Invasive fungal infections (IFIs) have become increasingly prevalent in the recent decade along with the increasing populations of immunocompromised patients and widespread use of the broad-spectrum antibiotics. The morbidity and the mortality of IFIs remain high while the diagnosis and treatment of IFIs are highly challenging. Recent advances in diagnostic methods and antifungal agents provide the potential to improve the outcomes of these infections. Conventional diagnostic methods including microbiological cultures and histopathological diagnosis have the disadvantages of either insensitivity or requiring invasive procedures. The innovative techniques of detecting circulating fungal antigens and detecting fungal genomic DNA represent improvements in the diagnosis of invasive aspergillosis. Several antifungal agents have been developed in recent years, such as lipid formulations of amphotericin B, newer azoles, and echinocandins. These agents have either lower toxicities or greater activities against certain fungi compared with older treatments. With the availability of diverse antifungal agents, their use in combination has the potential to produce additive or synergistic effects, leading to better treatment outcomes. Large-scale randomized clinical trials are needed to confirm the efficacy of combination strategies.

Key words: Antifungal agents, diagnosis, laboratory techniques and procedures, mycoses, review

Introduction

Infection is a constant threat to human health. Remarkable progress has been made in the prevention and treatment of infectious diseases. At the same time, patterns of human infections have undergone significant changes during the past several decades. Bacterial infection was the predominant issue before the availability of antibiotics. The use of penicillins and cephalosporins led to an increase in Gram-negative bacterial infections in the 1960s and 1970s [1]. Thereafter, fungal infection started to emerge as a major clinical issue [2-4]. Two main factors contributed to the steady increase of fungal infection, namely, the widespread use of antibacterial agents and rapid increase in numbers of immunocompromised populations [3,4].

Clinically important fungi consist of yeasts, molds, and dimorphic species. Each poses a different challenge to clinicians. Overall, diagnosis of fungal infection is more difficult compared with bacterial infections by conventional culture, and treatment is also difficult because of the limited range of antifungal agents.

Modern therapeutic modalities such as cancer chemotherapy and organ transplantation have a greatly increased risk of invasive fungal infections (IFIs) [2,3]. Currently, morbidity and mortality from IFIs remain high in patients with hematologic malignancies who receive intensive myelosuppressive chemotherapy or undergo bone marrow transplantation [2,5-11]. Thus, there is considerable scope for improvement both in the diagnosis and management of fungal infection.

Advances in Laboratory Diagnosis

Early diagnosis of IFIs remains a great challenge. The symptoms and signs are often nonspecific and microbiological cultures are usually negative. Histopathological diagnosis, which requires invasive procedures to obtain the specimens, is often hindered by the grave conditions in these patients. The high
mortality of IFI has contributed to the difficulties in establishing timely diagnosis and initiating prompt antifungal therapy. Recently, more rapid diagnosis has been achieved by use of detection of the circulating markers, including fungal cell wall components and fungal genomic DNA. These techniques have advanced the diagnosis of aspergillosis. Non-culture-based diagnosis for candidiasis and noninvasive diagnosis for molds other than *Aspergillus* are still mainly investigational [12].

**Detection of Fungal Antigens**

**Galactomannan detection**

Galactomannan (GM) is a polysaccharide cell wall component that is released by the fungus into the serum during its growth in tissues [13]. A sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of GM antigen of *Aspergillus* was commercialized and has been applied in the diagnosis of invasive aspergillosis (IA). The ELISA test (Platelia *Aspergillus*; Biorad, Marnes-La-Coquette, France) uses the EB-A2 monoclonal antibody to recognize the galactofuran epitopes of the GM molecules [14]. The number of epitopes on the GM antigen released by the fungi may vary between strains, between species, and over time. Angioinvasion is assumed to be required for the GM antigens released from fungal hyphae to reach the circulation [14]. The degree of angioinvasion varies in relation to the underlying conditions and toxic damage caused by cytotoxic drugs or irradiation. In certain underlying diseases, such as chronic granulomatous disease, in which the formation of abscess predominates and may hamper the leakage of GM antigens into the circulation, IA may occur with the absence of the antigenemia [14]. In contrast, antigen detection has excellent sensitivity and specificity (up to 89.7% and 98.4%, respectively) in stem cell transplant recipients and in patients with prolonged neutropenia [15]. This result might indicate rapid progression of disease caused by the angioinvasive growth of *Aspergillus* in damaged lung epithelia [14].

Once the GM reaches the circulation, it might bind to substances present in the blood, including *Aspergillus* antibodies and other human proteins. Such binding could interfere with the performance of the ELISA test and cause false-negative results [14]. Clearance of the GM from the blood due to renal excretion and uptake by macrophage was demonstrated in an animal model [14]. Renal clearance depends on the renal function of the patient and the size of the GM antigen.

The performance of the GM ELISA test was reported to range from 50% to 100% in sensitivity and from 92% to 100% in specificity (Table 1) [13-20]. In these reports, sensitivity varied considerably, while specificity remained more consistent and was usually greater than 85%. When the test is applied to clinical diagnosis, double-checking of positive samples might be necessary because of the possible lack of reproducibility [17]. As mentioned by the manufacturer, IA

<table>
<thead>
<tr>
<th>No. of patients (episodes)</th>
<th>Underlying conditions</th>
<th>IA patients</th>
<th>GM cut-off index value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>135 (193)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HeM with neutropenia</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0</td>
<td>100</td>
<td>92</td>
<td>64</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>191 (362)</td>
<td>HeM with neutropenia</td>
<td>39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0</td>
<td>89.7</td>
<td>98.1</td>
<td>87.5</td>
<td>98.4</td>
<td>15</td>
</tr>
<tr>
<td>74</td>
<td>SCT</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5</td>
<td>75</td>
<td>100</td>
<td>97</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>807&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HeM or admitted to an</td>
<td>34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0</td>
<td>50</td>
<td>99.6</td>
<td>-</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>intensive care units</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>67</td>
<td>SCT</td>
<td>24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0</td>
<td>54.2</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>797&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HeM, SCT</td>
<td>53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5</td>
<td>90.6</td>
<td>94</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HeM with neutropenia</td>
<td>27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0</td>
<td>92.6</td>
<td>95.4</td>
<td>93</td>
<td>95</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>and/or steroid treatment CGD, SCT</td>
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</tr>
</tbody>
</table>

Abbreviations: PPV = positive predictive value; NPV = negative predictive value; HeM = hematological malignancies; SCT = stem cell transplant; CGD = chronic granulomatous disease

<sup>a</sup>Includes pediatric patients.

<sup>b</sup>Includes proven, probable, and possible IA.

<sup>c</sup>Includes proven and probable IA.

<sup>d</sup>Includes proven IA.
should be considered when 2 consecutive positive samples from a patient have been obtained. In patients with only single positive samples, the sensitivity is low, perhaps around 40% or less [14]. The cut-off index value for a positive result suggested by the manufacturer was above 1.5. Some studies suggested that a cut-off value of 1.5 was too high and that lowering the cut-off to 1.0 could improve sensitivity without compromising specificity [13,20]. However, it also had been reported elsewhere that lowering the cut-off value to 1.0 did not improve sensitivity [19]. In patients with IA, the ELISA test usually gives positive results before the clinical symptoms and signs become detectable [18,19]. While serving as an early diagnostic tool, some study suggested decreasing the cut-off value further to 0.5 in order to increase the duration of test positivity before diagnosis could be established by clinical means [18]. Moreover, serial determination of serum GM index values is useful in order to evaluate the prognosis of IA. A 1.0 increase in GM level above baseline was a marker of disease progression and predictive of treatment failure in allogeneic stem cell transplant recipients [21]. Nevertheless, it is important to recognize the causes of false-positives and false-negatives when the ELISA test is utilized in the clinical setting.

The false-positive rate ranged from 5% [13] to 14% [15]. The occurrence of false positivity frequently coincided with mucositis, cytotoxic chemotherapy, and/or graft-versus-host disease [13,15]. GM was detected in various kinds of foods [14,17,19]. It is postulated that translocation of dietary GM via damaged or immature intestinal mucosa could result in false-positive results [15,17]. The false-positive rate was reported to be high in up to 83% of newborn babies [14]. Besides GM of food origin, lipoteichoic acid of Bifidobacterium spp., which heavily colonize the neonatal gut, might cause ELISA reactivity in infants after translocation through immature intestinal mucosa [22]. Cross-reactivity to other fungi or bacteria was not reported, with the exceptions of Penicillium chrysogenum, Penicillium digitatum, and Paecilomyces variotii, fungi that rarely cause human infections [23]. Some drugs of fungal origin, such as antibiotics, could also cause persistent or transient antigenemia [17]. False-positive results had been described in some patients receiving piperacillin-tazobactam [24,25] and in a patient receiving amoxicillin-clavulanic acid from a case report [26]. However, the nature of false-positives remains undetermined in many cases and the causes may be multifactorial.

False-negatives may result from low-level release of the GM of the growing fungi, the use of prophylactic antifungal agents, and limited angioinvasion. Exposure to antifungal agents such as amphotericin B (AmB) might reduce the mycelial growth and/or alter the hyphal release of GM [18], causing the false-negative results. Because of the risk of false-negative results, GM antigen detection does not replace other diagnostic tools, such as computed tomography imaging, in the exploration of IFIs in high-risk patients [16].

The utility of the GM antigen ELISA in specimens other than serum has been evaluated [27]. Detection of GM antigen in bronchoalveolar lavage (BAL) fluid had good sensitivity and was more sensitive than antigen detection in serum. Increased GM index in cerebrospinal fluid indicated IA of the central nervous system. The utility of the ELISA in urine remains controversial and needs further validation.

Noninvasive testing of GM ELISA has advanced the diagnosis of IA. In practice, routine follow-up of patients at high risk for aspergillosis should include serum GM determination twice per week during neutropenia and when patients have additional risk factors, such as graft-versus-host-disease, and/or prolonged corticosteroid therapy. GM detection remains one of the major criteria to establish the IA diagnosis even when mycological detection is negative, according to the consensus group of the European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [28]. Because of the overall good performance of the ELISA test, a positive result should trigger further evaluation for disease by radiography, and lead to prompt treatment if indicated, while a negative result should lead to the aggressive search for other etiologies.

Glucan detection

(1-3)-Beta (β)-D-glucan ([1-3]-BDG) is a component of the cell wall of a variety of fungi [29] and can be utilized as a nonspecific marker for IFIs. It can be detected by its ability to activate factor G of the horseshoe crab coagulation cascade [29]. Commercially available BDG assays (e.g., Fungitec G; Seikagaku, Tokyo, Japan) allow determination of serum BDG by colorimetric or kinetic assay [30]. The BDG assay had good performance in patients infected with Candida, Aspergillus, and Fusarium spp., but failed to detect patients infected with zygomycetes and Cryptococcus, which contain little or no BDG [31]. Sensitivity and
Invasive fungal infection

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Conclusions

Laboratory diagnosis of IFIs has progressed in recent years, with advances mainly occurring in the area of diagnosis of IA. In the evaluation of the diagnostic methods for IA, the determination of sensitivity and specificity has been confounded by the uncertainty of the disease status; inclusion of probable cases of IA in the evaluation would affect performances of diagnostic tests. Non-culture-based diagnosis for candidiasis and noninvasive diagnosis for molds other than *Aspergillus* mostly remain investigational. New methods or better strategies are needed to improve diagnostic methods for IFIs.

There have been many advances in antifungal treatment in the last decade. The availability of more potent and less toxic antifungal agents, such as second-generation triazoles and echinocandins, has greatly improved the treatment of IFIs. However, the mortality of IFIs remains high. Combination therapy is promising conceptually as a means to increase the success rate of treatment, but more controlled clinical trials are needed to verify the efficacy of this approach.

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References

Review article
Transpl Infect Dis, 2003, Vol. 5, pp. 158-166

Rapid diagnosis of invasive aspergillosis by antigen detection

Abstract: Aspergillosis is a serious and often fatal infection in the bone marrow or organ transplant patient, for which improved methods of diagnosis are desperately needed. Currently, the diagnosis is most often made based on clinical findings and radiographic findings, which are nonspecific, and toxic therapies are initiated empirically, often without ever establishing the diagnosis. Without a definitive diagnosis, physicians often withhold or reduce the doses of the antifungal agent when toxicity develops or the patient improves, permitting progression of disease in those with invasive aspergillosis. The Platelia Aspergillus galactomannan antigenemia assay may assist physicians in making these decisions. With a sensitivity of 81% and a specificity of 89% in the studies leading to its FDA clearance, physicians still must be aware of the potential for false-positive and false-negative results; the test does not replace careful microbiological and clinical evaluation. This report will review the relevant literature and provide guidelines for use of the test in patient management.

Aspergillosis continues to be a serious and common opportunistic infection in immunocompromised subjects. Invasive aspergillosis (IA) occurs in 5–20% of individuals who undergo allogeneic stem cell transplantation, while a lower proportion occurs in solid organ allograft recipients (1–4). Furthermore, mortality remains high, above 50% in most studies (1). While the effect of the serious underlying disease has a profound impact on outcomes, delayed diagnosis contributes to mortality.

Diagnosis of IA may be difficult (5). Although air-crescent and halo signs seen on radiographs or computed tomography (CT) scans suggest IA (6), they are neither specific nor sensitive, and often are not correctly identified (7, 8). Often, definitive diagnosis by biopsy is not feasible because of coagulation abnormalities. Bronchoscopy to obtain specimens for cytology or culture may be possible in such patients, but the sensitivity for diagnosis is only about 25% (9). More often, antifungal therapy is initiated empirically, and the diagnosis is not proven.

Today, empiric therapies may include voriconazole (8) or caspofungin (10, 11), which, while active against Aspergillus, may not be effective...
prospective studies will be reviewed more fully to provide an understanding of the indications and limitations of the assay in patient management (Table 1) (7, 18–21). Other studies, summarized in Table 2, are presented to provide a comprehensive review of the literature (15, 22–35).

Maertens et al. (7) prospectively monitored allogeneic hematopoietic stem cell transplantation (HSCT) patients. Patients underwent aggressive evaluation for IA, including frequent chest radiographs and CT scans, weekly surveillance cultures, and Aspergillus antigen testing twice weekly. An assay cut-off of 1.5 was considered to be positive, and consecutive positive results were required to be classified as a true-positive assay result. Based on the autopsy-updated classification, the sensitivity was 94.4%. These findings substantiate their earlier experience, where they reported a sensitivity of 92.6% in autopsy-confirmed cases (28). Diagnosis by detection of antigenemia was more sensitive and specific than other procedures, including radiography, CT scan, and culture (Table 3).

