J Antimicrob Chemother 2017; **72** Suppl 1: i29–i38
doi:10.1093/jac/dkx031

Journal of Antimicrobial Chemotherapy

Clinical considerations in the early treatment of invasive mould infections and disease

Toine Mercier and Johan Maertens*

Department of Hematology, Acute Leukemia and Stem Cell Transplantation Unit, University Hospitals Leuven, Leuven, Belgium

*Corresponding author. Department of Hematology, Acute Leukemia and Stem Cell Transplantation Unit, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium. Tel: +32-16-34-68-89. E-mail: johan.maertens@uzleuven.be

Different therapeutic strategies for invasive fungal diseases have been explored, each with particular strengths and weaknesses. Broad-spectrum antifungal prophylaxis seems logical, but selective use is important due to its substantial disadvantages, including interference with diagnostic assays, selection for resistance, drug toxicity and drug–drug interactions. Antimould prophylaxis should be restricted to high-risk groups, such as patients undergoing intensive chemotherapy for acute myeloid leukaemia or myelodysplastic syndrome, allogeneic HSCT patients with prior invasive fungal infection, graft-versus-host-disease or extended neutropenia, recipients of a solid organ transplant, or patients with a high-risk inherited immunodeficiency. An empirical approach, whereby mould-active therapy is started in neutropenic patients with fever unresponsive to broad-spectrum antibiotics, is widely applied but incurs the clinical and cost penalties associated with overtreatment. A benefit for all-cause mortality using empirical therapy has not been shown, but it is recommended for high-risk patients who remain febrile after 4–7 days of broad-spectrum antibiotics and in whom extended neutropenia is anticipated. There is growing interest in delaying antifungal treatment until an invasive fungal infection is confirmed ('pre-emptive' or 'diagnostics-driven' management), prompted by the development of more sensitive diagnostic techniques. Comparisons of empirical versus pre-emptive regimens are sparse, particularly with modern triazole agents, but treatment costs are lower with pre-emptive therapy and the available evidence has not indicated reduced efficacy. Pre-emptive treatment may be appropriate in neutropenic patients who remain febrile after administration of broad-spectrum antibiotics but who are clinically stable. Further work is required to define accurately the specific patient subgroups in which each management approach is optimal.

Introduction

Crude mortality rates for invasive fungal disease have improved dramatically over the last three decades. One comparison of outcomes in studies of invasive aspergillosis in patients with acute leukaemia concluded that overall mortality declined from 48% in the 1980s/1990s to 24% in the mid-2000s,[1] with similar results reported for invasive aspergillosis after HSCT.[2] A major contributing factor has been the development of new antimould agents offering improved efficacy with reduced toxicity, notably the introduction of voriconazole in the early 2000s, with its improved bioavailability and potency compared with earlier antifungal therapies. Table 1 summarizes the key agents now available in the treatment of invasive mould infections, and their licensed indications.

Despite this, and substantial advances in diagnostic techniques, the management of invasive fungal diseases remains complex. These life-threatening infections usually occur in immunodeficient patients, such as those with haematological malignancies, solid organ transplant recipients, patients with chronic granulomatous disease or other inherited immunodeficiency disorders, and other risk groups that are less well quantified, including patients with advanced HIV infection and critically ill patients with chronic obstructive lung disease, liver failure, or after influenza complicated by acute respiratory distress syndrome treated with high-dose corticosteroids.[3,4] Usually these patients mount only a limited immune response and show muted signs and symptoms.[5] Prompt detection can be problematic, particularly in the context of prolonged neutropenia, graft-versus-host disease (GvHD), organ failure or graft rejection, where the clinician is focused more on the underlying disease than on infectious complications. The adverse consequences of delayed treatment, however, can be profound. A uniform approach to treatment is inappropriate in view of variations in the patient's underlying disorder, disease status and risk level, as well as local differences in fungal epidemiology and diagnostic capability. Hence, a tailored approach to when and how to treat is essential. Different strategies (prophylaxis, empirical treatment, diagnostics-based therapy) have been explored, each of which has particular strengths and weaknesses. Various patterns of invasive mould disease have been defined in neutropenic patients and haematopoietic cell transplant recipients based on

Table 1. Overview of indications for key drugs in the management of invasive mould infections

Drug (brand name)	Europe	USA
Amphotericin B deoxycholate (Fungizone®)	• Treatment of potentially life-threatening fungal infections including aspergillosis and mucormycosis	• As for Europe
Liposomal amphotericin B (L-AmB) (Ambisome®)	• Treatment of severe systemic and/or deep mycoses (including aspergillosis and mucormycosis) • Empirical treatment for presumed fungal infections	• Treatment of patients with *Aspergillus* species refractory to amphotericin B deoxycholate, or in patients where renal impairment or unacceptable toxicity precludes the use of amphotericin B deoxycholate • Empirical therapy for presumed fungal infection
Amphotericin B lipid complex (ABLC) (Abelcet®)	• Second-line treatment of severe systemic fungal infections in patients who have not responded to conventional amphotericin B or other systemic antifungal agents, in those who have renal impairment or other contraindications to conventional amphotericin B, or in patients who have developed amphotericin B nephrotoxicity	• Treatment of invasive fungal infections in patients who are refractory to, or intolerant of, conventional amphotericin B therapy
Voriconazole (Vfend®)	• Prophylaxis of fungal infections in high-risk bone marrow transplant recipients • Treatment for invasive aspergillosis • Treatment of serious infections caused by *Scedosporium apiospermum* and *Fusarium* species in patients intolerant of, or refractory to, other therapies	• Treatment for invasive aspergillosis • Treatment of serious infections caused by *Scedosporium apiospermum* and *Fusarium* species in patients intolerant of, or refractory to, other therapies
Posaconazole (Noxafil®, Posanol®)	• Prophylaxis of invasive fungal infections • Treatment of invasive aspergillosis or fusariosis in patients who are intolerant of, or refractory to, other antifungal agents	• Prophylaxis of invasive *Aspergillus* • Treatment of invasive aspergillosis or fusariosis in patients who are intolerant of, or refractory to, other antifungal agents
Itraconazole (Sporanox®, Onmel®)	• Treatment of invasive aspergillosis when first-line therapy is inappropriate or has proved ineffective	• Prophylaxis of invasive *Aspergillus*
Isavuconazole (Cresemba®)	• Treatment of invasive aspergillosis • Treatment of mucormycosis if amphotericin B is inappropriate	• Treatment of invasive aspergillosis • Treatment of invasive mucormycosis
Caspofungin (Cancidas®)	• Treatment of invasive aspergillosis in patients who are refractory to, or intolerant of, amphotericin B, lipid formulations of amphotericin B and/or itraconazole • Empirical therapy for presumed fungal infections (e.g. *Aspergillus*)	• Treatment of invasive aspergillosis in patients who are refractory to, or intolerant of, other therapies • Empirical therapy for presumed fungal infections

