

Carlos Vallejo¹
Lourdes Vázquez²
José Rafael Cabrera Martín³
Enric Carreras⁴
Julio García Rodríguez⁵
Isabel Ruiz Camps⁶
Jesús Fortún⁷
Josep Mensa⁸
José Barberán⁹

Treatment of invasive fungal infections in high-risk haematological patients: What have we learnt in the past 10 years?

¹ Hematology Dpt, Hospital Central de Asturias, Oviedo

² Hematology Dpt, Hospital Clínico Universitario. Salamanca.

³ Hematology Dept, Hospital Universitario Puerta de Hierro, Madrid

⁴ Fundació Josep Carreras contra la Leucèmia. Barcelona.

⁵ Microbiology Dpt, Hospital La Paz. Madrid.

⁶ Infectious Diseases Dpt, Hospital Val d'Hebron, Barcelona.

⁷ Infectious Diseases Dpt, Hospital Ramón y Cajal. Madrid

⁸ Infectious Diseases Dpt., Hospital Clínic i Provincial, Barcelona.

⁹ Internal Medicine Dpt, Hospital Montepríncipe, Universidad San Pablo-CEU, Madrid

ABSTRACT

Invasive fungal infection (IFI) caused by filamentous fungi remains a very severe infectious complication in patients with onco-haematological diseases. Last advances in the diagnostic and therapeutic fields, today we know that their contributions are limited. Something similar can be said of clinical trials especially in relation to some changes in the characteristics of the host. The development of promising diagnostic techniques and the relative expansion in the number of antifungal agents has been associated with diversification of therapeutic strategies (prophylaxis with extended-spectrum azoles and preemptive antifungal treatment). However, the low sensitivity of AGA testing in some circumstances, and the potential delay in starting treatment due to logistic reasons, has been reflected by a greater mortality in certain type of patients and a significant increase in the days of treatment. All these circumstances has once again focus attention to the empirical approach as a central strategy in high-risk patients. The objective of this article is to review the clinical experience in the treatment of IFI in onco-haematological patients according to data published in the literature in the last decade and to present a set of recommendations.

Key words: onco-haematological patients, diagnosis, prophylaxis, treatment, liposomal amphotericin B, voriconazole

Tratamiento de las infecciones fúngicas invasoras en pacientes hematológicos de alto riesgo: ¿Qué hemos aprendido en los pasados 10 años?

RESUMEN

La infección fúngica invasora (IFI) por hongos filamentosos (HF) sigue constituyendo una complicación infecciosa muy grave en

los pacientes con enfermedades onco-hematológicas. Las últimas aportaciones en el campo del diagnóstico y la terapéutica, hoy sabemos que son limitadas. Algo parecido se puede decir de los ensayos clínicos, en especial por algunos cambios en las características del huésped. La aparición de técnicas diagnósticas esperanzadoras y la relativa ampliación en el número de antifúngicos, dio lugar a una diversificación de las estrategias terapéuticas (profilaxis y tratamiento anticipado). Pero la falta de sensibilidad del AGA bajo algunas circunstancias y el potencial retraso en el inicio del tratamiento por motivos logísticos en su realización, se ha traducido en una mayor mortalidad en determinados tipos de pacientes y en un aumento significativo de los días de tratamiento. Todas estas circunstancias han vuelto a colocar el abordaje empírico como una estrategia central en los pacientes de alto riesgo. El objetivo de este artículo es revisar la experiencia clínica en el tratamiento de las IFI en el paciente onco-hematológico publicada en el curso de la última década y hacer unas recomendaciones en base a ésta.

Palabras clave: paciente oncohematológico, diagnóstico, profilaxis, tratamiento, anfotericina B Liposomal, voriconazol

RATIONALE

Invasive fungal infection (IFI) caused by filamentous fungi remains a very severe infectious complication in patients with onco-haematological diseases, particularly, in allogenic stem cell transplant recipients mostly due to the lack of rapid and highly reliable diagnostic tests allowing an early diagnosis and directed antifungal treatment¹.

Last advances in the diagnostic and therapeutic fields have been the detection of *Aspergillus* galactomannan antigen testing (AGA in 2003) and the introduction of echinocandins in 2001, respectively^{2,3}. However, after a decade of clinical experience, today we know that their contributions are limited⁴⁻⁶. Something similar can be said of clinical trials especially in relation to some changes in the characteristics of the host (age, risk factors, new immunosuppressive treatments, prophylactic regimens, etc.)⁷⁻⁹. In fact, a large body of evidence supporting current decisions regarding antifungal therapy is based on data from observational studies and expert recommendations¹⁰⁻¹³.

Correspondence:

Carlos Vallejo

Hematology Dept, Hospital Central de Asturias, Oviedo, Spain.

E-mail: carlosvallej@gmail.com

In the last decade, the increase in high-risk onco-haematological patients, the development of promising diagnostic techniques and the relative expansion in the number of antifungal agents has been associated with diversification of therapeutic strategies. Among these strategies, prophylaxis with extended-spectrum azoles (EEA) has resulted in a decrease in mortality of high-risk patients and changes in the epidemiological characteristics of IFI¹⁴⁻¹⁹. However, interactions of azoles with some drugs (chemotherapeutic and immunosuppressive agents) that are increasingly used in this type of patients, as well as difficulties in reaching therapeutic serum concentrations in some clinical situations (mucositis, diarrhoea, etc.) when the drug is administered by the oral route, has prompted the introduction of other antifungal classes in the prophylaxis setting^{8,20-23}. On the other hand, based on the possibility of AGA detection, preemptive antifungal treatment has been strongly positioned against empirical treatment, the objective of which was to prevent unnecessary overexposure to antifungals and to reduce the economic cost²⁴. However, the low sensitivity of AGA testing in some circumstances, such as in patients receiving prophylaxis with EEA²⁴⁻²⁶ and the potential delay in starting treatment due to logistic reasons, has been reflected, according to the experience of some authors, by a greater mortality in certain type of patients and a significant increase in the days of treatment¹⁰. All these circumstances has once again focus attention to the empirical approach as a central strategy in high-risk patients, as stated in the recommendations of some of the most recent therapeutic guidelines^{13,17}. At present, it is accepted that empirical antifungal treatment of the high-risk onco-haematological patient should be started as soon as possible, should offer a broad spectrum of antifungal activity and should be preferably fungicidal with an acceptable toxicity.

