J Antimicrob Chemother 2013; **68** Suppl 3: iii5–iii15
doi:10.1093/jac/dkt389

Journal of Antimicrobial Chemotherapy

Preventing invasive fungal disease in patients with haematological malignancies and the recipients of haematopoietic stem cell transplantation: practical aspects

Hamdi Akan[1]*, Vistasp P. Antia[2], Michal Kouba[3], János Sinkó[4], Alina Daniela Tănase[5], Radovan Vrhovac[6] and Raoul Herbrecht[7]

[1]Department of Hematology, Ankara University Medical Faculty, Ankara, Turkey; [2]Department of Hematological Medicine, Breach Candy Hospital Trust, Mumbai, India; [3]The Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [4]Department of Hematology and Stem Cell Transplantation, St István and St László Hospital, Budapest, Hungary; [5]Department of Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania; [6]Department of Hematology, University Hospital Centre, Zagreb, Croatia; [7]Department of Oncology and Hematology, Hôpital de Hautepierre, Strasbourg, France

*Corresponding author. Tel: +90-5324-242640; Fax: +90-3123-625422; E-mail: hamdiakan@gmail.com

Invasive fungal disease (IFD), predominantly aspergillosis, is associated with significant morbidity and mortality in immunocompromised patients, especially those with haematological malignancies and recipients of allogeneic haematopoietic stem cell transplantation. There has been a great deal of scientific debate as to the effectiveness of antifungal prophylaxis in preventing infection in different patient groups and in which patients it is an appropriate management option. Deciding on an appropriate prophylaxis regimen for IFD is challenging as the incidence varies among different patient groups, due to the varied nature of their underlying haematological disease, and in different regions and centres. Attempts have been made to define risk factors and include them in treatment protocols. Impaired immune status of the patient, especially neutropenia, is a key risk factor for IFD and can sometimes be related to specific polymorphisms of genes controlling innate immunity. Risk factors also vary according to the type of fungal pathogen. Consequently, prophylaxis needs to be tailored to individual patient groups. Furthermore, the choice of antifungal agent for prophylaxis depends on the potential for drug–drug interactions with the patients' concomitant medications. Additional challenges are optimal timing of antifungal prophylaxis, when to change from prophylaxis to antifungal treatment and how to prevent recurrence of IFD. This article considers the use of antifungal prophylaxis for patients at risk of IFD in daily clinical practice, with clinical profiles that may be distinct from those covered by guidelines, and aims to provide practical advice for treatment of these patient groups.

Keywords: prophylaxis, aspergillosis, candidiasis, haematological malignancy, immunocompromised hosts

Introduction

Invasive fungal disease (IFD) is one of the most prevalent causes of morbidity and mortality in immunocompromised patients.[1–3] Prevention is a rational strategy for patients at high risk of IFD. Consequently, there has been a great deal of research and discussion examining whether prophylaxis improves disease outcomes.[4–9] As the group of patients at high risk of IFD is heterogeneous, with different underlying diseases, risk factors and demographic characteristics, it is not surprising that more-tailored prophylactic measures are needed. Recent research has allowed some distinctions to be made between different patient groups and various guidelines and prophylactic protocols have been developed.[10–19] However, there are still some patient groups, such as those with acute lymphoblastic leukaemia or patients at the aplastic phase of allogeneic stem cell transplantation, in which the use of prophylaxis needs to be better assessed. In addition, the availability of drugs and socioeconomic issues (reimbursement policies, high drug costs and regulatory restrictions) are also important factors to be considered when choosing the optimal prophylactic approach. Despite the widespread use of antifungal prophylaxis, there are several issues that remain to be answered, such as timing and monitoring of prophylaxis.

This article considers patients at risk of IFD who may have clinical profiles that are distinct from those covered by management guidelines. We give insights based on our experience of the use of antifungal prophylaxis in daily clinical practice.

Risk factor assessment and targets for prophylaxis

IFD is one of the most serious complications seen in immunocompromised patients, especially those with haematological malignancies and the recipients of allogeneic haematopoietic stem cell transplantation (HSCT). IFD not only increases mortality and morbidity, but may also lead to delayed administration of

chemotherapy, prolonged hospitalization and additional costs associated with antifungal therapy.[20]

Many publications suggest that there have been advances in antifungal therapy and prophylaxis,[1,21–26] but it is still difficult to establish a diagnosis of IFD, particularly for patients in the early phase of HSCT and for those who are critically ill.[26,27] Against this background, stratification based on the known risk factors for IFD can help to identify patients who are at high risk.[27]

The incidence of IFD is known to vary among different patient groups and in different regions and centres.[2,22,27–29] Patients with acute myeloid leukaemia (AML) are considered to be at highest risk of developing IFD, particularly during remission–induction chemotherapy.[10,11,28] Paradoxically, in a recent study the incidence of mould infections was higher in patients with chronic lymphocytic leukaemia (CLL), possibly because of the widespread and prolonged use of the monoclonal antibody alemtuzumab.[28] However, that study has several weaknesses, such as lack of regular biomarker screening, use of empirical antifungal therapy, a catheter-related *Candida parapsilosis* outbreak and conclusions that were drawn from a group of patients with CLL that constituted only 1.7% of the total patient cohort.

IFD also constitutes a common problem for patients undergoing allogeneic HSCT. Mortality rates attributed to IFD were significantly higher for recipients of allogeneic HSCTs than for patients with AML, particularly for patients who had developed mould infection within 100 days of the transplantation.[12]

Risk factors for IFD differ according to the type of infection and, consequently, it is important to have an understanding of the various universal and distinct risk factors for the more common invasive fungal infections.[13] For example, most IFD attributed to yeasts is due to *Candida* spp. Risk factors for invasive disseminated or visceral candidiasis include intensive colonization of the gastrointestinal tract and other mucosal surfaces by *Candida* spp., and disruption of the protective mucocutaneous membrane barriers due to chemotherapy and/or radiotherapy, decreased activity of phagocytic cells in the blood and tissues and lack of an effective antifungal therapy.[13]

As opposed to invasive candidiasis, invasive aspergillosis is usually acquired from the inhalation of airborne spores. It is rare for invasive aspergillosis to occur unless there is a large burden of spores present from an environmental source seeding onto susceptible membranes or there is lack of an effective phagocytic host defence response in the tissues.[14] Neutropenia is the main risk factor for invasive aspergillosis, with both the depth and duration of neutropenia being key factors. After allogeneic HSCT, the use of steroids and other immunosuppressants is a common risk factor for invasive aspergillosis. Other important risk factors are advanced age, advanced disease phase, alternative donor sources (mismatched or unrelated donor) and absence of a protective environment for isolation of patients.[13,14]

IFD caused by zygomycetes is an emerging problem and is probably related to the use of more aggressive chemotherapy, better diagnostics for their identification or the use of antifungals without anti-*Mucor* activity.[28] *Mucor* represents the third leading cause of IFD after *Aspergillus* spp. and *Candida* spp.[3] In a retrospective analysis, prior use of steroids, found to be more frequent in patients with qualitative or quantitative defects in their phagocytic cells and metabolic acidosis, was also considered to be an important risk factor for zygomycete infections.[3,14]