Herbrecht et al. (18) found the test to be less sensitive. They prospectively evaluated neutropenic patients undergoing workup for fever, patients under investigation for suspected IA, and patients undergoing routine monitoring following HSCT. Their cut-off for a positive result was 1.5, and a single positive result was considered to be diagnostic. The sensitivity was 64.5% with definite, 16.4% with probable, and 25.5% with possible IA. If the cut-off was reduced to 0.7 and the analysis was restricted to adult non-allogeneic HSCT patients, the sensitivity increased to 73.1% in definite cases.

### Early diagnosis

The detection of antigenemia during twice-weekly monitoring facilitates early diagnosis of IA. Antigenemia preceded CT findings by 1 week and preceded initiation of antifungal therapy in nearly 90% of cases (7), confirming earlier findings (36) (Table 4). Sulahian et al. (32) reported that antigenemia preceded CT evidence of IA by more than a week in 65% of cases. Others reported similar findings in retrospective cohorts (21, 37), while some investigators have not shared this experience (25, 26, 30, 34, 38).

### Specificity

False-positive results have been observed in all studies, but the prevalence has varied considerably. Major variables affecting specificity include the selection of the cut-off to define positivity and the requirement for demonstration of persistent positivity for classification as true-positive. Maertens et al. (7) reported a specificity of 98.8% if two or more positive results were required vs. 85.4% if only one was required for classification as true-positive. However, false-positive results also have been reported in up to 20% of cases in studies requiring consecutive positive results (7, 17, 23, 24, 28, 32). Herbrecht et al. reported a specificity of 99.4% using a cut-off of 1.5, 93.9% with a cut-off of 0.7, but only 88.7% with a cut-off of 0.6. McLaughlin et al. (21), however, reported only a small drop in specificity from 99.7% to 97% when the cut-off was reduced from 1.5 to 0.5.

### Table 1

Sensitivity and specificity of Platelia® Aspergillus galactomannan immunoassay

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sensitivity Positive/total (%)</th>
<th>Specificity Negative/total (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad package insert</td>
<td>Prov/prob 25/31 (81)</td>
<td>132/148 (89)</td>
<td>Retrospective, stored specimens from bone marrow transplant and leukemia patients at 3 US centers, adults and children, cut-off 0.5, single positive</td>
</tr>
<tr>
<td>Maertens (7)</td>
<td>Prov 17/18 (94)</td>
<td>72/73 (99)</td>
<td>Prospective monitoring allo-HSCT, 2 × /week, cut-off 1.5, consecutive positive</td>
</tr>
<tr>
<td></td>
<td>Prob 0/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos 0/6 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbrecht (18)</td>
<td>Prov 20/31 (64)</td>
<td>607/640 (95)</td>
<td>Prospective monitoring and diagnostic evaluation of suspected IA, neutropenic and HSCT, cut-off 1.5, single positive</td>
</tr>
<tr>
<td></td>
<td>Prob 11/67 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos 14/55 (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinel (19)</td>
<td>Prov 0/3 (0)</td>
<td>748/751 (99)</td>
<td>Prospective monitoring during neutropenia and ICU, cut-off 3.0, consecutive positive</td>
</tr>
<tr>
<td></td>
<td>Prob 14/31 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Becker (20)</td>
<td>Serum 8/17 (47)</td>
<td>134/143 (93)</td>
<td>Prospective diagnostic evaluation during neutropenia, CT-guided BAL, blinded, cut-off 1.0</td>
</tr>
<tr>
<td></td>
<td>BAL 17/17 (100)</td>
<td>143/143 (100)</td>
<td></td>
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<tr>
<td></td>
<td>BAL1 19/22 (85)</td>
<td>176/176 (100)</td>
<td></td>
</tr>
<tr>
<td>McLaughlin (21)</td>
<td>Prov 12/13(92)¹</td>
<td>589/607 (97)²</td>
<td>Retrospective, HSCT patients, cut-off 0.5, single positive</td>
</tr>
<tr>
<td></td>
<td>Prob 8/11 (73)</td>
<td></td>
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</tbody>
</table>

¹Second non-blinded study.  
²Specimens.

Prov, proven; Prob, probable; Pos, possible; HSCT, hematopoietic stem cell transplantation; IA, invasive aspergillosis; CT, computed tomography; BAL, bronchoalveolar lavage; ICU, intensive care unit.
False-positive results may occur more frequently in children. Herbrecht et al. (18) reported a specificity of 48% of children vs. 98% of adults. Sulahian et al. (32) noted a specificity of 97.5% in adult patients compared with 89.9% in children. Others noted false-positive results in 83% of premature infants (27). Hayden et al. (37), however, reported the specificity to be 98.4% in pediatric cases using a cut-off of 0.5. Some suggest that galactomannan present in milk, rice, or protein-rich nutrients is the cause of false-positive results in children (27, 32, 39, 40), a conclusion that cannot explain the high rate of false-positivity in premature infants who do not receive cereal (27) (Table 5).

False-positive results may be more common in allogeneic HSCT patients (6.6%) than in other patient groups (0.6%) (18). When used for monitoring for IA following allogeneic HSCT, false-positive results have occurred most often during the first 2 weeks after cytoreductive therapy (28). This higher positive rate was ascribed to increased absorption of dietary galactomannan made possible by the breakdown of the intestinal mucosa caused by chemotherapy and irradiation (18).

Cyclophosphamide metabolites were suspected to cause false-positive results (41), and perhaps explained positive results before infection in an experimental model of aspergillosis in which the animals received cyclophosphamide 2 days before infection (42). Others, however, failed to observe false-positive results in patients who had received cyclophosphamide (28).

One concern is that *Aspergillus* colonization could cause antigenemia, resulting in the incorrect diagnosis of aspergillosis. Of note, however, is that Maertens et al. (36) did not observe false-positivity in patients who

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sensitivity Positive/total (%)</th>
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<tr>
<td>Stynen (15)</td>
<td>9/9 (100)</td>
<td>81/88 (92)</td>
<td>Retrospective, neutropenia and bone marrow transplant, cut-off 4 SD above mean of negative controls</td>
</tr>
<tr>
<td>Verweij (22)</td>
<td>9/10 (90)</td>
<td>43/51 (84)</td>
<td>Retrospective, neutropenia, cut-off 1 ng/mL galactomannan standard, multiple sera/patient</td>
</tr>
<tr>
<td>Rohrich (23)</td>
<td>10/10 (100)</td>
<td>21/27 (78)</td>
<td>Prospective, neutropenia, 2 × /week, cut-off mean negative controls plus 4 SD, consecutively positive</td>
</tr>
<tr>
<td>Sulahian (24)</td>
<td>Prov 19/25 (76)</td>
<td>138/169 (82)</td>
<td>Retrospective, bone marrow transplant, cut-off mean negative controls plus 5 SD</td>
</tr>
<tr>
<td>Bretagna (25)</td>
<td>Prov or Prob 6/6 (100)</td>
<td>31/35 (89)</td>
<td>Prospective, hematologic patients, once weekly testing, cut-off &gt; 1 ng/mL galactomannan, single positive</td>
</tr>
<tr>
<td>Bretagna (26)</td>
<td>Prov 6/6 (100)</td>
<td>18/19 (95)</td>
<td>Retrospetive, hematologic patients, cut-off &gt; 1 ng/mL, single positive</td>
</tr>
<tr>
<td>Siemann (27)</td>
<td>Prov 5/5 (100)</td>
<td>Unlikely IA</td>
<td>Retrospective, hematologic malignancy or ICU, cut-off 1.5, single specimen</td>
</tr>
<tr>
<td>Maertens (28)</td>
<td>Prov 25/27 (93)</td>
<td>42/44 (94.4)</td>
<td>Prospective monitoring, neutropenia or steroids, cut-off 1.0, consecutive positive</td>
</tr>
<tr>
<td>Kawamura (29)</td>
<td>4/4 (100)</td>
<td>90/90 (100)</td>
<td>Inadequate description of diagnostic criteria or patient groups, cut-off 1.0</td>
</tr>
<tr>
<td>Ulusakarya (30)</td>
<td>Prov 8/10 (80)</td>
<td>118/135 (96)</td>
<td>Prospective monitoring, neutropenia, cut-off 1.5, consecutive positive</td>
</tr>
<tr>
<td>Salonen (31)</td>
<td>Prov 6/6 (100)</td>
<td>118/135 (96)</td>
<td>Prospective, hematologic malignancy or HSCT, cut-off 1.5, consecutive positive</td>
</tr>
<tr>
<td>Sulahian (32)</td>
<td>Prov or Prob 48/53 (91)</td>
<td>700/744 (94)</td>
<td>Prospective, children with hematologic malignancy or HSCT, cut-off 1.5, consecutive positive</td>
</tr>
<tr>
<td>Fortun (33)</td>
<td>Prov or Prob 5/9 (56)</td>
<td>31/33 (94)</td>
<td>Retrospective, liver transplantation, cut-off 1 ng, consecutive positive</td>
</tr>
<tr>
<td>Kami (34)</td>
<td>19/33 (58)</td>
<td>86/89 (97%)</td>
<td>Retrospective and prospective, hematologic malignancy, cut-off 1.5</td>
</tr>
<tr>
<td>Sanguinetti (35)</td>
<td>BAL 20/20 (100)</td>
<td>BAL 0/24 (100)</td>
<td>Retrospective, cut-off 1.5</td>
</tr>
</tbody>
</table>

**Table 2**

SD, standard deviation; Prov, proven; Prob, probable; IA, invasive aspergillosis; ICU, intensive care unit; HSCT, hematopoietic stem cell transplantation; BAL, bronchoalveolar lavage.
Comparison of diagnostic tests in proven invasive aspergillosis

<table>
<thead>
<tr>
<th>Finding</th>
<th>Sensitivity (N = 18)</th>
<th>Specificity (N = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest radiograph</td>
<td>94%</td>
<td>60%</td>
</tr>
<tr>
<td>CT of lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any abnormality</td>
<td>78%</td>
<td>7%</td>
</tr>
<tr>
<td>&quot;Halo sign&quot;</td>
<td>28%</td>
<td>93%</td>
</tr>
<tr>
<td>BAL growing Aspergillus</td>
<td>50%</td>
<td>92%</td>
</tr>
<tr>
<td>Aspergillus galactomannan EIA:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 result</td>
<td>94%</td>
<td>85%</td>
</tr>
<tr>
<td>≥ 2 results</td>
<td>94%</td>
<td>99%</td>
</tr>
</tbody>
</table>

1. Data from Maertens et al. (7).
2. Computed tomography (CT) done in 15 cases and 15 controls.
3. Bronchoscopy and bronchoalveolar lavage (BAL) performed in 16 patients and 26 controls.

Temporal onset of galactomannan antigenemia in invasive aspergillosis

<table>
<thead>
<tr>
<th>Finding</th>
<th>Antigen first2</th>
<th>Days prior3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiogram chest</td>
<td>12/15 (80%)</td>
<td>8</td>
</tr>
<tr>
<td>CT lung</td>
<td>12/15 (80%)</td>
<td>6</td>
</tr>
<tr>
<td>Positive culture</td>
<td>16/18 (89%)</td>
<td>9</td>
</tr>
<tr>
<td>Definite diagnosis IA</td>
<td>16/18 (89%)</td>
<td>14</td>
</tr>
<tr>
<td>Initiation therapy</td>
<td>16/18 (89%)</td>
<td>6</td>
</tr>
<tr>
<td>Death</td>
<td>17/18 (94%)</td>
<td>14</td>
</tr>
</tbody>
</table>

1. Data from Maertens et al. (7).
2. The data depict the number of cases in which the positive antigen result preceded the finding.
3. Median number of days by which the positive antigen preceded the finding.

False-positive results have also been reported in neutropenic patients with bacteremia caused by staphylococci, enterococci, Corynebacterium jeikeium, Pseudomonas, Escherichia coli, and fungemia with Candida albicans (17). Interestingly, exoantigens from these organisms were not reactive in the assay, and others failed to observe false-positive results in bacteremic patients (28).

Anti-animal antibodies may cause false-positive results in sandwich assays (44). However, pre-treatment with EDTA and boiling should overcome this problem in the Platelia® assay. Nevertheless, a false-positive result was reported in a patient with graft-versus-host disease (GVHD) who had auto-reactive antibodies (45).

Finally, miscellaneous other causes for false-positive results have been observed or proposed. Glucopyranose present in cellulose was postulated to cause false-positive results when material on a cotton swab was tested in the latex agglutination format of the Aspergillus antigen assay (46). Galactomannan was detected in several drugs that originated from fungal organisms, including co-amoxiclav, piperacillin (39), piperacillin/tazobactam (Thomas Walsh, unpublished communication, 2003), and uricase (19). Undoubtedly, other causes for false-positive results will be discovered as the test is used more often in the US.

Other body fluids

Antigen has been detected in body fluids other than serum. Although these specimens were not included in the FDA clearance, they may be superior to serum for testing in certain circumstances. The detection of antigen in bronchoalveolar lavage fluid (BAL) was described in the initial report of the test (47). Becker et al. (20) reported detection of antigen in the BAL of all 18 cases of IA, while antigenemia was present in only 47%. Others have also reported the detection of antigen in BAL (48, 49). More recently, Sanguinetti et al. (55) described the detection of galactomannan in BAL of all 20 hematology patients with IA, but did not compare the results to antigenemia testing. The results were negative in all control specimens in that study. Airway colonization, however, may cause false-positive results in BAL specimens.

The central nervous system (CNS) is often involved in patients with disseminated aspergillosis, and four studies have reported detection of antigen in the cerebrospinal fluid (CSF) (50–53). However, antigen in the CSF was also found in control patients with pulmonary IA who lacked...
were colonized. Furthermore, Rohrlch et al. (23) evaluated patients with cystic fibrosis, who exhibited persistent airway colonization with *Aspergillus*, and failed to detect antigenemia. However, occult *Aspergillus* infection may occur in some colonized patients with presumed false-positive results, as shown by Maertens et al. (7): three patients presumed to have false-positive results during life were proven to have IA at autopsy.

False-positive results also may be caused by infection with organisms that share cross-reacting antigens with *Aspergillus*. The monoclonal antibody used in the assay reacts with antigens from members of several fungal genera besides *Aspergillus* (14). Kappe and Schulze-Berger (16) reported a false-positive result caused by contamination of a specimen with *Penicillium chrysogenum*, and cautioned laboratories to inspect carefully for contamination. Bretagne et al. (25) described a false-positive result in a patient with pneumonia and sinusitis caused by *Phialophora americana*, although antigens extracted from this organism did not react in the assay. Positive results using the same monoclonal antibody in a latex agglutination assay were observed in guinea pigs that were infected with *Penicillium marneffei* (43).

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Review Articles

Update on the Laboratory Diagnosis of Invasive Fungal Infections.

