Outcomes of invasive aspergillosis are substantially improved with early initiation of therapy. Unfortunately, the diagnosis remains difficult to establish so that early suspicion of infection is imperative. Identification of high risk patients and use of non-culture-based diagnostics and radiographic studies can facilitate earlier recognition of infection. It is important to realize that the timing and spectrum of risk for invasive aspergillosis have expanded. While patients with hematological malignancies and hematopoietic stem cell transplants make up the highest risk groups, patients such as those receiving steroids and other immunosuppressive therapies are also at risk. Non-culture-based methods (including galactomannan, 1,3-beta-D-glucan, and PCR-based methods) are actively being pursued to improve early diagnosis. Thus, identifying patients at high risk for infection and utilizing non-culture-based methods and radiologic studies to assist in establishing a likely diagnosis of invasive aspergillosis will further enhance the role of new agents in the early, effective treatment and prophylaxis of invasive aspergillosis.

Keywords invasive aspergillosis, epidemiology, non-culture-based diagnostics, diagnosis, therapy

Introduction
The diagnosis of invasive aspergillosis remains difficult as cultures may not be positive even with extensive or disseminated infection [1]. Non-culture-based methods have been developed to improve the early diagnosis of invasive aspergillosis but these methods have limited sensitivity, especially in early infection and in the presence of antifungal therapy or prophylaxis [2]. New antifungal agents, such as the extended spectrum triazoles, including voriconazole and posaconazole, and lipid formulations of amphotericin B, have increased the therapeutic options for the management of invasive infection. Outcomes of invasive aspergillosis are substantially improved with early therapy so that early diagnosis and prompt institution of therapy is critical [3–5]. Posaconazole prophylaxis has been shown to reduce breakthrough invasive fungal infection including Aspergillus in graft-versus-host-disease patients and in neutropenic patients with acute myelogenous leukemia or myelodysplastic syndrome [6,7]. A survival benefit was also seen in the patients with acute myelogenous leukemia. However, increased utility of antifungal prophylaxis is seen in patients with higher rates of infection so that identification of highest risk patients is key [8,9]. Thus, the clinician is challenged with identifying patients at high risk for infection based on risk stratification and to utilize non-culture-based methods and radiologic studies to assist in establishing a likely diagnosis of invasive aspergillosis. Successful use of these strategies to identify patients at high risk for invasive aspergillosis will further enhance the role of new agents in the early, effective treatment and prophylaxis of this often lethal disease.
Epidemiology and risk factors for invasive aspergillosis

The spectrum of patients at risk for invasive aspergillosis is broad [10], including an expanding population of patients receiving immunosuppressive therapies and patients with other risk factors for this infection. These include patients undergoing hematopoietic stem cell transplantation (HSCT), recipients of solid organ transplants, those receiving corticosteroids and other immunosuppressive agents and those with a variety of other conditions, including uncommon patients with no documented immunosuppression [11]. However, in considering risk of invasive aspergillosis, patients undergoing HSCT and those with hematological malignancies remain groups at highest risk.

For infectious complications in hematological malignancy or HSCT and for invasive aspergillosis in particular, it is useful to use the approach described by Fishman and Rubin for immunocompromised patients [12,13]. These investigators describe a ‘Net State of Immunosuppression’ which is a complex function determined by the interaction of a number of factors which influence the patient’s risk of opportunistic infection (Table 1). These factors include dose, temporal sequence and intensity of immunosuppressive therapy or chemotherapy; duration and depth of neutropenia; compromise of the mucocutaneous barrier – such as mucositis, indwelling vascular and urinary catheters; host factors – such as malnutrition and co-morbid illnesses; immunomodulating viruses – especially CMV; and age. Specific risk factors for invasive aspergillosis have been described for patients with hematological malignancies and those with HSCT: profound and persistent neutropenia combined with refractory malignancy requiring a second (or more) induction course of chemotherapy substantially increases risk for invasive aspergillosis as does graft failure or graft-versus-host disease and the therapies required for treated that complication [14–17].

Moulds, particularly Aspergillus, pose the greatest risk for invasive fungal infection in patients undergoing intensive chemotherapy for hematological malignancies and HSCT. The incidence and etiology of invasive mycoses in hematological malignancies were reported by Pagano and colleagues from a multi-centered study in Italy reviewing 11,802 patients with hematological malignancies, of whom 3012 were diagnosed with acute myeloid leukemia [14]. Overall incidence of mould infections was 2.9% while yeast infections occurred in 1.6%. Highest rates occurred in patients with acute myeloid leukemia, with moulds diagnosed in 7.9%. The most common moulds were Aspergillus (90%), followed by Zygomycetes and Fusarium (4% each). Attributable mortality associated with Aspergillus was 48%.