The innate immune status of the patient plays a key and pivotal role in predisposition to fungal infections. Studies have suggested that specific polymorphisms in 'innate immunity' genes, such as those encoding interleukin 10, tumour necrosis factor receptor superfamily member 1A and toll-like receptor 4, are associated with an increased relative risk for invasive aspergillosis in HSCT recipients and haematology patients.[30] The immune status of patients, particularly haemato-oncology patients with neutropenia, is a more critical risk factor for mould infections than for *Candida* spp. Neutrophils are essential in the initiation and execution of the acute inflammatory response and the subsequent resolution of infiltrates caused by IFD.[3] The most common causes of neutropenia are cytotoxic or radiation therapy for a malignancy, although it is also associated with autoimmune diseases, HIV infection, myelodysplastic syndromes, aplastic anaemia and drug-related bone marrow toxicity.[3] A study conducted by Bodey[31] revealed that the frequency of infections in patients with acute leukaemia was related to the levels of circulating neutrophils and the prevalence of all types of infection was inversely proportional to the neutrophil level.[32] It is interesting to note that the neutrophil count also impacts on the outcome of both bacterial and fungal infections, with the highest fatality rate observed among patients with long-standing severe neutropenia.[32] Lymphocytopenia, occurring after an allogeneic transplant procedure, is also considered to be another important risk factor for invasive aspergillosis.[33]

Environmental factors constitute an additional risk factor for IFD, although their impact varies markedly for different invasive fungal infections. *Aspergillus* spp. are ubiquitous environmental moulds and saprophytic moulds, such as zygomycetes, are also frequently present in air, soil and water to which many individuals are exposed. Many hundreds of spores are inhaled daily but may not always lead to any clinical consequence,[30] although a depressed immune system may present risks for invasive aspergillosis, which could result in systemic dissemination.[30,33] Meteorological conditions have also been thought to influence the epidemiology of mould infections. For example, a high incidence of invasive aspergillosis is often seen just after seasonal periods of dry weather and high temperatures.[14,34]

In addition to weather conditions, there are many other direct and indirect environmental factors, such as personal habits or lifestyle, that have an effect on the risk of fungal infections. Consequently, a substantial number of patients with haematological malignancies were found to be already colonized with fungi at the time of the first clinical manifestation of their underlying disease. Smokers, people living in rural areas or those who have been exposed to high concentrations of fungal spores have been found to be at increased risk of developing IFD.[14,35] Environmental factors also predispose patients to yeast infections. Careful handwashing and avoidance of colonized food can minimize exposure to and colonization by exogenous yeasts, while contact with airborne mould spores can be reduced by effective air-quality maintenance of the hospital environment.[36]

Most literature suggests a positive association between environmental factors and the incidence of IFD, but in a recent study of patients with IFD there were low levels of contamination of air, water and surface samples.[28]

Timing of antifungal prophylaxis

Optimal timing of antifungal prophylaxis remains a matter of debate. Should it be started immediately when the patient is admitted, when chemotherapy is started or when the chemotherapy course is completed? The time at which to discontinue antifungal prophylaxis is even more challenging, with resolution of neutropenia not being the only indicator that prophylactic treatment may be discontinued. In order to be effective when neutropenia occurs, antifungal prophylaxis is started in most cases at the time of initiation of chemotherapy.

For patients with haematological malignancies undergoing chemotherapy or autologous HSCT, antifungal prophylaxis should typically be discontinued once the neutrophil count has recovered (absolute neutrophil count $>500/mm^3$). In allogeneic HSCT recipients, the optimal duration of antifungal prophylaxis has not been defined as clearly. There is evidence suggesting that continuation of antifungal prophylaxis after engraftment is required.[37] Although it remains unclear if this continuation is necessary in all cases, $\geq$3 months of antifungal prophylaxis following transplantation is usually advised for prophylaxis against moulds.[38] Besides local epidemiology and the availability of resources to perform specific diagnostic tests, a number of variables related to the transplant procedure, such as the drugs used for conditioning and immunosuppression and the source of stem cells, influence the clinician's decision on the appropriate duration of antifungal prophylaxis. Transplanted patients with graft-versus-host disease (GvHD), those receiving high-dose corticosteroids, antithymocyte globulin or alemtuzumab and patients with lung involvement or active cytomegalovirus infection are at higher risk of IFD and should receive antifungal prophylaxis for a longer period of time.[39] Long-term prophylaxis can, however, not only lead to treatment-related side effects and increased cost, but also has an important impact on fungal epidemiology, leading to the selection of more-resistant pathogens.[40]

Antifungal prophylaxis should be stopped if judged ineffective or if adverse events occur and changing to another class of antifungal drug is not an option. While evidence of IFD is a clear reason to stop prophylaxis and start a different antifungal treatment, positivity of a biomarker alone should primarily warrant a detailed diagnostic investigation, particularly if plasma drug levels are within an acceptable range. Gastrointestinal intolerance and abnormal liver function tests are generally the most frequent toxicity-related reasons that lead to cessation of antifungal prophylaxis.[23]

Choice of antifungal agents for prophylaxis

Owing to the broad use of antifungal prophylaxis, clinicians have to consider many different interactions of antifungal drugs, including the influence of food on the absorption of oral antifungals and direct interactions with other drugs, such as antineoplastic agents, immunosuppressants, concomitant cardiovascular drugs and benzodiazepines. There are also indirect interactions, whereby impairment of organ function (renal or hepatic) by the prophylactic agent may be additive to the action of concomitant medication. Triazoles are particularly prone to drug–drug interactions via cytochrome (CYP) P450 inhibition. Echinocandins have barely any clinically significant interactions, while for polyenes a cumulative nephrotoxicity, which is additive to the effects of other drugs, is the main problem.[41,42]

Azoles

The bioavailability of triazoles in their oral forms is influenced by food intake. Voriconazole capsules should be taken without food, while posaconazole reaches its highest plasma levels when taken with fat-rich food. For itraconazole, higher bioavailability is achieved under fasting conditions, an effect that is more evident with the oral solution than with capsules. In the case of fluconazole, food has no effect on absorption.[43] Coadministration of itraconazole and posaconazole with histamine H_2-receptor antagonists or proton pump inhibitors decreased their bioavailability, an effect that has not been seen for voriconazole.[43]

Azoles are inhibitors of the CYP P450 isoenzymes. However, the interaction profile is not the same for all triazoles, as they exhibit differential affinity for CYP3A4, CYP2C9 and CYP2C19. Itraconazole and posaconazole are also substrates and/or inhibitors of ATP-dependent drug transporters such as P-glycoprotein, an efflux pump that decreases gastrointestinal absorption of many drugs.[44] The pharmacokinetics of many drugs can be modified by concomitant azole administration—some of those with the highest clinical significance are shown in Table 1. A genetic polymorphism in P450 isoenzymes plays an important role in azole metabolism, as described for the influence of CYP2C19 polymorphism on voriconazole metabolism.[45,46] In Table 2, we present the seven most common and potentially dangerous interactions.

