Medical Mycology September 2009, **47**, 571–583

informa healthcare

Invasive mould infections: a multi-disciplinary update

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Systemic fungal infections remain a significant cause of mortality in neutropenic and immunocompromised patients, despite advances in their diagnosis and treatment. The incidence of such infections is rising due to the use of intensive chemotherapy regimens in patients with solid tumours or haematological cancers, the increasing numbers of allogeneic haematopoietic stem cell and solid organ transplants, and the use of potent immunosuppressive therapy in patients with autoimmune disorders. In addition, the epidemiology of systemic fungal infections is changing, with atypical species such as *Aspergillus terreus* and zygomycetes becoming more common. Treatment has traditionally focused on empirical therapy, but targeted pre-emptive therapy in high-risk patients and prophylactic antifungal treatment are increasingly being adopted. New treatments, including lipid formulations of amphotericin B, second-generation broad-spectrum azoles, and echinocandins, offer effective antifungal activity with improved tolerability compared with older agents; the potential impact of these treatments is reflected in their inclusion in current treatment and prophylaxis guidelines. New treatment strategies, such as aerosolized lipid formulations of amphotericin B, may also reduce the burden of mortality associated with systemic fungal infections. The challenge is to identify ways of coupling potentially effective treatments with early and reliable identification of patients at highest risk of infection.

Keywords amphotericin B, azoles, echinocandins, transplantation, neutropenia, aspergillosis, zygomycosis

Introduction

The incidence of systemic fungal infections such as invasive aspergillosis (IA) has increased during the past two decades, largely due to the use of intensive chemotherapy regimens in patients with haematological malignancies, increasing numbers of allogeneic haematopoietic stem cell and solid organ transplants, and the broad use of potent immunosuppressive therapy in many different patient groups [1]. This increase has been facilitated by improvements in the management of such infections resulting from the introduction of more effective drugs, and the advent of new diagnostic approaches, such as high-resolution computed tomography (CT) [2] and *Aspergillus* galactomannan antigen assays [3], that may provide early identification and treatment. Despite these advances, however, mortality and morbidity remain high. For example, in a meta-analysis of 50 trials including a total of 1941 patients, the overall case-fatality rate associated with IA was 58%, and mortality rates ranged from 49.3% in patients

Received 28 November 2008; Final revision received 30 March 2009; Accepted 3 April 2009

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DOI: 10.1080/13693780902946559

with leukaemia or lymphoma to 86.7% in patients undergoing allogeneic bone marrow transplantation [4]. In contrast, starting effective systemic antifungal therapy (AFT) pre-emptively, based upon early diagnosis of pulmonary infiltrates by means of serial thoracic CT scans, may reduce aspergillosis-related mortality to below 20% [5]. However, the cultural or histological proof of invasive mould infection (IMI) is lacking in almost all of these patients, so that an undefined number of patients may also have had non-fungal causes of their pulmonary infiltrates.

The importance of prompt and effective treatment of systemic fungal infections is highlighted by a study that compared primary treatment with voriconazole *versus* intravenous amphotericin B, both followed by oral licensed AFT, in patients with IA, the majority of whom had haematological disorders [6]. The overall response rate (both complete and partial responses) was 52.8% with voriconazole and 31.6% with amphotericin B, and the survival rates at 12 weeks were 70.8% and 57.9%, respectively (hazard ratio [HR] 0.59, 95% confidence interval [CI] 0.40–0.88, $P = 0.02$). Importantly, the study showed that unsuccessful first-line therapy can markedly affect the response to subsequent treatment: among patients who were switched to other antifungal agents because of insufficient clinical response, only 26% of those originally randomized to voriconazole, and 19% of those randomized to amphotericin B, showed a successful outcome at 12 weeks [7].

Some important aspects of the epidemiology and current management of invasive fungal infections are updated in this paper.

Epidemiology and risk factors of invasive fungal infections

Although *Aspergillus* and *Candida* species remain the principal pathogens associated with invasive fungal infections, the epidemiology of such infections has changed considerably during the past three decades, and is continuing to evolve. Prior to the 1980s, the majority of deaths from systemic fungal infections were associated with *Candida* species, but following the introduction of fluconazole, *Candida*-related deaths have decreased while infections caused by moulds, particularly *Aspergillus* species, have increased [8,9]; indeed, *Aspergillus* is now the most common cause of systemic fungal infections in patients undergoing allogeneic bone marrow transplantation and those with acute leukaemia undergoing intensive chemotherapy [10]. Patients with haematological malignancies account for approximately 50–60% of systemic fungal infections (Fig. 1) [11]. Moreover, the incidence of fungal infections in these patients is increasing (Fig. 2) [12–14] and retrospective autopsy data show that approximately 75% of such infections are not diagnosed prior to death [13].

Among 395 patients undergoing allogeneic haematopoietic stem cell transplantation, 2-year survival among those with a non-*Candida* systemic fungal infection was 20%, compared with 55% among non-infected patients ($P < 0.0001$); the development of a non-*Candida* infection was the strongest independent risk factor for death, with an odds ratio of 5.6 (95% CI 3.7–8.6, $P < 0.0001$) [15]. The principal risk factors for the development of such infections in these patients

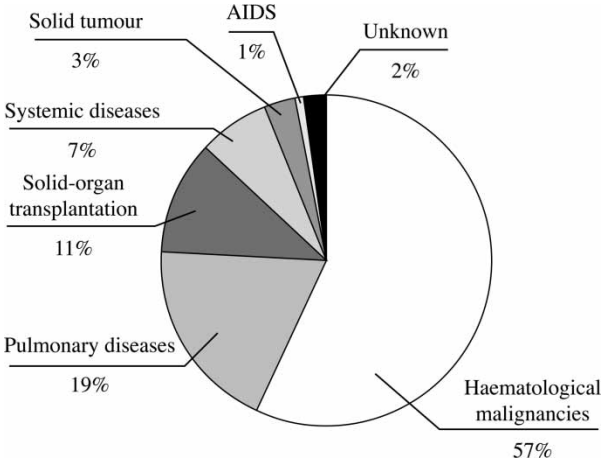

Fig. 1 Underlying diseases in 88 patients with invasive aspergillosis [11].

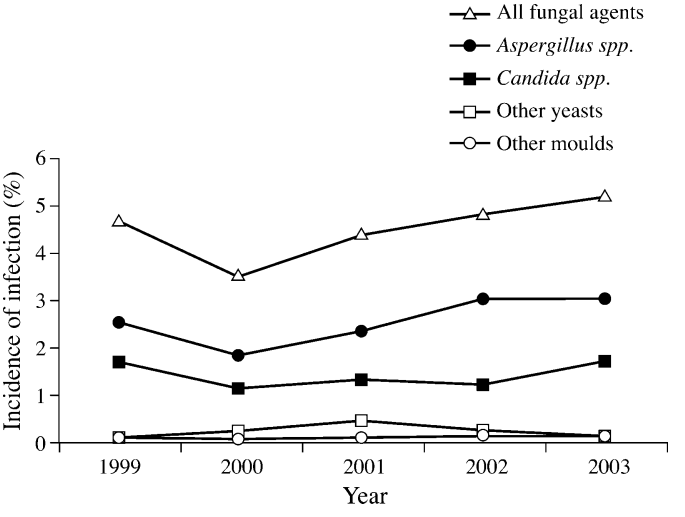

Fig. 2 Incidence of mould and yeast infections in patients with haematological malignancies between 1999 and 2003 [12].

were moderate-to-severe graft-versus-host disease (GVHD; odds ratio [OR] 4.6, 95% CI 2.2–9.7, $P<0.0001$) and corticosteroid treatment for GVHD (OR 2.1, 95% CI 1.1–4.1, $P=0.04$). The risk of infection increased from 4–11% when one of these risk factors was present, and to 33% when both were present [15]. Cytomegalovirus (CMV) infection is also a significant risk factor for IA in patients undergoing allogeneic stem cell transplants; in a study in 1682 patients, those infected with this virus were seven times (95% CI 4.5–10.8) more likely to develop IA than those without CMV [14]. The susceptibility of patients with CMV disease to opportunistic fungal infections reflects the known immunosuppressant effects of CMV [16–18], particularly against cell-mediated immune responses, and is probably also due to myelosuppression from CMV treatment with ganciclovir.