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Abstract: Recent advances in the management of patients with haematological malignancies and transplant recipients have paralleled an increase in the incidence of fungal diseases due to pathogenic genera such as Candida and Aspergillus and the emergence of less common genera including Fusarium and Zygomycetes. Despite availability of new antifungal agents these opportunistic infections have high mortality. Rapid and reliable species identification is essential for antifungal treatment, but detection of the increasing diversity of fungal pathogens by conventional phenotypic methods remains difficult and time-consuming, and the results may sometimes be inconclusive, especially for unusual species. New diagnostic techniques (e.g., 1,3-beta-d-glucan detection) could improve this scenario, although further studies are necessary to confirm their usefulness in clinical practice.

Introduction: Despite the development of new techniques and new antifungal agents, diagnosis of invasive fungal infection (IFI), which still relies upon a combination of clinical observation and laboratory investigation, remains a challenge especially for immunocompromised patients with haematological disease.¹ This has important clinical repercussions since delayed diagnosis and therapy contribute significantly to the high mortality rates associated with IFIs,² whereas early intervention with antifungal drugs may result in more effective management of high-risk patients.³ While superficial and subcutaneous fungal infections often produce characteristic lesions that suggest the diagnosis, a thorough knowledge of potential causative organisms is yet required to aid the diagnostic process, mainly in situations where systemic fungal infection is suspected but the clinical presentation is nonspecific and then ascribable to a wide range of infections, underlying illnesses, or complication of treatments.⁴

The exact identification of the infecting organism is became essential in light of the increased use of prophylactic schedules that predispose the patient not just to fungal infection, but also to the selection of fungal species such as non-albicans Candida (e.g., C. glabrata and C. krusei), Aspergillus terreus, Scedosporium species, and Zygomycetes, many of
Molecular-based detection methods: A range of polymerase chain reaction (PCR)-based methods have been developed with the prospect of giving highly specific, highly sensitive, and rapid means for fungal detection and identification. Most of them have focused on *Aspergillus* and *Candida* species, using different specimens types (e.g., serum, plasma, or BAL fluid), even though pan-fungal PCR amplification technology may be able to detect a broad range of fungal targets. Although PCR has been studied for years, the lack of standardization and clinical validation has led to its exclusion from consensus criteria for defining IFI. Nevertheless, a recent prospective evaluation of serial PCR assays against Con Andrew  and along with GM and computed tomography was carried out in haematological patients, thus showing acceptable sensitivity and specificity. In such one study, the combination of serial PCR and GM detected 100% of aspergillosis cases, with a positive predictive value of 75.1%. Of note, in a systematic review and meta-analysis of *Aspergillus* PCR tests for diagnosis of IA, the authors proposed that a single PCR-negative test is sufficient to exclude IA, whereas two PCR-positive results are required to confirm disease. Compared with *Aspergillus* PCR, only a few *Candida* PCR methods have received major clinical evaluation. As confirmed by a national consensus evaluation, performance of these tests is generally good, with sensitivities and specificities consistently >90%. Although addressed to critically ill patients, a prospective clinical trial published in 2008 reported positive predictive values and negative predictive values of >90% for a PCR method that detects several species of *Candida*.

**Conclusion:** Molecular detection methods, combined with additional microbiological and clinical information, has the potential not only to accurately and rapidly identify fungal pathogens, but also to indicate whether the pathogen is likely to respond to conventional antifungal treatment. Inclusion of these methods in a diagnostic surveillance strategy to exclude IFI in high-risk patients with haematological malignancy should result in improved clinical management, thus allowing more rational use of antifungal drugs.

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mucormycosis. \textsuperscript{25,26} Measurement of serum BG has been shown to be an aid in the diagnosis of fungaemia and deep-seated mycoses, including IA.\textsuperscript{27,28} Among commercially available assays, the Fungitell, which is also an ELISA technique, is widely used to detect serum BG concentrations as low as 1 pg/mL.\textsuperscript{27} The cutoff for a positive result is >80 pg/ml. As with GM, variable results have been reported for BG assay, with a slightly higher sensitivity and specificity, ranging from 70\% to 90\%.\textsuperscript{28,29} When performances of both GM and BD tests were compared to determine their diagnostic usefulness for high-risk haematological malignancy patients, GM assay was significantly better for detecting non-	extit{fumigatus} \textit{Aspergillus} species, whereas BG was shown to have a higher sensitivity in detecting IA and other mould infections.\textsuperscript{30}

Non-culture based methods for diagnosis of candidiasis are of limited value because the levels of circulating antigens are low and the transient nature of the antigenaemia requires sensitive assays and frequent sampling of at-risk patients.\textsuperscript{1,31} However, the use of Platelia Candida, an ELISA that combines the detection of mannann antigen and anti-mannan antibodies in serum, led to earlier diagnosis of \textit{Candida} infection when compared with blood cultures.\textsuperscript{31} In haematological patients with hepatosplenic lesions, assessing mannann/anti-mannan antibodies shortened significantly the median time of diagnosis of candidiasis when compared with imaging.\textsuperscript{32}

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\textbf{Conclusion:} Molecular detection methods, combined with additional microbiological and clinical information, has the potential not only to accurately and rapidly identify fungal pathogens, but also to indicate whether the pathogen is likely to respond to conventional antifungal treatment.\textsuperscript{9} Inclusion of these methods in a diagnostic surveillance strategy to exclude IFI in high-risk patients with haematological malignancy\textsuperscript{40} should result in improved clinical management, thus allowing more rational use of antifungal drugs.

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References:

PMid:16127033
PMCID:1195428
PMid:12384841
PMid:15653818
PM CID:544171
PMid:17287333
PMCID:1865843
PMid:11835304

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Using the Galactomannan Assay in the Diagnosis of Invasive Aspergillosis

By

Robert Wood-Morris, MD; Glenn Wortmann, MD [AIDS Reader. 2008;18:318-320]

June 1, 2008

Aspergillus species are ubiquitous molds to which humans are commonly exposed. Of approximately 180 species, it is estimated that 34 are medically significant. Most persons who come in contact with the fungus remain asymptomatic. However, some experience mild morbidity demonstrated by recurrent sinusitis, asthma exacerbations, or allergic bronchopulmonary aspergillosis.

Patients who are immunocompromised, however, are susceptible to more severe invasive disease, usually marked by an acute progressive infection, often resulting in death. A survey of 89 physicians whose experience with a combined 595 patients with proven or probable invasive aspergillosis (IA) showed that 32% of patients had undergone bone marrow transplantation, 29% had a hematological malignancy, 9% had undergone solid organ transplant or had another condition requiring immunosuppressive therapy, 9% had pulmonary disease, and 8% had AIDS.
The prognosis of IA is grim, with a case mortality rate of 58%. Although newer, less toxic antifungal agents have been developed, successful management of IA is contingent on early detection, which, unfortunately, can be difficult.

DIAGNOSIS OF IA
IA is usually suspected when signs of infection refractory to broad-spectrum antibiotics develop in an immunocompromised patient. The gold standard for the diagnosis of IA is tissue biopsy demonstrating invasion on histopathological examination and identification of the organism in culture. The finding of acute-angle branching, septated, nonpigmented hyphae on histopathological examination is not specific for IA because other molds, including *Fusarium*, *Paecilomyces*, and *Pseudallescheria boydii*, can have a similar appearance. For unclear reasons, growth of *Aspergillus* in culture occurs in only 30% to 50% of histopathologically suggestive cases. Obtaining tissue specimens for the diagnosis of IA is often difficult, because patients in whom IA is suspected often have medical conditions, such as thrombocytopenia, that preclude biopsy.

GALACTOMANNAN ANTIGEN TESTS
A recent diagnostic modality for IA is the galactomannan (GM) assay. GM is a cell wall component of many fungi, including *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Geotrichum* species. An enzyme-linked immunosorbent assay-based kit is commercially available as the Platelia Aspergillus EIA (Bio-Rad Laboratories, Redmond, Wash) and was cleared by the FDA for diagnostic use in May 2003. GM antigen positivity is among the microbiological criteria proposed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group for the diagnosis of IA. The Platelia EIA is an immunoenzymatic sandwich microplate assay that uses monoclonal antibodies that bind to side-chain residues of the GM molecule. A sandwich enzyme immunoassay format is used for detection. The performance of the assay varies according to the threshold value used.

When the Platelia EIA became available in Europe a decade ago, the manufacturer recommended an optical density (OD) index (also called a GM index) of 1.5 as the cutoff between positive and negative results. Lowering the threshold to between 0.7 and 1.5 in an effort to improve sensitivity was validated, and an analysis of 986 serum samples determined that a cutoff of 0.5 increased sensitivity with minimal loss of specificity.

In the United States, the suggested OD index threshold is 0.5; in Europe, a cutoff of 0.7 is commonly used. The overall reported sensitivity of the Platelia EIA ranges from 30% to 90%, with a reported specificity of greater than 93%.

Causes of false-positive GM assay results
False-positive test results with the GM assay have been reported by a number of investigators (Table). Because *Penicillium* produces GM, it is not surprising that a number of β-lactam antibiotics, including piperacillin (Drug information on piperacillin)/tazobactam, amoxicillin/clavulanate, ampicillin, and phenoxymethylpenicillin (Drug information on phenoxymethylpenicillin), have yielded positive Platelia EIA results.
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persisted for more than 72 hours. Nine patients had proven or possible IA. The GM test demonstrated a sensitivity of 100% and specificity of 93%, and the sensitivity of chest CT was comparable (100% had abnormal findings). Only 2 of the 9 patients died as a result of IA, leading to the conclusion that Aspergillus GM surveillance and early chest CT should be considered to detect IA in its initial stages.

CONCLUSION
The timely diagnosis of IA is problematic, and its delayed diagnosis is associated with high mortality rates. The GM assay offers the potential of securing a diagnosis through relatively noninvasive means. Clinicians should consider using this test when they suspect IA in immunocompromised patients. The test may prove especially helpful in patients in whom invasive tissue biopsy is contraindicated. There are published shortcomings of the GM test, and questions remain about GM detection in tissue and fluid other than serum and whether surveillance sampling in at-risk populations can both decrease use of empiric antifungal therapy and improve survival.

References
Issues with galactomannan testing

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Within the past decade detection of the aspergillus antigen galactomannan has become an important and reliable tool for the early diagnosis of invasive aspergillosis. The galactomannan molecule, that is detected by the commercial sandwich ELISA (Platelia Aspergillus, Biorad), was found not to be a single molecule, but a family of molecules that have the epitope that reacts with the monoclonal antibody. Also the cut off level is now world-wide lowered to 0.5 which will help to further standardize and compare this diagnostic tool. Despite the advantages of galactomannan detection, there are several issues that have impact on its use in clinical practice. Both false negative and false positive reactivity is encountered and although the causes of false reactivity are not fully understood, new insights have become available which help us to optimize the use of the assay. This review discusses present issues with galactomannan testing with a view to future research and management.

Keywords  Galactomannan, diagnosis, invasive aspergillosis

Introduction

The detection of circulating galactomannan (GM) has become an important tool in the early diagnosis of invasive aspergillosis (IA). GM is part of the outer layer of the aspergillus cell wall, and is released during growth of the fungus at the tips of the hyphae [1,2]. The antigen can be detected using a commercially available sandwich ELISA (Platelia Aspergillus, BioRad, France)(PA-ELISA), which employs a monoclonal antibody (EB-A2) that binds to the galactofuran epitope of the GM antigen [3,4]. The assay has been extensively studied and is now commonly used to monitor patients at high risk for invasive aspergillosis [2,5–8]. There are several issues that hamper the use of the assay, which will be addressed in this review.

One area of controversy has recently been resolved. The PA-ELISA was originally marketed with a cut-off for positive of 1.5. There was no evidence to support this cut off level and in the past years many researchers have proposed lower cut off levels. The assay was released in the USA with a cut off of 0.5 and the producer of the assay recently decided to lower the cut off to 0.5 in all other countries based on ROC analysis of European data sets of GM monitoring in patients with hematological malignancy [9].

The GM antigen

Unlike the name suggests, the so-called ‘GM antigen’ is not a single molecule but a family of molecules which are better called galactofuranose(galf)-antigens. In addition to GM, fungal glycoproteins also react with the EB-A2 antibody, including phospholipase C and phytase, which were shown to have only one terminal galactofuranose unit that was essential for binding with the EB-A2 antibody [10]. These molecules might not only show different PA-ELISA reactivity’s but their expression might also be modulated by the fungal environment. A recent study showed that the release of PA-ELISA reactive antigens in vitro is influenced by the growth phase of the fungus [11]. Not only are reactive antigens released during early logarithmic growth, mycelial breakdown during the lytical phase results in a further increase of reactive antigens in the culture supernatant. However, the actual galf antigens that circulate in vivo in the
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Strategy and value of GM monitoring

Regular monitoring of patients with hematological malignancy, who are neutropenic and do not receive mould-active antifungal drugs is the setting in which the PA-ELISA appears to be of most value in the management of high risk patients [7,8,15]. In other patient groups the benefit of this assay and the optimal strategy which incorporates GM detection is less well established [37]. In hematology patients, the most appropriate strategy appears to be a combination of GM monitoring and high resolution CT scan, although some investigators find no additional value of GM-monitoring [38]. This pre-emptive approach was recently found to be feasible in hematology patients [39]. Patients with IA were better identified compared with a historic control group which received empiric antifungal therapy [39]. The number of patients requiring antifungal drugs was also lower in those undergoing the pre-emptive strategy than those receiving empiric antifungal therapy [17% vs 35%]. Only one patient, who died of invasive zygomycosis, was not diagnosed using this approach [39]. Although these results are encouraging, there is at present no study that shows that GM monitoring or the pre-emptive management strategy leads to a survival benefit.

Conclusions

GM detection remains a useful tool in the diagnosis of IA despite the current drawbacks as discussed above. Future research and improved GM detection techniques, such as described with filtration as part of the pre-treatment procedure, will certainly further contribute to early diagnosis of IA. At present monitoring of circulating GM is the only noninvasive tool with proven usefulness. However, new biological markers like (1→3)-β-D-glucan and fungal DNA might show an additional value. Management strategies should include all possible options for early diagnosis in order to increase the chance of successful treatment of patients with this disease.

References

Current therapeutic approaches to fungal infections in immunocompromised hematological patients

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Keywords:
Moulds
Yeasts
Leukemia
Hematopoietic stem cell transplantation

SUMMARY

Invasive fungal infections are significant causes of morbidity and mortality in patients with hematological malignancies. Patients with acute myeloid leukemia and those who have undergone allogeneic hematopoietic stem cell transplantation are at especially high risk. Various fungal agents are responsible for this complication, but Aspergillus spp. and Candida spp. are the most frequently isolated micro-organisms; less commonly, infections could be caused by Zygomycetes or other rare molds or yeasts.

Several new systemically-administered antifungal agents have been approved for clinical use since 2001; these agents include liposomal amphotericin B, voriconazole, caspofungin, and posaconazole, and they represent a major advance in antifungal therapy and have improved the prognosis of patients with hematological malignancies.

