fluconazole, most of the literature on voriconazole is from humans, rather than from experimental animals.

Penetration of voriconazole into the eye was studied in 14 patients who had noninflamed eyes and who were undergoing elective pars plana vitrectomy. Two doses of 400 mg voriconazole were given orally 12 hr apart on the day before surgery, and voriconazole concentrations were measured in blood, aqueous humor, and vitreous 3 h after the second dose of voriconazole [35]. The mean voriconazole concentrations achieved were 2.13 ± .93 μg/mL for plasma, 1.13 ± .57 μg/mL for aqueous humor, and .81 ± .31 μg/mL for vitreous (38% of plasma). In a single patient receiving systemic voriconazole for infection other than endophthalmitis, voriconazole levels 8 h post mortem were 1.52 μg/mL in the aqueous humor and 1.12 μg/mL in the vitreous [33].

There is a growing body of data on intravitreal injection of voriconazole. An in vitro study using human retinal pigment epithelium cells exposed to voriconazole at concentrations from 25 up to 10,000 μg/mL showed that concentrations <250 μg/mL had no toxic effects [36]. Rats underwent a single intravitreal injection of voriconazole to achieve concentrations that ranged from 5 to 500 μg/mL [37]. Three weeks later electroretinographic studies showed no toxic effects at any dosage level, and histologic examination showed focal necrosis in the outer retina only in those eyes in which the concentrations were ≥50 μg/mL. Thus, it is suggested that voriconazole concentrations of up to 25 μg/mL in the vitreous are safe. When an injection of 100 μg is given into the vitreous, which has a volume of approximately 4 mL, the voriconazole concentration will be 25 μg/mL. The concentration of voriconazole given by intravitreal injection in normal rabbit eyes was found to exhibit exponential decay, and the half-life was 2.5 h [38]. Whether this process is similar in humans is not known. The rationale for the injection of voriconazole is that it may be safer than AmB-d and that it immediately achieves high levels of the drug in the vitreous, whereas serum levels from systemic administration are gradually reaching a steady state.

Clinical efficacy of systemic voriconazole has been reported in a small number of patients who had Candida endophthalmitis and more who had mold endophthalmitis [33, 34, 39, 40]. Of 7 patients with Candida endophthalmitis treated with voriconazole (200 mg twice daily in most patients), 6 survived, and visual acuities at the end of therapy ranged from 20/20 to 20/100. One of these patients also received an intravitreal injection of voriconazole, several were given caspofungin, and 1 received intravitreal AmB-d, so it is difficult to evaluate the efficacy of voriconazole alone. In our practice, we have noted that systemic voriconazole, with or without intravitreal injection, seems to lead to a more rapid response than other antifungal agents.

One added advantage of voriconazole over fluconazole is that it has activity against Aspergillus species and fluconazole resistant Candida species, such as Candida glabrata and Candida krusei. Aspergillus endophthalmitis often involves the macula and is especially difficult to treat [18, 20]; response rates for AmB-d (intravitreal and systemic) have been as poor as 8% [41]. There are a few cases reports in which voriconazole was used; most reports were of exogenous Aspergillus endophthalmitis, but one documented resolution of endogenous Aspergillus terreus endophthalmitis [42]. Although it seems appropriate to use voriconazole for Aspergillus endophthalmitis, the true response rate is not known.

When voriconazole is used to treat endophthalmitis, serum levels should be monitored, because of their high variability among patients. Trough levels between 2 and 5 μg/mL are the goal. Serum levels within this range have been associated with a better outcome in invasive mold infections [43, 44]. Higher levels are associated with increasing toxicity [44].

**Posaconazole**

Very few data are available regarding the use of posaconazole for ocular infections. No animal data have been published regarding intraocular concentrations of oral posaconazole. One patient,
who had *Fusarium* keratitis and endophthalmitis, was successfully treated with oral posaconazole (200 mg 4 times daily) plus topical posaconazole and vitrectomy. Sampling at the time of surgery yielded a posaconazole concentration of .25 μg/mL in the vitreous, and .9 μg/mL in the aqueous humor [45]. Another report demonstrated resolution of *Fusarium* endophthalmitis in 2 patients in whom voriconazole treatment had failed [46].