The objective of this article is to review the clinical experience in the treatment of IFI in onco-haematological patients according to data published in the literature in the last decade and to present a set of recommendations.

Epidemiological changes of fungal infections

Aspergillus spp. and to a lesser extent *Candida* spp. are the main causative agents of IFIs in the onco-haematological patient. *Mucor* spp. ranks third but its prevalence seems to be increasing as shown by some North American and European studies^{1,27,28}. In our country, the incidence of mucormycosis appears to be low and poorly characterized²⁹, although the occurrence of this infection may be underestimated due to difficulties in establishing a definitive diagnosis^{30,31}. It should be noted that invasive zygomycosis may develop in patients exposed to antifungals without activity against these fungi, such as fluconazole, voriconazole or candins, or in those exposed to antifungals with activity but frequently given underdoses, such as the case of posaconazole²⁹⁻³².

During this time period, an increase in *Candida* spp. resistant to azoles has also been observed, favoured by the use of these antifungals in prophylaxis or treatment regimens^{11,33}. Moreover, secondary resistances of *Aspergillus* spp. to azoles have been reported in some European countries, a fact which has not been confirmed in national studies³⁴⁻⁴⁰. A similar phenomenon occurred with candins^{41,42}, although it is possible that resistance may be underestimated because of the lack of sensitivity of *in vitro* techniques, which hampers the correct identification of resistant isolates⁴¹⁻⁴³.

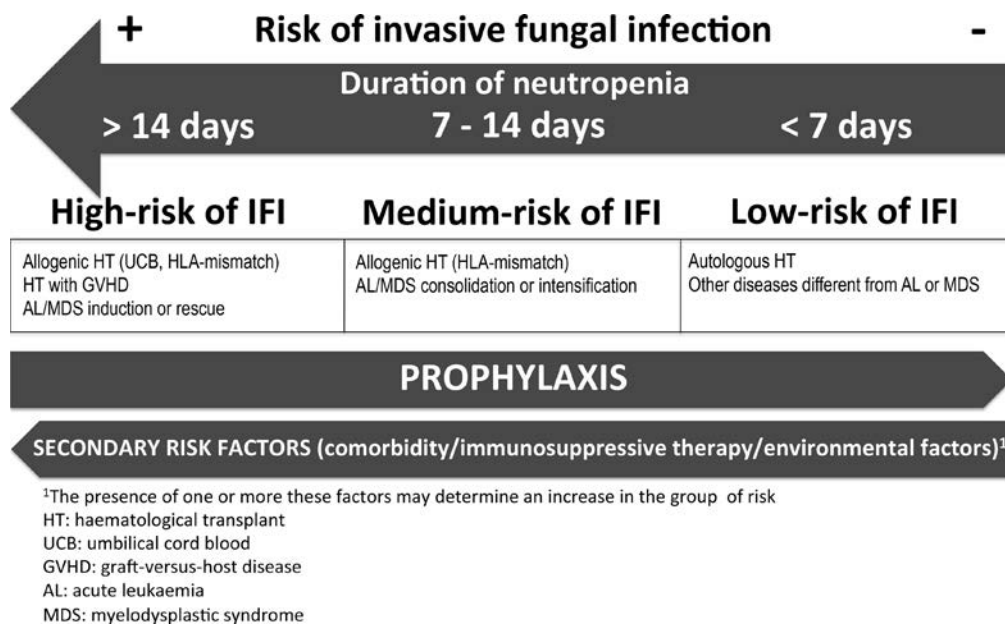


Figure 1

Classification of patients according to the risk of invasive fungal infection (IFI)

Comorbidity	Immunosuppressive treatment	Environmental factors
Age > 65 years Advanced disease Previous invasive fungal infection Iron overload Metabolic acidosis Non-controlled hyperglycemia Cytomegalovirus infection Infection caused by respiratory virus Chronic obstructive pulmonary disease (COPD) Renal failure Liver failure Malnutrition Genetic polymorphisms (MBL, TLR4-2 ...)	Prolonged corticosteroid treatment Alemtizumab Citarabine at high doses Anti-TNF agents High doses of total body irradiation	Building work in the neighboring Rooms without HEPA filters

HEPA: High-efficient-particulate-air; TNF: Tumor necrosis factor; MBL: Mannan binding lectin; TLR4-2: Toll-like receptors

Figure 2 Secondary risk factors of invasive fungal infection

High-risk haematological patient-related changes

With the aim of selecting the most adequate therapeutic strategy, the risk of IFI in onco-haematological patients has been stratified into high-risk, medium-risk and low-risk groups^{13,44}. Although profound and/or sustained neutropenia continues to be the main and most frequent risk factor for IFI, the different types of allogenic stem cell transplantation have gain an increasing interest in recent years. Also, changes in the host in association with the use of peripheral blood progenitors, among others, have increase the prevalence and duration of graft-versus-host disease (GVHD) in some groups of patients, who need immunosuppressive treatment and specifically the use of corticosteroids. The association of GVHD and steroids has become one of the main risk factors of IFI in the haematological patient^{7,45-48}. In general, the incidence of proven or probable IFI caused by filamentous fungi in haematological patients at risk ranges between 4% and 22%⁴⁹, although the extent of the problem may be even greater as shown by a recent study of 1213 autopsies in patients with malignant haematological diseases in whom *ante mortem* diagnosis of IFI was only established in 51% of the cases⁴.

Therefore, in the risk assessment of IFI in the onco-haematological patient both primary factors and other aspects related to comorbidities and concomitant treatments (purine analogues, immunosuppressants, mono or polyclonal antibodies, etc.) should be considered. The presence of one or the combination of several factors are determinants to increase the patient's risk category, initially included in the medium-risk or low-risk group^{7,14,50}. The design of a large epidemiological and multi-centre study would be ideal to develop a *risk score* including all and each individual risk factors of IFI, both primary and secondary, in order to define better which patients may benefit from an earlier antifungal treatment (figures 1 and 2).