In a retrospective study of 1850 patients admitted to a medical intensive care unit, 127 (6.9%) showed microbiological or histopathological evidence of *Aspergillus* [19]. Of these, 89 (70.1%) did not have haematological malignancies: the most common underlying medical conditions in these patients were chronic obstructive pulmonary disease (COPD) in 42%, autoimmune disorders treated with immunosuppressants in 19%, and solid organ transplants in 10%. Overall mortality in patients without haematological malignancy was 80%. *A. fumigatus* is the most commonly isolated species [20], although other species are increasingly being encountered. In one study, for example, the incidence of *A. terreus*, expressed as a percentage of all *Aspergillus* isolates, increased from 1.5% in 1996 to 15.4% in 2001 ($P<0.001$) [21]. This has important clinical implications because *A. terreus* is resistant to amphotericin B [22,23], and is associated with rapid disease progression and high mortality; in one series, six of 11 immunocompromised patients with invasive pulmonary aspergillosis caused by *A. terreus* developed disseminated disease, and 10 died [24].

The incidence of infections caused by zygomycetes is increasing, as are those of uncommon hyalohyphomycoses (caused by *Fusarium* and *Scedosporium* spp.) and phaeohyphomycoses (*Bipolaris*, *Exophiala* and *Wangiella* spp.). The increase in zygomycosis has been observed in patients without underlying malignancies as well as in patients with cancer and those undergoing bone marrow transplantation [25]. In a review of 929 patients with zygomycosis, patients with diabetes accounted for the highest proportion of cases (36%), followed by those without underlying disorders (19%) and those with malignancies (17%) [25]. Zygomycosis is highly aggressive and associated with mortality ranging from 76% in patients with pulmonary infection to 96% in those with disseminated disease [25]. It has been discussed that the rising incidence of zygomycosis may be associated with increasing use of voriconazole to prevent and treat aspergillosis (Fig. 3) [26,27]; for example, in a prospective comparison of patients with IA or zygomycosis, the OR for the development of zygomycosis associated with voriconazole prophylaxis was 20.3 (95% CI 3.85–108.15, $P=0.0001$) [26].

Diagnosis of systemic fungal infections

Early diagnosis is critical for a favourable outcome in patients with systemic fungal infections. However, this remains challenging due to the low sensitivity of microbiological culture techniques and the low specificity of radiological procedures, particularly in neutropenic patients [28,29]. At best, *Aspergillus* can be isolated in 34% of sputum specimens and 62% of bronchoalveolar lavage (BAL) specimens from transplant patients with pulmonary aspergillosis [30,31]. Therefore, histopathological confirmation of the diagnosis is usually

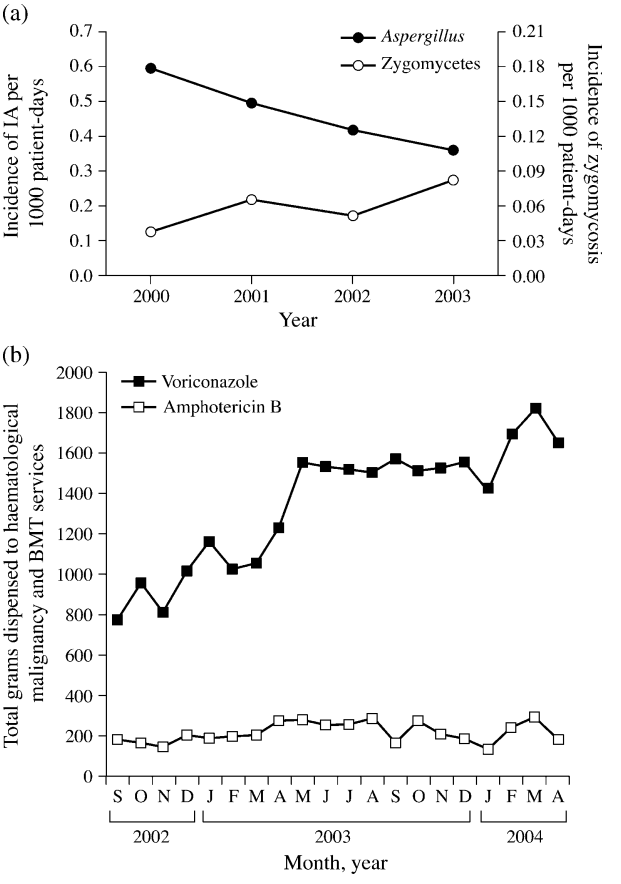

Fig. 3 Increasing incidence of zygomycosis (a) in relation to the use of voriconazole prophylaxis (b) [26]. BMT, bone marrow transplantation; IA, invasive aspergillosis.

required, but thrombocytopenia may preclude transbronchial or open lung biopsies [31].

Typical radiographic signs of invasive pulmonary mould infection include the halo sign and the air crescent sign. The halo sign is a sensitive but non-specific marker of IA, and is short-lived: its prevalence on computed tomography (CT) scans decreases from 68% at 3 days after diagnosis to 22% at 7 days [5]. The air crescent sign is detected in 8% of cases at 3 days after diagnosis, in 28% at 7 and 63% at 14 days, indicating that characteristic CT signs change with time, and that early and repeated imaging is essential for diagnosis. Importantly, the presence of a halo sign is associated with a favourable outcome among those treated on the basis of this finding. In a subanalysis of a randomized trial on IA treatment with voriconazole [6], 61% of patients had halo signs at the time of presentation, whereas other CT signs, such as consolidations, cavitary lesions and air crescent signs, were present in 10–30% of cases [32]. The response to treatment was higher in patients with halo signs, compared with those with other imaging findings (52% versus 29%, $P < 0.001$), and survival rates at 84 days were also higher (71% versus 53%, $P < 0.01$). In clinical practice, mould-active AFT is started pre-emptively in at-risk febrile patients with thoracic CT findings compatible with early pulmonary filamentous fungal infection, while microbiological and/or non-culture based confirmation of mould infection is sought from blood or respiratory tract specimens (see below). However, in patients who have been pre-treated with voriconazole, or who have multiple pulmonary nodules and pleural effusion on CT scans, zygomycosis may be more likely than in patients without these characteristics [26,33].

Diagnostic laboratory testing

Current diagnostic tests for systemic fungal infections include measurement of *Aspergillus* galactomannan (GM) or (1,3)-β-D-glucan, and polymerase chain reaction (PCR) assays for *Aspergillus* DNA. GM is a polysaccharide cell wall component of *Aspergillus* that is released into the circulation during fungal growth in tissues, and can be measured by sensitive enzyme-linked immunosorbent assay (ELISA) techniques [3,34,35]. In patients undergoing cancer chemotherapy or haematopoietic stem cell transplantation, GM assays have shown a sensitivity of 67–100% and a specificity of 86–99% for the detection of *Aspergillus*, whereas lower specificities (15–30%) have been reported in non-neutropenic patients [3,34,36–40]. When serial GM testing was performed, a positive GM test typically preceded the proven diagnosis of IA by 6–14 days.

(1,3)-β-D-glucan is an integral cell wall component of a number of pathogenic yeasts and filamentous fungi [41]. Sensitivities of 67–100%, and specificities of 84–100%, have been reported with the standard *Limulus* colorimetric assay [[41–44]]. In a recent study, a positive test for (1,3)-β-D-glucan was found to precede the diagnosis of IA by a median of 3 days [45].