For details, refer to prescribing information.

clinical symptoms, radiological findings and mycological results, and in theory the different strategies can be assigned accordingly (Figure 1).[6] Recent years have seen a shift towards the more closely directed use of antimould intervention as diagnostic resources have become more widely available, but wide differences in practice remain and intense debate continues regarding the optimal approach. Importantly, these strategies have mainly been explored and validated in neutropenic patients and cannot be uniformly applied to other at-risk groups, including solid organ transplant recipients and critically ill patients. Indeed, these latter groups frequently present with less-specific radiological findings,[7] and non-culture-based microbiological tests perform less well in these non-neutropenic populations.[8]

Defining risk

Decisions regarding antifungal prophylaxis or empirical antifungal therapy require an assessment of the patient's risk for invasive fungal disease. 'High risk' is imperfectly defined in this setting, but it has been proposed that subpopulations with >10% incidence of invasive fungal disease fall into this category.[9] The Infectious Diseases Society of America points out that patients are widely considered to be at high risk if they have prolonged and profound neutropenia (absolute neutrophil count $\leq$100 cells/mm^3 expected to last >7 days) following cytotoxic chemotherapy, or if they have significant comorbidity, including hypotension, pneumonia, new-onset abdominal pain

	Prophylaxis	Empirical	Diagnostics-driven (pre-emptive)				Directed
			I	II	III	IV	
Radiological signs & clinical symptoms	No	Persistent febrile neutropenia	No	Clinical (any new infiltrate not fulfilling the EORTC/MSG criteria)		Radiological signs on CT (dense, well-circumscribed lesions with or without a halo sign, air-crescent sign, or cavity)	
Mycology results	Negative	Negative	Positive biomarker or culture	Negative	Positive biomarker or culture	Negative	Positive biomarker or micrososcopy or culture
Evidence of IFD	No	No	No	No	No	Yes	Yes
Evidence of IFI	No	No	Yes	No	Yes	No	Yes
EORTC/MSG	–	–	–	–	–	Possible	Probable
Final diagnosis	No IFD		IFD cannot be excluded			IFD	

Figure 1. Patterns of IFD. (Adapted with permission from Maertens *et al.*[6]) EORTC/MSG, 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;[5] IFI, invasive fungal infection; IFD, invasive fungal disease.

or neurological changes.[10] Low-risk patients include those in whom neutropenia is expected to be short-lived (no more than 7 days) and who have no or few morbidities. European guidelines on invasive fungal diseases in paediatric patients state that patients are at high risk if they have *de novo* or recurrent acute myeloid leukaemia, acute lymphatic leukaemia, GvHD after allogeneic HSCT, or admission to the ICU.[9]

Based on the net state of immunosuppression, fungal exposure, genetic susceptibilities and organ function, patients can generally be stratified into high- and low-risk groups for invasive fungal disease, and risk-adapted antifungal strategies can be applied accordingly.[3] Importantly, risk assessment is a dynamic process and patients may gradually move to a higher or lower risk category (e.g. patients initially considered low risk who have refractory disease and require intensive chemotherapy may become high-risk patients).[11]

Prophylactic antifungal therapy

Prophylactic administration of broad-spectrum antifungal agents to lower the risk of invasive fungal disease seems logical in view of high mortality rates and the difficulty of ensuring early diagnosis. Universal prophylaxis, however, is inadvisable. The disadvantages of prophylaxis include interference with some diagnostic assays such as the galactomannan assay with the consequent risk for false-negative results, increased selection for resistance, drug toxicity and drug–drug interactions in this heavily medicated population, as well as the cost. Breakthrough infections can develop if the prophylactic agent has no activity against the invading fungal pathogen or if resistance has developed, or in particular circumstances such as profound immunosuppression or low serum levels of the prophylactic drug. One multicentre French survey found that of 423 patients who developed invasive fungal disease during 2007–08, 44% had received prophylaxis.[12] Of note, the impact of antimould prophylaxis has been evaluated predominantly in high-risk haemato-oncological patients, with limited data (e.g. solid organ transplant recipients) or no data (e.g. complicated influenza or decompensated cirrhosis) for other risk groups.

Clinical evidence

An early double-blind randomized trial of fluconazole prophylaxis in 356 patients undergoing bone marrow transplantation reported systemic fungal infections in 2.8% of fluconazole-treated patients versus 15.8% in the placebo arm ($P < 0.001$), with fewer deaths related to fungal infections under fluconazole.[13] Recent randomized trials have generally compared different prophylactic agents against one another, but in 2008 Rijnders *et al.*[14] randomized 271 patients with haematological disease and neutropenia expected to last $\geq$10 days to prophylaxis with aerosolized liposomal amphotericin B (L-AmB) or inhaled placebo (both arms also received fluconazole to prevent invasive *Candida* infection). Assessed over a total of 407 neutropenic episodes, the active treatment group had a decreased rate of invasive pulmonary aspergillosis (Figure 2). Systemic reviews of randomized trials in patients with chemotherapy-induced neutropenia have confirmed that antifungal prophylaxis reduces the risk of invasive fungal infections versus untreated controls or placebo,[15,16] with reduced mortality attributed to fungal infections.[15] One analysis suggested that

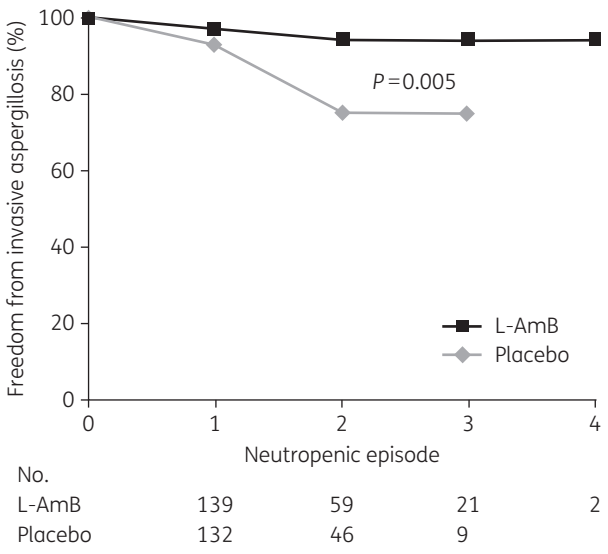

Figure 2. Incidence of invasive pulmonary aspergillosis in patients with haematological disease and chemotherapy-induced neutropenia expected to last ≥10 days, randomized to prophylactic aerosolized L-AmB or placebo inhalation twice a week until the neutrophil count increased to > 300 cells/mm^3. Reproduced with permission from Rijnders *et al.*[14]

recipients of HSCT experienced the most marked benefit in terms of avoiding infections and infection-related deaths.[15]