This review focuses on therapeutic aspects of the management of fungal infections in hematological patients.

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Introduction

The proportion of patients with malignancies who develop invasive fungal infections (IFI) has increased dramatically worldwide over the past few decades. The majority of these infections occur in patients with hematological malignancies (HM), particularly in patients with acute myeloid leukemia (AML) and those who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT).1–3

Because of difficulties in early and accurate diagnosis, systemic antifungals have been used for universal prophylaxis and/or on an empirical basis in high-risk populations; this use results in increased costs, toxicity, and concerns about emergent drug resistance. In the past, health providers largely relied on amphotericin B deoxycholate (d-AMB) for the target treatment of proven IFI, but recently, this treatment has been replaced with lipid formulations, new triazoles, and a novel class of echinocandins. The availability of new treatments offers an opportunity to revise traditional approaches to antifungal therapy and to perhaps improve outcomes.

Epidemiology and risk factors

Historically, systemic Candida infections have been primarily responsible for IFI in HM. However, autopsy and epidemiologic studies have confirmed that over the last ten years mold infections have increased.1–6 This change in epidemiologic trends of IFI may be related to a real increase in the incidence of molds or to the introduction of more accurate diagnostic procedures. Furthermore, particularly in HSCT patients, the widespread use of fluconazole prophylaxis could also play a role; fluconazole targets many Candida spp., but not Aspergillus spp. or other molds.4

Aspergillus spp. infections remain the most common cause of death in HM; the overall incidence of Aspergillus spp., ranges from 0.3% to 2.8% depending on the underlying hematological condition.1,3,6–9 Patients undergoing allo-HSCT appear to have two time-frames for the peak occurrence of invasive aspergillosis (IA). The first period is before engraftment, during the neutropenic state. The second period occurs later during the post-engraftment period, when patients are on immunosuppressive therapy or suffer for graft-versus-host disease (GVHD).5

Yeast infections are less common than mold infections, and Candida is still the predominant yeast pathogen. Recently, the emergence of other opportunistic mold pathogens (e.g., Fusarium spp. and Zygomycetes) has been reported, while infections due to other fungal pathogens remain rare.10–14

In general, the patient’s immune status, degree of organ damage (i.e., mucositis or GVHD), microbial exposure (i.e., colonization,
Current therapeutic approaches to fungal infections in immunocompromised hematological patients

Livio Pagano, *, Morena Caira, Caterina Giovanna Valentini, Brunella Posteraro, Luana Fianchi

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Aspergillus spp. infections remain the most common cause of death in HM; the overall incidence of Aspergillus spp. ranges from 0.3% to 12.8% depending on the underlying hematological condition. Patients undergoing allo-HSCT appear to have two timeframes for the peak occurrence of invasive aspergillosis (IA). The first period is before engraftment, during the neutropenic state. The second period occurs later during the post-engraftment period, when patients are on immunosuppressive therapy or suffer for graft-versus-host disease (GVHD).

Yeast infections are less common than mold infections, and Candida is still the predominant yeast pathogen. Recently, the emergence of other opportunistic mold pathogens (e.g., Fusarium spp. and Zygomycetes) has been reported, while infections due to other fungal pathogens remain rare.

In general, the patient’s immune status, degree of organ damage (i.e., mucositis or GVHD), microbial exposure (i.e., colonization,
environment, and prior infection), older age, type of chemotherapy agent received (i.e., cytarabine) and the use of new drugs (such as monoclonal antibodies) are major factors influencing the likelihood of IFI. However, deep and prolonged granulocytopenia is the greatest risk factor for IFI. The risk progressively increases over time and reaches a plateau of 70% among patients who are granulocytopenic for more than 34 days. Another key risk factor for IFI is steroid use; such use modifies phagocyte migration, decreases the antifungal activity of monocytes and macrophages, and enhances the growth of Aspergillus spp. Some reported risk factors are related to specific pathogens. For example, indwelling catheters, prior exposure to broad-spectrum antimicrobial agents, and prior surgery have been well-recognized to facilitate yeast access to the bloodstream and cause invasive candidiasis.

The clinical signs and symptoms of IFI strictly depend on the patient’s immune status and the etiological agent and range (i.e., from focal localization to hematogenous dissemination in which almost all organs are involved).

During the earliest phase of infection, IFI may be asymptomatic, or fever may be the only clinical symptom observed. This lack of symptoms may be related in part to the masking of clinical symptoms by treatments (i.e., steroids) administered for the underlying malignancy. Other symptoms, when present, are usually highly aspecific and are related to the site of infection. The lungs are most frequently affected by Aspergillus or other mold infections, and cough, dyspnea, and chest pain are often reported during the early course of pulmonary infections; in advanced stages, severe manifestations (including hemoptysis or pneumothorax) may also occur.

Pneumonia due to Zygomycetes or other molds is clinically indistinguishable from that caused by Aspergillus, except for a higher frequency of rhinosinus localization. Dissemination to secondary sites of infection (i.e., skin or the central nervous system) may cause additional clinical symptoms.

Diagnosis of invasive fungal infections

Laboratory diagnosis of IFI is based on the direct microscopic detection of the etiologic agent in clinical specimens, isolation and identification of the pathogen in culture, or non-culture-based methods involving detection of a serologic response to the pathogen or other markers of its presence (i.e., fungal antigens or metabolites). Microbiological cultures are often insensitive or of limited value; even with modern blood culture systems candidemia can be transient and not detected, and Aspergillus cannot be cultured from a significant proportion of sputum or bronchoalveolar lavage (BAL) samples from patients with IA.

To facilitate the early diagnosis of IFI, important advances have been made in the development of laboratory markers (i.e., galactomannan [GM] and 1,3-beta-D-glucan [BG] assays); these advances have created the need for new paradigms regarding prevention and early treatment of IFIs. Among fungal markers, GM has been approved by the US Food and Drug Administration as a diagnostic adjunct for IA (at a serum cut-off of 0.5). However, this technique has shown contradictory results in terms of sensitivity and specificity due to several factors, including prior antifungal therapy and false-positive results due to antibiotic treatments or different positivity cut-offs. Thus, a recently published meta-analysis of 27 studies showed an overall sensitivity of 71% and specificity of 89% for proven cases of IA when GM is used for surveillance.

The measurement of serum BG has also been shown to aid in the diagnosis of fungemia and deep-seated mycoses, including IA. The cut-off for a positive result is over 80 pg/ml. As with GM, variable results have been reported for BG assays, though they have a slightly higher sensitivity and specificity that range from 70% to 90%.

Non-culture-based methods for the diagnosis of candidiasis are of limited value because the levels of circulating antigens are low and the transient nature of the antigenemia requires sensitive assays and frequent sampling of at-risk patients. The use of Platelia Candida, an ELISA that combines the detection of mannan antigen and anti-mannan antibodies in serum, allows the earlier diagnosis of Candida infection than do blood cultures. A range of polymerase chain reaction (PCR)-based methods has been developed with the goal of offering a highly specific, sensitive, and rapid method of fungal detection and identification. Most of these methods have focused on Aspergillus and Candida species in various specimen types (i.e., serum, plasma, or BAL fluid), even though pan-fungal PCR amplification technology may be able to detect a broad range of fungal targets. Although PCR has been studied for years, the lack of standardization and clinical validation has led to its exclusion from consensus criteria for defining IFI. Nevertheless, a recent prospective evaluation of serial PCR assays against or along with GM and chest-computed tomography was carried out in HM; the PCR method showed acceptable sensitivity and specificity. Notably, in a systematic meta-analysis of Aspergillus PCR tests for IA diagnosis, the authors proposed that a single PCR-negative test is sufficient to exclude IA, whereas two PCR-positive results are required to confirm disease. Compared with Aspergillus PCR, only a few Candida PCR methods have received major clinical evaluation.

A national consensus evaluation confirmed that the performance of these tests is generally good, with sensitivities and specificities consistently above 90%.

Recent studies have focused attention on the role of adaptive immunity in the host defense against Aspergillus species. In particular, a preliminary report on ELISPOT, Aspergillus-specific IFNgammaTH1, and Aspergillus-specific IL-10-producing T-cells (IL-10-TH2) through an ex vivo enzyme-linked immunosassay showed interesting results in the diagnosis and follow-up of IA.

Radiological exams are one of the most important diagnostic tools, particularly for pulmonary-localized mold infections. A standard chest X-ray is the first-line approach, even if it is often not diagnostic. In fact, HM typically have a decreased inflammatory response and, consequently, clinical features may not be manifested until the infection is quite advanced. A chest CT-scan is better, particularly a high resolution CT-scan (HRCT-scan); various studies have shown a sensitivity and specificity near 100%.

PET-scans can give us more information about the type, number, and localization of lesions, as well as their morphologic characteristics. These scans can demonstrate the presence of the “halo sign,” a typical precocious lesion, or the presence of the “air crescent sign,” which usually becomes more prevalent later in the course of the disease. Furthermore, Caillot et al. in a following prospective study demonstrated that serial chest CT-scans depict more precisely the evolution of lesion volumes of pulmonary aspergillosis; this finding emphasizes that these scans are a useful tool for the the diagnosis and outcome evaluation of IA.

Despite their importance, characteristic radiological patterns do not allow us to diagnose a specific aspergillosis because of their similarity to other angioinvasive fungi such as Zygomycetes, Fusarium spp., or Scedosporium spp. This fact makes biopsy necessary to clarify the diagnosis. A study evaluated the utility of CT-guided lung biopsy for the diagnosis of IFI; this study combined Calcofluor white staining, GM, and PCR on biopsy specimens. This combination resulted in the fast and reliable identification of the fungus with a specificity and sensitivity of 100%. However, the utility of such a diagnostic platform is doubtful, because of the many contraindications for the performance of an invasive procedure in HM (i.e., thrombocytopenia and hemodynamical instability). The usefulness of PET-scans has been evaluated, but data are presently scant.
Table 6
Characteristics of different approaches in the therapy of invasive fungal infections in patients with hematological malignancies and literature suggestions.

<table>
<thead>
<tr>
<th>Description</th>
<th>Pro</th>
<th>Con</th>
<th>Suggested antifungal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>Applicable to uninfected patients who are at risk for IFI</td>
<td>• Possible reduction of the incidence of IFIs</td>
<td>Voriconazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Possible reduction of fungal infection-related mortality</td>
<td>Fluconazole (in allo-HSCT)</td>
</tr>
<tr>
<td>Empirical approach</td>
<td>Early treatment of occult fungal infection when patients have clinical signs and symptoms of infection but no clearly identifiable pathogen or radiological signs</td>
<td>• Suggested by many international guidelines</td>
<td>L-AmB Caspofungin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Early therapy for patients with an undetected but possible IFI, because survival seems to be affected by the early initiation of treatment</td>
<td>Not specific indication</td>
</tr>
<tr>
<td>Pre-emptive approach</td>
<td>Administered in neutropenic patients with persistent fever who show image-documented pneumonia, acute sinusitis, or a positive galactomannan test</td>
<td>• Early detection of asymptomatic infections thanks to the intensive use of screening markers</td>
<td>L-AmB for zygomycosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduction in the use of antifungal drugs compared with an empirical approach</td>
<td>Voriconazole for aspergillosis</td>
</tr>
<tr>
<td>Target therapy</td>
<td>Administered in patients with a clear evidence of fungal infection</td>
<td>• Possibility to administer an antifungal treatment really effective against the pathogen</td>
<td>Voriconazole for candidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduction in the use of antifungal drugs compared with an empirical approach</td>
<td>L-AmB for zygomycosis</td>
</tr>
</tbody>
</table>

Practice points

• Effective treatment options are available for the majority of mold and yeast infections.
• Echinocandins represent the drugs with a higher efficacy against Candida in non-neutropenic patients. In spite of little experience in HM, they could be considered a good choice in these patients also.
• At present, voriconazole has been recognized as the gold standard among anti-Aspergillus treatments.
• Zygomycosis must be considered a rare, not emerging fungal infection. Treatment with L-AMB or posaconazole improves the prognosis.

Conclusions

Over the last ten years, new antifungal agents have become available for the treatment of IFIs. These drugs, along with better supportive care and more effective diagnostic tools, have significantly reduced the mortality rate linked to fungal complications. The differences among the various therapeutic approaches and the best antifungal agent suggested for each strategy have been summarized in Table 6.

IFIs are still a significant cause of morbidity and mortality, and future efforts should focus on further improvements in diagnostic techniques, which would allow for the timely application of antifungal therapy and would reduce the use of treatments in inappropriate settings.

Conflict of Interest

L.P. has received research grants and honoraries as speaker and consultant from Mercks, Gilead, Pfizer and Schering Plough. L.F., G.V., B.P. and M.C. reported no potential conflicts of interest.

Acknowledgments

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References

REVIEW

Positive-pressure isolation and the prevention of invasive aspergillosis. What is the evidence?

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KEYWORDS
Aspergillosis; Positive-pressure; High-efficiency particulate air; Isolation

Summary Positive-pressure ventilation implies a sealed room, usually with an anteroom to facilitate the donning of protective clothing, airflows of at least 12 air changes per hour and high-efficiency particulate air (HEPA) to prevent infection in susceptible patients. Laminar airflow (LAF) involves much greater air changes, expense and inconvenience to the patient due to noise and draughts. There are few, if any, truly controlled trials on the impact of positive-pressure ventilation and the prevention of invasive aspergillosis (IA); most are observational studies conducted during an outbreak or retrospective analyses of the incidence of IA over periods of time when a variety of preventative interventions were introduced. Therefore, it is often difficult to determine the specific impact of positive-pressure ventilation with HEPA in leading to a reduction in IA. During periods of hospital demolition or construction, HEPA significantly reduces the aspergillus spore counts and in many studies, the incidence of IA, but other measures such as enhanced cleaning, the sealing of windows and the use of prophylactic anti-fungal agents are also important. On balance, the additional expense and inconvenience of LAF does not appear to be justified. Where positive-pressure ventilation is installed, it is imperative that the system be monitored to ensure that the pressure differentials and air changes are appropriate. Whilst there is a role for positive-pressure ventilation in reducing the incidence of IA, we need a better definition of the importance of hospital-acquired IA compared with community-acquired infection and of the relationship between strains of Aspergillus species isolated from the environment and those strains causing infection.

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This review is largely based on a presentation at the spring meeting of the Hospital Infection Society, March 2003. The author acts as a professional advisor for Regent Medical (UK).