PCR-based tests for *Aspergillus* are not yet commercially available and not standardized. They have a high (92–99%) negative predictive value in blood or BAL samples [34], however, the positive predictive value in BAL samples is low, apparently due to transient colonization of the respiratory tract by *Aspergillus* [46]. In blood-based assays, sensitivities of 79–100% and specificities of 81–93% have been reported [47–49]. PCR assays are considerably more sensitive than culture techniques: in one study, PCR was 19 times more sensitive than culture for the detection of *A. fumigatus* [50].

Treatment and prevention of systemic fungal infections

Empirical AFT has been demonstrated to prevent overt invasive fungal infection in the majority high-risk neutropenic patients with fever refractory to broad-spectrum antibiotics [51–53]. Concerns about unselected administration of systemic antifungals resulting in significant costs, adverse events, and possible emergence of resistance to broad-spectrum antifungals have, however, led to studies on alternative clinical approaches, restricting AFT to patients with clinical, imaging and/or laboratory findings more specific for invasive mould infection than just persisting fever.

Current guidelines on empirical antifungal treatment and primary treatment of invasive aspergillosis in haematological patients

Evidence-based guidelines for the empirical administration of antifungals in high-risk patients, and for primary treatment of IA, have been published by the Infectious Diseases Society of America (IDSA) [54], and the European Conference on Infections in Leukaemia (ECIL) (Table 1) [55,56]. For empirical therapy, liposomal amphotericin B (L-AmB) and caspofungin are supported by the strongest evidence.

In patients with IA, both the ISDA guidelines [54] and the ECIL-2 guidelines [56] recommend voriconazole as first-line therapy [6]. However, the intravenous form of this agent is not appropriate for all patients, as

Table 1 Recommendations of the 2nd European Conference on Infections in Leukaemia (ECIL-2) for empirical therapy in patients with systemic fungal infections [55]

	Dose	Level of recommendation*
Liposomal amphotericin B	3 mg/kg	A
Caspofungin	50 mg	A
Amphotericin B lipid complex	5 mg/kg	B
Amphotericin B colloidal dispersion	4 mg/kg	B
Voriconazole	2 ×3 mg/kg i.v.	B
Itraconazole	200 mg i.v.	B
Amphotericin B deoxycholate	0.5–1.0 mg/kg	B/D†
Fluconazole	400 mg i.v.	C

*Strength of evidence is graded A–E. For all agents, both efficacy and safety are graded I for quality of evidence (i.e., supported by at least one properly designed, randomized, trial).
†B in the absence of risk factors for renal toxicity (e.g., impaired renal function at baseline, concomitant nephrotoxic medication, or history of previous toxicity); D in the presence of such risk factors.

it is subject to numerous drug interactions, mainly due to inhibition of cytochrome P450 activity [57], and its use is contraindicated in patients with moderate or severe renal dysfunction (creatinine clearance <50 ml/minute). Lipid formulations of amphotericin B (LF-AB) may be an appropriate alternative to voriconazole as first-line therapy of IA. In a randomized, double-blind, trial involving 201 patients with confirmed systemic fungal infections (IA in 97%), the response rates achieved with liposomal amphotericin B (L-AmB), 3 mg/kg and 10 mg/kg, were 50% and 46%, respectively, and the 12-week survival rates were 72% and 59%, respectively [58]. There was no significant difference in efficacy between the two doses, although nephrotoxicity and hypokalaemia were significantly more common with the higher dose. Among 398 patients who had received amphotericin B lipid complex (ABLC) for IA, the overall response rate was 65%: 44% were cured or improved, and 21% had stabilization of infection at the end of therapy (Fig. 4) [20].

In patients not showing a favourable clinical response to this first-line therapy, there is a paucity of data allowing for a clear proof of treatment failure [59,60]. Switching to second-line or "salvage" AFT should be considered in patients in whom insufficient dosing [61], inhibitory drug-drug interactions [62] or differential diagnoses such as immune reconstitution syndrome [63] have been properly excluded. There are, however, few data to guide the choice of second-line AFT in patients with IA. Both the ECIL-2 and IDSA guidelines recommend class switches (i.e., LF-AB in patients primarily treated with voriconazole, voriconazole or posaconazole in patients primarily treated with LF-AB), and combinations with echinocandins such as caspofungin as an experimental option [54,56]. Among 83 patients with IA (73% of whom had haematological malignancies or had undergone stem cell or bone marrow transplantation), who were refractory to or intolerant of amphotericin B or triazoles, the overall success rate with caspofungin was 45%; the response rate was 50% in patients with pulmonary aspergillosis and 23% in those with disseminated aspergillosis [64]. Salvage therapy with posaconazole resulted in an overall success rate of 42%, compared with 26% in historical controls (OR 4.1, 95% CI 1.5–11.0, $P=0.006$) [65].

Primary antifungal treatment of zygomycosis

Treatment options in patients with zygomycosis include LF-AB and posaconazole. The overall response rate in patients with zygomycosis treated with ABLC was 72% [66]. Impaired renal function stabilized or improved in the majority of patients with pre-existing renal disease, indicating that this formulation is less nephrotoxic than conventional amphotericin B. In a retrospective review of 91 patients with zygomycosis treated with posaconazole, the rate of complete plus partial response at 12 weeks was 60%, and a further 21% of patients had stable disease. [67].

Pre-emptive antifungal therapy

An alternative to empirical AFT, or targeted AFT restricted to proven invasive mycoses, may be pre-emptive AFT in high-risk patients with clinical, laboratory and/or imaging findings indicative for invasive fungal infection. This approach was evaluated in 136 high-risk neutropenic patients, all of whom received prophylaxis with fluconazole and underwent screening by means of daily GM assays [28]. Seropositive patients, and those with positive microbiological tests plus supportive radiological findings, received L-AmB, 5 mg/kg/day. This approach reduced the use of AFT in patients with neutropenic fever by 78%, from 35% to 7.7%; furthermore, it led to the early initiation of AFT in 10 episodes (7.3%) where invasive infection was not suspected on clinical criteria. These findings suggest that pre-emptive AFT might provide effective antifungal control, while reducing exposure to potentially toxic antifungal drugs: however, this approach has not yet been validated in patients who are on mould-active antifungal prophylaxis, and in this study the pre-emptive approach failed to detect non-*Aspergillus* infections. A recently published study

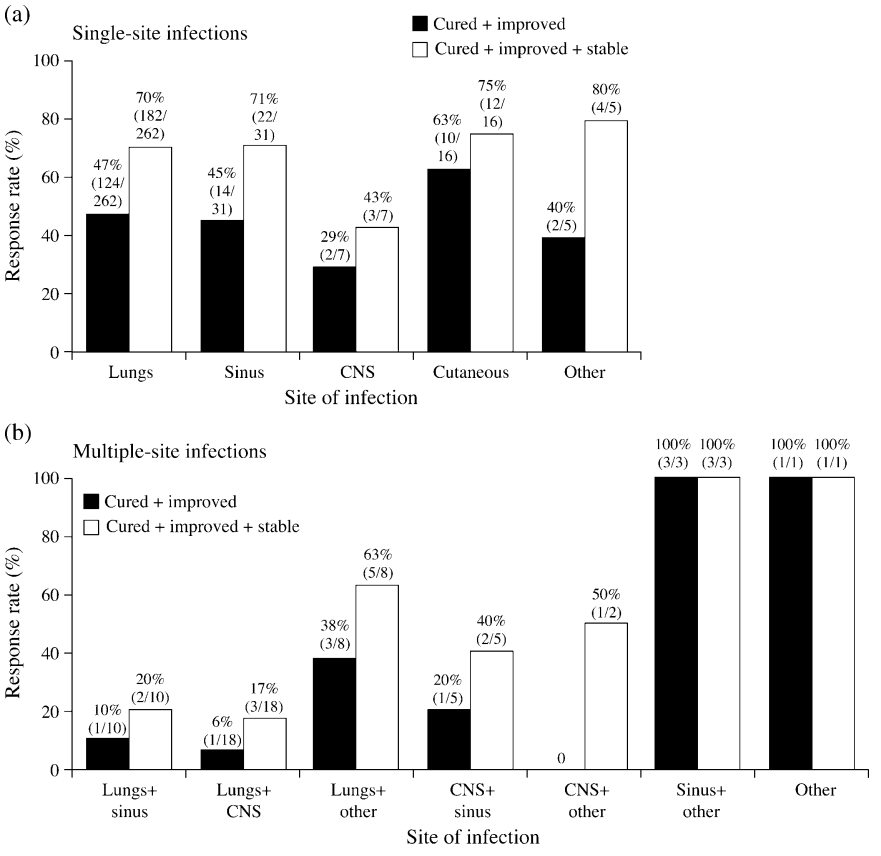

Fig. 4 Clinical response to amphotericin B lipid complex in 398 patients with IA [20].

comparing pre-emptive with empirical AFT in high-risk, febrile neutropenic patients showed that the number of proven IMIs increases in patients with pre-emptive as compared with empirical AFT, but that overall mortality rates are not significantly affected, while acquisition costs for antifungals are reduced [68].