Usefulness of diagnostic tools in the high-risk haematological patient

The current diagnostic techniques are neither sufficiently sensitive nor specific to detect early the development of IFI caused

by filamentous fungi in high-risk patients; also, research and advances in this field up to the present time have been limited. Culture continues to be the "gold standard" of microbiological diagnosis but its reliability for filamentous fungi is very low, and false negative results are common in most cases of IFI⁵¹. Other disadvantages are the length of time needed for diagnosis and the difficulty in distinguishing between colonization and infection.

AGA detection has become a key test for early diagnosis of invasive aspergillosis. However, the AGA test is not free of false positive and false negative results^{24,25,52}. It has been shown that prophylaxis with EEA can reduce the sensitivity of the test up to 30%^{24,25}. Also, in a necropsy study of transplant recipients, 49% of patients with proven aspergillosis had various and successive negative AGA tests; it should be noted that in this population non-myceloablative conditioning regimens were used, so that the lower degree of neutropenia may account for the low sensitivity of the test^{4,53}. On the other hand, it has been found that the sensitivity of the AGA test varies according to the fungal species⁵⁴. False positive results of the AGA tests have been reported with the use of candidins^{54,55} and some *Fusarium* spp. (containing galactomannan)⁵⁶. Moreover, in the analysis of the usefulness of the AGA test, some logistic aspects such as the periodicity of the performance of the test at each centre should be considered. In any case, an eventual delay in performing the test and having the results available should not affect the time of starting antifungal treatment in a high-risk patient.

Chest CT demonstration of halo or the reserve halo sign are suggestive of aspergillosis or mucormycosis and, therefore these radioimaging findings are not useful for the differentiation of these entities, and even may be observed in other non-fungal respiratory infections⁵⁷⁻⁶⁰. In addition, the presence of radiologically visible pulmonary lesions is frequently a sign of advanced disease.

In summary, diagnostic techniques currently available in the hospitals are not sufficiently early or reliable to be considered

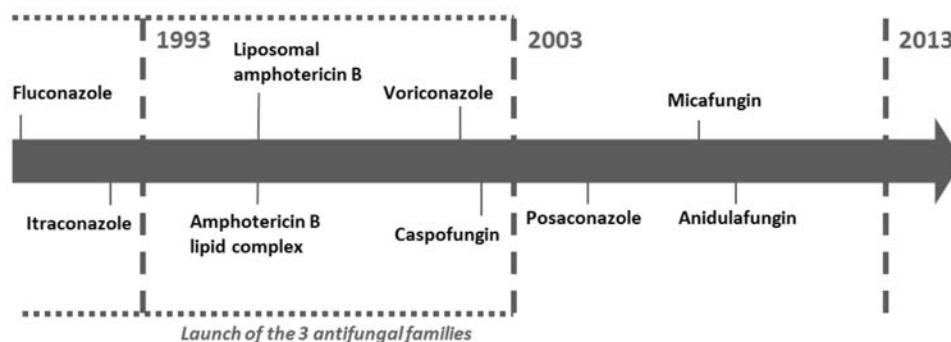


Figure 3 Time course in the development of antifungal agents.

Table 1 Efficacy according to the clinical experience (IV therapies)^{49,101}.

Efficacy	<i>Aspergillus</i>	<i>Zygomycetes</i>	<i>Non-albicans Candida</i>
L-Amph B	+++	+++	+++
Echinocandins	+ / ++	+ / -	+++
Voriconazole	+++	-	++

L-Amph B: Liposomal amphotericin B

unequivocal to exclude or to establish a definitive diagnosis of IFI, as well as to indicate or discard the beginning of specific antifungal treatment.

Changes related to antifungal knowledge

The therapeutic armamentarium has not changed substantially in the last decade, which continues to include the three classical antifungal families: polyenes, azoles and echinocandins (figure 3).

Amphotericin is the antifungal agent with the broadest spectrum of activity, including both yeasts and the majority of filamentous fungi against which is a fungicidal drug^{61,62}. The intrinsic resistance is against the most prevalent fungi is scarce⁶³ and secondary resistances, up to the present time, are very rare despite the fact that this compound has been used for over 50 years⁶⁴. On the other hand, some observational studies have documented a low incidence of breakthrough IFI⁶⁵. The incidence of infusion reactions and nephrotoxicity, the most frequent adverse events of amphotericin B, has been significantly reduced with lipid formulations and, in particular, with the liposomal formulation probably in relation to the higher stability of the liposome at the body temperature⁶⁶⁻⁶⁸. Liposomal amphotericin B is the formulation recommended by the majority of guidelines and experts opinion due to its tolerability and lower toxicity^{12,13,69,70}. Liposomal amphotericin B is used at doses of 3 mg/kg/day for the treatment of *Aspergillus*

infection⁷¹ and 5-7 mg/kg/day in *Mucorales* infection⁷². The most appropriate dose for the use in prophylactic therapy is currently being assessed in an ongoing clinical trial⁷³.

EEA, voriconazole and posaconazole, are fungicides against filamentous fungi and have fungistatic activity against yeasts⁶². These drugs are currently the agents of choice for prophylaxis, and voriconazole together with liposomal amphotericin B are the best agents for the treatment of aspergillosis. Voriconazole has no activity against *Mucorales*. Conversely, posaconazole is active against some species *Mucorales*, (*Lichtheimia*, *Rhizomucor*), however, amphotericin remains the treatment of choice in infections caused by these fungal species^{72,74}. The most remarkable disadvantages of the use of EEA in prophylactic regimens are as follows: a) potential interactions with other drugs (vincristine, cyclophosphamide, cyclosporine, sirolimus and drugs that cause QT prolongation) through modification of the activity of cytochromes²⁰⁻²³ and, b) the probability of not reaching effective serum concentrations⁷⁵⁻⁷⁷, with the associated risk of breakthrough infections^{11,78-80}. This disadvantage is more noticeable in the case of posaconazole due to its limited oral absorption and large interindividual variability. The problem is further complicated if the patient is treated with antacids or proton pump inhibitions, or presents mucositis or diarrhoea. To solve this inconvenience it is advisable to increase the doses and to administer the drug with fat-rich meals⁸¹⁻⁸⁴. Voriconazole has a more favourable bioavailability but currently it is recommended to reach serum concentrations > 1 mg/dL for which doses of 300 mg/12 h should be administered^{85,86}. Hepatotoxicity, usually moderate and reversible, is the main adverse effect of azoles. Cases of photosensitivity and squamous cell carcinoma as a result of the prolonged use of voriconazole have been reported, although at present are anecdotic cases and the mechanism of action is unknown^{87,88}.