At this time, there are too few data to suggest that posaconazole should be considered for the treatment of endophthalmitis, although it might possibly be an alternative option in cases of intolerance to other antifungal agents. It cannot be recommended because of its relatively poor penetration into ocular structures combined with the variable absorption of the oral suspension, which is currently the only available formulation.

**Echinocandins**

The echinocandins, micafungin, caspofungin, and anidulafungin, penetrate ocular compartments poorly [47–49]. In studies in experimental animals, it has been shown that concentrations of micafungin in the vitreous in noninflamed eyes are very low, ranging from undetectable to .034 μg/mL [48]. Anidulafungin levels in the vitreous have ranged from undetectable to .184 μg/mL when very high dosages were used [49]. A rabbit uveitis model was used to evaluate caspofungin penetration into various ocular compartments. At a dose of 1 mg/kg/day, no drug was detectable in the vitreous of inflamed eyes at any time point during the study [50]. In rabbit models of disseminated candidiasis and *Candida* meningitis, micafungin reduced the fungal burden in the vitreous, but only when doses higher than those used clinically were given [51, 52]. In rabbits, intravitreal injection of 15 μg micafungin was nontoxic [53]; intravitreal echinocandin use has not been reported in humans.

A single case report of possible *Candida* endophthalmitis in a patient who appeared to have mild vitritis noted success with caspofungin therapy [54], but others have documented failure with caspofungin and found undetectable levels in the vitreous [55]. Review of the 5 randomized controlled treatment trials that studied echinocandins for candidemia and invasive candidiasis revealed that 21 of the 1028 patients who received an echinocandin were noted to have endophthalmitis [56–60]. Among these 21 patients, 12 were said to have had resolution of their eye infection. However, these data are not terribly useful, because the studies were designed to exclude patients who had known endophthalmitis, the reports did not differentiate between those who had vitritis and those who had only chorioretinitis, and only scant data were provided on individual cases.

It is difficult to draw any conclusions about the efficacy of systemic echinocandins for the treatment of endophthalmitis. It is possible that isolated chorioretinitis without vitreous extension could respond to echinocandin therapy, but there are no firm data to verify this hypothesis. At this time it seems prudent to not use echinocandins for treatment of endophthalmitis.

**VITRECTOMY**

Vitrectomy is recommended for sight-threatening *Candida* and *Aspergillus* endophthalmitis with vitritis [1, 2]. Sampling the vitreous at the time of vitrectomy provides important culture data to guide treatment. Vitrectomy allows removal of loculated areas of infection that would not respond to systemic antifungal agents and decreases the overall burden of organisms. The procedure is usually combined with the administration of intravitreal antifungal agents. Outcomes for early vitrectomy combined with systemic antifungal therapy with AmB-d or fluconazole have been favorable for *Candida* endophthalmitis [61, 62]. When difficult-to-treat fungal organisms are present in a protected space in which antifungal penetration is poor, surgical resection of the involved tissue (vitreous) with intravitreal administration of antifungal agents is a logical therapeutic intervention. It is important to recognize that the half-life of antifungal agents administered directly into the vitreous at the time of vitrectomy will be shortened and that repeated administration may be necessary.

**SUGGESTED RECOMMENDATIONS FOR TREATMENT OF ENDOGENOUS ENDOPHTHALMITIS**

For *Candida* endophthalmitis, we favor the use of systemically administered agents that are known to achieve adequate concentrations in the vitreous. Fluconazole, voriconazole, and flucytosine achieve therapeutic intravitreal concentrations, whereas the echinocandins and all formulations of AmB do not. Most experience has accumulated with fluconazole. There is less experience with voriconazole, but there are data on the efficacy and safety of intravitreal injection of this agent. Flucytosine should be used in combination with AmB and not as sole therapy. We feel that the role for echinocandins and posaconazole in the management of endogenous endophthalmitis is minimal. These suggestions differ from the IDSA guidelines in encouraging an increasing role for fluconazole and voriconazole and a decreasing role for AmB-d, and also in suggesting that echinocandins should not be used as alternative agents.