Primary and secondary antifungal prophylaxis

Fluconazole has been standard antifungal prophylaxis in patients undergoing allogeneic haematopoietic stem cell transplantation [69–73], while itraconazole has not been shown to provide more effective antifungal prophylaxis in a direct randomized comparison [74]. Recent studies have shown that posaconazole provides more effective prophylaxis than these agents in neutropenic patients with acute myeloid leukaemia or myelodysplastic syndrome [75], and is superior to fluconazole in patients with severe GVHD [76]. However, the use of azoles for prophylaxis may preclude their therapeutic use in patients with established infections, due to concerns about the emergence of atypical pathogens and the potential for antifungal resistance [23,77–80]. Current ECIL-2 recommendations for antifungal prophylaxis are summarized in Table 2 [81]. In patients undergoing repeated episodes of intensive myelosuppression, preferably those with acute myeloid leukaemia, bridging systemic AFT from hospital discharge to re-admission by administration of secondary prophylaxis with a broad-spectrum azole has been reported to minimize IMI relapses [82,83].

Table 2 ECIL-2 recommendations for antifungal prophylaxis [81]

	Strength and quality of evidence*	
	Allogeneic haematopoietic stem cell transplantation	Induction chemotherapy of acute leukaemia
Fluconazole i.v./oral	AI	CI
Itraconazole i.v. then oral	BI	CI
Posaconazole oral	AI	AI
Micafungin i.v.	CI	
Candins i.v.		Insufficient data
Polyenes i.v.	CI – CII	

*Strength of evidence is graded A–E; quality of evidence is graded I–III.

Systemic mould infections in solid organ transplant patients

Epidemiology and risk factors

The increasing number of solid organ transplants has resulted in a concomitant rise in the incidence of associated systemic fungal infections, such that these procedures now account for approximately 11% of cases of IA (Fig. 1) [11]. In patients undergoing liver or lung transplants, the incidence ranges from 1–8% and 3–14%, respectively, compared with 0.4% for kidney transplant recipients and 1–3% for pancreas transplant patients [31]. A mortality rate of 87% is seen in liver transplant patients, compared with 70–80% after other transplants [31].

Infection with *Aspergillus* can be detected in airway sample cultures from approximately 25–30% of lung transplant recipients, with IA developing in 3–15% [31]. The most common types of infection are tracheobronchitis or bronchial anastomotic infections, which account for 58% of IA cases; invasive pulmonary aspergillosis occurs in 32% of cases and disseminated infection in 22% [31]. The median time to onset of aspergillosis is 3.2 months after transplantation; 51% of cases occur within 3 months, and 72% occur within 6 months [31,84]. Risk factors for bronchial anastomotic infections include airway ischaemia, reperfusion injury, bilateral lung transplantation, T cell-depleting induction therapy, and sirolimus-based immunosuppression [85,86].

Aspergillus infections in patients undergoing liver transplantation typically occur during the early post-operative period: median times to onset of 16–17 days have been reported [31,87,88], however, delayed occurrences have been documented [89]. Disseminated infection is more common after liver transplantation than after other transplants, occurring in 50–60% of cases, compared with 12–20% of lung transplant recipients and 20–35% of heart transplant recipients [31].

Renal failure, particularly when renal replacement therapy is required, is associated with a 15–25-fold increase in the risk of IA [31]. This has important implications because the number of liver transplants in patients with renal dysfunction has increased exponentially since the introduction of MELD (Model for End-stage Liver Disease) [90]. Re-transplantation is associated with a 30-fold increase in the risk of IA [91,92], and accounts for 25% of all IA cases, and 21% of related deaths, among liver transplant recipients. The mortality rate was 82% among patients who developed IA after re-transplantation, compared with 72% among those developing the infection after their primary transplant ($P = 0.4$) [93]. In the same study, the outcome of IA was worse after late re-transplantation (≥ 30 days after primary transplant) than after earlier re-transplantation. Other risk factors include transplantation for fulminant liver failure, repeated intra-abdominal or intrathoracic surgery, and preoperative steroid treatment [31,91,92].

An increasingly high proportion of mould infections in organ transplant recipients are due to organisms other than *Aspergillus*: 27% of all mould infections are due to other hyaline and dematiaceous moulds and zygomycetes [94].

Antifungal chemoprophylaxis

Antifungal prophylaxis in liver transplant recipients has typically involved fluconazole or itraconazole or, less commonly, LF-AB. A meta-analysis of six randomized studies comparing these agents with placebo (five studies) or oral nystatin, showed that prophylactic treatment reduced the incidence of fungal colonization (relative risk [RR] 0.45, 95% CI 0.37–0.55), total proven fungal infections (RR 0.31, 95% CI 0.21–0.46), and invasive fungal infections (RR 0.33, 95% CI 0.18–0.59), and decreased mortality attributable to fungal infections (RR 0.30, 95% CI 0.12–0.75); however, there was no effect on overall mortality or the use of empirical treatment for suspected infections [95]. It should be noted, however, that the number needed to treat (NNT) to prevent one case of invasive fungal infection was 5, and to prevent attributable mortality in one case was 26; indeed, with regards to side-effects, the NNT actually favoured the control arm. Moreover, no beneficial effect on invasive *Aspergillus* infection was observed.

Several studies have investigated the use of targeted prophylaxis, mainly with LF-AB, in high-risk liver transplant patients. In a study in 280 liver transplant recipients who were treated with ABLC or L-AmB, the incidences of systemic fungal infections and IA were 6% and 4%, respectively, compared with 17% ($P < 0.01$) and 10% ($P = 0.08$), respectively, in a historical control group [96]. Among patients with four or more risk factors for systemic fungal infections, the risk of any systemic fungal infection was reduced from 36% to 14% ($P = 0.07$), and that of IA from 23% to 5% ($P = 0.08$), in patients receiving the lipid formulations. Moreover, in patients undergoing renal dialysis, the risk of aspergillosis was reduced from 32% to 0% ($P = 0.03$). In a further study, the use of ABLC prophylaxis in patients at high or intermediate risk of systemic fungal infection was associated with a reduction in the incidence of IMI from 5% to 1% ($P = 0.08$), compared with the preintervention period when antifungal prophylaxis was not provided [97].

The risk of IA in lung transplant recipients persists beyond 3 months post-transplant. Antifungal prophylaxis during the first 3 months after transplantation would prevent 62% of tracheobronchitis cases, but only 36% of pulmonary infections and 50% of disseminated infections [84]. Long-term antifungal prophylaxis is therefore necessary in lung transplant patients. As a result, voriconazole is increasingly the agent of choice because it can be given orally. In a retrospective study, the incidence of IA 1 year after transplantation was 1.5% in patients receiving universal prophylaxis with voriconazole, compared with 23% ($P=0.001$) in high-risk patients receiving targeted prophylaxis with itraconazole alone or with aerosolized amphotericin B [98]. However, more patients had to discontinue treatment because of adverse effects with voriconazole than with targeted prophylaxis (14% versus 8%). In 88% of cases, treatment discontinuations are due to elevations of liver enzymes. The use of aerosolized lipid formulations of amphotericin B may provide an alternative approach to prophylaxis in lung transplant recipients, with the potential for improved tolerability (see below).