Echinocandins constitute a pharmacological group with excellent *in vitro* activity and efficacy against *Candida* spp., according to which the Infectious Diseases Society of America (IDSA)¹⁴ and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)⁸⁹ recommend echinocandins as the antifungals of

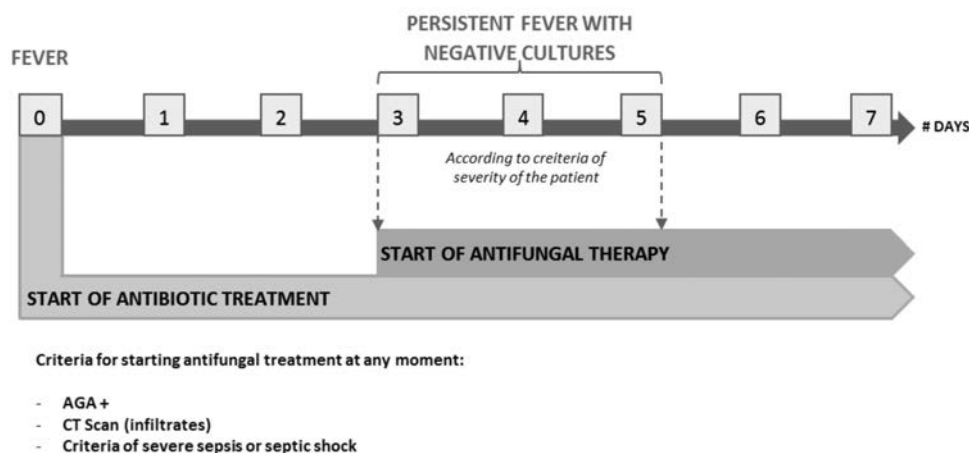


Figure 4 Time-out/starting antifungal treatment intervals in the high-risk patient

choice for the treatment of candidemia and invasive candidiasis. However, the activity against the large majority of filamentous fungi is scarce. In the particular case of *Aspergillus* spp., the efficacy is not related to the *in vitro* activity probably due to its fungistatic effect⁶². In relation to caspofungin, two clinical studies of first-line treatment in onco-haematological patients with invasive aspergillosis have shown a lower efficacy than the target objective (relative risk [RR] > 35%)^{5,90}. A further dose escalation study to assess the tolerability in haematological patients with 50% of possible IFIs⁹¹, the 200 mg dose was more effective than 50 mg, although the results obtained were not superior to those reported with voriconazole in the study of Herbrecht et al.⁹² in 2002, and with liposomal amphotericin B in the the AmBiLoad study of Cornely et al.⁷¹ in 2007. Recently, different studies of caspofungin have shown the appearance of breakthrough fungemias caused by *Aspergillus*^{6,65,93-95}. Micafungin is the only candidin approved by the FDA for the prophylaxis of candidiasis in haematopoietic transplant recipients, although the dose required for the prophylaxis of infections caused by filamentous fungi may be higher than that recommended⁹⁶⁻⁹⁹. Finally, echinocandins have shown an excellent safety profile¹⁰⁰. The efficacy of liposomal amphotericin B, echinocandins and voriconazole according to the clinical experience is shown in table 1^{48,101}.

ACCORDING TO THIS KNOWLEDGE, SHOULD WE CHANGE THE CURRENT TREATMENT STRATEGY IN THE HIGH-RISK PATIENT?

Prophylaxis

The indication of EEA as antifungal prophylaxis in high-risk patients is a highly recommendable strategy due to the high mortality associated with IFI. The possibility of oral administration makes these agents the antifungals of choice^{8,14-19}. However, other alternatives such as micafungin or liposomal amphotericin B are necessary in some circumstances, including the

following: 1) Concomitant treatment with vincristine, cyclophosphamide and sirolimus, or drugs that induce biosynthesis of cytochrome CYP3A4 or QT prolongation²⁰⁻²³; 2) severe liver failure¹⁰²; and 3) absorption- or metabolism-related problems limiting the drug bioavailability^{75-77,85}.

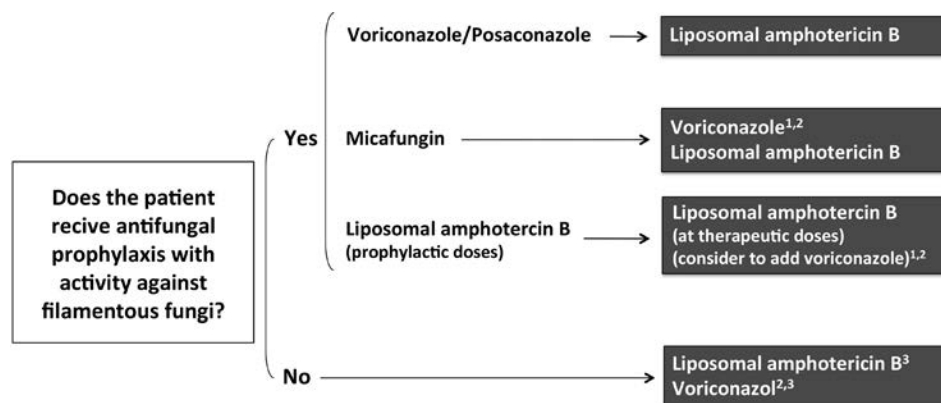
Preemptive treatment

The antifungal of choice for preemptive therapy, based on the positivity of the AGA test, is voriconazole. Liposomal amphotericin B is an option for cases in which voriconazole cannot be administered or appearance of adverse events that discourage its use. If the patient meets criteria of severe sepsis (signs of poor peripheral perfusion or functional failure of an organ), presents disseminated infection, involvement of the central nervous system (CNS) or radiological documentation of extensive respiratory involvement, then it is advisable to add a second antifungal drug in order to ensure the efficacy of treatment from the beginning. Approximately 20% of patients treated with voriconazole may have suboptimal serum concentrations during the first days of treatment^{86,103}.