Randomized trials have compared the outcomes of prophylaxis with different antifungal agents in at-risk groups[17–20] with the results indicating a better risk–benefit profile for mould-active triazoles versus fluconazole. Cornely *et al.*[17] randomized 304 patients with acute myelogenous leukaemia or myelodysplastic syndrome and prolonged neutropenia to posaconazole or to fluconazole or itraconazole prophylaxis. The posaconazole group experienced a lower rate of probable or proven invasive fungal infection overall, and invasive aspergillosis specifically, with significantly longer survival versus the fluconazole/itraconazole group. However, drug-related serious adverse events were more frequent with posaconazole. A recent meta-analysis of studies published up to early 2013 found posaconazole to be the most effective of the antifungal agents examined in terms of lower risk for invasive fungal infections and all-cause mortality.[16] Expert guidelines have recommended various clinical situations in which use of triazole prophylaxis, including posaconazole, is appropriate.[9,21,22]

The optimal duration of antifungal prophylaxis is unknown.[23] Many recipients of allogeneic HSCT receive azole prophylaxis for an extended period, but it should be noted that there is evidence to suggest that extended administration may increase toxicity. For instance, the incidence of photosensitivity reactions, while generally rare, have been reported to increase to 5.6% when voriconazole is given for >12 weeks[24] and longer treatment may increase risk for aggressive cutaneous squamous cell carcinoma (SCC) in immunocompromised patients.[25] Wojenski *et al.*[26] retrospectively analysed 381 adults with allogeneic HSCT given voriconazole for a median of 214 days and found that the cumulative number of days of voriconazole use was an independent risk factor for SCC on multivariate analysis. Retrospective studies in lung transplant patients have also demonstrated a significant association between duration or cumulative dose of voriconazole and risk for SCC.[27–29]

Candidates for antifungal prophylaxis

Antifungal prophylaxis is recommended for HSCT patients and for cancer patients with no symptoms of fever or active infection if they are considered to be at increased risk for invasive fungal disease.[9,10,22] Specific clinical scenarios have been proposed (Table 2),[9,10,21,22,30] but accurate identification of patients in whom prophylaxis is merited—and the point at which prophylaxis should be initiated—can be difficult. Firm criteria for what constitutes 'high risk' for invasive fungal disease are still unconfirmed and although the local prevalence of mould infections would ideally be taken into account, such data are frequently lacking. At present, the prevalence of antifungal prophylaxis varies between centres due to variations in selection criteria and differing views on where the balance of advantages versus disadvantages lies.

Secondary prophylaxis to help prevent relapse for a previous documented invasive fungal infection when the patient enters a new at-risk period (e.g. prolonged neutropenia) is recommended.

Empirical antifungal therapy

An empirical approach to treatment, whereby mould-active therapy is started in neutropenic patients with persistent or relapsing fever unresponsive to broad-spectrum antibiotics, has been widely used for many years. However, its use remains a matter of debate due to overtreatment triggered by the non-specific symptom of fever. Fever is a frequent occurrence in patients with chemotherapy-induced neutropenia; as many as 80% of patients with haematological malignancies develop fever at least once.[31] In the majority of cases, however, there is no infectious aetiology. Fungal infections, in particular, rarely cause the initial fever but instead develop after the first week of neutropenia following empirical antibiotic therapy. Aspergillosis and other moulds are typically detected only after ≥2 weeks of neutropenia.[10] Thus, broad-spectrum intravenous or oral antibiotics are usually the first management step. Where neutropenic patients remain febrile despite broad-spectrum antibiotics, empirical antifungal therapy could be applied universally or selectively in high-risk individuals. The downsides of empirical treatment are similar to those of universal prophylaxis and include the cost, increased risk of resistance and potential toxicity for the patient. As many as a third of cancer patients with prolonged neutropenia receive empirical antifungal therapy,[10] but clinical trials have found that only ~4% have proven invasive fungal infection.[17,32] Furthermore, breakthrough infections have been reported in up to 16% of patients given modern antimould therapies,[33,34] and in up to one-quarter of patients given fluconazole (which has no antimould activity).

Clinical evidence

Evidence in support of empirical antifungal therapy is surprisingly limited given its widespread use. Many of the trials comparing empirical treatment versus untreated controls or placebo did not apply modern diagnostic techniques, and were often performed

Table 2. Overview of treatment strategies for invasive fungal disease (in haemato-oncology patients)[10,22,30]

Treatment strategy	Candidate patients	Clinical triggers to start treatment
Prophylaxis	• Patients undergoing intensive chemotherapy for AML or MDS • Allogeneic HSCT patients with prior invasive fungal infection, or expected neutropenia duration ≥2 weeks, GvHD treated with prolonged immunosuppression, or prolonged neutropenia immediately prior to allogeneic HSCT • Not generally required for patients undergoing autologous HSCT • Not recommended for patients with an anticipated duration of neutropenia <7 days	• Not applicable
Empirical	• Patients with persistent or recurrent fever unresponsive to broad-spectrum antibiotics with an expected duration of neutropenia >7 days • Not recommended in low-risk patients	• Initiate empirical iv antifungal therapy with antimould coverage if febrile neutropenia does not respond to broad-spectrum antibiotics after 4–7 days and myeloid recovery is not imminent. If the patient is already receiving oral antifungal prophylaxis, switch to empirical iv therapy • Possibly initiate iv antifungal therapy with antimould coverage earlier in patients with a documented infection who are clinically unstable with worsening signs of infection under antibiotic therapy, while fungal or other pathogens are being sought and identified
Pre-emptive	• Patients who remain febrile after 4–7 days of broad-spectrum antibiotics but are clinically stable, with no clinical symptoms of fungal infection, or signs of fungal infection on chest and sinus CT scans, or negative serological assay results for invasive fungal infection, and no recovery of fungi from any of the body sites	• Initiate iv antifungal therapy with antimould coverage immediately on receipt of a positive biomarker test (e.g. serum galactomannan) for invasive fungal infection, or clinical signs of fungal infection, or chest or sinus CT evidence of fungal infection (e.g. halo sign and/or macronodule)

iv, intravenous; MDS, myelodysplastic syndrome.