It is also important to recognize that even among these high risk hematological patients there is substantial heterogeneity of risk. For example, in patients with acute myelogenous leukemia, the incidence of invasive aspergillosis has been reported to range from as low as 2% to rates as high as 25–28% or more (Table 2) [8]. These differences in rates of invasive aspergillosis are likely due to host, environmental, and treatment-related issues. Thus, when considering the diagnosis of invasive aspergillosis in these patients it is important to evaluate whether they are indeed at increased risk of infection. The underlying rate of infection also significantly impacts the benefits of prophylaxis against invasive aspergillosis [8]. In patients who are anticipated to have lower rates of infection, the benefits of antifungal prophylaxis are reduced, whereas in patients with higher rates of infection, the benefits of prophylaxis are likely to outweigh the risk of developing infection [8].

It is also important to recognize the evolution of the epidemiology of invasive aspergillosis in HSCT

Table 1  Net state of immunosuppression.

<table>
<thead>
<tr>
<th>Complex function determined by the interaction of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Previous therapies (e.g., chemotherapy, antimicrobial agents)</td>
</tr>
<tr>
<td>● Dose, temporal sequence, intensity of immunosuppressive therapy</td>
</tr>
<tr>
<td>● Neutropenia, lymphopenia</td>
</tr>
<tr>
<td>● Underlying immuno deficiencies</td>
</tr>
<tr>
<td>● Compromise of primary mucocutaneous barrier (e.g., burns, drains, intravascular catheters, urinary catheters, surgery)</td>
</tr>
<tr>
<td>● Host factors (e.g., protein-calorie malnutrition, cirrhosis, uremia, diabetes)</td>
</tr>
<tr>
<td>● Immunomodulating viruses (CMV, HHV-6, HBV, HCV, HIV)</td>
</tr>
<tr>
<td>● Age</td>
</tr>
</tbody>
</table>


Table 2  Incidence of invasive aspergillosis in acute myelogenous leukemia.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>No. cases/Total (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerson et al.</td>
<td>1985</td>
<td>15/560 25</td>
<td>[46]</td>
</tr>
<tr>
<td>Talbot et al.</td>
<td>1987</td>
<td>22/79 28</td>
<td>[47]</td>
</tr>
<tr>
<td>Winston et al.</td>
<td>1993</td>
<td>5/241 2</td>
<td>[48]</td>
</tr>
<tr>
<td>Rotstein et al.</td>
<td>1999</td>
<td>9/304 3*</td>
<td>[49]</td>
</tr>
<tr>
<td>Pagano et al.</td>
<td>2006</td>
<td>213/3012 7+</td>
<td>[14]</td>
</tr>
<tr>
<td>De Pauw et al.</td>
<td>2007</td>
<td>– 4.5</td>
<td>[8]</td>
</tr>
<tr>
<td>Cornely et al.</td>
<td>2007</td>
<td>20/298 7</td>
<td>[6]</td>
</tr>
</tbody>
</table>

*Eight probable pulmonary invasive fungal infections; one proven pulmonary IA; 17 moulds (90% Aspergillus).
recipients recent years. While the epidemiology and risk factors for invasive aspergillosis in hematological malignancies and stem cell transplantation continue to evolve, profound and prolonged neutropenia remains a major risk factor for developing *Aspergillus* infection [18,19]. Nevertheless, Wald and colleagues demonstrated the late development of invasive aspergillosis in patients undergoing HSCT [18]. In their study of 2496 marrow transplant patients, 158 had invasive aspergillosis. The incidence of invasive aspergillosis increased from 5.7% to 11.2% during the study. The onset of infection was bimodal, peaking 16 and 96 days after transplant. For patients within 40 days after transplant, risk factors for infection included underlying disease, donor type, season, and transplant outside of laminar air flow rooms. For patients >40 days after transplant, age, underlying disease, donor type, graft-versus-host disease, neutropenia, and corticosteroid use were associated with increased risk of aspergillosis. Only 31% of infected patients were neutropenic at the time of diagnosis. Those authors concluded that risk factors for aspergillosis depend on the time after marrow transplant and include both host and environmental characteristics.

In patients undergoing solid organ transplantation, invasive aspergillosis is also a significant fungal pathogen. *Aspergillus* is the most common mould infection and second to only *Candida* species in importance for invasive mycoses in those patients, comprising overall around 20% of fungal pathogens in large epidemiological surveys [20]. However, similar to the heterogeneity of infection discussed for patients with hematological malignancies, there is a great difference between rates of invasive aspergillosis in various organ transplant populations as shown in Table 3. Generally, more experimental transplants, those requiring more extensive immunosuppression and/or associated with surgical complications, are usually associated with greater risk for invasive aspergillosis.