Empirical treatment

Starting empirical treatment is considered adequate in case of persistence of fever and/or absence of clinical improvement despite antibiotic therapy, and negative microbiological studies. According to the risk level of fungal infection and clinical severity, treatment should be considered from the third day (high-risk patient and/or clinical worsening) or the fifth day (medium-risk and/or clinical stability)^{13,49} (figure 4). The selection of the antifungal agent depends on whether the patient is being treated prophylactically and the antifungal drug used for prophylaxis.

If prophylaxis is being carried out with: 1) an EEA (posaconazole or voriconazole), liposomal amphotericin B is the treatment of choice; 2) in case of prophylaxis with micafungin, treatment with liposomal amphotericin B or voriconazole can be indica-



¹If the patient was on prophylaxis with micafungin probably there is some contraindication for the use of triazoles.

²If the patient meets criteria of severe sepsis (signs of poor peripheral perfusion or functional failure of an organ), it is necessary that antifungal treatment should be effective as soon possible. Up to 20% of patients treated with voriconazole, optimal serum concentrations during the first week of treatment are not reached, so that initial treatment may include the association of voriconazole and liposomal amphotericin B or liposomal amphotericin B as monotherapy.

³If the AGA test is unavailable or negative, liposomal amphotericin B should be used. Caspofungin is an alternative option for cases in which the recommended regimens of choice cannot be used. Its activity and clinical efficacy against filamentous fungi are lower than those of triazoles and polyenes.

Figure 5 Selection of early antifungal treatment

ted. The antifungal drug should be selected taking into account the reasons for which the patient was treated prophylactically with micafungin instead of a triazole; and 3) in case of the patient given prophylactic treatment with low or intermittent doses of liposomal amphotericin B given intravenously or inhaled, treatment may include switching liposomal amphotericin to therapeutic doses in association (or not) to a second antifungal, voriconazole or a candin, although it is likely that some contraindication for the use of voriconazole may have been present if the patient was receiving triazole antifungals for prophylaxis.

Finally, if the patient was not treated with prophylactic antifungals and the AGA test is negative or unavailable, treatment with liposomal amphotericin B, voriconazole or caspofungin can be administered. However, when the antifungal agent is directed to treat a filamentous fungus, candin is considered the second choice drug following liposomal amphotericin and voriconazole (figure 5)^{6,10, 65,93-95}.

REFERENCES

1. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007;44(4):531-40.
2. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus galactomannan* detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002; 20:1898-906.
3. Mora-Duarte J, Betts R, Rotstein C, Colombo AL, Thompson-Moya L, Smietana J, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *New Engl J Med* 2002; 347: 2020-9.
4. Lewis RE, Cahyame-Zuniga L, Leventakos K, Chamilos G, Ben-Ami R, Tamboli P et al. Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study. *Mycoses* 2013; 56:638-45.
5. Viscoli C, Herbrecht R, Akan H, Baila L, Sonet A, Gallamini A et al. Infectious Disease Group of the EORTC. An EORTC Phase II study of caspofungin as first-line therapy of invasive aspergillosis in haematological patients. *J Antimicrob Chemother* 2009; 64:1274-81.
6. Madureira A, Bergeron A, Lacroix C, Robin M, Rocha V, de Latour RP et al. Breakthrough invasive aspergillosis in allogeneic haematopoietic stem cell transplant recipients treated with caspofungin. *Int J Antimicrob Agents* 2007; 30:551-4.
7. Herbrecht R, Bories P, Moulin JC, Ledoux MP, Letscher-Bru V. Risk stratification for invasive aspergillosis in immunocompromised patients. *Ann N Y Acad Sci* 2012; 1272:23-30.
8. Vázquez L, Carreras E, Serrano D, Jarque I, Mensa J, Barberán J. Antifungal prophylaxis in the haematological patient: a practical approach. *Rev Esp Quimioter* 2012; 25:299-304.
9. Zabalza A, Gorosquieta A, Equiza EP, Olavarria E. Voriconazole and its clinical potential in the prophylaxis of systemic fungal infection in patients with hematologic malignancies: a perspective review. *Ther Adv Hematol* 2013; 4:217-30.
10. Pagano L, Caira M, Nosari A, Cattaneo C, Fanci R, Bonini A et al. The use and efficacy of empirical versus pre-emptive therapy in the management of fungal infections: the HEMA e-Chart Project. *Haematologica* 2011; 96:1366-70.
11. Auberger J, Lass-Flörl C, Aigner M, Clausen J, Gastl G, Nachbaur D. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. *J Antimicrob Chemother* 2012; 67:2268-73.
12. Fortún J, Carratalá J, Gavalda J, Lizaola M, Salavert M, de la Cámara R et al. Recomendaciones sobre el tratamiento de la enfermedad fúngica invasiva por