For patients who have *Candida* chorioretinitis with no vitreal involvement, systemic antifungal agents are appropriate as long as repeated examinations show no extension into the vitreous or the macula. Either fluconazole (12 mg/kg loading dose, then 6–12 mg/kg daily), or voriconazole (6 mg/kg for 2 doses, then 4 mg/kg twice daily) can be used. Initial intravenous administration seems prudent for voriconazole, and serum concentrations should be monitored carefully to ensure adequate...
exposure and to prevent toxicity. No studies have defined the appropriate duration of therapy. A reasonable approach, consistent with the IDSA guidelines, is to treat for at least 4–6 weeks, with the final duration dependent on the response observed in repeated ophthalmologic examinations.

For sight-threatening macular involvement and vitritis due to *Candida* species and for all cases of *Aspergillus* endophthalmitis, in addition to systemic therapy, intravitreal injection of an antifungal agent should be performed to ensure immediate achievement of appropriate levels in the posterior segment. Either voriconazole (100 µg) or AmB-d (5–10 µg) can be given by intravitreal injection. Voriconazole may be safer than AmB-d, but there is more experience with AmB-d, which also has the advantage of having a longer half-life after intravitreal injection. The need for repeated injections is dependent on the response to therapy noted at ophthalmologic examinations. Vitrectomy should be considered to decrease the burden of organisms and to allow the removal of fungal abscesses that are inaccessible to systemic antifungal agents.

It is important to emphasize that a team approach involving both ophthalmology and infectious diseases is essential to ensure the most efficacious treatment and preservation of visual acuity. Vitrectomy and intravitreal injection of an antifungal agent require the patient to be seen by an ophthalmologist who has experience in treating fungal endophthalmitis. The nuances of drug absorption, drug-drug interactions, and toxicity associated with the azole agents require an infectious diseases physician who has experience using these agents. Careful follow-up to assess the response to therapy is essential to detect macular or vitreal extension as early as possible and initiate aggressive therapy to preserve visual acuity.

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**References**


Guidance for the laboratory investigation, management and infection prevention and control for cases of *Candida auris*

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Introduction: *Candida auris*

*Candida auris* is a recently identified *Candida* species that has been associated with infection and outbreaks in healthcare settings on five continents. It has been isolated from a range of body sites, including skin (very common), urogenital tract (common), and respiratory tract (occasional), and resulted in invasive infections, such as candidaemia, pericarditis, urinary tract infections and pneumonia. *C. auris* affects both paediatric and adult populations, and has predominantly been identified in critically unwell patients in high dependency settings. As with other organisms associated with nosocomial outbreaks, it appears to be highly transmissible between patients and from contaminated environments, highlighting the importance of instituting effective infection prevention and control practices. Significantly, all *C. auris* isolates from the UK have demonstrated reduced susceptibility to the first line antifungal therapy, fluconazole, and variable susceptibility to other antifungal agents. Difficulties with identification of this organism in the laboratory and uncertainty about routes of transmission have impacted significantly on outbreak detection and management. By the end of July 2017, there have been over 200 patients with *C. auris* initially detected in 20 NHS Trusts and independent healthcare providers (first detection only), and over 35 additional hospitals in the United Kingdom have received patients with a known *C. auris* detection. Approximately one quarter of reported *C. auris* detections are clinical infections, including 27 candidaemias. There have been three large nosocomial intensive care unit outbreaks in England, which despite intensive infection prevention and control measures have been difficult to control. Given limitations in typing methodology these may include several novel introductions, including after periods without any new detections.