Targeted antifungal prophylaxis can significantly reduce the risk of systemic fungal infections in high-risk liver transplant recipients. Indeed, the approach of targeted prophylaxis is becoming widely adopted: a recent survey of liver transplant centres in the USA found that 91% were using antifungal prophylaxis, of whom 72% directed prophylaxis specifically to high-risk patients [99].

Treatment

For AFT of IA in solid organ transplant patients, the use of combination therapy with voriconazole and caspofungin was studied [100]. The survival rate at 90 days was 67.5%, compared with 51% ($P=0.11$) in a control group receiving an LF-AB. Among patients with renal failure, mortality was significantly lower in the combination therapy group than in the control group (HR 0.32, 95% CI 0.12–0.85, $P=0.022$). In a retrospective study of 251 lung transplant recipients, all three patients receiving combination therapy with voriconazole and caspofungin survived, compared with two of 14 patients receiving amphotericin B alone or with itraconazole ($P=0.014$) [101].

IMIs in paediatric patients

Epidemiology

The incidence of systemic fungal infections in immunocompromised children is increasing, particularly among children with cancer and those receiving solid organ or allogeneic haematopoietic stem cell transplants [25,102–107]. A review of 666 paediatric cases of IA found that the children at greatest risk were those undergoing allogeneic bone marrow transplantation and those with acute myeloid leukaemia [103]. However, the incidence of IA in these high-risk groups was approximately 5% only: thus, empirical therapy or universal prophylaxis creates the risk of over-treatment in a substantial proportion of patients. The overall attributable mortality rate associated with systemic fungal infections in patients up to 20 years of age is approximately 68% for IA, compared with approximately 55–60% of patients in other age groups [4]. Effective early identification and treatment of high-risk paediatric patients is therefore essential.

Systemic antifungal treatment

Many antifungal agents have not been studied extensively in paediatric patients. Among 69 children with IA (median age 7 years) who received voriconazole for a median of 93 days, the overall response rate was 45% and a further 7% had stable disease; the highest response rates were achieved in children with chronic granulomatous disease, and the lowest in patients with haematological malignancies [108]. In this study, however, the voriconazole doses used were those developed for use in adults, and it is now recognized that the pharmacokinetics of voriconazole differ markedly in children and adults [109,110]. Higher doses on a per kilogram basis are required in younger children to achieve comparable AUCs to adult patients, highlighting the importance of specific paediatric studies and appropriate dose strategies. It is possible that voriconazole may have greater effectiveness when dosed appropriately in children. Conversely, increased effectiveness is unknown but increasing toxicity at higher per kilogram doses is also possible. Further studies with this agent in children are needed.

In an open-label, compassionate use study in 551 patients with invasive fungal infections, which included 111 episodes in paediatric patients, the response rate achieved with ABLC in patients with IA was 56%, and the overall response rate was 70% [111]. In a further study, involving 46 children (mean age 9.7 years) who received amphotericin B lipid complex (ABLC) as salvage therapy, response rates in patients with aspergillosis or candidiasis were 78% and 89%, respectively [112]. Among 548 paediatric patients (age $\leq$20 years) who received ABLC for a median of 15 days (range 1–182 days), 300 (54.7%) were transplant recipients, and most were refractory to or intolerant of

conventional AFT. Among this subset, 41.7% were recipients of haematopoietic stem cell transplants, 83.0% of whom from an allogeneic donor. In the overall transplant group, 71.7% had received one or more nephrotoxic agents. The overall response rate was 54.9%, and a further 16.9% of patients had a stable outcome. Among patients with confirmed *Aspergillus* or *Candida* infection, the overall response rates were 59% and 72%, respectively. In the subgroup of patients with *Aspergillus* infection, there was no significant difference in the response rates (complete or partial response) in transplant and non-transplant patients (40.5% versus 37.5%, respectively); however, when patients with stable disease were included, the overall response rate was significantly higher in the non-transplant group (71.9% versus 48.6%, $P=0.05$) [113]. In the total patient population, there was no significant change in serum creatinine during ABLC therapy although a slight increase was recorded in patients aged 12–20 years, from a median of 1.2 mg/dl at baseline to 1.5 mg/dl at the end of treatment. These findings are encouraging for the use of ABLC in paediatric patients, including high-risk groups such as transplant recipients.

Aerosolized amphotericin B: a new approach to antifungal prophylaxis?

Inhaled AFT offers potential advantages, as this approach delivers the drug directly to the site of infection while reducing the risk of systemic toxicity and drug interactions. Amphotericin B has been the most widely used agent in inhalation therapy, but the results have been variable [114–116]. Nevertheless, a survey in 2001 revealed that 76% of lung transplant centres in the USA were using antifungal prophylaxis, of which 61% were using inhaled amphotericin B [117]. Approximately 75% of the aerosol particles are 1–8 μm in diameter, and thus can enter the bronchioles, while approximately 13% are less than 1 μm in diameter and can penetrate the alveoli [118].

Clinical studies have investigated the efficacy and tolerability of aerosolized LF-AB in lung transplant or stem cell patients, and in patients with haematological malignancies. An early study, involving 51 lung or heart–lung transplant patients, showed that aerosolized ABLC was well tolerated in such patients [119]. In a study in 100 lung transplant recipients receiving aerosolized amphotericin B deoxycholate (D-AmB), 25 mg, or aerosolized ABLC, 50 mg, given once daily for 4 days and then once weekly for 7 weeks for prophylaxis [120], the incidence of systemic fungal infections within 2 months was similar in both groups: 11.8% with ABLC and 14.3% with D-AmB. However, patients receiving D-AmB were significantly more likely to experience adverse events than those receiving ABLC (OR 2.16, 95% CI 1.10–4.24, $P=0.02$). The most common adverse event was worsening dyspnoea, which occurred in 19.9% of patients receiving D-AmB and 2.1% of ABLC-treated patients, followed by cough (10.6% versus 2.1%, respectively) and taste disturbances (10.6% versus 7.7%). Decreases of 20% or more in forced expiratory volume in 1 second (FEV_1) or forced vital capacity (FVC) occurred in approximately 11% of patients in both groups. A further study in 102 lung transplant patients treated with aerosolized L-AmB also showed good tolerability [121].

In a study in 382 neutropenic patients with leukaemias, non-Hodgkin's lymphoma or solid tumours, there was no significant difference in the incidence of IA between patients who received aerosolized D-AmB or placebo for antifungal prophylaxis (4% versus 7%, respectively, $P=0.37$); moreover, there were no significant differences in overall mortality or infection-related mortality between the two groups [116]. More positive results were obtained in an open-label pilot study with aerosolized ABLC that included 40 patients undergoing allogeneic haematopoietic stem cell transplantation [122]. Patients received aerosolized ABLC once daily for 4 days and then once weekly for 13 weeks, with daily oral fluconazole throughout the study. Three proven systemic fungal infections occurred during the study, only one of which developed during study treatment. There were no cases of aspergillosis; two cases of *C. glabrata* fungaemia occurred, which were attributed to the use of fluconazole prophylaxis. Aerosolized ABLC was well tolerated: the incidence of adverse events such as cough, taste disturbances, nausea or vomiting was 2.2%. Decreases in FEV_1 or FVC of 20% or more occurred after 5.2% of inhaled treatments. In a further study, involving 271 high-risk neutropenic patients, treatment with aerosolized L-AmB significantly reduced the incidence of IA, compared with placebo (4% versus 14%, $P=0.003$) [123].

Aerosolized amphotericin B may provide an effective and safe alternative to systemic therapy for the prevention of systemic fungal infections in high-risk patients. However, large comparative studies are needed to evaluate the potential benefits of this approach. [124].