One organ particularly vulnerable to aspergillosis is that of lung transplant recipients [21]. In those patients, rates of aspergillosis range as high as 10–15% in several series [22]. The reasons for this increased rate of infection as compared to other solid organ transplants are several and include the fact that the organ is constantly exposed to the environment, ciliary clearance mechanisms are altered, patients may have been previously colonized with *Aspergillus* (such as patients with cystic fibrosis and chronic lung conditions), development of *Aspergillus* at the anastamosis site and others. *Aspergillus* infection in these patients can present in a variety of clinical presentations including disseminated nodular infiltrates, cavitary lung disease, a distinct tracheobronchitis, and anastamotic infection [22], so that the clinician should be especially concerned about aspergillosis in lung transplant recipients and be aware of the variety of presentations. Because the rate of infection is substantial, several groups have employed prophylaxis in lung transplantation due to the high risk of infection. Methods of prophylaxis include aerosol delivery of lipid forms of amphotericin as well as systemic itraconazole, voriconazole and others [23,24].

In contrast to the high rates of invasive aspergillosis documented in patients undergoing lung transplantation, *Aspergillus* infections are significantly less common in patients receiving other solid organ transplants. For example, the rates of invasive aspergillosis in patients undergoing kidney transplantation are very low, ranging from 0–4% with a mean reported incidence of only 1%. However, it should be realized that while rates of invasive aspergillosis are lower in some organ transplants, when infection occurs, high rates of mortality occur, ranging from 66–87% in a recent series [22].

Invasive aspergillosis in other patient groups remains less common, although it is important to recognize that other populations are also at risk, including the growing number of patients receiving corticosteroids and other immunosuppressive therapies (such as monoclonal antibody therapies) for a variety of medical conditions. Additionally, new risk groups such as patients in intensive care units have recently been reported [25]. Thus, the clinician must at least entertain the possibility of invasive aspergillosis in these settings as establishing a confirmed diagnosis may be difficult and may not be recognized due to lack of suspicion of the diagnosis.

**Table 3** Incidence and mortality of invasive aspergillosis in solid organ transplantation.

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Incidence (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>3–14</td>
<td>68</td>
</tr>
<tr>
<td>Liver</td>
<td>1–8</td>
<td>87</td>
</tr>
<tr>
<td>Heart</td>
<td>1–15</td>
<td>78</td>
</tr>
<tr>
<td>Kidney</td>
<td>0–4</td>
<td>77</td>
</tr>
<tr>
<td>Small bowel</td>
<td>0–10</td>
<td>66</td>
</tr>
</tbody>
</table>

Data from Ref: [22].

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**Recent advances in diagnosis**

The diagnosis of invasive mycoses remains a significant challenge. Cultures are frequently not positive early in the course of infection and invasive procedures are
reluctantly undertaken in highly immunosuppressed (often pancytopenic) patients. Recent efforts have focused on radiography and non-culture based methods to establish an early diagnosis of infection.

Radiography

Radiographic findings are now well-established as an important tool for the diagnosis of invasive mould infections, particularly invasive pulmonary aspergillosis [3,26,27]. Although plain chest radiographs are insensitive and non-specific, computed tomography (CT) of the chest can be very useful for establishing a diagnosis of invasive aspergillosis. While the presence of a 'halo' of low attenuation surrounding a nodular lesion is not found exclusively in invasive mould infections, it is an early finding in invasive pulmonary aspergillosis and has been used as a marker for initiating early antifungal therapy [3,27,28]. An 'air-crescent' is also suggestive of invasive aspergillosis but it is a finding that occurs late. These CT findings have been validated in high risk, neutropenic patients and HSCT patients but in other populations, including solid organ transplant recipients, these findings are not as useful. Other etiologic agents such as Nocardia and other opportunistic pathogens as well as non-infectious etiologies such as pulmonary embolism can be associated with similar findings, but in patients for whom the probability of invasive aspergillosis is high, it can be a very useful finding. Recent studies have also shown that nodular lung lesions, even without a 'halo', are present in >95% of patients with invasive aspergillosis, so that the absence of such a finding in a neutropenic patient suggests other diagnoses should be considered [29].