Fluconazole

Fluconazole [400 mg daily intravenously (iv)/orally,[52] according to European Conference on Infections in Leukaemia Guidelines (ECIL)][16] is a generally well-tolerated drug without significant drug–drug interactions. In the current environment of prevailing mould diseases, fluconazole has been used less for antifungal prophylaxis, but has an important place in therapy. Its favourable pharmacokinetics, toxicity and interaction profile often lead to better results for the treatment of susceptible yeasts than the use of second-generation azoles, as shown in our experience in mucosal candidiasis (authors' personal observations).

Itraconazole

Itraconazole (200 mg daily iv followed by 200 mg oral solution twice daily,[6] according to ECIL[16]) has many reported drug–drug interactions and toxicities. Visceral neurotoxicity with ileus is a well-described and life-threatening sequela of itraconazole–vincristine interaction.[50] Moreover, as an inhibitor of P-glycoprotein, itraconazole can increase the efflux of vincristine across the blood–brain barrier, leading to increased neurotoxicity of the drug.[53] It also exhibits gastrointestinal toxicity, gynaecomastia and adrenocortical insufficiency, due to inhibition of the CYP3A4-mediated metabolism of human steroid hormones.[48] Itraconazole also interacts with cyclophosphamide- or busulfan-based conditioning regimens.

Voriconazole

Voriconazole (200 mg orally twice daily,[6] according to ECIL[16]) has some side effects, such as altered vision, fever, rash, teratogenicity and possibly increased risk of skin cancer in patients with previous phototoxicity, gastrointestinal intolerance and/or abnormal liver function test results. Hepatic toxicity and CNS toxicity have been associated with higher voriconazole plasma concentrations. As voriconazole has non-linear pharmacokinetics, the use of a loading dose (6 mg/kg every 12 h on the first day iv/oral) is indicated. There are numerous drug–drug interactions with voriconazole and certain drug combinations should be avoided, e.g. voriconazole plus sirolimus, where toxic levels of sirolimus have to be expected, or voriconazole plus phenytoin or rifampicin, where ineffective levels of voriconazole are the issue.[54,55]

Posaconazole

Posaconazole (200 mg orally three times daily,[4] according to ECIL[16]) is a generally well-tolerated second-generation azole that is frequently used for antifungal prophylaxis during induction therapy of AML/myelodysplastic syndrome and during GvHD in HSCT patients. Absorption can be decreased significantly in patients who are fasting or have gastrointestinal tract mucositis. In these patients, posaconazole could be given at a dose of 200 mg four times daily, which is also the dosing that would be considered for patients not reaching target serum levels.

Echinocandins

The echinocandins are a group of drugs with a favourable toxicity and drug–drug interaction profile.[56] Micafungin (50 mg daily iv, according to ECIL[16]) is the only drug in this class that has been identified to be beneficial for primary antifungal prophylaxis in the neutropenic phase of HSCT by a composite endpoint (absence of suspected, probable or proven fungal infection on prophylaxis and absence of probable and proven fungal infections 4 weeks after prophylaxis).[57] Micafungin is a weak inhibitor of CYP3A4 and could increase the plasma levels of sirolimus, cyclosporin A or nifedipine.[41] In the echinocandin group, the most important consideration is to take care of renal function and electrolyte and fluid balance.

Table 1. Most-critical drug–drug interactions with voriconazole and posaconazole

Levels increased by voriconazole	Levels increased by posaconazole
Cyclophosphamide	cyclosporin A
Cyclosporin A	tacrolimus
Tacrolimus	sirolimus
Sirolimus	tyrosine kinase inhibitors
Warfarin	astemizole
Tyrosine kinase inhibitors	quinidine
Omeprazole	cisapride
Astemizole	terfenadine
Quinidine	ergot alkaloids
Cisapride	midazolam
Terfenadine	
Efavirenz	
Ergot alkaloids	
Midazolam, triazolam, alprazolam	

Polyenes

The polyenes are not widely used for antifungal prophylaxis, although there are limited data available on the use of either iv

Table 2. The most common and potentially dangerous interactions between azoles and other drugs

Drug group	Interaction
QT-prolonging medication (e.g. quinidine and terfenadine[47])	triazoles have occasionally led to potentially dangerous QT interval prolongation with risk of torsades de pointes arrhythmias and should not be combined with other QT-prolonging medication
Statins	combination of statins with azoles can lead to severe rhabdomyolysis
Warfarin	coadministration of azoles can significantly prolong prothrombin time, so dose adjustment of warfarin is required
Benzodiazepines	dose adjustment of midazolam and other hypnotics is needed when used concomitantly with an azole
Rifampicin	as potent inducers of CYP4A4, these compounds should not be used concomitantly with azoles if there is a reasonable alternative
Antineoplastic drugs	concomitant use of azoles and antineoplastic drugs used in leukaemia management should be avoided; azole treatment should be initiated after chemotherapy whenever possible;[48] studies have shown that there can be toxic interactions between itraconazole and busulfan- and cyclophosphamide-based conditioning regimens, which may also be applicable to some of the other newer azoles;[23,49] azoles should be initiated 1 day after these conditioning regimens; azole/vincristine combinations should also be avoided due to significant neurotoxicity and gastrointestinal toxicity[50,51]
Immunosuppressants	monitoring of calcineurin inhibitor levels and dose adjustments are necessary when coadministered with azoles; combination of voriconazole with sirolimus is contraindicated[46]

CYP4A4, cytochrome P450 4A4.

or aerosolized liposomal amphotericin B as an antifungal prophylactic agent.[58,59] Polyenes do not have any important enzymatic interactions, but are nephrotoxic. Deoxycholate amphotericin B is by far the most nephrotoxic of all the polyene formulations and is rarely used. Most authorities do not recommend the use of deoxycholate amphotericin B in prophylaxis and/or therapy because of the problem with nephrotoxicity. The nephrotoxicity of amphotericin B formulations can be additive to the nephrotoxic effects of other drugs, including aminoglycosides, glycopeptides and cyclosporin A.[60]

Amphotericin B also directly stimulates the release of proinflammatory cytokines, often resulting in fever, rigor and chills during infusion.[48] Another potentially dangerous adverse effect is hypokalaemia. Vigorous hydration, electrolyte supplementation and therapeutic drug monitoring (TDM) of concomitant medication (glycopeptides, aminoglycosides and cyclosporin A) are necessary. Liposomal amphotericin B and amphotericin B lipid complex are less nephrotoxic then amphotericin B deoxycholate, but also require the aforementioned steps in handling.[60]

TDM during antifungal prophylaxis

The objective of monitoring antifungal prophylaxis is to maximize the probability of a successful outcome and to minimize the probability of toxicity. Recently, there has been increased interest in the utility of TDM to optimize the safety and efficacy of antifungals in an attempt to improve patient outcomes. TDM is frequently recommended for mould-active triazole antifungal agents (itraconazole, voriconazole and posaconazole)[61–63] Drug efficacy and safety could theoretically be improved by utilizing serum drug concentrations to individualize antifungal regimens.[61,62,64] However, the role of TDM in the antifungal prophylaxis setting has not yet been clearly established and randomized controlled trials addressing the relationship between exposure and efficacy/toxicity are required.