Conclusions

Despite important advances in the diagnosis, treatment, and prevention of systemic fungal infections, mortality from such infections remains high. The

potential impact of new treatments, including lipid formulations of amphotericin B, second-generation triazoles, and the echinocandins, is reflected in the inclusion of these agents in current guidelines for treatment and prophylaxis. New treatment strategies, such as the use of aerosolized lipid formulations of amphotericin B, may also have an important role to play in reducing the burden of mortality associated with systemic fungal infections. The challenge is to identify ways of coupling potentially effective treatments with early and reliable identification of patients at highest risk of infection.

Funding statement

The content of this manuscript is based on a satellite symposium of the 3rd Trends in Medical Mycology Congress, sponsored by Cephalon Europe. The initial draft of the manuscript was prepared by Dr Ann McIlhinney of Anagram Communications, funded by Cephalon Europe. The final text was written and approved by all listed authors without funding or any external influence.

Conflicts of interest

G.M. has received grants/research support from Novartis and Pfizer, consultancy fees from Gilead, MSD, Pfizer, Essex (Schering-Plough), and Novartis, and honoraria for speaking from Pfizer, MSD, Gilead, and Cephalon. T.C. has received grant support from Merck, Pfizer, and Schering-Plough, consultancy fees from Pfizer, Merck, Schering-Plough, and Novartis, and honoraria for speaking from Pfizer, Merck, Novartis, Schering-Plough, and Cephalon. N.S. has received research support from Schering-Plough, Astellas, and Enzon. J.W. has received honoraria for speaking from Cephalon and Enzon. J.P. has received research grants, consulting fees and honoraria from Enzon, Astellas, Pfizer, Merck, and Schering-Plough.

References

1 Maschmeyer G, Haas A, Cornely OA. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. *Drugs* 2007; **67**: 1567–1601.
2 Gotway MB, Dawn SK, Caoili EM, *et al*. The radiologic spectrum of pulmonary *Aspergillus* infections. *J Comput Assist Tomogr* 2002; **26**: 159–173.
3 Maertens J, Verhaegen J, Lagrou K, *et al*. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; **97**: 1604–1610.
4 Lin S-J, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001; **32**: 358–366.
5 Caillot D, Couaillier JF, Bernard A, *et al*. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; **19**: 253–259.
6 Herbrecht R, Denning DW, Patterson TF, *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–415.
7 Patterson TF, Boucher HW, Herbrecht R, *et al*. Strategy of following voriconazole versus amphotericin B therapy with other licensed antifungal therapy for primary treatment of invasive aspergillosis: impact of other therapies on outcome. *Clin Infect Dis* 2005; **41**: 1448–1452.
8 Groll AH, Shah PM, Mentzel C, *et al*. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J Infect* 1996; **33**: 23–32.
9 McNeil MM, Nash SL, Hajjeh RA, *et al*. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. *Clin Infect Dis* 2001; **33**: 641–647.
10 Bowden RA. Fungal infections after marrow transplantation. In: Bowden RA, Ljungman P, Paya CV (eds). *Transplant Infections*. Philadelphia: Lippincott-Raven, 1998: 339–350.
11 Cornillet A, Camus C, Nimubona S, *et al*. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. *Clin Infect Dis* 2006; **43**: 577–584.
12 Pagano L, Caira M, Candoni A, *et al*. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica* 2006; **91**: 1068–1075.
13 Chamilos G, Luna M, Lewis RE, *et al*. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989–2003). *Haematologica* 2006; **91**: 986–989.
14 Marr KA, Carter RA, Boeckh M, *et al*. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; **100**: 4358–4366.
15 Martino R, Subirá M, Rovira M, *et al*. Invasive fungal infections after allogeneic peripheral blood stem cell transplantation: incidence and risk factors in 395 patients. *Br J Haematol* 2002; **116**: 475–482.
16 Boland GJ, Hene RJ, Ververs C, *et al*. Factors influencing the occurrence of active cytomegalovirus (CMV) infections after organ transplantation. *Clin Exp Immunol* 1993; **94**: 306–312.
17 Schrier RD, Rice GP, Oldstone MB. Suppression of natural killer cell activity and T cell proliferation by fresh isolates of human cytomegalovirus. *J Infect Dis* 1986; **153**: 1084–1091.
18 Husni RN, Gordon SM, Longworth DL, *et al*. Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients. *Clin Infect Dis* 1998; **26**: 753–755.
19 Meersseman W, Vandecasteele SJ, Wilmer A, *et al*. Invasive aspergillosis in critically ill patients without malignancy. *Am J Respir Crit Care Med* 2004; **170**: 621–625.
20 Chandrasekar PH, Ito JI. Amphotericin B lipid complex in the management of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* 2005; **40**(Suppl. 6): S392–S400.
21 Baddley JW, Pappas PG, Smith AC, *et al*. Epidemiology of *Aspergillus terreus* at a university hospital. *J Clin Microbiol* 2003; **41**: 5525–5529.
22 Sutton DA, Sanche SE, Revankar SG, *et al*. *In vitro* amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a