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Introduction

As the number of patients undergoing chemotherapy for neoplastic disease increases, accompanied by a rise in the number of patients with neutropenia, infection control teams are often asked about the requirement for positive-pressure isolation facilities, either within an existing unit, or during the planning stages for a new haematology/oncology unit. Many believe that the scientific evidence is convincing in favour of positive-pressure isolation, specifically in preventing invasive aspergillosis (IA). However, the requirement to balance the needs of patients, the expense of both constructing and maintaining a positive-pressure isolation facility, and the need for other infection preventative measures, make the assessment complicated in the absence of well-conducted trials. Some knowledge of the principles and design of positive-pressure isolation facilities and what they offer is also required to make such a decision.

What follows is a combination of a literature search (MedLine), a review of various guidelines and official documents published in English in the area, and the author’s own experience and perspectives. In the absence of controlled trials, as discussed below, the conclusions and recommendations are not graded or ranked in terms of priority and are personal opinions only.

Positive-pressure ventilation

Positive-pressure isolation, sometimes referred to as ‘protective environment’ or ‘positive-pressure rooms,’ is where a patient is placed in a separate environment because the patient is more susceptible to infection, and therefore, needs protection from both other patients and from the hospital environment. Air pressures within such a facility are higher than in the surrounding areas to prevent the ingress of potentially contaminated air, and there is also an anteroom with a handwash basin. Categories of patients for which this usually applies are those with significant immunodeficiency, e.g. neutropenia, or patients with extensive burns. Whilst these patients are at risk from infection acquired from an external source, such as the hands and clothes of a member of staff, the purpose of protective isolation is to prevent acquisition from other patients or from the environment. Endogenous infection is still possible and common.

Bishop Joscelin of Bath and Wells in 1219 was perhaps the first to describe protective isolation, but this was in the context of preventing patients with infectious diseases from being admitted to hospital in the first place and spreading infection.1 Throughout the early and mid decades of the 20th century, there were various recommendations from the USA and the UK which identified the need for private rooms to prevent infection, in some instances mechanically ventilated to provide clean air.1 Protective isolation facilities, especially those that consist of high efficiency particulate air (HEPA) and laminar airflow (LAF) have been advocated for the prevention of IA in immunocompromised patients or for patients with extensive burns, who are non-infected.2,4 However, it could be argued that for burns patients who are already infected, negative-pressure ventilation is required to prevent the organisms causing infection spreading to other parts of the unit.3

The protected environment of a room or unit commissioned for the prevention of infection in susceptible patients (Table I) usually consists of HEPA filters (99.97% efficient at 0.3 μm particles), at least 12 air changes per hour, sealed rooms, air direction from the patient to the exhaust systems, with re-circulation of air.4 When LAF is provided, the air is usually swept across the room parallel to the floor driving contaminants out through the ducts. This usually involves 400 air changes per hour or more and is expensive, uncomfortable due to draughts and noise, and may not appreciably add to the degree of protection, as discussed later. Apart from regional or referral units where burns patients are usually cared for, most of the issues pertaining to protective isolation relate to patients with malignancy, patients on chemotherapy, or patients after bone marrow or solid organ transplantation. In this group of patients, the purpose of protective isolation is to reduce or remove, in particular, the risk of fungal infection caused by moulds such as Aspergillus species, Mucorales/Rhizopus species, Scedosporium species, Acremonium species and Penicillium species. Most commonly it is the risk of IA that motivates the requirement for positive-pressure isolation.

Epidemiology and pathogenesis of IA

Aspergillus spores are ubiquitous, and in most normal healthy individuals are removed by the innate defences of the upper respiratory system, e.g. nasal hairs and ciliated respiratory epithelium. Probably only a minority of spores that enter the lower respiratory tract actually remain there and only some of these will germinate. Deposition and germination are probably pre-requisites for IA,
Imaging Findings in Acute Invasive Pulmonary Aspergillosis: Clinical Significance of the Halo Sign

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(See the editorial commentary by Vandewoude and Vogelaers on pages 380–1)

Background. Computed tomography (CT) of the chest may be used to identify the halo sign, a macronodule surrounded by a perimeter of ground-glass opacity, which is an early sign of invasive pulmonary aspergillosis (IPA). This study analyzed chest CT findings at presentation from a large series of patients with IPA, to assess the prevalence of these imaging findings and to evaluate the clinical utility of the halo sign for early identification of this potentially life-threatening infection.

Methods. Baseline chest CT imaging findings from 235 patients with IPA who participated in a previously published study were systematically analyzed. To evaluate the clinical utility of the halo sign for the early identification and treatment of IPA, we compared response to treatment and survival after 12 weeks of treatment in 143 patients who presented with a halo sign and in 79 patients with other imaging findings.

Results. At presentation, most patients (94%) had ≥1 macronodules, and many (61%) also had halo signs. Other imaging findings at presentation, including consolidations (30%), infarct-shaped nodules (27%), cavitary lesions (20%), and air-crescent signs (10%), were less common. Patients presenting with a halo sign had significantly better responses to treatment (52% vs. 29%; P<.001) and greater survival to 84 days (71% vs. 53%; P<.01) than did patients who presented with other imaging findings.

Conclusions. Most patients presented with a halo sign and/or a macronodule in this large imaging study of IPA. Initiation of antifungal treatment on the basis of the identification of a halo sign by chest CT is associated with a significantly better response to treatment and improved survival.

Severely immunocompromised patients, particularly those with hematopoietic stem cell transplants (HSCTs) or hematological malignancies, are at high risk for invasive fungal infection [1, 2]. Invasive pulmonary aspergillosis (IPA) is increasing in incidence in this population [3] and is associated with unacceptably high morbidity and mortality [4–6], especially when diagnosis and treatment are delayed [3, 7, 8]. Despite recent advances in antifungal treatment, clinical outcomes are still generally considered to be inadequate [5, 8–10], with the best reported response rates in the range of 50%–60% [9, 11, 12].

Response to treatment and survival can, potentially, be improved if specific antifungal treatment for IPA is initiated at an early stage of infection. However, an early diagnosis of IPA based on histopathological or mycological evidence can be difficult to establish [9, 13–15]. Sputum cultures are neither sensitive nor specific: positive sputum culture results do not always correlate with invasive disease, and negative sputum culture results are common in patients with proven IPA [16–18]. Coagulation abnormalities and coexisting thrombocytopenia may preclude invasive procedures for tissue har-
Imaging Findings in Acute Invasive Pulmonary Aspergillosis: Clinical Significance of the Halo Sign

Reginald E. Greene, Haran T. Schlamm, Jürg-W. Oestmann, Paul Stark, Christine Durand, Olivier Lortholary, John R. Wingard, Raoul Herbrecht, Patricia Ribaud, Thomas F. Patterson, Peter F. Troke, David W. Denning, John E. Bennett, Ben E. de Pauw, and Robert H. Rubin

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Current Status of Nonculture Methods for Diagnosis of Invasive Fungal Infections

Siew Fah Yeo and Brian Wong*

Infectious Disease Section, Department of Internal Medicine, Yale University School of Medicine, New Haven, and the Infectious Diseases Section, VA Connecticut Healthcare System, West Haven, Connecticut

INTRODUCTION

The frequency of invasive fungal infections has risen dramatically in recent years (209, 216). Early and accurate diagnosis of these infections is important for several reasons, including timely institution of antifungal therapy (164) and to decrease the unnecessary use of toxic antifungal agents. In addition, the availability of accurate and timely diagnoses could reduce the use of empirical antifungal therapy, thereby reducing antifungal selection pressure and the emergence of antifungal resistance. Unfortunately, a major obstacle to the successful treatment of invasive fungal infections is the lack of sensitive and specific methods for the early diagnosis of invasive fungal infections. Standard approaches to the laboratory diagnosis of invasive fungal infections include (i) direct microscopic visualization for the presence of organisms in freshly obtained body fluids, (ii) histopathologic demonstration of fungi within tissue sections, and (iii) cultivation of the causative fungus and its subsequent identification. However, these approaches often are not sufficiently sensitive and/or specific to diagnose invasive fungal infections, and they sometimes require invasive procedures to obtain the necessary specimens.

This work reviews recent advances of nonculture methods for the diagnosis of invasive aspergillosis, invasive candidiasis, cryptococcosis, blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, and penicilliosis. Among the nonculture methods we review, detection of a specific host antibody response is attractive because such tests can be performed rapidly and do not require invasive sampling procedures. However, presence of host antibodies does not always correlate with presence of invasive disease, especially in patients whose abilities to produce specific immunoglobulin responses may be impeded by immunosuppressive drugs and/or serious underlying diseases. Detection of macromolecular microbial antigens generally requires a relatively large microbial burden, which may limit assay sensitivity. Nonetheless, several
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However, immunoassays employing either crude antigen preparation or whole fungal cell have limitations. Hence, attempts have been made to circumvent this situation.

A *P. marneffei* gene that encodes a highly antigenic cell wall mannoprotein, Mp1p, has been cloned (35), and antibodies to Mp1p have been demonstrated by immunoprecipitation in *P. marneffei*-inoculated guinea pigs and in patients with penicilliosis (36). Another approach that used the purified recombinant Mp1p protein in an enzyme-linked immunosorbent assay (ELISA)-based antibody test detected 14 of 17 (82%) HIV-infected patients with penicilliosis. Moreover, the specificity of this test appeared to be very good; no false-positive reactions were noted in serum samples from healthy donors, patients with typhoid fever, or patients with tuberculosis (36). Although these studies showed good sensitivity and specificity with the purified recombinant Mp1p protein, prospective studies are required before this approach can be applied to routine usage.

**Summary**

Early attempts at antibody detection relied on the use of crude, unfractionated mixtures of antigens, and this resulted in major problems with cross-reactivity. Much of this cross-reactivity was due to nonprotein components (e.g., carbohydrates and phospholipid choline) that are present in crude antigens prepared directly from yeasts and mycelial stage, fungal cytoplasm, or fungal cell walls. Moreover, tests that rely on crude extracts may be impeded by difficulties in producing reproducible reagents. Hence, most recent efforts have focused on (i) developing more-defined antigens that are derived from the application of recombinant techniques and (ii) using more-advanced assay systems. The use of recombinant antigens prepared in a prokaryotic host can eliminate cross-reactivity due to posttranslational antigen modification. However, problems associated with false positives generated through prior exposure either through skin testing or to the organism itself, and with false negatives resulting from the development of infection in immunocompromised patients, such as those with advanced HIV infection, still exist.

**DETECTION OF FUNGAL ANTIGENS**

Another approach to diagnosis of invasive fungal infections is to use immunologic reagents to detect and quantify macromolecular fungal antigens in host body fluids. Ideal antigenic markers for invasive fungal infections should not be present too transiently, and they should be associated with infection rather than colonization. They should be conserved within the fungal species of interest, they should not cross-react with other human and microbial antigens, and they should be present sufficiently early for the starting of antifungal therapy. Moreover, tests for these antigens should be adaptable to formats that can be used in routine clinical laboratories, they should be easy to perform, and they should not be subject to significant interlaboratory variation.

A number of early studies focused on using cell wall components of fungal species as antigenic markers (3, 54, 116, 168, 220, 221). Among these markers are mannan and galactomannans, which have been shown to be useful in the diagnosis of invasive aspergillosis and candidiasis (see Tables 1 and 2).

Early efforts to detect antigenemia were often hampered by the use of insensitive methods with low detection limits (48, 49, 114, 132). Moreover, fungal mannans or galactomannans may be rapidly removed from circulation by the formation of immune complexes and by receptor-mediated endocytosis by Kupffer’s cells in the liver (48), thereby limiting the sensitivity of these diagnostic approaches. Hence, attempts have been made toward development of immunoassays with increased specificity and sensitivity for candidiasis and aspergillosis, and there has been much less interest in diagnosis of other systemic fungal infections.

These studies of antigenic markers were not limited to mannoprotein but also included polysaccharide capsular antigens, carbohydrates other than mannoproteins, and soluble proteins. However, some antigen detection assays described in the past employed either poorly standardized reagents or insensitive methodology (26, 48, 49, 80). In general, antigen tests that use polyclonal antibodies raised against crude fungal antigens have significant cross-reactivities with several pathogenic fungi (48, 80). For example, the radioimmunoassay (RIA) based on anti-*H. capsulatum* rabbit polyclonal antibody as capture and detector antibody to detect *H. capsulatum* polysaccharide antigen (HPA) exhibited some degree of cross-reaction with other organisms (71, 80, 228). In addition, assays that use polyclonal antibodies may be subject to variability among different batches of antisera. For instance, detection of cryptococcal capsular polysaccharide antigen by latex agglutination with polyclonal antibodies has proven highly sensitive since its inception in 1960s (11), but may be subject to the type of variation reported for polyclonal serum-based latex bead assays (242). Monoclonal antibody-based immunodiagnostic assays have several advantages over polyclonally based assays, which make the former likely to reduce such variability since they are readily available in unlimited quantities as appropriate and since monoclonal antibodies do not exhibit as much batch-to-batch variability.

This section will review how far the above requirements have been fulfilled and recent development of antigen detection in the diagnosis of invasive candidiasis, invasive aspergillosis, cryptococcosis, and some endemic mycoses.

**Invasive Aspergillosis**

Invasive aspergillosis is an increasingly recognized condition in immunocompromised hosts. However, the major problem associated with invasive aspergillosis is the difficulty in diagnosing this infection early enough to be of value in patient management. Cultures of blood or respiratory specimens are seldom positive, especially during the early stages of the disease. The reliable diagnosis of invasive aspergillosis requires invasive procedures to obtain biopsy material for histological and microbiological examination. However, the performance of invasive diagnostic procedures is often precluded by the critical condition of the patients. Antibody detection tests are used as an adjunct to microbiological methods for the diagnosis of invasive aspergillosis, but these tests are often negative because of the fulminating nature of the disease and/or the poor immunological status of the host (26, 49, 113, 114, 115, 216). Therefore, the detection of various antigenic markers of invasive aspergillosis is currently an area of great interest.
Uncommon yeast infections in hematological patients: from diagnosis to treatment


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Keywords: Blastoschizomyces; cryptococcosis; Rhodotorula spp.; trichosporonosis; yeast infection

Invasive fungal diseases (IFDs) have been recognized with increasing frequency as major pathogens in patients with malignancies over the past few decades, as a result of new and more aggressive anticancer treatments and supportive care [¹-⁴]. Patients with hematological malignancies (HMs), and in particular those with acute myeloid leukemia and those who have undergone allogeneic hematopoietic stem cell transplantation, are at highest risk for these infections [¹-⁶]. *Candida* spp. and *Aspergillus* spp. are the most common recognized causes of IFDs in patients with HMs [¹-²,⁷-⁹]. However, other typically less common yeasts and filamentous fungi (e.g., *Blastomyces* spp., *Cryptococcus* spp., *Trichosporon* spp. and Zygomycetes) are emerging in patients with HMs as significant human pathogens, frequently as breakthrough infections in patients receiving empirical antifungal therapy or antifungal prophylaxis [¹,²,¹⁰]. Although relatively rare, crude mortality rates up to approximately 70% have been reported for these fungal infections; in addition, the role of recently available antifungal agents, such as novel triazoles and echinocandins, on treatment outcome has not been clarified [¹¹,¹²]. This article is intended to discuss certain aspects of the approach to IFDs due to uncommon yeasts in patients with HMs, focusing on epidemiology, diagnosis, and treatment strategies and outcomes.