Voriconazole

Patients treated with voriconazole at a given dose may exhibit a wide range of drug concentrations in the plasma.[61] It is recommended to wait 5 days before measuring the serum concentration of voriconazole, as steady-state plasma concentrations are most commonly reached after several days of treatment.[65] Published data suggest that efficient antifungal treatment can be achieved with a wide range (0.35–2.2 mg/L) of voriconazole trough concentrations, but 1–2 mg/L may be considered optimal, as the voriconazole MIC for most fungi is 0.5–1 mg/L.[66] The first report of a possible relationship between voriconazole trough levels and efficacy appeared in the FDA briefing document on voriconazole, which included 280 patients with proven or probable IFD.[61] That study showed a higher treatment success rate with mean voriconazole concentrations >0.5 mg/L (56%) compared with concentrations <0.5 mg/L (46%). It is important to emphasize that food, especially high-fat meals, delays absorption and lowers bioavailability; thus, voriconazole should be taken on an empty stomach.

The upper limit of the therapeutic range (5–6 mg/L) for voriconazole is dictated by the occurrence of serious adverse effects (neuropathy, visual disturbance, hepatic toxicity and skin rash) at higher doses.[67] Pascual *et al.*[61] showed that encephalopathy occurred in approximately one-third of patients with voriconazole plasma concentrations >5.5 mg/L. Discontinuation of voriconazole treatment due to adverse events has been found to be significantly lower in patients with TDM than in those without TDM (4% versus 17%; $P=0.02$).[61] The following voriconazole therapy modifications may be beneficial: (i) a 50% increase in the daily dose in patients with trough levels <1 mg/L and lack of response to therapy; and (ii) discontinuation of therapy in patients with trough levels >5.5 mg/L, whether or not they exhibit adverse events that may be related to overdosing.[61]

Posaconazole

Compared with voriconazole, posaconazole has a more favourable pharmacokinetic profile,[68] except for its saturable absorption, but achievement of recommended posaconazole levels remains a challenge in patients undergoing HSCT and in those with leukaemia. The oral absorption of a posaconazole suspension can be unpredictable in patients with gastrointestinal symptoms (e.g. diarrhoea, mucositis due to chemotherapy or GvHD in the gastrointestinal tract) or in patients taking proton pump inhibitors.[7] Patients with diarrhoea due to gastrointestinal GvHD experienced a 33% reduction in the posaconazole plasma drug trough concentrations compared with those with GvHD in other locations.[62,69] Stopping proton pump inhibitor treatment can increase posaconazole absorption in patients;[63] hence, proton pump inhibitors should be avoided in all patients receiving posaconazole, in the absence of an established indication.

TDM of posaconazole during prophylaxis may be beneficial in these specific groups of patients, in patients with compliance concerns and also in patients at the highest risk of development of fungal infection (e.g. with relapsed or refractory disease or >14 days anticipated duration of neutropenia).[62] Counselling of patients on ways to increase absorption by taking posaconazole with a high-fat meal, nutritional supplement or carbonated beverage is also important.

The FDA recommendation was for treatment with a target average serum posaconazole concentration of >0.7 mg/L for treatment, but the exposure–response relationship and the value of TDM for posaconazole in prophylaxis remains controversial.[62]

Itraconazole

Exposure–response relationships for itraconazole have been established in laboratory animal models.[70] A steady-state itraconazole trough concentration of 0.5 mg/L is considered appropriate for the prevention of IFD in patients with neutropenia.[71] The absorption of itraconazole is facilitated by an acidic environment, which is the basis for the administration of itraconazole with food. Absorption is compromised in patients receiving histamine H_2-receptor antagonists or proton pump inhibitors, or in those with achlorhydria. It should also be considered that generic formulations of itraconazole may have different bioavailabilities.[64]

Monitoring drug levels during prophylaxis

Monitoring prophylaxis requires surveillance of the local epidemiology and appropriate use of diagnostic tools. Surveillance is essential to determine the burden of fungal disease in different centres, as well as the efficacy of prevention and control strategies. When patients are receiving mould-active antifungal agents, the results

of the diagnostic tests should always be interpreted with caution. Diagnostic tests have been reported to exhibit decreased sensitivity during prophylaxis with the mould-active antifungal agents posaconazole and voriconazole. A meta-analysis investigating the accuracy of the galactomannan (GM) assay concluded that a major cause of variable test performance may be prior antifungal therapy, due to the decreased fungal burden lowering the sensitivity and specificity of the GM assay.[72]

It is also important to monitor prophylaxis to enable early detection of severe adverse events that may indicate that a change or termination of the prophylactic drug is required. Stopping prophylaxis may be related to patient intolerability or to drug toxicity; the most common reasons for stopping prophylaxis include hepatotoxicity and neurotoxicity, depending on the antifungal agent.[4,5,61,68]

Prophylaxis will only be beneficial in conditions where the risk of a life-threatening IFD outweighs the risks of toxic effects caused by the antifungal agent.[38]

Antifungal therapy in patients receiving prophylaxis

The decision to initiate antifungal therapy in patients who developed IFD whilst receiving prophylaxis is always challenging and no guidelines for this circumstance exist so far. Even though prophylaxis has shown efficacy in a number of trials, several reports demonstrate that there is still a failure rate with mycological infections as well as a high rate of lung infiltrates and persistent fever has been documented.[4–9,21] Thus, there is a need for an antifungal therapy strategy following prophylaxis failure. The most critical parameters to consider are listed below and recommendations that can be made based on these parameters are presented in Figure 1.

Clinical aspects

Owing to the large variety of potential fungal pathogens, clinical symptoms remain critical in the decision of which prophylactic treatment to choose. Sinus disease, pulmonary nodules and necrotic skin lesions suggest aspergillosis, mucormycosis, fusariosis or other rare invasive mould diseases, while small non-necrotic skins lesions or multivisceral small abscesses suggest disseminated invasive yeast disease due to organisms such as resistant *Candida* spp. or *Trichosporon* spp.

Type of prophylaxis

The failure of antiyeast prophylaxis with fluconazole is often the result of invasive mould diseases or fluconazole-resistant candidiasis. The failure of antimould prophylaxis (with e.g. itraconazole, posaconazole or voriconazole) frequently leads to possible mould infections with the development of pulmonary nodules and negative mycological tests, and also to breakthrough azole-resistant *Candida* spp. infections.[76,77]

Nature of the agent

Failure to achieve appropriate levels of the antifungal agent is most commonly seen with posaconazole and oral itraconazole and can result in breakthrough fungal diseases.[7] Most of these prophylaxis failures are due to lung infiltrates, are not mycologically documented and are therefore classified as possible fungal infections. It is not known whether all these infiltrates need antifungal treatment, but in most cases clinicians feel it is appropriate to initiate treatment.[8] These possible breakthrough IFDs are associated with a significant fatality rate. In a recent study, 28/100 patients experienced breakthrough infections during posaconazole prophylaxis and there were four fatalities that were clinically attributed to IFD, although only 24 patients were possible cases of IFD and three of the fatalities had possible IFD.[8] Breakthrough mucormycosis