head-to-head comparison to voriconazole. *J Clin Microbiol* 1999; **37**: 2343–2345.
23 Hachem RY, Kontoyiannis DP, Boktour MR, *et al*. *Aspergillus terreus*: an emerging amphotericin B-resistant opportunistic mold in patients with hematologic malignancies. *Cancer* 2004; **101**: 1594–1600.
24 Iwen PC, Rupp ME, Langnas AN, *et al*. Invasive pulmonary aspergillosis due to *Aspergillus terreus*: 12-year experience and review of the literature. *Clin Infect Dis* 1998; **26**: 1092–1097.
25 Roden MM, Zaoutis TE, Buchanan WL, *et al*. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis* 2005; **41**: 634–653.
26 Kontoyiannis DP, Lionakis MS, Lewis RE, *et al*. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005; **191**: 1350–1360.
27 Imhof A, Balajee SA, Fredricks DN, *et al*. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin Infect Dis* 2004; **39**: 743–746.
28 Maertens J, Theunissen K, Verhoef G, *et al*. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* 2005; **41**: 1242–1250.
29 Hope WW, Denning DW. Invasive aspergillosis: current and future challenges in diagnosis and therapy. *Clin Microbiol Infect* 2004; **10**: 2–4.
30 Paterson DL, Singh N. Invasive aspergillosis in transplant patients. *Medicine* 1999; **78**: 123–138.
31 Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. *Clin Microbiol Rev* 2005; **18**: 44–69.
32 Greene RE, Schlamm HT, Oestmann JW, *et al*. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis* 2007; **44**: 373–379.
33 Chamilos G, Marom EM, Lewis RE, *et al*. Predictors of pulmonary zygomycosis versus invasive pulmonary aspergillosis in patients with cancer. *Clin Infect Dis* 2005; **41**: 60–66.
34 Maertens J, Verhaegen J, Demuynck H, *et al*. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive Aspergillosis. *J Clin Microbiol* 1999; **37**: 3223–3228.
35 Stynen D, Goris A, Sarfati J, *et al*. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. *J Clin Microbiol* 1995; **33**: 497–500.
36 Bretagne S, Costa J-M, Bart-Delabesse E, *et al*. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. *Clin Infect Dis* 1998; **26**: 1407–1412.
37 Machetti M, Feasi M, Mordini N, *et al*. Comparison of an enzyme immunoassay and a latex aggregation system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. *Bone Marrow Transplant* 1998; **21**: 917–921.
38 Rohrlich P, Sarfati J, Mariani P, *et al*. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. *Pediatr Infect Dis J* 1996; **15**: 232–237.
39 Ulusakarya A, Chachaty E, Vantelon J-M, *et al*. Surveillance of *Aspergillus* galactomannan anitgenemia for invasive aspergillosis by enzyme-linked immunosorbant assay in neutropenic patients treated for haematological malignancies. *Hematol J* 2000; **1**: 111–116.
40 Verweij PE, Stynen D, Rijs AMMM, *et al*. Sandwich enzyme-linked immunosorbant assay compared with Pastorex latex agglutination test for diagnosing invasive aspergilloisis in immunocompromised patients. *J Clin Microbiol* 1995; **33**: 1912–1914.
41 Miyazaki T, Kohno S, Mitsutake K, *et al*. Plasma (1-3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *J Clin Microbiol* 1995; **33**: 3115–3118.
42 Obayashi T, Yoshida M, Mori T, *et al*. Plasma (1–3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995; **345**: 17–20.
43 Obayashi T, Yoshida M, Tamura H, *et al*. Determination of plasma (1–3)-β-D-glucan: a new diagnosis aid to deep mycosis. *J Med Vet Mycol* 1992; **30**: 275–280.
44 Yuasa K, Goto H, Iguchi M, *et al*. Evaluation of the diagnostic value of the measurement of (1–3)-β-D-glucan in patients with pulmonary aspergillosis. *Respiration* 1996; **63**: 78–83.
45 Senn L, Robinson JO, Schmidt S, *et al*. 1,3-Beta-D-glucan antigenemia for early diagnosis of invasive fungal infections in neutropenic patients with acute leukemia. *Clin Infect Dis* 2008; **46**: 878–885.
46 Bart-Delabesse E, Marmorat-Khuong A, Costa JM, *et al*. Detection of *Aspergillus* DNA in bronchoalveolar lavage fluid of AIDS patients by the polymerase chain reaction [letter]. *Eur J Clin Microbiol Infect Dis* 1997; **16**: 24–25.
47 Buchheidt D, Baust C, Skladny H, *et al*. Detection of *Aspergillus* species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis* 2001; **33**: 428–435.
48 Hebart H, Bollinger C, Fisch P, *et al*. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individual and patients with haematological malignancies. *Blood* 2003; **100**: 4521–4528.
49 Kami M, Fukui T, Ogawa S, *et al*. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* 2001; **33**: 1504–1512.
50 Loeffler J, Kloepfer K, Hebart H, *et al*. Polymerase chain reaction detection of *Aspergillus* DNA in experimental models of invasive aspergillosis. *J Infect Dis* 2002; **185**: 1203–1206.
51 Walsh TJ, Finberg RW, Arndt C, *et al*. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med* 1999; **340**: 764–771.
52 Walsh TJ, Pappas P, Winston DJ, *et al*. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002; **346**: 225–234.
53 Walsh TJ, Teppler H, Donowitz GR, *et al*. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Engl J Med* 2004; **351**: 1391–1402.
54 Walsh TJ, Anaissie EJ, Denning DW, *et al*. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **46**: 327–360.
55 Marchetti O, Cordonnier C, Calandra T. Empirical antifungal therapy in neutropaenic cancer patients with persistent fever. *Eur J Cancer Supplements* 2007; **5**: 32–42.
56 Herbrecht R, Flückinger U, Gachot B, *et al*. Treatment of invasive *Candida* and invasive *Aspergillus* infections in adult haematological patients. *Eur J Cancer Supplements* 2007; **5**: 49–59.

57 Ullmann AJ. Review of the safety, tolerability, and drug interactions of the new antifungal agents caspofungin and voriconazole. *Curr Med Res Opin* 2003; **19**: 263–271.
58 Cornely OA, Maertens J, Bresnik M, *et al*. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007; **44**: 1289–1297.
59 Maschmeyer G, Haas A. Defining clinical failure for salvage studies. *Med Mycol* 2006; **44**: S315–S318.
60 Nucci M, Perfect JR. When primary antifungal therapy fails. *Clin Infect Dis* 2008; **46**: 1426–33.
61 Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009; **53**: 24–34.
62 Maschmeyer G, Haas A. Voriconazole: a broad spectrum triazole for the treatment of serious and invasive fungal infections. *Future Microbiol* 2006; **1**: 365–385.
63 Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. *Lancet Infect Dis* 2007; **7**: 395–401.
64 Maertens J, Raad I, Petrikkos G, *et al*. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004; **39**: 1563–1571.
65 Walsh TJ, Raad I, Patterson TF, *et al*. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin Infect Dis* 2007; **44**: 2–12.
66 Larkin JA, Montero JA. Efficacy and safety of amphotericin B lipid complex for zygomycosis. *Infect Med* 2003; **20**: 201–206.
67 van Burik JA, Hare RS, Solomon HF, *et al*. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. *Clin Infect Dis* 2006; **42**: e61–e65.
68 Cordonnier C, Pautas C, Maury S, *et al*. Empirical versus preemptive antifungal therapy for high risk, febrile, neutropenic patients: a randomized, controlled trial. *Clin Infect Dis* 2009; **48**: 1042–1051.
69 Slavin MA, Osborne B, Adams R, *et al*. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation – a prospective, randomized, double-blind study. *J Infect Dis* 1995; **171**: 1545–1552.
70 Marr KA, Seidel K, Slavin MA, *et al*. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000; **96**: 2055–2061.
71 Goodman JL, Winston DJ, Greenfield RA, *et al*. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992; **326**: 845–851.
72 Schaffner A, Schaffner M. Effect of prophylactic fluconazole on the frequency of fungal infections, amphotericin B use, and health care costs in patients undergoing intensive chemotherapy for hematologic neoplasias. *J Infect Dis* 1995; **172**: 1035–1041.
73 Rotstein C, Bow EJ, Laverdiere M, *et al*. Randomized placebo-controlled trial of fluconazole prophylaxis for neutropenic cancer patients: benefit based on purpose and intensity of cytotoxic therapy. The Canadian Fluconazole Prophylaxis Study Group. *Clin Infect Dis* 1999; **28**: 331–340.
74 Glasmacher A, Cornely O, Ullmann AJ, *et al*. An open-label randomized trial comparing itraconazole oral solution with fluconazole oral solution for primary prophylaxis of fungal infections in patients with haematological malignancy and profound neutropenia. *J Antimicrob Chemother* 2006; **57**: 317–325.
75 Cornely OA, Maertens J, Winston DJ, *et al*. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007; **356**: 348–359.
76 Ullmann AJ, Lipton JH, Vesole DH, *et al*. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007; **356**: 335–347.
77 Marr KA, Lyons CN, Ha K, *et al*. Inducible azole resistance associated with a heterogeneous phenotype in *Candida albicans*. *Antimicrob Agents Chemother* 2001; **45**: 52–59.
78 Uzun O, Anaissie EJ. Antifungal prophylaxis in patients with hematologic malignancies: a reappraisal. *Blood* 1995; **86**: 2063–2072.
79 Lionakis MS, Lewis RE, Torres HA, *et al*. Increased frequency of non-fumigatus *Aspergillus* species in amphotericin B- or triazole-pre-exposed cancer patients with positive cultures for aspergilli. *Diagn Microbiol Infect Dis* 2005; **52**: 15–20.
80 Verweij PE, Mellado E, Melchers WJG. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 2007; **356**: 1481–1483.
81 Maertens J, Frére P, Lass-Flörl C, *et al*. Primary antifungal prophylaxis in leukaemia patients. *Eur J Cancer Supplements* 2007; **5**: 43–48.
82 Cordonnier C, Maury S, Pautas C, *et al*. Secondary antifungal prophylaxis with voriconazole to adhere to scheduled treatment in leukemic patients and stem cell transplant recipients. *Bone Marrow Transplant* 2004; **33**: 943–948.
83 Cornely OA, Böhme A, Reichert D, *et al*. Multinational Case Registry of the Infectious Diseases Working Party of the German Society for Hematology and Oncology. Risk factors for breakthrough invasive fungal infection during secondary prophylaxis. *J Antimicrob Chemother* 2008; **61**: 939–946.
84 Singh N, Husain S. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. *J Heart Lung Transplant* 2003; **22**: 258–266.
85 Hadjiliadis D, Howell DN, Davis RD, *et al*. Anastomotic infections in lung transplant recipients. *Ann Transplant* 2000; **5**: 13–19.
86 Groetzner J, Kur F, Spelsberg F, *et al*. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. *J Heart Lung Transplant* 2004; **23**: 632–638.
87 Selby R, Ramirez CB, Singh R, *et al*. Brain abscess in solid organ transplant recipients receiving cyclosporine-based immunosuppression. *Arch Surg* 1997; **132**: 304–310.
88 Singh N, Arnow PM, Bonham A, *et al*. Invasive aspergillosis in liver transplant recipients in the 1990s. *Transplantation* 1997; **64**: 716–720.
89 Singh N, Avery RK, Munoz P, *et al*. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. *Clin Infect Dis* 2003; **36**: 46–52.
90 Gonwa TA, McBride MA, Anderson K, *et al*. Continued influence of preoperative renal function on outcome of orthotopic liver transplant (OLTX) in the US: where will MELD lead us? *Am J Transplant* 2006; **6**: 2651–2659.
91 Fortún J, Martín-Dávila P, Moreno S, *et al*. Risk factors for invasive aspergillosis in liver transplant recipients. *Liver Transpl* 2002; **8**: 1065–1070.
92 Singh N. Invasive mycoses in organ transplant recipients: controversies in prophylaxis and management. *J Antimicrob Chemother* 2000; **45**: 749–755.