**Trichosporonosis**

**Trichosporon spp.**

*Trichosporon* spp. are widely distributed in nature and can predominantly be found in environmental substrates, such as soil, birds, vegetables, water and decomposing wood. These fungi can colonize skin and, less frequently, respiratory and GI tracts of humans [¹³].
Uncommon yeast infections in hematological patients: from diagnosis to treatment


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Probable Invasive Aspergillosis without Prespecified Radiologic Findings: Proposal for Inclusion of a New Category of Aspergillosis and Implications for Studying Novel Therapies

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(See the editorial commentary by Neofytos, on pages 1281–1283.)

Background. The European Organization for Research and Treatment of Cancer (EORTC) and the Mycosis Study Group (MSG) definition of invasive aspergillosis used in clinical trials lacks sensitivity. We hypothesize that giving lower weight to the prespecified radiologic findings in patients with a positive serum galactomannan index test result will improve the definition’s diagnostic sensitivity.

Methods. The medical records of 121 patients with 125 cases of invasive aspergillosis treated at a referral cancer institute from January 2003 through December 2009 were reviewed. Aspergillosis was diagnosed as EORTC-MSG proven or probable (controls, 83) or probable invasive aspergillosis without prespecified radiologic criteria (cases, 42). The latter differed from the former by the inclusion of patients whose pulmonary infiltrates, although well described in invasive aspergillosis, do not fulfill EORTC-MSG invasive aspergillosis requirements. The host, clinical, and mycologic characteristics and survival of cases and controls served as end points.

Results. A total of 114 (91%) of 125 patients had multiple myeloma. Patients had a median age of 65 years (range, 26–81 years), and 74 were male. All had received antineoplastic therapy, including stem cell transplantation (58 [46%]). Aspergillosis involved lungs (88 patients), sinuses (9 patients), or both (28 patients). Except for higher median baseline platelet count and shorter duration of neutropenia among cases, there were no statistically significant differences between groups on all predefined end points, including 4-, 6-, and 12-week survival. Eleven of 26 cases were reclassified as controls on the basis of subsequent imaging.

Conclusions. Except for less well-circumscribed consolidations, the host, clinical, radiologic, and mycologic characteristics and outcome of patients with probable invasive aspergillosis but without prespecified radiologic criteria are similar to those with EORTC-MSG invasive aspergillosis. Enrolling such patients in clinical trials of novel therapies will increase the pool of eligible study participants and improve trial speed and efficiency.

Invasive aspergillosis is a leading cause of invasive mycosis, affecting mostly patients with prolonged and profound neutropenia. The disease usually manifests with fever, cough, chest pain, and typical radiologic findings, and the diagnosis is confirmed by culture and histo-pathologic testing and/or by Aspergillus-specific serologic testing (serum Aspergillus galactomannan antigen index [GMI]). Despite the availability of effective drugs, the burden of invasive aspergillosis remains significant [1]. Attempts at improving the prognosis of invasive aspergillosis have relied on the early application of more sensitive diagnostic tests, such as chest computed tomography (CT) [2] and serodiagnosis with serial GMI [3] and/or 1-3-β–d-glucan (BDG) [4], and the evaluation of novel antifungal strategies in randomized clinical trials [5].

Because the lack of standard diagnostic criteria for invasive aspergillosis constituted a major hurdle for the conduct of randomized clinical trials, the European Organization for Research and Treatment of Cancer...
(EORTC) and the Mycosis Study Group (MSG) developed the EORTC-MSG consensus definitions for the diagnosis of invasive aspergillosis [6, 7]. These definitions require the presence of host factors and clinical, radiologic, and mycologic criteria, including culture and serodiagnosis (serum GMI or BDG). By making it possible to conduct randomized clinical trials worldwide, these definitions represented a major advance in the field. However, their diagnostic performance is low (25%–50%), compared with the gold standard of autopsy examination [8–11]. This carries important implications for the conduct of randomized clinical trials of novel therapies. Several factors explain the limited performance of these expert-derived diagnostic criteria.

The first factor is a lack of prospective validation. The second factor is skewing toward patients with advanced disease. These definitions were derived from older publications (1985–1997) [12], before the availability of serum GMI [3] and more effective therapies [5], and were more representative of patients with advanced disease. The third factor is poor test attributes. The pulmonary CT findings of the patients with EORTC-MSG–proven or –probable invasive aspergillosis are given a significant diagnostic weight despite the fact that they are nonspecific [13], observer dependent [5], not easily quantifiable, and transient, such as the halo sign [2]. They may even be misleading; indeed, worsening findings may represent an immune reconstitution and inflammatory syndrome in a patient whose invasive aspergillosis is resolving [14], and the air crescent sign and cavitory lesions may be seen with responding infection [15]. Furthermore, the radiologic findings at extrapulmonary sites are poorly defined and cannot be relied on to suggest invasive aspergillosis [7]. Unlike CT imaging, GMI serodiagnosis fulfills all criteria as a surrogate marker for the diagnosis and outcome evaluation of invasive aspergillosis [16], with high test performance in patients with hematologic malignant neoplasms [17] and very strong correlation with findings at autopsy examination [18]. Serial GMI monitoring has been shown to significantly shorten the time to diagnosis [17] and timely preemptive antifungal therapy [19]. This, in turn, is thought to positively alter the disease and slow the development of the more overt manifestations that are required by the EORTC-MSG invasive aspergillosis. Fourth, the EORTC-MSG invasive aspergillosis criteria have been derived from patients with profound and prolonged neutropenia and may not apply to those with severe T-cell immunodeficiency, such as solid organ or allogeneic hematopoietic stem cell transplant (HCT) recipients, patients with chronic hematologic malignant neoplasms, and others [20, 21]. These patients may present with more inconspicuous images, including ill-defined consolidations, ground-glass attenuation, small nodules (≤1 cm), and other findings [20, 22], none of which qualifies as a diagnostic criterion in the EORTC-MSG invasive aspergillosis [7].

Because of the expanding population at risk, the major clinical consequences of invasive aspergillosis, and the limited number of effective therapies, it is crucial that enrollment in randomized clinical trials of potentially effective therapies of invasive aspergillosis be facilitated for these trials to be performed quickly, efficiently, and at minimal costs [16].

The objective of this study was to identify criteria that would improve on the 25%–50% diagnostic sensitivity [8–11] of the EORTC-MSG invasive aspergillosis definitions. We hypothesized that patients at risk for invasive aspergillosis who fulfill all criteria for EORTC-MSG invasive aspergillosis (plus a requirement for a positive GMI test result), but who do not exhibit the prespecified radiologic criteria for EORTC-MSG invasive aspergillosis, in fact have invasive aspergillosis. In this report of 125 consecutive cases of invasive aspergillosis in 121 high-risk patients cared for in a myeloma center with frequent serial serologic testing, we show that host factors, clinical characteristics, mycologic criteria, and survival at 4, 6, and 12 weeks after diagnosis in 42 such patients (referred to as probable invasive aspergillosis without prespecified radiologic criteria) are almost identical to those of 83 patients with proven or probable EORTC-MSG invasive aspergillosis.

PATIENTS AND METHODS

This is a retrospective evaluation of a cohort of 125 consecutive episodes of invasive aspergillosis in 121 cancer patients who were prospectively followed up by one of us (E.A.) and cared for at the Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, from January 2003 through December 2009. The study was approved by the Institutional Review Board. Subjects consisted of patients with cancer at risk for invasive aspergillosis because of immunosuppressive therapies. All patients were managed according to the predefined standards of care at the Myeloma Institute for Research and Therapy. For the purpose of the present study, 2 of the authors (S.A.N. and M.N., both physicians with expertise in invasive aspergillosis) reviewed patients’ medical records, including all images (chest radiographs, CTs, and positron emission tomograms).

Cases were classified as proven or probable according to the revised EORTC-MSG criteria [7] (control group) or as probable invasive aspergillosis without prespecified radiologic criteria (study group). This latter category fulfilled the same criteria as the former (plus a required positive serum GMI test result), except that the abnormal pulmonary infiltrates findings did not fulfill the 3 following EORTC-MSG radiologic criteria: dense, well-circumscribed lesions, air crescent sign, or cavity. A diagnosis of sinusitis was made in the presence of clinical findings (dry cough, postnasal dripping, nasal obstruction, nasal discharge, or sinus pain) and CT images compatible with sinusitis. All findings were temporarily related to the period at risk, and
Infectious diseases of the respiratory system which can easily result to Pneumonia


Fonom Theophilus Makama

Aspergillosis 3 years ago

Background

Aspergillus species are ubiquitous molds found in organic matter. Although more than 100 species have been identified, the majority of human illness is caused by Aspergillus fumigatus and Aspergillus niger and, less frequently, by Aspergillus flavus and Aspergillus clavatus. The transmission of fungal spores to the human host is via inhalation. Also see the eMedicine articles Aspergillosis (dermatology focus), Aspergillosis (pediatric focus), and Aspergillosis, Thoracic (radiology focus).

Aspergillus may cause a broad spectrum of disease in the human host, ranging from hypersensitivity reactions to direct angioinvasion. Aspergillus primarily affects the lungs, causing 4 main syndromes, including allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing Aspergillus pneumonia (or chronic necrotizing pulmonary aspergillosis [CNPA]), aspergilloma, and invasive aspergillosis. However, in patients who are severely immunocompromised, Aspergillus may hematogenously disseminate beyond the lung, potentially causing endophthalmitis, endocarditis, and abscesses in the myocardium, kidney, liver, spleen, soft tissue, and bone. Aspergillus is second to Candida species as a cause of fungal endocarditis. Aspergillus -related endocarditis and wound infections occur in the context of cardiac surgery.

ABPA is a hypersensitivity reaction to A fumigatus colonization of the tracheobronchial tree and occurs in conjunction with asthma and cystic fibrosis (CF). Allergic fungal sinusitis may also occur alone or with ABPA. Bronchocentric granulomatosis and malt worker's lung are 2 hypersensitivity lung diseases that are caused by Aspergillus species, but they are rare.

An aspergilloma is a fungus ball (mycetoma) that develops in a preexisting cavity in the lung parenchyma. Underlying causes of the cavitary disease may include treated tuberculosis or other necrotizing infection, sarcoidosis, CF, and emphysematous bullae. The ball of fungus may move within the cavity but does not invade the cavity wall; however, it may cause hemoptysis.

CNPA is a subacute process usually found in patients with some degree of immunosuppression, most commonly that associated with underlying lung disease, alcoholism, or long-term
corticosteroid therapy. Because it is uncommon, CNPA often remains unrecognized for weeks or months and can cause a progressive cavitary pulmonary infiltrate.

Invasive aspergillosis is a rapidly progressive, often fatal infection that occurs in patients who are severely immunosuppressed, including those who are profoundly neutropenic, those who have received bone marrow or solid organ transplants, and patients with advanced AIDS1 or chronic granulomatous disease. This infectious process is characterized by invasion of blood vessels, resulting in multifocal infiltrates, which are often wedge-shaped, pleural-based, and cavitary. Dissemination to other organs, particularly the central nervous system, may occur.

Pathophysiology

Aspergillus causes a spectrum of disease, from colonization to hypersensitivity reactions to chronic necrotizing infections to rapidly progressive angioinvasion, often resulting in death. Rarely found in individuals who are immunocompetent, invasive Aspergillus infection almost always occurs in patients who are immunosuppressed by virtue of underlying lung disease, immunosuppressive drug therapy, or immunodeficiency.

Aspergillus hyphae are histologically distinct from other fungi in that the hyphae have frequent septae, which branch at 45° angles. The hyphae are best visualized in tissue with silver stains. Although many species of Aspergillus have been isolated in nature, A fumigatus is the most common cause of infection in humans. A flavus and A niger are less common. Likely, this relates to the ability of A fumigatus, but not most other Aspergillus species, to grow at normal human body temperature.

Human host defense against the inhaled spores begins with the mucous layer and the ciliary action in the respiratory tract. Macrophages and neutrophils encompass, engulf, and eradicate the fungus. However, many species of Aspergillus produce toxic metabolites that inhibit macrophage and neutrophil phagocytosis. Corticosteroids also impair macrophage and neutrophil function. Underlying immunosuppression (eg, HIV disease, chronic granulomatous disease, pharmacologic immunosuppression) also contributes directly to neutrophil dysfunction or decreased numbers of neutrophils. In individuals who are immunosuppressed, vascular invasion is much more common and may lead to infarction, hemorrhage, and necrosis of lung tissue. Persons with CNPA typically have granuloma formation and alveolar consolidation. Hyphae may be observed within the granulomata.
Invasive aspergillosis (IA) still remains difficult to diagnose and hard to treat in immunocompromised patients. The mortality rate due to this life-threatening opportunistic mycosis is as high as 50-100% and early diagnosis and prompt initiation of antifungal therapy remain as the crucial factors that may aid in reduction of the mortality rates. Definitive diagnosis of IA by cultivation of the infecting strain requires surgical biopsy of a sterile site for optimal results, may take as long as 4 weeks, and may be confounded by contamination. Although biopsy and culture will doubtless remain as gold standard tests, there is a remarkable demand for additional tests that may help in early diagnosis of this infection.

Among these, detection of galactomannan antigen in serum and body fluids has been intensively studied in recent years. Galactomannan is a component of the fungal cell wall and an exoantigen of *Aspergillus*. The first test to detect this antigen used a latex agglutination (LA) method. Subsequently, an ELISA-based method was developed which provided higher sensitivity and specificity compared to LA [2125]. The ELISA-based kit is commercially available as "Platelia Aspergillus EIA" (Bio-Rad Laboratories). Galactomannan antigen positivity is among the microbiological diagnostic criteria proposed by European Organization for Research and Treatment of Cancer (EORTC) and Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) for diagnosis of IA. The kit has been available for some time in Europe and was cleared for diagnostic use in the United States by the FDA on 16 May 2003. The cut-off used in the European kit was an index of 1.0 for indeterminate and 1.5 for positive. The FDA cleared version of the test kit uses a cut-off of 0.5 index value and demonstrated good sensitivity and specificity claims in the clinical testing from three separate medical centers.