before today's prophylactic strategies were in place. Goldberg *et al.*[35] performed a meta-analysis of five randomized controlled trials of empirical antifungal therapy versus no treatment or placebo, all but one of which employed amphotericin B preparations. They observed no significant effect of empirical therapy on all-cause mortality (relative risk 0.93; 95% CI 0.55–1.58).[35] Invasive fungal infections were less frequent under empirical therapy, but event numbers were low.[35]

Placebo-controlled trials of triazoles or echinocandin agents for empirical therapy are rare, but one double-blind randomized trial, published in 1987, compared intravenous miconazole versus placebo in 180 patients receiving cytotoxic therapy with an expected neutropenia duration of at least 2 weeks.[36] Miconazole was started simultaneously with empirical antibiotics, at the onset of fever, in contrast to current practice where it is delayed to determine whether the patient responds to antibiotics. Fungal sepsis was less frequent with miconazole ($P = 0.03$) with a trend to fewer fatal fungal sepsis events ($P = 0.08$). More recently, Maschmeyer *et al.*[37] undertook a randomized, double-blind trial in which 147 high-risk haematology patients with neutropenia received either voriconazole immediately at onset of neutropenic fever, or placebo with voriconazole started only if fever persisted for 4 days; all patients were given broad-spectrum antibacterial therapy. Immediate voriconazole did not reduce the incidence of probable or proven invasive fungal disease by week 4 compared with delayed voriconazole. Delaying antifungal therapy until fever has persisted despite antibiotic therapy appears rational.

Randomized trials comparing empirical therapy with voriconazole[38] or the echinocandin caspofungin[32,39] versus L-AmB have shown no significant difference in success rates but some secondary advantages and a superior safety and tolerability profile, particularly for caspofungin compared with L-AmB. Of note, mould-active triazoles should not be used empirically in a patient already receiving mould-active prophylaxis.

Candidates for empirical antifungal therapy

If an empirical treatment strategy is used, it should be given to high-risk patients who have no identified fever source and who continue to have fever after 4–7 days of broad-spectrum antibiotics, with an expected duration of neutropenia of >7 days.[10] If the patient is already receiving oral antifungal prophylaxis, switching to empirical therapy with an intravenous agent is recommended.[10] Earlier introduction of empirical therapy can be advisable in patients with a documented infection who are deteriorating clinically with worsening signs of infection under antibiotic

therapy. Routine use of empirical therapy is not appropriate in low-risk patients.[10]

These criteria, however, will not detect patients with invasive mould infections who do not develop fever, e.g. highly immunosuppressed patients or those receiving high-dose steroids.

Pre-emptive antifungal therapy

Empirical treatment was adopted in the 1980s prior to the advent of reliable non-invasive diagnostic techniques, since clinicians were unwilling to obtain tissue-based evidence in this frail population. More recently there has been growing interest in delaying antifungal treatment until an invasive fungal infection is confirmed ('pre-emptive' or 'diagnostics-driven' management). This more rigorous strategy has evolved following advances in the early detection of fungal infections, notably development of galactomannan and the β-(1–3)-D-glucan serum tests, in combination with high-resolution CT scanning. Pre-emptive treatment represents a more targeted treatment strategy than empirical therapy, and is usually applied to the subpopulation of neutropenic patients who are still febrile after 4–7 days of broad-spectrum antibiotics but who are clinically stable without clinical or other signs of fungal infection.[40] Such patients should have no indication of fungal infection on CT scans of the chest or sinus, negative serology for invasive fungal infections and no recovery of fungi from any of the body sites. In these cases, antifungal therapy can be withheld until evidence of infection is provided by CT scans of the chest and sinuses, and/or serum tests. Since the sensitivity of a single serum test is low, serial tests are required before a fungal infection can be excluded for high-risk patients in whom empirical therapy has been withheld. Monitoring based on clinical and radiological signs with biweekly blood screening using galactomannan antigenaemia and a quantitative PCR assay has been proposed.[41,42] Self-evidently, pre-emptive management is not feasible unless sensitive diagnostic testing and CT scanning are readily available. Hence, it should not be used in populations in which these tests appear to be less sensitive and predictive (e.g. critically ill patients).

Clinical evidence

Pre-emptive therapy in selected patients (Table 2) allows treatment in cases of possible invasive fungal disease, achieving higher response rates than if treatment is withheld until the diagnosis is probable or proven. Cornely *et al.* re-evaluated outcomes in a prospective trial of L-AmB in patients with haematological malignancies with a halo or air crescent sign on chest CT, categorizing patients according to 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 (EORTC/MSG) 2008 criteria.[43] Survival was compared between patients treated for 'possible' invasive disease (i.e. on the basis of radiological signs on CT but negative mycology results; $n = 118$) or only once when invasive infection was probable or proven (i.e. both radiological CT and mycological evidence; $n = 83$). Survival at week 6 was higher in the 'possible' cohort (Figure 3). Herbrecht *et al.*, similarly, re-categorized patients in a *post-hoc* analysis of data from a randomized comparative study of voriconazole versus amphotericin B[44] by applying the EORTC/MSG 2008 criteria.[45] Of the 277 patients considered to have definite or probable invasive aspergillosis in the original study,[44] 59 proven, 178 probable and 106 possible cases were identified after re-categorization.[45] The primary endpoint of partial or complete response at week 12 was more frequent with voriconazole than amphotericin B in the possible cases as well as in mycologically documented episodes, with a similar magnitude of difference (26.2% and 24.3%, respectively).[45]