- Diagnostic investigations should always be extensively conducted in patients failing prophylaxis (with clinical signs or symptoms indicative of invasive fungal disease):
  - Identification of the pathogen as precisely as possible. Mycological tests should include, whenever feasible:
    - Microscopy and culture of relevant samples. *In vitro* susceptibility tests are indicated when a pathogen is identified
    - Wherever possible, bronchoalveolar lavage samples should be taken in the presence of a lung lesion at CT scan
    - Histopathology in the case of a biopsy
    - Indirect tests (galactomannan in serum, bronchoalveolar lavage fluid or CSF for *Aspergillus* spp., mannan or β-D-glucan detection tests for *Candida* spp., or real-time PCR for *Aspergillus* spp. or *Candida* spp.[73,74]
- Analysis of specific risk factors (e.g. prior respiratory disease for aspergillosis risk,[75] central venous lines in patients increasing the risk for *Candida* infections and diabetes for mucormycosis) remains critical in the treatment choice
- As a general rule, a change in class of antifungal agent should be considered if invasive fungal disease is suspected:
  - During oral mould-active azole prophylaxis, intravenous voriconazole can be used if aspergillosis is suspected, provided low serum levels of itraconazole or posaconazole have been documented
  - Serum levels of the prophylaxtic drug within the expected range (>0.5 mg/L for posaconazole or itraconazole) or inability to monitor the levels in plasma are indicators to switch to another class for antifungal therapy, such as a lipid formulation of amphotericin B or caspofungin

Figure 1. Recommendations for treatment in patients failing antifungal prophylaxis.

has been reported with voriconazole, itraconazole, posaconazole and echinocandin prophylaxis and aspergillosis has been diagnosed in patients receiving caspofungin prophylaxis, despite appropriate serum levels and *in vitro* susceptibility to the antifungal agents.[78–80]

Route of administration

Oral administration of an antifungal agent is more frequently associated with insufficient serum levels, especially in patients with mucositis, vomiting or inability to take oral food. Inhaled antifungal agents such as amphotericin B or the lipid formulation of amphotericin B have limitations, having no protective effects against IFD that has a non-pulmonary mode of entry.

Timing and duration of prophylaxis

Long-term prophylaxis is more likely to be associated with pathogens presenting a constitutive or an acquired resistance pattern.[77]

Preventing recurrence of prior IFD: secondary antifungal prophylaxis

Patients with recent history of an IFD are at increased risk of reactivation of the mycotic process during subsequent episodes of neutropenia, HSCT or immunosuppression.[81,82] To prevent a negative outcome in relapsing cases, strategies for secondary prophylaxis are warranted.[16,82,83] The term 'secondary prophylaxis' is usually applied to patients who had fully recovered from the previous episode of IFD or who are asymptomatic but might have residual foci on imaging considered to be inactive. Practical points for the management of this risk group are suggested below.

Evaluation of overall risk

The localization, extent and causative pathogen of IFD, as well as the duration of the preceding antifungal therapy should be evaluated.[84] The estimated cumulative risk calculated from these factors should be carefully considered against the anticipated benefits of ongoing treatment of the underlying disease that is causing the additional immunosuppression. In cases with a history of uncomplicated candidaemia, secondary prophylaxis is not justified.[60] Patients with chronic disseminated (hepatosplenic) candidiasis, however, need preventive antifungal treatment.[85]

Decreasing the burden of infection

An attempt to remove solitary, surgically approachable lesions before initiating further chemotherapy or conditioning regimen is advisable.[86] However, contraindications to the intervention (decreased pulmonary reserve, poor general condition and high bleeding risk) and possible delay caused by post-operative recovery should be taken into account.

Considering less-immunosuppressive treatment modalities

In the allogeneic HSCT setting, reduced intensity conditioning and peripheral stem cell grafts are usually preferred.[87] From the clinical point of view, it may be an appropriate decision to decrease corticosteroid use, to avoid alemtuzumab and antithymocyte globulin in GvHD therapy, as well as to administer foscarnet rather than ganciclovir for cytomegalovirus reactivation.

Secondary antifungal chemoprophylaxis

It is generally accepted that antifungal drugs successfully used in primary therapy are the compounds of choice for secondary antifungal prophylaxis. At times when there has been a likelihood of drug–drug interactions (e.g. with the chemotherapy or conditioning agents), treatment with amphotericin B lipid formulations (1–3 mg/kg/day)[88,89] or caspofungin (70/50 mg/day)[90] has usually been preferred. In other instances, iv/oral voriconazole has been given at the therapeutic dose.[91–93] It should be noted that caspofungin is inactive against *Cryptococcus* spp. and voriconazole lacks activity against zygomycetes.

Adjunctive therapies

To boost immune function, granulocyte colony-stimulating factor (G-CSF) and, in very selected cases, transfusions of G-CSF-mobilized granulocytes can be administered.[94]

Monitoring for reactivation of breakthrough IFD

Blood cultures, high-resolution imaging and bronchoalveolar lavage are diagnostic tools used clinically to detect relapses or new episodes of IFD. Although the performance of tests detecting biomarkers such as GM, β-D-glucan or fungal DNA is hampered by concomitant antifungal therapy, these methods are often used to seek evidence of infection.[95,96]

Duration of secondary antifungal prophylaxis

The optimal duration of secondary antifungal prophylaxis is still unknown. In clinical practice, antifungal prophylaxis is usually given either until neutrophil recovery, until there is initiation of a new antifungal for relapse/breakthrough infections or until the end of immunosuppression for GvHD.

Controlling environmental sources of IFD

The environment is a potential source of IFD in at-risk patient groups, both in the hospital as well as in the outpatient setting. As IFD is often preceded by fungal colonization, especially in cases of candidiasis, it is difficult to distinguish between nosocomial and endogenous sources of infection. Standardized methodology should be used across monitoring periods in order to avoid sensitivity bias.

Awareness of IFD incidence

Institutions should monitor their incidence of IFD on a regular basis. Care should be taken to use consistent methodological standards for measuring levels of 'incidence' to avoid methodology-related changes in levels. Any unexpected rise should prompt immediate action to detect and control possible environmental sources.

- A dedicated multidisciplinary team should be established to plan and guide preventive strategy
- Healthcare and construction workers should be educated about antifungal risks
- Measures for dust control should be implemented, including relocation of patients, institution of additional air filtration, sealing of areas, wet handling of construction debris
- Patients should be provided with respiratory protection when leaving their protective environment
- There should be constant surveillance of environmental contamination and for nosocomial mould infection

Figure 2. Key strategies for preventing outbreaks of invasive fungal disease during construction work.

Preventing nosocomial candidiasis

The exact origin of nosocomial candidiasis is often unknown; however, patient-to-patient transmission and outbreaks can occur.[97,98] To prove true nosocomial transmission and to rule out pseudo-outbreaks, molecular genetic tests (e.g. DNA fingerprinting) should be utilized. In general, the enforcement of hand disinfection and proper hygiene in vascular catheter care is crucial.[99] In outbreak situations, the role of infected or colonized healthcare workers, contaminated medication or material and breaches in hygiene standards should be investigated.