- Aspergillus* spp. y otros hongos filamentosos de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Actualización 2011. *Enferm Infecc Microbiol Clin* 2011; 29:435-54.
13. Barberán J, Mensa J, Llamas JC, Ramos IJ, Ruiz JC, Marín JR *et al*; Spanish Society of Chemotherapy. Recommendations for the treatment of invasive fungal infection caused by filamentous fungi in the hematological patient. *Rev Esp Quimioter* 2011; 24:263-70.
 14. Pappas PG, Kauffman CA, Andes D, Benjamin DJ Jr, Thierry F, Calandra TF, Edwards JE Jr *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.
 15. Cornely OA, Böhme A, Buchheidt D, Einsele H, Heinz WJ, Karthaus M *et al*. Primary prophylaxis of invasive fungal infections in patients with hematologic malignancies. Recommendations of the Infectious Diseases Working Party of the German Society for Haematology and Oncology. *Haematologica* 2009; 94:113-22.
 16. Marr KA, Bow E, Chiller T, Maschmeyer G, Ribaud P, Segal B *et al*. Fungal infection prevention after hematopoietic cell transplantation. *Bone Marrow Transplant* 2009; 44: 483-7.
 17. Maertens J, Marchetti O, Herbrecht R, Cornely OA, Flückiger U, Frère P *et al*. European guidelines for antifungal management in leucemia and hematopoietic stem cell transplant recipients: summary of the ECIL 3 2009 Update. *Bone Marrow Transplant* 2011; 46:709-18.
 18. NCCN Clinical Practices guidelines in oncology. Prevention and treatment of cancer-related infections version 1.2012. http://www.nccn.org/professionals/physician_gls/pdf/infections.pdf
 19. Ullmann AJ, Cornely OA, Donnelly JP, Akova M, Arendrup MC, Arikan-Akdoglu S *et al*. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: developing european guidelines in clinical microbiology and infectious diseases. *Clin Microbiol Infect* 2012; 18 (Suppl. 7): 1-8.
 20. Moriyma B, Henning SA, Leung J, Falade-Nwulia O, Jarosinski P, Penzak SR *et al*. Adverse interactions between antifungal azoles and vincristine: review and analysis of cases. *Mycoses* 2012; 55:290-7.
 21. Surowiec D, DePestel DD, Carver PL. Concurrent administration of sirolimus and voriconazole: a pilot study assessing safety and approaches to appropriate management. *Pharmacotherapy* 2008; 28:719-29.
 22. Kubiak DW, Koo S, Hammond SP, Armand P, Baden LR, Antin JH *et al*. Safety of posaconazole and sirolimus coadministration in allogeneic hematopoietic stem cell transplants. *Biol Blood Marrow Transplant* 2012; 18:1462-5.
 23. Alkan Y, Haefeli WE, Burhenne J, Stein J, Yaniv I, Shalit I. Voriconazole-induced QT interval prolongation and ventricular tachycardia: a non-concentration-dependent adverse effect. *Clin Infect Dis* 2004; 39:49-52.
 24. Pagano L, Caira M, Cuenca-Estrella M. The management of febrile neutropenia in the posaconazole era: a new challenge? *Haematologica* 2012; 97:963-5.
 25. Marr KA, Laverdiere M, Gugel A, Leisenring W. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. *Clin Infect Dis* 2005; 40:1762-9.
 26. Jha AK, Bansal D, Chakrabarti A, Shivaprakash MR, Trehan A, Marwaha RK. Serum galactomannan assay for the diagnosis of invasive aspergillosis in children with haematological malignancies. *Mycoses* 2013; 56:442-8.
 27. Neofytos D, Horn D, Anaissie E, Steinbach W, Olyaei A, Fishman J *et al*. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin Infect Dis* 2009; 48:265-73. Erratum in: *Clin Infect Dis* 2009; 48:690.
 28. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, Walsh TJ *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.
 29. Llorente A, Perez-Valero I, García E, Heras I, Fraile V, García P *et al*. Mortality risk factors in patients with zygomycosis: a retrospective and multicentre study of 25 cases. *Enferm Infecc Microbiol Clin* 2011; 29:263-8.
 30. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. Zygomycosis in the 1990s in a tertiary-care cancer center. *Clin Infect Dis* 2000; 30:851-6.
 31. Lewis RE, Kontoyiannis DP. Epidemiology and treatment of mucormycosis. *Future Microbiol* 2013; 8:1163-75.
 32. Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C *et al*. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005; 191:1350-60.
 33. Sipsas NV, Lewis RE, Tarrand J, Hachem R, Rolston KV, Raad II, Kontoyiannis DP. Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001-2007): stable incidence but changing epidemiology of a still frequently lethal infection. *Cancer* 2009; 115: 4745-52.
 34. Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M. Current section and species complex concepts in *Aspergillus*: recommendations for routine daily practice. *Ann N Y Acad Sci* 2012; 1273:18-24.
 35. Escribano P, Peláez T, Muñoz P, Bouza E, Guinea J. Is azole resistance in *Aspergillus fumigatus* a problem in Spain? *Antimicrob Agents Chemother* 2013; 57:2815-20.
 36. Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Med Mycol* 2011; 49 Suppl 1:S90-5.
 37. van der Linden JW, Snelders E, Kampinga GA, Rijnders BJ, Mattsson E, Debets-Ossenokop YJ *et al*. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007-2009. *Emerg Infect Dis* 2011; 17:1846-54.
 38. Snelders E, Huis In 't Veld RA, Rijs AJ, Kema GH, Melchers WJ, Verweij PE. Possible environmental origin of resistance of *Aspergillus fumigatus* to medical triazoles. *Appl Environ Microbiol*. 2009; 75:4053-7.
 39. Snelders E, van der Lee HA, Kuijpers J, Rijs AJ, Varga J, Samson RA *et al*. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008; 5:e219.
 40. Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC, Pasqualotto AC *et al*. Frequency and evolution of Azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009; 15:1068-76.
 41. Blanchard E, Lortholary O, Boukris-Sitbon K, Desnos-Ollivier M, Dromer F, Guillemot D; French Mycosis Study Group. Prior caspofungin exposure in patients with hematological malignancies is a risk factor for subsequent fungemia due to decreased susceptibility in *Candida* spp.: a case-control study in Paris, France. *Antimicrob Agents Chemother* 2011; 55:5358-61.
 42. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R *et al*. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin Infect Dis* 2013; 56:1724-32.
 43. Gamaletsou MN, Daikos G, Walsh TJ *et al*. Phenotypic and molecular resistance