Investigation in clinical laboratories

*C. auris*, on microscopy, is indistinguishable from most other *Candida* species, it is a germ tube test negative budding yeast, however some strains can form rudimentary pseudohyphae on cornmeal agar. Growth at 42 - 45°C (used at the Mycology Reference Laboratory) may be useful to help differentiate it from many other *Candida* species,
especially those for which it is most commonly mis-identified such as Candida haemulonii. Most C. auris isolates are a pale purple or pink colour on the chromogenic agar, CHROMagar™ Candida, in common with several other non C. albicans species. Growth on this and other chromogenic agars (which may display a different colour) cannot be used as a primary identification method. However, chromogenic agars are useful for screening to identify suspicious colonies from mixed cultures including the presence of C. albicans. If there is evidence of non– C. albicans species on chromogenic agar these should be sub-cultured onto Sabouraud's agar and identified according to local laboratory protocols. It is unlikely that any of the currently available biochemical-based tests will include C. auris in their database, as it is a newly recognised species, so laboratories are advised to check the databases provided for their current methods. According to published data, commercially available biochemical-based tests, including API AUX 20C, VITEK-2 YST, BD Phoenix and MicroScan, used in many front line diagnostic laboratories can misidentify C. auris as a wide range of Candida species and other genera (most commonly as Candida haemulonii, Candida famata, Candida lusitaniae, Rhodotorula glutinis or Saccharomyces cerevisiae).

Therefore, it is important that any Candida spp isolates associated with invasive infections and isolates from superficial sites in patients from high intensity/augmented care settings and those transferred from an affected hospital (UK or abroad) should be analysed to species level. If suspected Candida spp are identified, further work should be undertaken to ensure that they are not C. auris. This would involve either molecular sequencing of the D1/D2 domain or MALDI-TOF Biotyper analysis with C. auris either already present or added to the database. This facility is available at the PHE Mycology Reference Laboratory. Please send pure isolates on Sabouraud's slopes accompanied by the appropriate form accessed from https://www.gov.uk/government/publications/mycology-identification-and-susceptibility-testing-request-form.

Laboratories should also ensure correct mapping of the species code for C. auris to facilitate reporting to PHE through Second Generation Surveillance System (SGSS).
Antifungal susceptibility testing

There are no established minimum inhibitory concentration (MIC) breakpoints at present for *C. auris*. Using breakpoints for other *Candida* spp the Centers for Disease Control and Prevention (CDC) demonstrated that, of the global outbreaks they investigated, nearly all of 54 isolates were highly resistant to fluconazole. In their analysis, more than half of *C. auris* isolates were resistant to voriconazole, one third were resistant to amphotericin B (MIC ≥2 mg/L), and a few were resistant to echinocandins. Some isolates have demonstrated elevated MICs to all three major antifungal classes, including azoles, echinocandins, and polyenes indicating that treatment options would be limited. A recent review of 123 global isolates showed that Clinical Laboratory Standard Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) MICs are very similar, with 7% resistance to echinocandins and 10% to amphotericin B. Multi-resistant isolates have been reported from South America. Whole genome sequencing of the organism has found resistant determinants to a variety of antifungal agents. Development of resistance to various antifungals has been observed in previously sensitive isolates. Experience to date from the PHE Mycology Reference Laboratory indicates that so far very few multi-drug resistant strains have been found in the UK but all isolates are resistant to fluconazole and often cross-resistant to other azoles, with variable resistance to polyenes (approximately 20% for amphotericin B) and echinocandins (approximately 10%).