There is limited evidence comparing empirical versus pre-emptive management strategies. Cordonnier and colleagues compared 14 day survival with empirical versus pre-emptive antifungal therapy in an open-label, multicentre randomized trial.[46] A total of 293 patients were enrolled within 2 days of developing fever, all of whom had haematological malignancies or had undergone autologous HSCT, with neutropenia expected to last at least 10 days. Antifungal prophylaxis was given as per local practice to 42% and 48% of patients in the empirical and pre-emptive groups, respectively. Empirical therapy (amphotericin B or L-AmB, depending on renal function) was started on day 4 of persistent fever despite antibacterial treatment. Pre-emptive treatment, triggered by various predefined clinical, laboratory and imaging criteria, also comprised amphotericin B or L-AmB. The primary endpoint, proportion of patients alive at 14 days after recovery from neutropenia was similar between groups (97.3% with empirical therapy, 95.1% with pre-emptive therapy).[46] As expected, fewer patients received antifungal therapy in the pre-emptive group (Figure 4), and when the cost of therapy was adjusted to assume that all treated patients received L-AmB, drug costs were ∼40% lower than with empirical treatment (mean €2509 versus €4261 at 2005 prices; $P < 0.001$). Invasive fungal infections were more frequent with pre-emptive therapy (Figure 4) but mortality related to fungal infections was similar (2.1% versus 0% in the empirical group; $P = 0.11$).[46] More recently, the costs associated with empirical therapy using amphotericin B, L-AmB or caspofungin compared with pre-emptive therapy using amphotericin B, voriconazole or L-AmB were analysed from a UK perspective.[47] Results indicated that the pre-emptive strategy incurred 32% lower total costs due to lower drug use and fewer adverse events. Contrasting results have been reported in a randomized, multicentre trial comparing empirical versus pre-emptive therapy with L-AmB in 409 patients after allogeneic HSCT.[48] Empirical therapy was started if fever continued for 5 days, while pre-emptive treatment was started in response to a positive PCR analysis or after 5 days of fever. Use of antifungal therapy was higher in the pre-emptive arm (57.1% versus 36.7% with empirical therapy; $P < 0.0001$), partly due to the high rate of single false-positive PCR results in the pre-emptive group. Perhaps the most robust data to date are derived from an open-label study from Australia in which 240 haemato-oncology patients at high risk for invasive aspergillosis were randomized to empirical therapy based on culture results and history, or to a pre-emptive strategy in which treatment was initiated based on galactomannan and PCR results.[49] Antifungal drugs were administered to 32% of the empirical therapy cohort compared with 15% of the pre-emptive therapy group ($P = 0.002$).

Randomized trials of empirical versus pre-emptive treatment using modern triazole agents have not been performed.

For pre-emptive management to be successful, laboratory and imaging tests must be performed promptly with results provided quickly (within 24 h, or a maximum of 48 h). In invasive pulmonary aspergillosis, after the early stage of infection following conidia

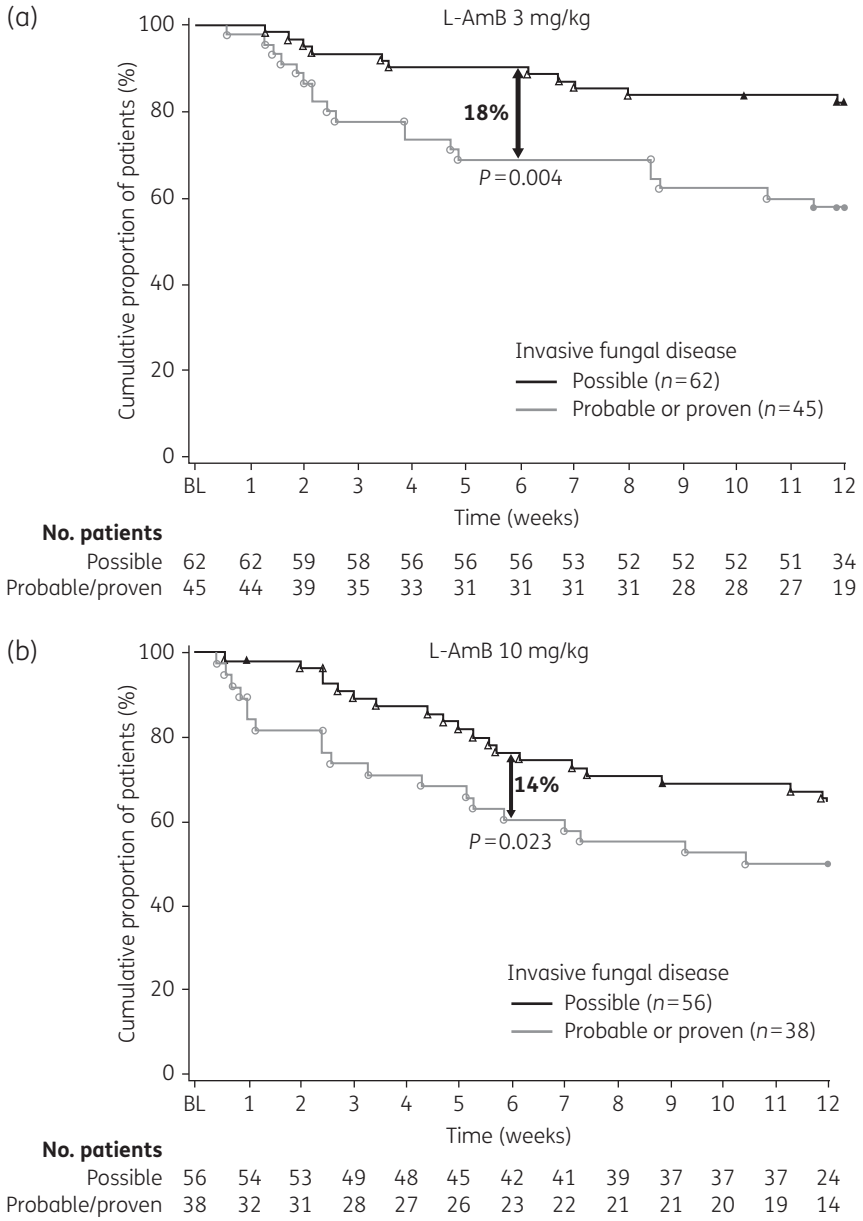

Figure 3. Survival in allogeneic HSCT patients from the time at which L-AmB at an initial dose of (a) 3 mg/kg or (b) 10 mg/kg was initiated for possible invasive fungal disease (i.e. radiological signs on CT with negative mycology results) or probable/proven invasive fungal disease (i.e. radiological signs on CT and possible biomarker, microscopy or culture). Reproduced with permission from from Cornely *et al.*[43]