Reducing the risk of mould diseases

Mould infections are mostly airborne. Contaminated water can also play a role when aerosolized (e.g. in showers) or in cases of submersion. Food, fomites and medications are less often the cause. However, healthcare-related outbreaks do occur.[100]

Protective environment

Patients undergoing allogeneic HSCT represent a population at the highest risk of developing IFD, particularly pulmonary aspergillosis. Placing individuals in a protective environment aims to minimize exposure to airborne fungal spores. A protective environment could be defined as housing patients in well-sealed single rooms with directed flow of high-efficiency particulate air-filtered air and positive pressure.[101] Fresh flowers, potted plants as well as carpeting and upholstered furniture should be avoided. The method and timing of cleaning is also important.[102,103] In other risk groups (patients undergoing autologous HSCT or induction chemotherapy for AML and those with aplastic anaemia), the advantages of a protective environment are less well defined. Special attention should be paid to infection control logistics during times of construction work taking place either in-house or in the area surrounding the facility.[103,104] Key strategies for preventing outbreaks of IFD during construction work are shown in Figure 2.

Safe living outside the hospital

After being discharged from their protective environment, allogeneic HSCT patients, especially those with chronic GvHD and immunosuppression, should be advised to keep away from places and situations with high risk of spore exposure. Vacuuming, dusting, gardening, contact with plants, soil or waste, staying in moist and mouldy areas and construction sites should all be avoided.[92] Their diet should not contain uncooked dried fruits, dried spices and herbs, nuts and soft cheese, and patients should refrain from smoking. The duration of these precautions should be tailored to the individual patient. In the allogeneic HSCT setting, general precautions should be taken for $\geq$6 months post-transplant or the end of immunosuppression for GvHD. Regarding food safety recommendations, HSCT physicians should have final responsibility for determining when the dietary adjustments mentioned above can be discontinued safely.[92]

Conclusions

There are a number of clinical studies and guidelines in the area of prophylaxis against IFD. The main problem with clinical trials and guidelines is that they do not reflect real-life clinical practice and may not address issues typically encountered on a day-to-day basis. Attempts to define which patients are at high risk of IFD on the basis of published studies of subsets of haematological malignancies may miss some individuals, as such studies, by definition, do not represent every indication. Also, the development of new tailored or targeted therapies, such as monoclonal antibodies, tyrosine kinase inhibitors and radioisotope-conjugated agents, can create other indications that may need antifungal prophylaxis.

Most of the efficacy data depend on a precise protocol, but in daily clinical practice an effective prophylactic drug can sometimes prove not to be very useful. This is the case especially for the azoles, which have a lot of interactions and bioavailability issues, thus making TDM a helpful tool. It is also important in situations where drug prophylaxis has been initiated that physical protection, education and team work, which are pivotal factors in eliminating the burden of IFD, should not be forgotten. These problems suggest that despite the availability of new drugs, there are still a number of issues to be resolved in this field.

Funding

The authors attended a meeting in Vienna to discuss the content for this article, for which they received financial compensation for their time and expenses. No reimbursement was received for drafting or reviewing the article. Logistical support and assistance was provided by HealthCare21 Communications Ltd and was funded by Pfizer Ilaclari Ltd Sti.

Transparency declarations

This article is part of a Supplement sponsored by Pfizer Ilaclari Ltd Sti. H. A. has received research grants and honoraria from Pfizer Inc., Gilead Sciences Inc., and Merck, Sharp and Dohme, and is a member of Pfizer Inc., Gilead Sciences Inc., and Merck, Sharp and Dohme advisory boards. J.S. has received funds as speaker's bureau for Merck, Sharp and Dohme, Pfizer Inc. and Astellas, and is a member of the Merck, Sharp and Dohme, Pfizer Inc. and Astellas advisory board. R.V. is a member of the Sanofi advisory

board. R.H. has received funds for speaking at symposia organised by Gilead Sciences Inc., Merck, Sharp and Dohme, and Pfizer Inc. and has received a research grant from Pfizer Inc. and is a member of Astellas, Basilea, Gilead Sciences Inc., Merck, Sharp and Dohme, Pfizer Inc. and, Schering-Plough advisory boards. V.P.A., M.K. and A.T. have no further declarations other than participating in the development of this manuscript (see Funding section).

References

1 Marr KA, Carter RA, Crippa F *et al*. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002; **34**: 909–17.

2 Kontoyiannis DP, Marr KA, Park BJ *et al*. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010; **50**: 1091–100.

3 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**: 1068–75.

4 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.

5 Wingard JR. Antifungal chemoprophylaxis after blood and marrow transplantation. *Clin Infect Dis* 2002; **34**: 1386–90.

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

7 Dolton MJ, Ray JE, Chen SC *et al*. Multicenter study of posaconazole therapeutic drug monitoring: exposure–response relationship and factors affecting concentration. *Antimicrob Agents Chemother* 2012; **56**: 5503–10.

8 Peterson L, Ostermann J, Rieger H *et al*. Posaconazole prophylaxis—impact on incidence of invasive fungal disease and antifungal treatment in haematological patients. *Mycoses* 2013; doi:10.1111/myc.12086.

9 Vehreschild JJ, Ruping MJ, Wisplinghoff H *et al*. Clinical effectiveness of posaconazole prophylaxis in patients with acute myelogenous leukaemia (AML): a 6 year experience of the Cologne AML cohort. *J Antimicrob Chemother* 2010; **65**: 1466–71.

10 Ruping MJ, Vehreschild JJ, Cornely OA. Patients at high risk of invasive fungal infections: when and how to treat. *Drugs* 2008; **68**: 1941–62.

11 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.

12 Caira M, Girmenia C, Fadda RM *et al*. Invasive fungal infections in patients with acute myeloid leukemia and in those submitted to allogeneic hemopoietic stem cell transplant: who is at highest risk? *Eur J Haematol* 2008; **81**: 242–3.

13 Martino R, Subira M. Invasive fungal infections in hematology: new trends. *Ann Hematol* 2002; **81**: 233–43.

14 Pagano L, Akova M, Dimopoulos G *et al*. Risk assessment and prognostic factors for mould-related diseases in immunocompromised patients. *J Antimicrob Chemother* 2011; **66** Suppl 1: i5–14.

15 Rickerts V, Bohme A, Just-Nubling G. Risk factor for invasive zygomycosis in patients with hematologic malignancies. *Mycoses* 2002; **45** Suppl 1: 27–30.

16 Maertens J, Marchetti O, Herbrecht R *et al*. European guidelines for antifungal management in leukemia and hematopoietic stem cell transplant recipients: summary of the ECIL 3—2009 update. *Bone Marrow Transplant* 2011; **46**: 709–18.

17 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.

18 Freifeld 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.

19 Pappas PG, Kauffman CA, Andes D *et al*. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 503–35.

20 Worth LJ, Slavin MA. Bloodstream infections in haematology: risks and new challenges for prevention. *Blood Rev* 2009; **23**: 113–22.

21 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.

22 Schwartz S, Ruhnke M, Ribaud P *et al*. Improved outcome in central nervous system aspergillosis, using voriconazole treatment. *Blood* 2005; **106**: 2641–5.

23 Marr KA, Crippa F, Leisenring W *et al*. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. *Blood* 2004; **103**: 1527–33.

24 Winston DJ, Maziarz RT, Chandrasekar PH *et al*. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. *Ann Intern Med* 2003; **138**: 705–13.

25 Robenshtok E, Gafter-Gvili A, Goldberg E *et al*. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: systematic review and meta-analysis. *J Clin Oncol* 2007; **25**: 5471–89.