Treatment

First-line therapy remains an echinocandin pending specific susceptibility testing which should be undertaken as soon as possible. However, there is evidence that resistance can evolve quite rapidly in this species, ongoing vigilance for evolving resistance is advised in patients who are found to be infected or colonised with *C. auris*. There is currently no evidence or experience to support combination therapy in bloodstream infections with this organism, although if the urinary tract or central nervous system (CNS) is involved dual therapy may be necessary, and some antifungal classes do not have bio-availability in either urine or CNS. Clinicians are advised to make decisions on
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a case by case basis depending on the site of infection. The PHE Mycology Reference Laboratory is able to undertake susceptibility testing for amphotericin B, fluconazole, voriconazole, itraconazole, posaconazole, isavuconazole, anidulafungin, caspofungin, and micafungin. If an isolate is found to be resistant to all of these agents the Reference Laboratory will also test for susceptibility to flucytosine, nystatin and terbinafine. Currently UK strains remain susceptible to the topical agents nystatin and terbinafine and it is possible that for the treatment of any future multi-drug resistant strains a regimen incorporating oral terbinafine could be considered.

**Colonisation**

Colonisation of patients has been reported from affected hospitals around the world. There is no evidence currently that reliably demonstrates whether *C. auris* is susceptible to chlorhexidine. More work is being done in this area. Clinical experience to date has shown that colonisation tends to persist and is difficult to eradicate making infection prevention and control strategies particularly important. However, it is still recommended that strategies to prevent and/or treat colonisation include:

- strict adherence to central and peripheral catheter care bundles, urinary catheter care bundle and care of the tracheostomy site
- prompt removal of venous cannulas if there is any sign of infection
- high standards of aseptic technique when undertaking wound care
- skin decontamination with chlorhexidine washes in critically ill patients.

There is not an evidence base to recommend the following, though these may be considered in individual settings:

- mouth gargles with chlorhexidine
- use of topical nystatin and terbinafine for targeted topical management of key sites such as venous cannula entry sites.

There is limited evidence that in *in vitro* settings, shorter contact times with chlorhexidine (without alcohol) may not be as effective as povo-iodine based topical
applications in reducing *C. auris* colonisation – this may be considered when performing invasive procedures such as line insertions or surgical procedures in colonised patients.

**Screening policies**

All Trusts are encouraged to develop a screening policy after local risk assessments are undertaken. Screening is recommended in units that have ongoing cases and/or colonisations, or identification of a new infected or colonised patient, as follows:

- any novel detection in a Trust should be an indicator to screen close contacts if on an intensive care setting
- if the patient has been isolated during admission on a ward other than an intensive care setting, Trusts are advised to speciate all candida isolates from the same unit to the species level using an appropriate method that will detect *C. auris* for the subsequent four weeks
- in all cases, in the four weeks prior to diagnosis in the index patient, hospitals should look back to see if there has been an increase in detection of *Candida* spp in the same intensive care setting or ward as this may represent unrecognised transmission
- if the index patient was not isolated, close contacts who have been in the same bay with an affected patient in the 48 hours prior to first identification should be isolated or cohorted with other contacts, and cared for with enhanced infection prevention and control measures as detailed below for cases. Close contacts can be de-isolated after three consecutive negative screens at least 24 hours apart.

Screening is advised for patients coming from other affected hospitals/units in the UK and abroad. Currently hospital outbreaks have been reported from the United States, India, Pakistan, Venezuela, Columbia, Israel, Oman, South Africa, and Spain, although UK and worldwide prevalence is still to be established due to problems with laboratory diagnosis. An updated PHE briefing note was disseminated in March 2017 to all Trusts
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listing UK Trusts with evidence of sustained outbreaks\(^1\). Time from initial exposure to colonisation as low as four hours have been reported by several affected hospitals.

Suggested screening sites, based on the predilection of Candida spp to colonise the skin and mucosal surfaces i.e. genitourinary tract, gastrointestinal, mouth and respiratory tract, are:

- groin and axilla (the most persistently positive in Trusts that have conducted screening)
- urine (there have been several cases of persistent urinary colonisation in catheterised patients)
- nose and throat
- perineal swab
- rectal swab or stool sample.

Other sites that may be considered if clinically indicated are:

- low vaginal swab
- sputum / endotracheal secretions
- drain fluid (abdominal/pelvic/mediastinal)
- cannula entry sites
- wounds.