93 Singh N, Pruett TL, Houston S, *et al*. Invasive aspergillosis in the recipients of liver retransplantation. *Liver Transpl* 2006; **12**: 1205–1209.
94 Husain S, Alexander BD, Munoz P, *et al*. Opportunistic mycelial fungal infections in organ transplant recipients: emerging importance of non-*Aspergillus* mycelial fungi. *Clin Infect Dis* 2003; **37**: 221–229.
95 Cruciani M, Mengoli C, Malena M, *et al*. Antifungal prophylaxis in liver transplant patients: a systematic review and meta-analysis. *Liver Transpl* 2006; **12**: 850–858.
96 Fortún J, Martín-Davila P, Moreno S, *et al*. Prevention of invasive fungal infections in liver transplant recipients: the role of prophylaxis with lipid formulations of amphotericin B in high-risk patients. *J Antimicrob Chemother* 2003; **52**: 813–819.
97 Hellinger WC, Bonatti H, Yao JD, *et al*. Risk stratification and targeted antifungal prophylaxis for prevention of aspergillosis and other invasive mold infections after liver transplantation. *Liver Transpl* 2005; **11**: 656–662.
98 Husain S, Paterson DL, Studer S, *et al*. Voriconazole prophylaxis in lung transplant recipients. *Am J Transplant* 2006; **6**: 3008–3016.
99 Singh N, Wagener MM, Cacciarelli TV, *et al*. Antifungal management practices in liver transplant recipients. *Am J Transplant* 2008; **8**: 426–431.
100 Singh N, Limaye AP, Forrest G, *et al*. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. *Transplantation* 2006; **81**: 320–326.
101 Solé A, Morant P, Salavert M, *et al*. *Aspergillus* infections in lung transplant recipients: risk factors and outcome. *Clin Microbiol Infect* 2005; **11**: 359–365.
102 Abbasi S, Shenep JL, Hughes WT, *et al*. Aspergillosis in children with cancer: A 34-year experience. *Clin Infect Dis* 1999; **29**: 1210–1219.
103 Zaoutis TE, Heydon K, Chu JH, *et al*. Epidemiology, outcomes, and costs of invasive aspergillosis in immunocompromised children in the United States, 2000. *Pediatrics* 2006; **117**: e711–e716.
104 Groll AH, Müller FM, Piscitelli SC, *et al*. Lipid formulations of amphotericin B: clinical perspectives for the management of invasive fungal infections in children with cancer. *Klin Padiatr* 1998; **210**: 264–273.
105 Gladdy RA, Richardson SE, Davies HD, *et al*. *Candida* infection in pediatric liver transplant recipients. *Liver Transpl Surg* 1999; **5**: 16–24.
106 Ringdén O, Andström EE, Remberger M, *et al*. Prophylaxis and therapy using liposomal amphotericin B (AmBisome) for invasive fungal infections in children undergoing organ or allogeneic bone-marrow transplantation. *Pediatr Transplant* 1997; **1**: 124–129.
107 Hovi L, Saarinen-Pihkala UM, Vettenranta K, *et al*. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. *Bone Marrow Transplant* 2000; **26**: 999–1004.
108 Walsh TJ, Lutsar I, Driscoll T, *et al*. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J* 2002; **21**: 240–248.
109 Walsh TJ, Karlsson MO, Driscoll T, *et al*. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. *Antimicrob Agents Chemother* 2004; **48**: 2166–2172.
110 Steinbach WJ, Benjamin DK. New antifungal agents under development in children and neonates. *Curr Opin Infect Dis* 2005; **18**: 484–489.
111 Walsh TJ, Seibel NL, Arndt C, *et al*. Amphotericin B lipid complex in pediatric patients with invasive fungal infections. *Pediatr Infect Dis J* 1999; **18**: 702–708.
112 Herbrecht R, Auvrignon A, Andrès E, *et al*. Efficacy of amphotericin B lipid complex in the treatment of invasive fungal infections in immunosuppressed paediatric patients. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 77–82.
113 Wiley JM, Seibel NL, Walsh TJ. Efficacy and safety of amphotericin B lipid complex in 548 children and adolescents with invasive fungal infections. *Pediatr Infect Dis J* 2005; **24**: 167–174.
114 Beyer J, Schwartz S, Barzen G, *et al*. Use of amphotericin B aerosols for the prevention of pulmonary aspergillosis. *Infection* 1994; **22**: 143–148.
115 Reichenspurner H, Gamberg P, Nitschke M, *et al*. Significant reduction in the number of fungal infections after lung-, heart-lung, and heart transplantation using aerosolized amphotericin B prophylaxis. *Transplant Proc* 1997; **29**: 627–628.
116 Schwartz S, Behre G, Heinemann V, *et al*. Aerosolized amphotericin B inhalations as prophylaxis of invasive *Aspergillus* infections during prolonged neutropenia: results of a prospective randomized multicenter trial. *Blood* 1999; **93**: 3654–3661.
117 Dummer JS, Lazariashvilli N, Barnes J, *et al*. A survey of antifungal management in lung transplantation. *J Heart Lung Transplant* 2004; **23**: 1376–1381.
118 Eisenberg RS, Oatway WH. Nebulization of amphotericin B. *Am Rev Respir Dis* 1971; **103**: 289–292.
119 Palmer SM, Drew RH, Whitehouse JD, *et al*. Safety of aerosolized amphotericin B lipid complex in lung transplant recipients. *Transplantation* 2001; **72**: 545–548.
120 Drew RH, Dodds Ashley E, Benjamin DK Jr, *et al*. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. *Transplantation* 2004; **77**: 232–237.
121 Monforte V, Ussetti P, López R, *et al*. Nebulized liposomal amphotericin B prophylaxis for *Aspergillus* infection in lung transplantation: pharmacokinetics and safety. *J Heart Lung Transplant* 2009; **28**: 170–175.
122 Alexander BD, Dodds Ashley ES, Addison RM, *et al*. Non-comparative evaluation of the safety of aerosolized amphotericin B lipid complex in patients undergoing allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis* 2006; **8**: 13–20.
123 Rijnders BJ, Cornelissen JJ, Slobbe L, *et al*. Aerosolized liposomal amphotericin B for the prevention of invasive pulmonary aspergillosis during prolonged neutropenia: a randomized, placebo-controlled trial. *Clin Infect Dis* 2008; **46**: 1401–1408.
124 Monforte V, Román A, Gavaldà J, *et al*. Contamination of the nebulization systems used in the prophylaxis with amphotericin B nebulized in lung transplantation. *Transplant Proc* 2005; **37**: 4056–4058.

This paper was first published online on iFirst on 10 August 2009.