- patterns of *Candida* species in patients with candidemia and hematologic malignancies. Berlin, 23rd European Congress for Clinical Microbiology and Infectious Diseases (ECCMID), 2013, abstract O465.
44. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA *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–e93.
 45. Fukuda T, Hackman RC, Guthrie KA, Sandmaier BM, Boeckh M, Maris MB *et al.* Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood* 2003; 102:2777–85.
 46. Pagano L, Caira M, Nosari A, Van Lint MT, Candoni A, Offidani M *et al.* Fungal infections in recipients of hematopoietic stem cell transplants: results of the SEI-FEM B-2004 study--Sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne. *Clin Infect Dis* 2007; 45:1161–70.
 47. Marr KA. Delayed opportunistic infections in hematopoietic stem cell transplantation patients: a surmountable challenge. *Hematology Am Soc Hematol Educ Program* 2012; 2012:265–70.
 48. Neofytos D, Lu K, Hatfield-Seung A, Blackford A, Marr KA, Treadway S *et al.* Epidemiology, outcomes, and risk factors of invasive fungal infections in adult patients with acute myelogenous leukemia after induction chemotherapy. *Diagn Microbiol Infect Dis* 2013; 75:144–9.
 49. Vallejo JC, Ruiz I. Infección fúngica invasora en los pacientes hematológicos. *Enferm Infecc Microbiol Clin* 2012; 30:572–9.
 50. Cornely OA, Aversa F, Cook P, Jones B, Michallet M, Shea T, Vallejo C. Evaluating the role of prophylaxis in the management of invasive fungal infections in patients with hematologic malignancy. *Eur J Haematol* 2011; 87:289–301.
 51. Tarrand JJ, Lichterfeld M, Warraich I, Luna M, Han XY, May GS, Kontoyiannis DP. Diagnosis of invasive septate mold infections. A correlation of microbiological culture and histologic or cytologic examination. *Am J Clin Pathol* 2003; 119:854–8.
 52. Jarque I, Andreu R, Salavert M, Gómez D, Pemán J, Gobernado M, Sanz MA. Valor de la detección del antígeno galactomano de *Aspergillus* en el diagnóstico y seguimiento de la aspergilosis invasora en pacientes hematológicos. *Rev Iberoam Micol* 2003; 20:116–8.
 53. Cordonnier C, Botterel F, Ben Amor R, Pautas C, Maury S, Kuentz M *et al.* Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clin Microbiol Infect* 2009; 15:81–6.
 54. Hachem RY, Kontoyiannis DP, Chemaly RF, Jiang Y, Reitzel R, Raad I. Utility of galactomannan enzyme immunoassay and (1,3) beta-D-glucan in diagnosis of invasive fungal infections: low sensitivity for *Aspergillus fumigatus* infection in hematologic malignancy patients. *J Clin Microbiol* 2009; 47:129–33.
 55. Steinmann J, Buer J, Rath PM. Caspofungin: cross-reactivity in the *Aspergillus* antigen assay. *J Clin Microbiol* 2010; 48:2313.
 56. Mikulska M, Furfaro E, Del Bono V, Gualandi F, Raiola AM, Molinari MP *et al.* Galactomannan testing might be useful for early diagnosis of fusariosis. *Diagn Microbiol Infect Dis* 2012; 72:367–9.
 57. Caillot D, Couaillier JF, Bernard A, Casasnovas O, Denning DW, Mannone L *et al.* Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001; 19:253–9.
 58. Greene RE, Schlamm HT, Oestmann JW, Stark P, Durand C, Lortholary O *et al.* Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis* 2007; 44:373–9.
 59. Parrón M, Torres I, Pardo M, Morales C, Navarro M, Martínez-Schmizcraft M. The halo sign in computed tomography images: differential diagnosis and correlation with pathology findings. *Arch Bronconeumol* 2008; 44:386–92.
 60. Georgiadou SP, Sipsas NV, Marom EM, Kontoyiannis DP. The diagnostic value of halo and reversed halo signs for invasive mold infections in compromised hosts. *Clin Infect Dis* 2011; 52:1144–55.
 61. Arkan S, Lozano-Chiu M, Paetznick V, Nangia S, Rex JH. Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of *Aspergillus* and *Fusarium* species. *J Clin Microbiol* 1999; 37:3946–51.
 62. Cuenca-Estrella M. Antifúngicos en el tratamiento de las infecciones sistémicas: importancia del mecanismo de acción, espectro de actividad y resistencias. *Rev Esp Quimioter* 2010; 23:169–76.
 63. Ellis D. Amphotericin B: spectrum and resistance. *J Antimicrob Chemother* 2002; 49 Suppl 1:7–10.
 64. Marks DI, Pagliuca A, Kibbler CC, Glasmacher A, Heussel CP, Kantecki M *et al.* IMPROVIT Study Group. Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation. *Br J Haematol* 2011; 155:318–27.
 65. Ellis M, Frampton C, Joseph J, Alizadeh H, Kristensen J, Hauggaard A, Shammass F. An open study of the comparative efficacy and safety of caspofungin and liposomal amphotericin B in treating invasive fungal infections or febrile neutropenia in patients with haematological malignancy. *J Med Microbiol* 2006; 55:1357–65.
 66. Fleming RV, Kantarjian HM, Husni R, Rolston K, Lim J, Raad I *et al.* Comparison of amphotericin B lipid complex (ABLC) vs. Am-Bisome in the treatment of suspected or documented fungal infections in patients with leukemia. *Leukemia and lymphoma* 2001; 40:511–20.
 67. Ullmann AJ, Sanz MA, Tramarin A, Barnes RA, Wu W, Gerlach BA *et al.* Prospective Study of Amphotericin B Formulations in Immunocompromised Patients in 4 European Countries. *Clin Infect Dis* 2006; 43:e29–38.
 68. Moosa MY, Alangaden GJ, Manavathu E, Chandrasekar PH. Resistance to amphotericin B does not emerge during treatment for invasive aspergillosis. *J Antimicrob Chemother* 2002; 49:209–13.
 69. Cifani C, Costantino S, Massi M, Berrino L. Commercially available lipid formulations of amphotericin b: are they bioequivalent and therapeutically equivalent? *Acta Biomed* 2012; 83:154–63.
 70. Azanza Perea JR, Barberán J. Amfotericina B liposomal: un único perfil farmacocinético. Una historia inacabada. *Rev Esp Quimioter* 2012; 25:17–24.
 71. Cornely OA, Maertens J, Bresnik M, Ebrahimi R, Ullmann AJ, Bouza E *et al.* AmBi-Load Trial Study Group. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007; 44:1289–97.
 72. Kontoyiannis DP, Lewis RE. How I treat mucormycosis. *Blood* 2011; 118:1216–24.
 73. <http://clinicaltrials.gov/show/NCT01259713>.
 74. Leventakos K, Lewis RE, Kontoyiannis DP. Fungal infections in leukemia patients: how do we prevent and treat them? *Clin Infect Dis* 2010; 50:405–15.
 75. Thompson GR 3rd, Rinaldi MG, Pennick G, Dorsey SA, Patterson TF, and Lewis JL 2nd. Posaconazole therapeutic drug monitoring: a referente laboratory experience. *Antimicrob Agents Chemother* 2009; 53:2223–4.