Routine wound swabs may be used to collect screening samples. Rectal swabs have been shown to be intermittently positive – they may be more useful to detect incident colonisation rather than transmission in hospital environments, but the role of gastrointestinal carriage is as yet unclear.

All screen positive patients should be isolated or cohorted as described below. There is currently no evidence to support the de-isolation of patients found to be colonised or infected with C. auris as the length of carriage is unknown. As there is clinical

\(^1\) Please write to candidaauris@phe.gov.uk if you need this resent
experience of recurrence of colonisation, the need for ongoing vigilance in the form of weekly (or more frequent) screens in clinical environments where *C. auris* colonised patients have been managed should be considered by performing local risk assessments.

All newly positive screens or clinical samples from patients unknown to be colonised should be reported to the local PHE Centre Health Protection Team (HPT) – a detailed Standardised Operating Procedure has been developed for HPTs to utilise which details specifics of cases definitions, isolation of cases and contacts, and ward screening for both single sporadic cases and potential outbreak scenarios.

Isolation and rescreening of patients known to be previously colonised is recommended on readmission as there is not enough evidence yet to exclude lifelong colonisation. De-isolation is not recommended apart from in units with experience of managing *C. auris*.

**Infection, prevention and control (IPC)**

Reports from India, Pakistan, Venezuela, Spain, UK, South Africa, Israel, and Colombia (CDC, personal communication) have described large healthcare outbreaks of *C. auris* infection and colonisation. The precise mode of transmission within the healthcare environment is not known, and is likely to be multifactorial. However, experience during these outbreaks suggests that *C. auris* might substantially contaminate the environment and equipment of colonised or infected patients. Transmission directly from fomites (such as blood pressure cuffs, stethoscopes and other equipment in contact with the patient) is a particular risk, however this does not preclude transmission via hands of healthcare workers and hand hygiene needs to be strictly adhered to. Where possible equipment used for the infected/colonised patient should not be shared with other patients on the ward unless between-patient decontamination can be assured. *C. auris* has been detected on settle plates around patient bedsides and on monitoring devices within the UK. Hospitals must ensure that the bed space requirements between patients comply with the Health Building Note regulations in order to minimise the likelihood of transmission. Adherence to hand hygiene needs to be consistently high and sustained. It is essential that all healthcare staff work in a multi-disciplinary team with their Clinical
Microbiologists and under the direction of their IPC team when dealing with care of patients colonised with *C. auris*.

**The patient**

Key infection prevention and control measures include:

- isolation of all patients colonised or infected with the organism in a single room, ideally with *ensuite* facilities, wherever possible
- isolation of all patients who have been transferred from an affected UK hospital or a hospital abroad until screening results are available
- strict adherence to standard precautions including hand hygiene using soap and water followed by alcohol hand rub on dry hands
- personal protective equipment in the form of gloves and aprons (or gowns if there is a high risk of soiling with blood or body fluids, or likely physical contact with patient’s skin)
- these should be donned after hand washing and before entering the room or patient area and removed and discarded in the room or patient environment followed by a thorough hand wash and application of alcohol hand rub on dry hands before exit
- visors and masks are not routinely required and should be worn only if there is a procedural risk of spillage or splashes
- patients and visitors of infected or colonised patients need to be briefed about the infection (possibly using the patient information leaflet) and infection prevention and control precautions reinforced; including the need for robust hand hygiene and use of protective aprons
- single-patient use items such as blood pressure cuffs and pillows should be considered, especially in outbreak situations.

Some Trusts have found the introduction of chlorhexidine impregnated protective disks for long lines useful in preventing invasive infection.
Terminal clean

Once the patient has left the environment a terminal clean should be undertaken. For terminal cleaning of a bedspace or room vacated by a *C. auris* colonised/infected patient, disinfection, preceded by cleaning, of horizontal surfaces plus all items that may have come into contact with the patient or staff hands should occur. The disinfectants used should be those for each item in compliance with the hospital’s policy. A hypochlorite is currently recommended for cleaning of the environment at 1000 ppm of available chlorine. As different staff groups may be responsible for different items, attention should be focused on all relevant items going undecontaminated. Application of disinfectant should be thorough ensuring good contact before the disinfectant dries. Privacy curtains should be changed. Consideration should be given to discarding less expensive items that are difficult to decontaminate, or using single-patient use devices such as blood pressure cuffs. Stocks of single use items in the immediate patient environment should be discarded.