inhalation, hyphae become sequestered within dense inflammatory foci, creating a barrier to adequate antifungal exposure. A neutropenic rabbit model of invasive aspergillosis in the lung has shown that pulmonary infarction develops as soon as 24 h after infection, and that even a 72 h delay in starting antifungal treatment obviates the improvement in fungal burden seen when treatment was started early (within 24–48 h of infection).[50] Greene *et al.*[51] demonstrated the importance of prompt treatment in an analysis of chest CT images from 235 patients with definite or probable acute invasive pulmonary aspergillosis taking part in a study of voriconazole versus amphotericin B as the primary therapy. Patients in whom treatment was started at the point where a halo sign was detected, an early radiological manifestation of invasive pulmonary aspergillosis,[31] showed significantly higher treatment response rates and improved survival compared to those in whom treatment was started after the halo sign stage. A delay of several days before performing a CT scan can miss onset of the halo sign, with a commensurate delay in intervention. Similarly, Chamilos *et al.*[52] retrospectively assessed 70 patients with haematological malignancy and mucormycosis and found a 2-fold increase in mortality for patients in whom the start of intravenous antimould therapy was delayed by ≥6 days from the onset of symptoms.

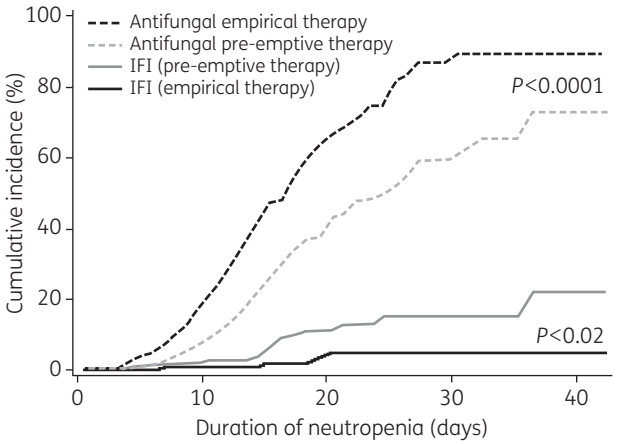

Figure 4. Cumulative incidence of antifungal therapy and IFI in febrile patients with haematological malignancies or autologous HSCT in whom neutropenia was expected to last ≥10 days, randomized to empirical therapy or pre-emptive therapy with amphotericin B or L-AmB. IFI, invasive fungal infection. Adapted with permission from Cordonnier *et al*.[46]

Candidates for diagnostics-driven antifungal therapy

Pre-emptive therapy may be an appropriate alternative to empirical therapy in neutropenic patients who remain febrile after 4–7 days of broad-spectrum antibiotics but who are clinically stable (Table 2). Patients should have negative CT scans and serological assays, with no fungal colonization of any of the body sites. Careful monitoring is essential, with intravenous antifungal treatment started immediately if a blood culture is positive for fungal pathogens, or if indicated by a positive biomarker test such as serum galactomannan, positive CT findings on chest or sinus scans (e.g. halo sign), histopathological evidence or highly suggestive clinical signs (e.g. pleuritic chest pain or periorbital swelling) of a fungal infection.

Areas for future research

Despite significant recent advances, the available tools for diagnosing invasive mould disease are far from perfect and clinicians still struggle to make a timely diagnosis. Therefore, the search for novel targets and platforms that could further improve our diagnostic capabilities continues. PCR-based methods have been developed for the diagnosis of many diseases, including mould disease. Real-time PCR is a highly sensitive technique that can be applied to many specimen types. However, lack of standardization between assays (either in-house or commercially available), and the use of varied protocols involving different specimens, extraction techniques, molecular targets, amplification platforms and detection techniques has hampered their acceptance. Fortunately, the European Aspergillus PCR Initiative has made tremendous progress in standardizing protocols for efficient DNA extraction and amplification.[52–56] Currently, clinical validation in multicentre prospective trials is ongoing. Other more investigational tools include: a lateral-flow device developed for point-of-care diagnosis of invasive aspergillosis;[57,58] an electronic nose (or eNose) device that can discriminate between various lung diseases through the analysis of exhaled volatile organic compounds;[59] and the detection of bis(methylthio)glyotoxin, a virulence factor that has been proposed as a diagnostic biomarker for invasive aspergillosis.[60,61]

Pending the outcomes of ongoing and planned validations of these surrogate markers for diagnosing invasive fungal infection or disease, it is likely that the future lies in combining diagnostic tools, either to rule out disease (based on their combined high negative predictive value) or to confirm disease (based on the positive predictive value of the combination). Ultimately, this should allow a smooth transition from universal antifungal prophylaxis or empirical antifungal therapy to an individualized approach.[11] Understanding the test performance in specific patient populations (e.g. haematology versus solid organ transplants) as well as in different clinical specimens (e.g. serum/plasma versus bronchoalveolar lavage) and acknowledging the strengths and limitations of different strategies is important to maximize the clinical benefit of antifungal drugs in an economically useful way.

Conclusions

Characterization of different patterns of invasive fungal disease is essential if the appropriate treatment strategy is to be applied (Figure 1). Risk assessment and close monitoring can then be applied to try to balance the opposing risks of over- and under-treatment in this vulnerable population. The indications for prophylaxis are relatively well defined in current expert guidelines, but in practice, identifying patients at high risk in whom prophylaxis should be instigated can be problematic. Furthermore, there is no accepted consensus on whether an empirical or pre-emptive strategy is preferable for neutropenic febrile patients who do not respond to broad-spectrum antibiotics. Empirical antifungal therapy is widely used but the evidence does not support a benefit for all-cause mortality. Although early studies found a reduction in invasive fungal infections, the conclusions were based on small numbers in trials performed before current antifungal prophylactic regimens, without baseline confirmation of fungal infection, and using outdated diagnostic criteria, and are not necessarily applicable today. Given the poor prognosis associated with invasive fungal infections, however, expert guidelines recommend that empirical therapy be implemented in high-risk groups with persistent neutropenic fever[6,20,27] and this approach is expected to continue at centres where the diagnostic capacity cannot support pre-emptive management. While pre-emptive treatment is appealing, the necessary monitoring and detailed treatment algorithms can be complex and require a multidisciplinary effort.

Many important questions are still unanswered. Which are the ideal patients for prophylaxis, empirical or pre-emptive therapy? What approach should be followed in neutropenic patients who develop persistent fever while receiving antimould prophylaxis—is empirical or pre-emptive therapy more suitable? If a pre-emptive strategy is followed, are clinical and radiological signs or a serum biomarker the optimal trigger for treatment, and which biomarker should be used? How long should maintenance therapy be continued after successful first-line therapy? While very considerable advances have been made in clinical decision-making, reducing the burden of invasive fungal infections in immunocompromised patients remains a work in progress.