26 Hachem R, Sumoza D, Hanna H *et al*. Clinical and radiologic predictors of invasive pulmonary aspergillosis in cancer patients: should the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria be revised? *Cancer* 2006; **106**: 1581–6.

27 Zhang P, Jiang EL, Yang DL *et al*. Risk factors and prognosis of invasive fungal infections in allogeneic stem cell transplantation recipients: a single-institution experience. *Transpl Infect Dis* 2010; **12**: 316–21.

28 Montagna MT, De Giglio O, Napoli C *et al*. Invasive fungal infections in patients with hematologic malignancies (AURORA Project): lights and shadows during 18-months surveillance. *Int J Mol Sci* 2012; **13**: 774–87.

29 Lewis RE, Cahyame-Zuniga L, Leventakos K *et al*. Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study. *Mycoses* 2013; doi:10.1111/myc.12081.

30 Barnes PD, Marr KA. Risks, diagnosis and outcomes of invasive fungal infections in haematopoietic stem cell transplant recipients. *Br J Haematol* 2007; **139**: 519–31.

31 Bodey GP. Infectious complications of acute leukemia. *Med Times* 1966; **94**: 1076–85.

32 Portugal RD, Garnica M, Nucci M. Index to predict invasive mold infection in high-risk neutropenic patients based on the area over the neutrophil curve. *J Clin Oncol* 2009; **27**: 3849–54.

33 Garcia-Vidal C, Upton A, Kirby KA *et al*. Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection according to time after transplantation. *Clin Infect Dis* 2008; **47**: 1041–50.

34 Panackal AA, Li H, Kontoyiannis DP *et al*. Geoclimatic influences on invasive aspergillosis after hematopoietic stem cell transplantation. *Clin Infect Dis* 2010; **50**: 1588–97.

35 Blum G, Eschertzhuber S, Auberger J *et al*. Airborne fungus exposure prior to hospitalisation as risk factor for mould infections in immunocompromised patients. *Mycoses* 2012; **55**: 237–43.

36 Munoz P, Burillo A, Bouza E. Environmental surveillance and other control measures in the prevention of nosocomial fungal infections. *Clin Microbiol Infect* 2001; **7** Suppl 2: 38–45.

37 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**: 2055–61.

38 Slavin MA, Heath CH, Thursky KA *et al*. Antifungal prophylaxis in adult stem cell transplantation and haematological malignancy. *Intern Med J* 2008; **38**: 468–76.

39 Thursky K, Byrnes G, Grigg A *et al*. Risk factors for post-engraftment invasive aspergillosis in allogeneic stem cell transplantation. *Bone Marrow Transpl* 2004; **34**: 115–21.

40 Bow EJ. Long-term antifungal prophylaxis in high-risk hematopoietic stem cell transplant recipients. *Med Mycol* 2005; **43** Suppl 1: S277–87.

41 Gubbins PO, Amsden JR. Drug–drug interactions of antifungal agents and implications for patient care. *Expert Opin Pharmacother* 2005; **6**: 2231–43.

42 Hamill RJ. Amphotericin B formulations: a comparative review of efficacy and toxicity. *Drugs* 2013; **73**: 919–34.

43 Bruggemann RJ, Alffenaar JW, Blijlevens NM *et al*. Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. *Clin Infect Dis* 2009; **48**: 1441–58.

44 Wang EJ, Lew K, Casciano CN *et al*. Interaction of common azole antifungals with P glycoprotein. *Antimicrob Agents Chemother* 2002; **46**: 160–5.

45 Dodds-Ashley E. Management of drug and food interactions with azole antifungal agents in transplant recipients. *Pharmacotherapy* 2010; **30**: 842–54.

46 Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. *Pharmacotherapy* 2006; **26**: 1730–44.

47 Elbey MA, Cil H, Onturk E *et al*. QTc prolongation and torsade de pointes ventricular tachycardia in a small dose voriconazole therapy. *Eur Rev Med Pharmacol Sci* 2012; **16**: 100–2.

48 Girmenia C, Iori AP. Safety and interactions of new antifungals in stem cell transplant recipients. *Expert Opin Drug Saf* 2012; **11**: 803–18.

49 Buggia I, Zecca M, Alessandrino EP *et al*. Itraconazole can increase systemic exposure to busulfan in patients given bone marrow transplantation. GITMO (Gruppo Italiano Trapianto di Midollo Osseo). *Anticancer Res* 1996; **16**: 2083–8.

50 Moriyama B, Henning SA, Leung J *et al*. Adverse interactions between antifungal azoles and vincristine: review and analysis of cases. *Mycoses* 2012; **55**: 290–7.

51 van Schie RM, Bruggemann RJ, Hoogerbrugge PM *et al*. Effect of azole antifungal therapy on vincristine toxicity in childhood acute lymphoblastic leukaemia. *J Antimicrob Chemother* 2011; **66**: 1853–6.

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

53 Nivoix Y, Leveque D, Herbrecht R *et al*. The enzymatic basis of drug–drug interactions with systemic triazole antifungals. *Clin Pharmacokinet* 2008; **47**: 779–92.

54 Park WB, Kim NH, Kim KH *et al*. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. *Clin Infect Dis* 2012; **55**: 1080–7.

55 Racil Z, Winterova J, Kouba M *et al*. Monitoring trough voriconazole plasma concentrations in haematological patients: real life multicentre experience. *Mycoses* 2012; **55**: 483–92.

56 Chen SC, Slavin MA, Sorrell TC. Echinocandin antifungal drugs in fungal infections: a comparison. *Drugs* 2011; **71**: 11–41.

57 van Burik JA, Ratanatharathorn V, Stepan DE *et al*. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. *Clin Infect Dis* 2004; **39**: 1407–16.

58 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.

59 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.

60 Ullmann AJ, Akova M, Herbrecht R *et al*. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). *Clin Microbiol Infect* 2012; **18** Suppl 7: 53–67.

61 Pascual A, Calandra T, Bolay S *et al*. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008; **46**: 201–11.

62 Ananda-Rajah MR, Grigg A, Slavin MA. Making sense of posaconazole therapeutic drug monitoring: a practical approach. *Curr Opin Infect Dis* 2012; **25**: 605–11.

63 Neubauer WC, Engelhardt M, Konig A *et al*. Therapeutic drug monitoring of posaconazole in hematology patients: experience with a new high-performance liquid chromatography-based method. *Antimicrob Agents Chemother* 2010; **54**: 4029–32.

64 Pasqualotto AC, Denning DW. Generic substitution of itraconazole resulting in sub-therapeutic levels and resistance. *Int J Antimicrob Agents* 2007; **30**: 93–4.

65 Purkins L, Wood N, Greenhalgh K *et al*. The pharmacokinetics and safety of intravenous voriconazole—a novel wide-spectrum antifungal agent. *Br J Clin Pharmacol* 2003; **56** Suppl 1: 2–9.

66 Howard A, Hoffman J, Sheth A. Application of voriconazole concentrations in the treatment of invasive aspergillosis. *Ann Pharmacother* 2008; **42**: 1859–64.