If any non-contact disinfection is used (e.g. gaseous hydrogen peroxide or UV), full cleaning and disinfection preceding it should still occur. Individual Trusts should adopt a local cleaning policy and regimen depending on the level of contamination and case load. Domestic staff will require training and supervision until declared competent. Cleaning staff should change gloves and aprons with appropriate hand decontamination after cleaning each *C. auris* area. There should be appropriate decontamination of dynamic mattresses.

If a patient needs to be taken out of the side room or bay to theatre, procedure room, or for imaging, they should be scheduled last on the list for the day and the environment cleaned as described above. Several hospitals have reported favourable use of gaseous hydrogen peroxide, following preparatory protocols.

Cleaning and decontamination of equipment

All equipment (including patient monitoring devices and mobility aids) should be cleaned in accordance with manufacturer’s instructions and where relevant returned to the company for cleaning. Particular attention should be paid to cleaning of reusable
equipment (e.g. pulse oximeters, thermometer probes, computers on wheels, ultrasound machines) from the bed space of an infected/colonised patient.

Waste and linen disposal

Trusts should follow their current waste and used linen policies as for any other multi-resistant healthcare-associated organism:

- attention should be paid to appropriate bagging and isolation of used linen and waste so that the environment is not contaminated
- in paediatric and neonatal units, specific attention should be paid to disposal of used nappies
- at no time should contaminated material be discarded / washed in the clinical hand wash basins.


Communications

An information leaflet for affected patients and relatives is available and can be accessed from https://www.gov.uk/government/publications/candida-auris-a-guide-for-patients-and-visitors.

*C. auris* colonisation information should be included in any discharge summary or patient transfer documents, ideally with direct communication to IPC representatives at receiving hospitals. If positive results become available after discharge or transfer, information should be relayed to the receiving hospital/GP for further communication to the patient and for relevant public health action.
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If a patient dies and the cause of death is attributable to *C. auris*, this must be included in the death certificate and should be relayed to the National Incident Team (Contact Details 6). To date there has been no attributable *C. auris* mortality within the UK.

Systems permitting, each hospital should label colonised patients with an infection control flag on the patient electronic case record, so healthcare professionals are immediately alerted to the *C. auris* status if or when that patient is readmitted in future.

**Useful contact details**

1. For mycology advice, and referral of candida isolates to PHE Mycology Reference Laboratory, please contact elizabeth.johnson@phe.gov.uk and andrew.borman@phe.gov.uk
2. For advice about decontamination, environmental screening, and cleaning please contact peter.hoffman@phe.gov.uk, jimmy.walker@phe.gov.uk and ginny.moore@phe.gov.uk
3. For clinical management advice, please contact s.schelenz@rbht.nhs.uk, a.hall@rbht.nhs.uk, surabhi.taori@nhs.net, and katie.jeffery@ouh.nhs.uk
4. For IPC advice, please contact the above and bharat.patel@phe.gov.uk, rohini.manuel@phe.gov.uk, and martina.cummins@phe.gov.uk
5. Trusts should contact their local Health Protection Team (HPT), however for HPTs who require advice from colleagues with *C. auris* experience, please contact yimmy.chow@phe.gov.uk, janice.lo@phe.gov.uk, louise.bishop@phe.gov.uk, and clare.humphreys@phe.gov.uk
6. For national incident advice please contact colin.brown@phe.gov.uk, rebecca.guy@phe.gov.uk, and PHE.candidaauris@nhs.net or candida.auris@phe.gov.uk
Further reading

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