76. Lebeaux D, Lanterrier F, Elie C, Suarez F, Buzyn A, Viard JP et al. Therapeutic drug monitoring of posaconazole: a monocentric study with 54 adults. *Antimicrob Agents Chemother* 2009; 53: 5224-9.
77. Hoenigl M, Raggam RB, Salzer HJ, Valentin T, Valentin A, Zollner-Schwetz I et al. Posaconazole plasma concentrations and invasive mould infections in patients with haematological malignancies. *Int J Antimicrob Agents* 2012; 39:510-3.
78. Egerer G, Geist MJ. Posaconazole prophylaxis in patients with acute myelogenous leukaemia--results from an observational study. *Mycoses* 2011; 54 Suppl 1:7-11.
79. Hahn J, Stifel F, Reichle A, Holler E, Andreesen R. Clinical experience with posaconazole prophylaxis--a retrospective analysis in a haematological unit. *Mycoses* 2011; 54 Suppl 1:12-6.
80. Pascual A, Csajka C, Buclin T, Bolay S, Bille J, Calandra T, Marchetti O. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin Infect Dis* 2012; 55:381-90.
81. Krishna G, Moton A, Ma L, Medlock MM, McLeod J. Pharmacokinetics and absorption of posaconazole oral suspension under various gastric conditions in healthy volunteers. *Antimicrob Agents Chemother* 2009; 53:958-66.
82. AbuTarif MA, Krishna G, Statkevich P. Population pharmacokinetics of posaconazole in neutropenic patients receiving chemotherapy for acute myelogenous leukemia or myelodysplastic syndrome. *Curr Med Res Opin* 2010; 26:397-405.
83. Kohl V, Muller C, Cornely OA, Abduljalil K, Fuhr U, Vehreschild JJ, et al. Factors influencing pharmacokinetics of prophylactic posaconazole in patients undergoing allogeneic stem cell transplantation. *Antimicrob Agents Chemother* 2010; 54:207-12.
84. Courtney R, Wexler D, Radwanski E, Lim J, Laughlin M. Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. *Br J Clin Pharmacol* 2003; 57:218-22.
85. Ananda-Rajah MR, Grigg A, Downey MT, Bajel A, Spelman T, Cheng A 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.
86. Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008; 46:201-11.
87. Haylett AK, Felton S, Denning DW, Rhodes LE. Voriconazole-induced photosensitivity: photobiological assessment of a case series of 12 patients. *Br J Dermatol* 2013; 168:179-85.
88. Epaulard O, Leccia MT, Blanche S, Chosidow O, Mamzer-Bruneel MF, Ravaud P et al. Phototoxicity and photocarcinogenesis associated with voriconazole. *Med Mal Infect* 2011; 41:639-45.
89. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O et al; ESCMID Fungal Infection Study Group. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. *Clin Microbiol Infect* 2012; 18 Suppl 7:19-37.
90. Herbrecht R, Maertens J, Baila L, Aoun M, Heinz W, Martino R et al. Caspofungin first-line therapy for invasive aspergillosis in allogeneic hematopoietic stem cell transplant patients: an European Organisation for Research and Treatment of Cancer study. *Bone Marrow Transplant* 2010; 45:1227-33.
91. Cornely OA, Vehreschild JJ, Vehreschild MJ, Würthwein G, Arenz D, Schwartz S et al. Phase II dose escalation study of caspofungin for invasive Aspergillosis. *Antimicrob Agents Chemother* 2011; 55:5798-803.
92. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW et al; Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; 347:408-15.
93. Lafaurie M, Lapalu J, Raffoux E, Breton B, Lacroix C, Socié G, Porcher R, Ribaud P, Touratier S, Molina JM. High rate of breakthrough invasive aspergillosis among patients receiving caspofungin for persistent fever and neutropenia. *Clin Microbiol Infect* 2010; 16:1191-6.
94. Kubiak DW, Bryar JM, McDonnell AM, Delgado-Flores JO, Mui E, Baden LR, Marty FM. 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.
95. Pang KA, Godet C, Fekkar A, Scholler J, Nivoix Y, Letscher-Bru V et al. Breakthrough invasive mould infections in patients treated with caspofungin. *J Infect* 2012; 64:424-9.
96. Hashino S, Morita L, Takahata M, Onozawa M, Nakagawa M, Kawamura T, et al. Administration of micafungin as prophylactic antifungal therapy in patients undergoing allogeneic stem cell transplantation. *Int J Hematol* 2008; 87:91-7.
97. Hiramatsu Y, Maeda Y, Fujii N, Saito T, Nawa Y, Hara M et al. Use of micafungin versus fluconazole for antifungal prophylaxis in neutropenic patients receiving-hematopoietic stem cell transplantation. *Int J Hematol* 2008; 88:588-95.
98. Hirata Y, Yokote T, Kobayashi K, Nakayama S, Oka S, Miyoshi T et al. Antifungal prophylaxis with micafungin in neutropenic patients with hematological malignancies. *Leuk Lymphoma* 2010; 51:853-9.
99. Heimann S, Cornely OA, Meintker L, Heinz W, Schroeder T, Vehreschild MJ et al. Different Doses of Micafungin for Prophylaxis of Invasive Fungal Diseases: A Web-based Non-Interventional Trial in Four Large University Hospitals in Germany [abstract O23]. Programme of the 23rd European Congress for Clinical Microbiology and Infectious Diseases (ECCMID); 2013.
100. Kofla G, Ruhnke M. Pharmacology and metabolism of anidulafungin, caspofungin and micafungin in the treatment of invasive candidosis: review of the literature. *Eur J Med Res* 2011; 16:159-66.
101. Cisterna R, Ezpeleta G, Telleria O, Guinea J, Regueiro B, Garcia-Rodriguez J et al. Nationwide sentinel surveillance of bloodstream Candida infections in 40 tertiary care hospitals in Spain. *J Clin Microbiol* 2010; 48:4200-6.
102. Solís-Muñoz P, López JC, Bernal W, Willars C, Verma A, Heneghan MA et al. Voriconazole hepatotoxicity in severe liver dysfunction. *J Infect* 2013; 66:80-6.
103. Hoenigl M, Duettmann W, Raggam RB, Seeber K, Troppan K, Fruhwald S, et al. Potential factors for inadequate voriconazole plasma concentrations in intensive care unit patients and patients with hematological malignancies. *Antimicrob Agents Chemother* 2013; 57:3262-7.