Here is a summary of the key data regarding use of the galactomannan antigen test in the diagnosis of IA:

1. The test can detect as little as 0.5-1 ng/ml of galactomannan in the tested sample [2003].

2. The most frequently tested sample and the one which provides highest sensitivity is serum [1840]. Other samples, such as bronchoalveolar lavage, urine [1840] and cerebrospinal fluid [2123, 2139] may also be beneficial in diagnosis of IA, but the kit is standardized and FDA cleared only for serum samples. The Platelia Aspergillus EIA has not been evaluated for use with plasma or other sample types such as urine, BAL or CSF in the US. In addition, the performance of the Platelia Aspergillus EIA has not been evaluated in neonate or pediatric serum samples in the US.

3. The galactomannan antigen test is a screening test and should be employed as such. Collection of serum samples should be initiated right after hospitalization in patients at high risk of developing IA. Samples should be collected (at least) twice weekly from then on [1284]. The concomitant use of mould-active antifungal therapy in some patients with IA may result in reduced sensitivity with the Platelia Aspergillus EIA.

4. Detection of positive results particularly in two consecutive serum samples provides strong support for the
by performing a treatment of a new aliquot of the serum sample and also collecting another sample for testing. Samples should be reported as negative or positive for galactomannan. The index value for positive samples can prove to be useful. Negative samples should just be reported as negative. A negative test cannot rule out the diagnosis of IA. Repeat testing of additional samples should be considered where there is clinical suspicion of IA or a procedural error.

5. Galactomannan detection can provide two types of information. First, early diagnosis is possible if the specimens are being tested upon collection. Galactomannan antigen positivity can be detected 5-8 days (average) before clinical signs develop (in 65.2% of patients), findings on chest X-ray are visible (in 71.5% of patients) and culture results become positive (in 100% of patients) [1283]. A second use is to provide negative data in patients with syndromes compatible with aspergillosis. In such settings, the absence of galactomannan positivity may provide support for more aggressive traditional diagnostic measures.

6. The sensitivity and specificity of the test are favorable. In the dataset evaluated by FDA, the overall sensitivity and specificity of the method were 80.7% and 89.2%, respectively. For this evaluation, 1890 blood samples collected from 170 patients were tested. Among these, 31 patients had proven or probable IA. Galactomannan antigen was detected in 25 of these 31 patients with a resulting sensitivity of 80.7%. Of 148 patients who did not have IA, 132 were negative for galactomannan antigen, yielding a specificity of 89.2%. Of these 148 patients, a total of 1362 sera were obtained and tested. 1343 of these 1362 sera were initially negative, resulting in a sample agreement of 98.6% with a 95% CI of 97.9-99.3. On repeat testing, 1355 of the 1362 sera were negative.

7. As with any serological test, false positive reactions may be observed. It has been detected in 1-18% of the tested samples [2264, 1284]. False positivity may originate from true antigenic cross reactivity with other fungi such as *Penicillium chrysogenum*, *Penicillium digitatum*, and *Paecilomyces variotii* [2024]. A pattern of false-positive antigenemia that is most common in children [1204] has been noted and may possibly develop after translocation of galactomannan antigen found in various foodstuffs (unsalted bread, macaroni, corn flakes, salted rice, dry cake, turkey slices, grilled sausage, fried potatoes, etc.) through the damaged intestinal mucosa [1204].

8. As well as early diagnosis, the test may also be helpful in assessment of the prognosis and the clinical response to antifungal therapy [1583, 1788, 283]. In cases of a favorable clinical response, the titer of the antigen tends to either decline or does not change significantly compared to the baseline titer. In contrary, it increases significantly in case of treatment failure [1840, 2123, 283]. Of note, and possibly due to the distinctive mode of action of the drug, galactomannan antigenemia persisted despite clinical improvement in animals who were treated with caspofungin [1609]. This finding suggests that same may apply to patients who are treated with an echinocandin compound.
Aspergillosis

Diagnostic Criteria

Diagnostic criteria for invasive aspergillosis

Confirmed invasive aspergillosis (IA)

- Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae are seen accompanied by evidence of tissue damage and/or recovery of mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid (BAL), a cranial sinus cavity specimen, and urine.

Probable IA

- Requires 1 host factor, 1 clinical criterion, and 1 mycological criterion to be present (see below).

Possible IA

- Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent are considered possible IA (see below).

Host factors

- Recent history of neutropenia (<0.5 x 10^9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of fungal disease.
- Receipt of an allogeneic stem cell transplant.
- Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for 13 weeks.
- Treatment with other recognized T-cell immunosuppressants, such as cyclosporine, TNF-alpha blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogs during the past 90 days.
- Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency).

Clinical criteria (must be consistent with the mycological findings, if any, and must be temporally related to current episode)

- Lower respiratory tract infection (presence of 1 of the following 3 signs on CT):
# Aspergillosis

## Diagnostic Criteria

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- Lower respiratory tract infection (presence of 1 of the following 3 signs on CT):
Utility of Galactomannan Enzyme Immunoassay and (1,3) β-D-Glucan in Diagnosis of Invasive Fungal Infections: Low Sensitivity for Aspergillus fumigatus Infection in Hematologic Malignancy Patients

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Previous studies have reported that galactomannan (GM) enzyme immunoassay and 1,3 beta-glucan (BG) assay may be useful diagnostic tools, but their sensitivities are variable. We compared the performances of both tests. Between October 2002 and May 2005, 82 patients were prospectively monitored for 12 weeks. A total of 414 samples were tested by GM assay and 409 samples were tested by BG assay for the following four groups of patients: those with invasive aspergillosis (IA), those with other mold infections (Fusarium, scedosporium, zygomycosis, etc.), those with candidemia, and control patients. Blood samples were obtained twice on week 1 and once every other week for a total of 12 weeks. Patients in the invasive fungal infection groups had comparable risk factors. The sensitivity of the GM test was significantly higher for patients with IA due to non-Aspergillus fumigatus species than for patients with IA due to Aspergillus fumigatus (49% versus 13%; \( P < 0.0001 \)) or with other mold infections (49% versus 6%; \( P < 0.0001 \)). However, the sensitivity range (47% to 64%) and specificity (88%) of the BG assay were comparable among all patients tested, regardless of the infecting pathogen. The performance of GM-based diagnosis appears to be better for detecting non-Aspergillus fumigatus species. The diagnostic marker BG was shown to have a higher sensitivity than that of GM in detecting IA and other mold infections in hematologic malignancy patients.

The incidence of invasive fungal infection (IFI) has increased dramatically during the last decade. These infections are associated with high morbidity and mortality, ranging from 60% to 90%, especially in hematologic malignancy patients in the setting of neutropenia and hematopoietic stem cell transplantation (1, 5, 9, 13, 24, 30). The critical problem is the difficulty in making the diagnosis. Unfortunately, delayed diagnosis and therapy for invasive aspergillosis (IA) are associated with poor outcomes and high mortality regardless of the therapeutic modalities used (11). Hence, there has been an increased search for better noninvasive diagnostic methods for IA. Galactomannan (GM) seems to be the most studied diagnostic marker, followed by 1,3 β-D-glucan (BG) (4, 10, 12, 16, 18, 21, 23, 25, 31, 32, 33). Moreover, until now, only a few prospective comparative studies of GM and BG have been performed (14, 25, 29). With regard to GM assay, the test has been commercially available in Europe since the mid-1990s and recently received FDA approval in the United States. However, the reported sensitivity rate has been variable, with a range from 30% to 100%, and the specificity ranges from 38% to 98% (28). This wide range of results may be due to several factors, including various numbers of serum samples collected from patients at different institutions, the severity of infections, and the impact of prior antifungal therapy on the levels of circulating fungal components in the serum (19, 20). Many studies were retrospective in nature and had a limited number of proven fungal infections. Also, the heterogeneity of the study populations is understudied. Similarly, variable results have been reported for BG assay, with a slightly higher sensitivity and specificity, ranging from 70% to 90% (14, 23).

We therefore conducted this study to determine the usefulness of GM and BG assays for diagnosis of hematologic malignancy patients.

MATERIALS AND METHODS

Study population. Between October 2002 and March 2005, 82 patients were prospectively monitored for 12 weeks and divided into the following groups: 22 patients with IA (proven or probable), chosen according to the criteria developed by the consensus of the European Organization of the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (2); 17 patients with other mold infections, such as Fusarium infection, zygomycosis, and scedosporium; and 23 patients with candidemia. In addition, we selected 20 control nonneutropenic patients with solid tumors and without any radiological or clinical evidence of IA or risk factors for IFI. Blood was obtained twice on week 1 and once every other week for a total follow-up of 12 weeks. Patient demographics and clinical characteristics were collected, including age, underlying disease, type of transplantation, steroid use, neutropenia, and antifungal therapy used during the study period. This study was approved by the M. D. Anderson Cancer Center Institutional Review Board.

BG analysis of serum. Blood samples were collected in sterile, BG-free clotting tubes. Serum was separated by centrifugation and stored at −80°C until testing. BG levels in the serum were assayed using a Fungitell kit (Associates of Cape Cod, East Falmouth, MA) according to the manufacturer’s specifications. BG levels were quantitated against a purified Pachyman standard, which includes a five-point twofold curve ranging from 100 pg/ml to 6.25 pg/ml. The cutoff was 80 pg/ml. In brief, 5 μl of serum per well was dispensed in triplicate and pretreated by addition of 20 ml of 0.125 M KOH–0.6 M KCl and incubation for 10 min at 37°C. This step inactivated protease and other inhibitors present in

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excluded from the analysis because his *Aspergillus* species information was unknown. The chi-square test indicated that the sensitivity of the GM test was significantly higher for patients with non-*fumigatus* Aspergillus species infection than for patients with *A. fumigatus* infection (49% versus 13%; *P < 0.0001*) and patients with other mold infection (49% versus 6%; *P < 0.0001*) (Table 3). However, the sensitivities of the BG test were comparable among these three groups of patients, regardless of the *Aspergillus* species or other molds as the source of the infection (*P = 0.14* (Table 3)). We tested 409 samples collected from patients with IA, other mold infections, and candidemia. The overall sensitivities of the BG test were 58%, 61%, and 47%, respectively, with a cutoff of 80 pg/ml.

In this study, all patients with IFI received antifungal therapy, except for two patients with candidemia. Antifungal agents included amphotericin B (Ambisome), caspofungin, fluconazole, voriconazole, itraconazole, and posaconazole. For patients with IA, the impact of different antifungal drugs on diagnostic performance was evaluated. For patients with other mold infections, we found that the sensitivity of the BG test was 69% higher for patients receiving piperacillin-tazobactam (*P = 0.09*). This may be due to false-positive testing in the presence of the drug. For patients with aspergillosis, the impact of polyenes or azoles as well as caspofungin on the diagnostic performance was also evaluated (data not shown). Although no significant difference in the impact on test performance between these agents was found, it was noted that 39% of the caspofungin group samples tested positive based on the GM test, compared to 21% of samples testing positive when patients were not receiving this drug (*P = 0.14*).

Of the patients infected by other molds, 16 were included for evaluating the sensitivity per patient, including 8 patients with disseminated fungal infections and 8 patients without disseminated fungal infections. Based on the BG tests, four patients (50%) with disseminated infections and six patients (75%) with nondisseminated fungal infections tested positive. On the other hand, only one patient with another mold infection tested positive by GM assay.

When we combined these two assays for diagnosis, the sensitivity did not increase much, as most patients (90%) who tested positive by the GM test were also positive by the BG test. For aspergillosis patients, the sensitivity increased to 71%, compared to 67% for the BG test alone. For candidemia patients, since the sensitivity based on the GM test was zero, the sensitivity based on the combination of tests was the same as that of the BG test (62%). Similarly, due to low sensitivity based on the GM test, the sensitivity of the combination of tests for the patients with other mold infections was also the same as that of the BG test alone (63%). The specificity was the same as that based on the BG test alone (90%).

### DISCUSSION

Making a definite diagnosis of IFI remains a challenge. Culture of nonsterile fluid, such as respiratory specimens, lacks sensitivity in the setting of invasive mold infection and specificity in the setting of invasive candidiasis (1, 33). Invasive procedures relying on tissue biopsy or histopathological specimens are still considered the gold standard for establishing the diagnosis. However, these procedures are rarely performed, especially in the setting of immunosuppression or patients with thrombocytopenia, where such invasive procedures can be life-threatening.

In our study, we demonstrated that for a population of high-risk hematologic malignancy patients already receiving antifungal therapy, GM assay was significantly better at diagnosing IFI due to non-*fumigatus* Aspergillus species than that due to *A. fumigatus*, whereas BG antigen detection was similar for most fungi.

This is the first clinical study of patients with hematologic malignancy showing that GM assay detection is more frequent for IA due to non-*fumigatus* Aspergillus species than for that due to *A. fumigatus*. This observation is supported by the in vitro study by Mennink-Kersten et al. (22) whereby they demonstrated that the quantity of GM released can vary according to *Aspergillus* species. Higher GM concentrations were seen with *A. terreus*, *A. niger*, and *A. nidulans* than with *A. fumigatus*.

GM assay sensitivity for IA has varied markedly among studies, from as low as 30% to as high as 100% (16, 17, 21, 26, 28). This variability in the assay may be related in part to the hosts and their exposure to antifungal agents. All patients in our study were on antifungal agents, which may explain the lower sensitivity of the GM assay (49%). Several studies reported the impact of antifungal agents lowering the antigen level by decreasing the fungal load (19, 20, 28), making the test less useful for patients receiving mold-active antifungal agents. Marr et al. showed that the sensitivity of GM was reduced from 80% for a nonexposed group to 20% for a group exposed to mold-active antifungal agents (19, 20). In our study, we further investigated the impact of polyenes and azoles, as well as caspofungin, on the diagnostic performance of the GM assay. Although no significant differences in the impact on test performance between these agents were found, it was noticed that testing of samples from patients receiving caspofungin had a slightly higher sensitivity (39% of samples were positive) than did testing of samples collected from patients not receiving this drug (21% of samples were positive) (*P = 0.14*). Similarly, Klont et al. reported a paradoxical increase in circulating GM after caspofungin treatment for proven IA (15).

Furthermore, we evaluated the correlation between the kinetics of serum GM and the clinical outcome of IFI in our study. The GM test was positive 56% of the time among the patients who failed antifungal therapy, compared to 25% for...
Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive

Potential conflicts of interest. B.d.P. has been an advisor/consultant for Basilea Pharmaceutica and Ipsat Therapies and has been on the speakers’ bureau for Gilead Sciences, Merck & Co (Merck), and Pfizer. J.P.D. has received grant support from AM-Pharma, Basilea Pharmaceutica, and Schering-Plough; has been an advisor/consultant for Gilead Sciences, Ipsat Therapies, and Pfizer; has been on the speakers’ bureau for Gilead Sciences, Janssen Pharmaceuticals, Pfizer, Schering-Plough, and Xian-Janssen; and has received travel grants from Merck Sharp & Dohme and UCB Pharma. J.E.E. has been an advisor/consultant for Cerexa, Merck, and Pfizer; has received grant support from Gilead Sciences, the National Institutes of Health, Merck, and Pfizer; and holds shares of NovoDigm Therapeutics. 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Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group

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Abstract

Background—Invasive fungal diseases are important causes of morbidity and mortality. Clarity and uniformity in defining these infections are important factors in improving the quality of clinical studies. A standard set of definitions strengthens the consistency and reproducibility of such studies.