67 Denning D, Ribaud P, Milpied N *et al*. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 2002; **34**: 563–71.

68 Ullmann AJ, Cornely OA, Burchardt A *et al*. Pharmacokinetics, safety, and efficacy of posaconazole in patients with persistent febrile neutropenia or refractory invasive fungal infection. *Antimicrob Agents Chemother* 2006; **50**: 658–66.

69 Tonini J, Thiebaut A, Jourdil JF *et al*. Therapeutic drug monitoring of posaconazole in allogeneic hematopoietic stem cell transplantation patients who develop gastrointestinal graft-versus-host disease. *Antimicrob Agents Chemother* 2012; **56**: 5247–52.

70 Berenguer J, Ali NM, Allende MC *et al*. Itraconazole for experimental pulmonary aspergillosis: comparison with amphotericin B, interaction with cyclosporin A, and correlation between therapeutic response and itraconazole concentrations in plasma. *Antimicrob Agents Chemother* 1994; **38**: 1303–8.

71 Glasmacher A, Hahn C, Leutner C *et al*. Breakthrough invasive fungal infections in neutropenic patients after prophylaxis with itraconazole. *Mycoses* 1999; **42**: 443–51.

72 Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006; **42**: 1417–27.

73 Maaroufli Y, DeBruyne JM, Duchteau V *et al*. Early detection and identification of commonly encountered *Candida* species from simulated blood cultures by using a real-time PCR-based assay. *J Mol Diagn* 2004; **6**: 108–14.

74 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.

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

76 Marr KA, Balajee SA, McLaughlin L *et al*. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004; **190**: 641–9.

77 Maertens J, Buve K, Anaissie E. Broad-spectrum antifungal prophylaxis in patients with cancer at high risk for invasive mold infections: counterpoint. *J Natl Compr Canc Netw* 2008; **6**: 183–9.

78 Skiada A, Lanternier F, Groll AH *et al*. Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). *Haematologica* 2013; **98**: 492–504.

79 Ruping MJ, Heinz WJ, Kindo AJ *et al*. Forty-one recent cases of invasive zygomycosis from a global clinical registry. *J Antimicrob Chemother* 2010; **65**: 296–302.

80 Pang KA, Godet C, Fekkar A *et al*. Breakthrough invasive mould infections in patients treated with caspofungin. *J Infect* 2012; **64**: 424–9.

81 Robertson MJ, Larson RA. Recurrent fungal pneumonias in patients with acute nonlymphocytic leukemia undergoing multiple courses of intensive chemotherapy. *Am J Med* 1988; **84**: 233–9.

82 Offner F, Cordonnier C, Ljungman P *et al*. Impact of previous aspergillosis on the outcome of bone marrow transplantation. *Clin Infect Dis* 1998; **26**: 1098–103.

83 Grigg A, Slavin M. Minimizing the risk of recurrent or progressive invasive mold infections during stem cell transplantation or further intensive chemotherapy. *Transpl Infect Dis* 2008; **10**: 3–12.

84 Cornely OA, Bohme A, Reichert D *et al*. Risk factors for breakthrough invasive fungal infection during secondary prophylaxis. *J Antimicrob Chemother* 2008; **61**: 939–46.

85 Bjerke JW, Meyers JD, Bowden RA. Hepatosplenic candidiasis—a contraindication to marrow transplantation? *Blood* 1994; **84**: 2811–4.

86 Nosari A, Ravini M, Cairoli R *et al*. Surgical resection of persistent pulmonary fungus nodules and secondary prophylaxis are effective in preventing fungal relapse in patients receiving chemotherapy or bone marrow transplantation for leukemia. *Bone Marrow Transplant* 2007; **39**: 631–5.

87 Martino R, Parody R, Fukuda T *et al*. Impact of the intensity of the pretransplantation conditioning regimen in patients with prior invasive aspergillosis undergoing allogeneic hematopoietic stem cell transplantation: a retrospective survey of the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Blood* 2006; **108**: 2928–36.

88 Allinson K, Kolve H, Gumbinger HG *et al*. Secondary antifungal prophylaxis in paediatric allogeneic haematopoietic stem cell recipients. *J Antimicrob Chemother* 2008; **61**: 734–42.

89 Mele L, Pagano L, Equitani F *et al*. Case reports. Secondary prophylaxis with liposomal amphotericin B after invasive aspergillosis following treatment for haematological malignancy. *Mycoses* 2001; **44**: 201–3.

90 de Fabritiis P, Spagnoli A, Di Bartolomeo P *et al*. Efficacy of caspofungin as secondary prophylaxis in patients undergoing allogeneic stem cell transplantation with prior pulmonary and/or systemic fungal infection. *Bone Marrow Transplant* 2007; **40**: 245–9.

91 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**: 943–8.

92 Tomblyn M, Chiller T, Einsele H *et al*. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009; **15**: 1143–238.

93 Cordonnier C, Rovira M, Maertens J *et al*. Voriconazole for secondary prophylaxis of invasive fungal infections in allogeneic stem cell transplant recipients: results of the VOSIFI study. *Haematologica* 2010; **95**: 1762–8.

94 Mousset S, Hermann S, Klein SA *et al*. Prophylactic and interventional granulocyte transfusions in patients with haematological malignancies and life-threatening infections during neutropenia. *Ann Hematol* 2005; **84**: 734–41.

95 Marr KA, Laverdiere M, Gugel A *et al*. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. *Clin Infect Dis* 2005; **40**: 1762–9.

96 Reinwald M, Hummel M, Kovalevskaya E. Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. *J Antimicrob Chemother* 2012; **67**: 2260–7.

97 Sanchez V, Vazquez JA, Barth-Jones D *et al*. Nosocomial acquisition of *Candida parapsilosis*: an epidemiologic study. *Am J Med* 1993; **94**: 577–82.

98 Asmundsdottir LR, Erlendsdottir H, Haraldsson G *et al*. Molecular epidemiology of candidemia: evidence of clusters of smoldering nosocomial infections. *Clin Infect Dis* 2008; **47**: e17–24.

99 O'Grady NP, Alexander M, Dellinger EP *et al*. Guidelines for the prevention of intravascular catheter-related infections. The Hospital Infection Control Practices Advisory Committee, Center for Disease Control and Prevention, US. *Pediatrics* 2002; **110**: e51.

100 Kainer MA, Reagan DR, Nguyen DB *et al*. Fungal infections associated with contaminated methylprednisolone in Tennessee. *N Engl J Med* 2012; **367**: 2194–203.

101 Sehulster L, Chinn RY. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep* 2003; **52**: 1–42.

102 Lee LD, Berkheiser M, Jiang Y *et al*. Risk of bioaerosol contamination with *Aspergillus* species before and after cleaning in rooms filtered with high-efficiency particulate air filters that house patients with hematologic malignancy. *Infect Control Hosp Epidemiol* 2007; **28**: 1066–70.

103 Tablan OC, Anderson LJ, Besser R *et al*. Guidelines for preventing health-care-associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep* 2004; **53**: 1–36.

104 Benet T, Nicolle MC, Thiebaut A *et al*. Reduction of invasive aspergillosis incidence among immunocompromised patients after control of environmental exposure. *Clin Infect Dis* 2007; **45**: 682–6.