Funding

This Supplement was funded by an unrestricted grant from Basilea Pharmaceutica International Ltd, Basel, Switzerland. Editorial support was provided by a freelance medical writer (C. Dunstall) with funding from Basilea.

Transparency declarations

T. M. has received speaker's honoraria from Gilead Sciences. J. M. has served as consultant to Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., Astellas Pharma, Basilea, Bio-Rad, Fujisawa Healthcare, Inc., Zeneus (Cephalon), Viropharma, and Boehringer-Ingelheim, Amgen and Shire; has been a member of the speaker's bureau for Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., Basilea, Bio-Rad, Fujisawa Healthcare, Inc., Astellas Pharma and Zeneus (Cephalon); and has received research funding from Bio-Rad, Merck, Sharp & Dohme, Gilead Sciences and Pfizer Inc.

This article forms part of a Supplement sponsored by Basilea Pharmaceutica International Ltd, Basel, Switzerland.

Author contributions

Both authors developed the article in collaboration with a freelance medical writer.

References

1 Pagano L, Caira M, Picardi M *et al*. Invasive aspergillosis in patients with acute leukemia: Update on morbidity and mortality—SEIFEM-C report. *Clin Infect Dis* 2007; **44**: 1524–5.

2 Upton A, Kirby KA, Carpenter P *et al*. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007; **44**: 531–40.

3 Herbrecht R, Bories P, Moulin JC *et al*. Risk stratification for invasive aspergillosis in immunocompromised patients. *Ann N Y Acad Sci* 2012; **1272**: 23–30.

4 Koulenti D, Garnacho-Montero J, Blot S. Approach to invasive pulmonary aspergillosis in critically ill patients. *Curr Opin Infect Dis* 2014; **27**: 174–83.

5 de Pauw BE, Viscoli C. Managing invasive fungal infections: relying on clinical instincts or a rational navigation system? *J Antimicrob Chemother* 2011; **66**: Suppl 1:i55–i58.

6 Maertens JA, Nucci M, Donnelly JP. The role of antifungal treatment in hematology. *Haematologica* 2012; **97**: 325–7.

7 Vandewoude KH, Blot SI, Depuydt P *et al*. Clinical relevance of *Aspergillus* isolation from respiratory tract samples in critically ill patients. *Crit Care* 2006; **10**: R31.

8 Meersseman W, Lagrou K, Maertens J *et al*. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008; **177**: 27–34.

9 Groll AH, Castagnola E, Cesaro S *et al*. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *Lancet Oncol* 2014; **15**: e327–40.

10 Freifield AG, Bow EJ, Sepkowitz KA *et al*. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011; **52**: e56–93.

11 Nucci M, Anaissie E. How we treat invasive fungal diseases in patients with acute leukemia: the importance of an individualized approach. *Blood* 2014; **124**: 3858–69.

12 Herbrecht R, Caillot D, Cordonnier C *et al*. Indications and outcomes of antifungal therapy in French patients with haematological conditions or recipients of haematopoietic stem cell transplantation. *J Antimicrob Chemother* 2012; **67**: 2731–8.

13 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**: 845–51.

14 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**: 1401–8.

15 Ziakas PD, Kourbeti IS, Voulgarelis M *et al*. Effectiveness of systemic antifungal prophylaxis in patients with neutropenia after chemotherapy: a meta-analysis of randomized controlled trials. *Clin Ther* 2010; **32**: 2316–36.

16 Pechlivanoglou P, Le HH, Daenen S *et al*. Mixed treatment comparison of prophylaxis against invasive fungal infections in neutropenic patients receiving therapy for haematological malignancies: a systematic review. *J Antimicrob Chemother* 2014; **69**: 1–11.

17 Cornely OA, Maertens J, Winston DJ *et al*. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007; **356**: 348–59.

18 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**: 335–47.

19 Wingard JR, Carter SL, Walsh TJ *et al*. Randomized double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood* 2010; **116**: 5111–8.

20 Marks DI, Pagliuca A, Kibbler CC, IMPROVIT Study Group *et al*. Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation. *Br J Haematol* 2011; **155**: 318–27.

21 European Society of Clinical Microbiology and Infectious Diseases/European Fungal Infection Study Group. European Conference on Infections in Leukemia 5; primary antifungal prophylaxis. https://www.ebmt.org/Contents/Resources/Library/ECIL/Documents/2014%20ECIL5/ECIL5antifungalprophylaxis%20%2020062014%20Final.pdf

22 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**: 327–60.

23 Marr KA, Bow E, Chiller T *et al*. Fungal infection prevention after hematopoietic cell transplantation. *Bone Marrow Transplant* 2009; **44**: 483–7.

24 Food and Drug Administration Antiviral Drugs Advisory Committee 2001. Briefing Document for Voriconazole (Oral and Intravenous Formulations). http://www.fda.gov/ohrms/dockets/ac/01/briefing/3792b2_01_Pfizer.pdf

25 Cowen EW, Nguyen JC, Miller DD *et al*. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. *J Am Acad Dermatol* 2010; **62**: 31–7.

26 Wojenski DJ, Bartoo GT, Merten JA *et al*. Voriconazole exposure and the risk of cutaneous squamous cell carcinoma in allogeneic hematopoietic stem cell transplant patients. *Transpl Infect Dis* 2015; **17**: 250–8.

27 Singer JP, Boker A, Metchnikoff C *et al*. High cumulative dose exposure to voriconazole is associated with cutaneous squamous cell carcinoma in lung transplant recipients. *J Heart Lung Transplant* 2012; **31**: 694–9.

28 Feist A, Lee R, Osborne S *et al*. Increased incidence of cutaneous squamous cell carcinoma in lung transplant recipients taking long-term voriconazole. *J Heart Lung Transplant* 2012; **31**: 1177–81.

29 Zwald FO, Spratt M, Lemos BD *et al*. Duration of voriconazole exposure: an independent risk factor for skin cancer after lung transplantation. *Dermatol Surg* 2012; **38**: 1369–74.

30 De Pauw B, Walsh TJ, Donnelly JP *et al*. Revised definitions of invasive fungal disease from 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 (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; **46**: 1813–21.