Non-culture-based methods

Non-culture-based methods in aspergillosis have focused on detection of galactomannan, a cell wall antigen, PCR and more recently detection of cell wall glucan. Detection of galactomannan is available as a commercial enzyme immunoassay (EIA) that utilizes a monoclonal antibody (Platelia Aspergillus EIA, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France; BioRad, Redmond, Washington) [30]. The sensitivity for detecting invasive aspergillosis was initially reported at more than 90% with a specificity that was more than 95% [31]. Other studies have found the assay to be less sensitive (40–50%), perhaps reflecting the impact of antifungal prophylaxis or therapy reducing the level of circulating galactomannan [32]. Other factors, including limited sampling, incidence of infection and others, also impact utility of the assay [30]. The low sensitivity has resulted in recommendations for a lower cut-off for a positive result with an optical index of 0.5 now recommended [33].

False positives may occur in pediatric patients and neonates, which may be due to dietary intake or even to cross-reacting cell wall motifs from bacteria such as Bifidobacterium spp. which heavily colonize the gut and may translocate across the gut wall in neonates or possibly in patients with mucositis [34]. False positives can also occur in patients receiving the antibiotic piperacillin/tazobactam and perhaps amoxicillin/clavulanic acid likely from lots of the antibiotics acquiring galactomannan in the antibiotic preparation [35,36]. In addition, other fungi (such as Penicillium spp. and even Histoplasma) may produce false positive results due to cross-reacting antigens [30].

Although detection of galactomannan has been used in other body fluids such as cerebrospinal fluid and in bronchoalveolar lavage (BAL) fluid, these samples have been less extensively evaluated compared to serum [37,38]. Detection of galactomannan in BAL fluid appears more sensitive than serum which may increase detection rates as much as 30% [37]. Despite the value of the galactomannan assay, several features limit its utility including the need for serial samples, impact of antifungal therapy in reducing circulating antigen, and correlation with clinical outcome [30].

Other non-culture-based methods include the non-specific fungal marker 1,3-β-D-glucan using a variation of the limulus assay which detects endotoxin. The 1,3-β-D-glucan assay is also commercially available (Fungitell [Associates of Cape Cod, Inc., Falmouth, MA], Fungitec G test MK [Seikagaku, Tokyo, Japan], Wako test [Wako Pure Chemical Industries Ltd., Tokyo, Japan], and others), and, like galactomannan, is accepted criteria for the diagnosis of invasive aspergillosis. These tests exhibit specific characteristics and vary in sensitivity [39]. They are not specific for Aspergillus, as all are also positive in other moulds and Candida, but not Cryptococcus or Zygomycetes which contain little or no β-D-glucan [40]. One study suggested the utility of the assay in early diagnosis of invasive fungal infection in a leukemic population, but validation of clinical utility remains limited [40].

PCR-based diagnosis which amplifies Aspergillus specific fungal-genes (usually ribosomal DNA genes) has shown considerable promise for invasive aspergillosis [41–43]. It may be that PCR is more useful as a very sensitive assay for excluding patients at risk for disease [44]. However, it also appears that fungal DNA from a lung lesion may require necrosis for its release which would be a significant barrier to the use of PCR for confirmation of early invasive disease [30]. However, these systems have not been standardized, are not
commercially available, and remain investigational [45]. Combining non-culture-based diagnostics (e.g., PCR and galactomannan, galactomannan and 1,3-β-D-glucan, etc) is an important research direction that may improve the overall predictive value of these systems.

In summary, non-culture based diagnostics have improved the early diagnosis of aspergillosis although limitations for each method are apparent. All these tests require serial assessments in high risk patients for optimal utility and may be best utilized in combination to increase diagnostic yield.

Approach to diagnosis and application to therapy and prophylaxis

Currently available methods for establishing a mycological diagnosis of invasive aspergillosis still have limited utility [2]. Galactomannan is relatively specific for Aspergillus but with low sensitivity, especially in the absence of serial samples or in patients on mould-active therapy; β-D-glucan is non-specifically positive for a variety of organisms including Aspergillus, but is associated with false positive results and is not locally available in many routine diagnostic laboratories. PCR remains investigational at the present time. A negative result from these tests does not rule out the possibility of fungal infection.

The importance of early and effective antifungal therapy in patients with invasive fungal infection is critical to a successful outcome, particularly in patients with significant immunosuppression. Therapy should be targeted to likely pathogens in high risk populations, recognizing the impact of prior antifungal therapy in reducing the sensitivity of surrogate markers (especially mould-active agents) and the emergence of less common moulds and other mycoses. Careful consideration of host susceptibility and risk of invasive aspergillosis is useful in determining empirical antifungal therapies while pursing a diagnosis of infection and is also useful when electing to initiate prophylaxis in high risk patients for whom the benefits outweigh the risks and costs of prophylaxis. Thus, establishing an early mycological diagnosis or determining the patient to be at high risk for invasive aspergillosis will allow prompt initiation of antifungal therapy or initiation of appropriate prophylaxis in a select group of high risk patients.

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