Methods—After the introduction of the original European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group definitions, advances in diagnostic technology and the recognition of areas in need of improvement led to a revision of this document. The revision process started with a meeting of participants in 2003, to decide on the process and to draft the proposal. This was followed by several rounds of consultation until a final draft was approved in 2005. This was made available for 6 months to allow public comment, and then the manuscript was prepared and approved.

Results—The revised definitions retain the original classifications of “proven,” “probable,” and “possible” invasive fungal disease, but the definition of “probable” has been expanded, whereas the scope of the category “possible” has been diminished. The category of proven invasive fungal disease can apply to any patient, regardless of whether the patient is immunocompromised, whereas the probable and possible categories are proposed for immunocompromised patients only.

Conclusions—These revised definitions of invasive fungal disease are intended to advance clinical and epidemiological research and may serve as a useful model for defining other infections in high-risk patients.
aspergillosis, were present. Indeed, the definitions were modified to allow enrollment of similar cases into clinical trials, because they are considered to represent likely invasive fungal disease even without supporting mycological evidence [2,16]. This pragmatic approach solved the problem of recruitment of representative cases, but it clearly highlighted the need to refine further the definitions, to distinguish dubious cases from the more likely cases when mycological evidence was not forthcoming. The growing body of evidence regarding the value of high-resolution CT of chest and abdomen [17] and of indirect diagnostic tests—such as the detection of galactomannan in body fluids other than serum and plasma, of $\beta$-D-glucan in serum, and of fungal DNA in body fluids by PCR—provided additional incentive to review the definitions [18,19]. The original definitions were also restricted to patients with cancer and to recipients of hematopoietic stem cell transplants; however, invasive fungal infections are known to affect other populations, including recipients of solid-organ transplants and patients with primary immunodeficiencies (e.g., chronic granulomatous disorder) [20,21]. Finally, it was considered appropriate to explore the possibility of formulating specific criteria for diseases caused by less common fungal pathogens.

**Revision Process**

The EORTC/MSG Consensus Group met in Chicago, Illinois, on 14 September 2003 during the 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) and included 13 members from the EORTC and 17 from the MSG. J. Powers also participated for the US Food and Drug Administration (FDA), and there were 5 observers from 4 pharmaceutical companies (J. Rex [Astra Zeneca], C. Sable [Merck], M. Bresnik [Gilead], and G. Triggs and A. Baruch [Pfizer]). B.d.P. and T.J.W. were confirmed as joint chairs, and J.P.D. was designated as secretary for the group. Three subcommittees were appointed to prepare proposals for mold infection, candidiasis, and endemic mycoses. The proposals were collated by the secretary, who integrated them into a general framework. They were then circulated by electronic mail to all group members. The ensuing comments were centrally combined for a subsequent round of electronic consultation. The remaining issues that appeared difficult to solve by the electronic route were addressed in open meetings during the 15th European Congress of Clinical Microbiology and Infectious Disease in Copenhagen, Denmark, and the 45th Annual ICAAC in Washington, DC. A majority vote was decisive when a consensus among the members could not be achieved. The final draft was made available to the wider community for comment at the Doctor Fungus Web site [22] and The Aspergillus Web site [23]. Thereafter, the manuscript was prepared and was circulated among all group members for their final approval.

At the first meeting, all group members agreed to the need to refine and revise the definitions. It was also agreed unanimously that the definition set should remain easily reproducible and should offer the opportunity for a reasonable comparison of future data sets with data sets that had been collected in clinical trials that involved patients with proven and probable invasive fungal infections according to the original definitions. Finally, the group set out to reexamine the feasibility of using the definitions for treatment purposes, to devise a means of extending their applicability to other patient groups, to review the relevance of the findings obtained from studies based on the definitions for clinical practice, and to attempt to incorporate all the available laboratory tests and imaging techniques into the definitions.

**Revised Definitions**

The term “invasive fungal disease” (IFD) was adopted to reflect more accurately the notion that we are dealing with a disease process caused by fungal infection. An adequate diagnostic evaluation of the infectious disease process, to exclude an alternative etiology, was deemed to be a necessary prerequisite to classify it as an IFD. The group reaffirmed that the definitions...
should be used only to assist in research and that the integrity of the original definitions with the classifications of proven, probable, and possible IFD would be preserved (Tables 1–3). Infections caused by *Pneumocystis jiroveci* are not included. The criteria for proven and probable IFD (Tables 1 and 2) were modified to reflect advances in indirect tests whereas the category of possible IFD (Table 3) was revised to include only cases that are highly likely to be caused by a fungal etiology, although mycological evidence is lacking. Hence, the definitions of probable and possible IFD were based on the same 3 elements as were the original definitions: host factors, clinical manifestations, and mycological evidence.

Host factors are not synonymous with risk factors but are characteristics by which individuals predisposed to acquire IFD can be recognized. Consequently, the presence of fever was removed as a host factor because it represents a clinical feature, not a host factor, and is nonspecific for IFD. The host factors were extended to receipt of a solid-organ transplant, hereditary immunodeficiencies, connective tissue disorders, and receipt of immunosuppressive agents—for example, corticosteroids or T cell immunosuppressants, such as calcineurin inhibitors, anti–TNF-α drugs, anti-lymphocyte antibodies, or purine analogues. The distinction between “minor” and “major” clinical criteria was abandoned in favor of more-characteristic and objectively verifiable evidence, such as the findings on medical imaging that indicated a disease process consistent with IFD by use of a standardized glossary of definitions. For example, in the case of chest CT imaging to categorize pulmonary lesions, the vast majority of immunocompromised patients with invasive pulmonary aspergillosis have focal rather than diffuse pulmonary infiltrates and present with at least 1 macronodule, with or without a halo sign [24]. These infections can also manifest as wedge-shaped infiltrates and segmental or lobar consolidation. Although none of the imaging findings is pathognomonic for IFD, the observation that, in the appropriate patient population, the outcome of antifungal therapy did not differ between febrile patients with nodular lesions and patients with mycological evidence of an IFD supports the use of this clinical criterion [17]. A similar consideration applies to patients with lesions on CT or ultrasound that are regarded as typical for chronic disseminated candidiasis. In the original definitions, patients with such lesions were defined as having probable hepatosplenic candidiasis without any need for mycological support. In the revised definitions, such cases are classified as possible IFD, thereby retaining the consistency of the definitions and preserving the distinction between probable IFD and possible IFD. For a patient with appropriate host factors and clinical evidence of pulmonary disease, bronchoalveolar lavage fluid that yields *Aspergillus*, *Zygomycetes*, *Fusarium*, or *Scedosporium* species or other pathogenic molds would constitute mycological support and would allow the case to be classified as probable pulmonary IFD.

As with the original definitions, indirect tests were considered for inclusion only if they were validated and standardized. Furthermore, because commercial tests for diagnostic use had to provide criteria for interpretation to gain approval, it was decided to rely entirely on the thresholds recommended by the manufacturer. On the basis of recent studies, the Platelia *Aspergillus* galactomannan EIA could be applied to CSF and bronchoalveolar lavage fluid, as well as plasma and serum. The β-D-glucan assay also was included as a marker for probable IFD, because this test detects other species of fungi besides *Aspergillus*, and a commercial test for it (Fungitell assay; Associates of Cape Cod) has been approved by the FDA. By contrast, molecular methods of detecting fungi in clinical specimens, such as PCR, were not included in the definitions because there is as yet no standard, and none of the techniques has been clinically validated.
These revised definitions of IFD categories are intended to advance clinical and epidemiological research and, as such, may serve as a useful model for defining other infections in high-risk patients. The definitions are not meant to be used to guide clinical practice but must be applied consistently if they are to continue to achieve their primary goal of fostering communication, furthering our understanding of the epidemiology and evolution of IFD, and facilitating our ability to test the efficacy of therapeutic regimens and strategies.

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We thank Chris Bentsen, Malcolm Finkelman, Richard Summerbell, Maiken Cavling Arendrup, Brigitte Crepin, and John H. Rex for their constructive comments.

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References

Table 2
Criteria for probable invasive fungal disease except for endemic mycoses

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<thead>
<tr>
<th>Host factors&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td>Recent history of neutropenia (&lt;0.5 × 10&lt;sup&gt;9&lt;/sup&gt; neutrophils/L [&lt;500 neutrophils/mm&lt;sup&gt;3&lt;/sup&gt;] for &gt;10 days) temporally related to the onset of fungal disease</td>
</tr>
<tr>
<td>Receipt of an allogeneic stem cell transplant</td>
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<tr>
<td>Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for &gt;3 weeks</td>
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<tr>
<td>Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days</td>
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<td>Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)</td>
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<tr>
<th>Clinical criteria&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Lower respiratory tract fungal disease&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>The presence of 1 of the following 3 signs on CT:</td>
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<tr>
<td>Dense, well-circumscribed lesions(s) with or without a halo sign</td>
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<tr>
<td>Air-crescent sign</td>
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<tr>
<td>Cavity</td>
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<tr>
<td>Tracheobronchitis</td>
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<tr>
<td>Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis</td>
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<tr>
<td>Sinonasal infection</td>
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<tr>
<td>Imaging showing sinusitis plus at least 1 of the following 3 signs:</td>
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<tr>
<td>Acute localized pain (including pain radiating to the eye)</td>
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<tr>
<td>Nasal ulcer with black eschar</td>
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<tr>
<td>Extension from the paranasal sinus across bony barriers, including into the orbit</td>
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<tr>
<td>CNS infection</td>
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<tr>
<td>1 of the following 2 signs:</td>
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<tr>
<td>Focal lesions on imaging</td>
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<tr>
<td>Meningeal enhancement on MRI or CT</td>
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<tr>
<td>Disseminated candidiasis&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks:</td>
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<tr>
<td>Small, target-like abscesses (bull's-eye lesions) in liver or spleen</td>
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<tr>
<td>Progressive retinal exudates on ophthalmologic examination</td>
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<th>Mycological criteria</th>
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<tr>
<td>Direct test (cytology, direct microscopy, or culture)</td>
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<tr>
<td>Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:</td>
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<tr>
<td>Presence of fungal elements indicating a mold</td>
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<tr>
<td>Recovery by culture of a mold (e.g., Aspergillus, Fusarium, Zygomycetes, or Scedosporium species)</td>
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<tr>
<td>Indirect tests (detection of antigen or cell-wall constituents)&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td><strong>Aspergillus</strong></td>
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<tr>
<td>Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF</td>
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<tr>
<td>Invasive fungal disease other than cryptococcosis and zygomycoses</td>
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<tr>
<td>β-D-glucan detected in serum</td>
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**NOTE.** Probable IFD requires the presence of a host factor, a clinical criterion, and a mycological criterion. Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent are considered possible IFD.

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We evaluated autopsy-proven invasive fungal infections (IFI) in patients with hematologic malignancies over three periods (1989-1993, 1994-1998, and 1999-2003). The autopsy rate declined significantly (67%-34%-26%, respectively, p<0.0001). IFI were identified in 314 (31%) of 1017 autopsies. Most IFI (75%) were not diagnosed ante-mortem. The prevalence of invasive mold infections increased significantly (19%-24%-25% p=0.05) in parallel with the emergence of Zygomycetes (0.9%-4%-3%; p=0.03). The prevalence of all other IFI remained relatively constant. Among patients with invasive pulmonary aspergillosis, those with graft-versus-host disease had a histopathological pattern distinct from those with neutropenia. The complex and evolving epidemiology of IFI in severely immunocompromised patients is not well captured by current diagnostic methods.

Key words: autopsy, aspergillus, Candida, Zygomycetes, hematologic malignancies.

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Trends in the Postmortem Epidemiology of Invasive Fungal Infections at a University Hospital

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Background. Due to the lack of reliable diagnostic tools, clinical data on the significance of most invasive fungal infections are difficult to assess and information on frequency, disease pattern and prognostic impact still largely relies on autopsy data.

Methods and results. To determine temporal trends in invasive fungal infections, we analyzed data from 8124 autopsies performed between 1978 and 1992 on patients who died at the University Hospital of Frankfurt/Main. During that period, a total of 278 invasive fungal infections were found. The prevalence rose from 2.2% (1978-82) and 3.2% (1983-87) to 5.1% in the most recent years (P<0.001). Besides the emergence of mixed and unclassified infections, this was mainly due to a significant increase in Aspergillus infections (P<0.001), whereas the prevalence of Candida infections was stable and even showed a declining trend within the last years. The highest infection rates were found in aplastic syndromes (68%), followed by AML (25%) and AIDS (19%). In the majority of cases (76%), invasive fungal infection was related to the immediate cause of death. However, the proportion of patients with endstage underlying conditions increased significantly over time from 53% to 80% (P<0.001). Accordingly, the number of patients who were not considered terminally ill but had died from fungal infection dropped from 35% to 17% within the last years (P<0.01).

Conclusions. These observations document significant changes in frequency, aetiology and underlying disease processes in invasive fungal infections at autopsy and underscore the continuing need for more effective prevention, diagnosis, and treatment.

Introduction

Along with the increased and prolonged survival of patients with life-threatening underlying diseases but impaired immunologic status, invasive fungal infections have emerged as a major cause of morbidity and mortality in the hospital.1,2 With the exception of cryptococcal meningitis and perhaps catheter-associated candidaemia, these infections remain difficult to diagnose, in particular at an early stage.3 Definitive diagnosis largely relies on biopptic procedures with histological and cultural confirmation of the organism from affected tissues, but unfortunately, biopsy specimen often can not be obtained because of the critical overall condition of the patient.1

The limitations of diagnostic methods and the overwhelming nature of invasive fungal infections in immunocompromised patients frequently necessitate the initiation of antifungal therapy on an empirical basis.8 In true or established infections, however, the response to currently available antifungal agents mainly depends on the correction of the underlying deficiency in host defenses and the prognostic significance of fungal disease in severely ill patients often remains unclear.1,5-7

Clinical data on incidence and impact of most invasive fungal infections are thus difficult to assess and many of these infections remain undetected antemortem.8 Although limited by the exclusion of patients who survive their infection or die and do not undergo examination, autopsy studies are still a major source for information on incidence and significance of invasive fungal infections.3 Most information has derived from autopsy studies in patients with malignant diseases, but studies on the epidemiology at a general hospital are currently lacking.

To determine temporal trends in invasive fungal infections at autopsy and to describe frequency, disease pattern and prognostic significance of these infections, we analyzed data from 8124 autopsies performed between 1978 and 1992 at a large university hospital with a relatively high autopsy rate.
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