31 Klastersky J. Management of fever in neutropenic patients with different risks of complications. *Clin Infect Dis* 2004; **39** Suppl 1: S32–7.

32 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**: 1391–402.

33 Kubiak DW, Bryar JM, McDonnell AM *et al*. Evaluation of caspofungin or micafungin as empiric antifungal therapy in adult patients with persistent febrile neutropenia: a retrospective, observational, sequential cohort analysis. *Clin Ther* 2010; **32**: 637–48.

34 Ananda-Rajah MR, Grigg A, Downey MT *et al*. Comparative clinical effectiveness of prophylactic voriconazole/posaconazole to fluconazole/itraconazole in patients with acute myeloid leukemia/myelodysplastic syndrome undergoing cytotoxic chemotherapy over a 12-year period. *Haematologica* 2012; **97**: 459–63.

35 Goldberg E, Gafter-Gvili A, Robenshtok E *et al*. Empirical antifungal therapy for patients with neutropenia and persistent fever: Systematic review and meta-analysis. *Eur J Cancer* 2008; **44**: 2192–203.

36 Wingard JR, Vaughan WP, Braine HG *et al*. Prevention of fungal sepsis in patients with prolonged neutropenia: a randomized, double-blind, placebo-controlled trial of intravenous miconazole. *Am J Med* 1987; **83**: 1103–10.

37 Maschmeyer G, Heinz WJ, Hertenstein B *et al*. Immediate versus deferred empirical antifungal (IDEA) therapy in high-risk patients with febrile neutropenia: a randomized, double-blind, placebo-controlled, multicenter study. *Eur J Clin Microbiol Infect Dis* 2013; **32**: 679–89.

38 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**: 225–34.

39 Maertens JA, Madero L, Reilly AF *et al*. A randomized, double-blind, multicenter study of caspofungin versus liposomal amphotericin B for empiric antifungal therapy in pediatric patients with persistent fever and neutropenia. *Pediatr Infect Dis J* 2010; **29**: 415–20.

40 Aguilar-Guisado M, Martín-Peña A, Espigado I *et al*. Universal antifungal therapy is not needed in persistent febrile neutropenia: a tailored diagnostic and therapeutic approach. *Haematologica* 2012; **97**: 464–71.

41 Barnes RA, White PL, Bygrave C *et al*. Clinical impact of enhanced diagnosis of invasive fungal disease in high-risk haematology and stem cell transplant patients. *J Clin Pathol* 2009; **62**: 64–9.

42 Aguado JM, Vázquez L, Fernández-Ruiz M *et al*. Serum galactomannan versus a combination of galactomannan and polymerase chain reaction-based *Aspergillus* DNA detection for early therapy of invasive aspergillosis in high-risk hematological patients: a randomized controlled trial. *Clin Infect Dis* 2015; **60**: 405–14.

43 Cornely OA, Maertens J, Bresnik M *et al*. Efficacy outcomes in a randomised trial of liposomal amphotericin B based on revised EORTC/MSG 2008 definitions of invasive mould disease. *Mycoses* 2011; **54**: e449–55.

44 Herbrecht R, Denning DW, Patterson TF *et al*. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–15.

45 Herbrecht R, Patterson TF, Slavin MA *et al*. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus amphotericin B for therapy of invasive aspergillosis: a collaborative study of the Mycoses Study Group (MSG 05) and the European Organization for Research and Treatment of Cancer Infectious Diseases Group. *Clin Infect Dis* 2015; **60**: 713–20.

46 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**: 1042–51.

47 Barnes R, Earnshaw S, Herbrecht R *et al*. Economic comparison of an empirical versus diagnostic-driven strategy for treating invasive fungal disease in immunocompromised patients. *Clin Ther* 2015; **37**: 1317–28.

48 Hebart H, Klingspor L, Klingebiel T *et al*. A prospective, randomized controlled trial comparing PCR-based and empirical treatment with liposomal amphotericin B in patients after allo-SCT. *Bone Marrow Transplant* 2009; **43**: 553–61.

49 Morrissey CO, Chen SC, Sorrell TC *et al*. Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial. *Lancet Infect Dis* 2013; **13**: 519–28.

50 Hope WW, Petraitis V, Petraitiene R *et al*. The initial 96 hours of invasive pulmonary aspergillosis: histopathology, comparative kinetics of galactomannan and (1→3) β-D-glucan and consequences of delayed antifungal therapy. *Antimicrob Agents Chemother* 2010; **54**: 4879–86.

51 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**: 373–9.

52 Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008; **47**: 503–9.

53 White PL, Wiederhold NP, Loeffler J *et al*. Comparison of nonculture blood-based tests for diagnosing invasive aspergillosis in an animal model. *J Clin Microbiol* 2016; **54**: 960–6.

54 Loeffler J, Mengoli C, Springer J *et al*. Analytical comparison of in vitro-spiked human serum and plasma for PCR-based detection of *Aspergillus fumigatus* DNA: a study by the European Aspergillus PCR Initiative. *J Clin Microbiol* 2015; **53**: 2838–45.

55 White PL, Barnes RA, Springer J *et al*. Clinical performance of *Aspergillus* PCR for testing serum and plasma: a study by the European *Aspergillus* PCR Initiative. *J Clin Microbiol* 2015; **53**: 2832–7.

56 Lyon GM, Abdul-Ali D, Loeffler J *et al*. Development and evaluation of a calibrator material for nucleic acid-based assays for diagnosing aspergillosis. *J Clin Microbiol* 2013; **51**: 2403–5.

57 Thornton C, Johnson G, Agrawal S. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. *J Vis Exp* 2012; **61**: pii=3721.

58 Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vaccine Immunol* 2008; **15**: 1095–105.

59 de Heer K, van der Schee MP, Zwinderman K *et al*. Electronic nose technology for detection of invasive pulmonary aspergillosis in prolonged chemotherapy-induced neutropenia: a proof-of-principle study. *J Clin Microbiol* 2013; **51**: 1490–5.

60 Domingo MP, Colmenarejo C, Martínez-Lostao L *et al*. Bis(methyl)gliotoxin proves to be a more stable and reliable marker for invasive aspergillosis than gliotoxin and suitable for use in diagnosis. *Diagn Microbiol Infect Dis* 2012; **73**: 57–64.

61 Vidal-García M, Domingo MP, De Rueda B *et al*. Clinical validity of bis(methylthio)gliotoxin for the diagnosis of invasive aspergillosis. *Appl Microbiol Biotechnol* 2016; **100**: 2327–34.