Diplomarbeit

Retrospective comparison of Micafungin versus Voriconazole during primary antifungal prophylaxis in patients with hematological malignancies undergoing allogeneic HSCT.

eingereicht von

Gulsina Murzabekova

zur Erlangung des akademischen Grades

Doktor(in) der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Klinik für Innere Medizin

unter der Anleitung von

unter der Anleitung von Prof.Neumeister Peter

DDr.Troppan Katharina

Graz, 29.04.2016.

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 29.04.2016 *Gulsina Murzabekova eh*

Zusammenfassung

Hintergrund: Invasive fungale Infektionen (IFI) sind eine Hauptursache für die Moratlität und Morbidität der Patienten, die mit einer allogenen Stammzelltransplantation (hematopoietic stem cell transplantation (HSCT)) behandelt werden. Die Frühdiagnose von IFI ist schwierig, wobei die Verzögerung in der Diagnostik die Mortalität erhöhen kann. Darum wird die prophylaktische Anwendung von anti-fungalen Medikamenten empfohlen. Das Ziel unserer Studie war die Effizienz von Micafungin und Voriconazole als Prophylaxe von fungalen Infektionen in einer retrospektiven Studie zu vergleichen.

Methoden: Jeweils 30 mit Micafungin behandelte Patienten mit hämatologischen Neoplasien, die sich an unserer Abteilung von 2011 bis 2014 einer allogenen HSCT unterzogen, wurden mit 30 Patienten verglichen die prophylaktisch Voriconazole erhielten. Primärer Endpunkt der Studie war das Auftreten von Durchbruchs-Infektionen (breakthrough IFI) während der anti-mikotischen Prophylaxe. Sekundäre Endpunkte waren fungalen Kolonisation, β-D-Glucan-, Galactomannan (GM)- und Voriconazole- Plasmakonzentrationen.

Ergebnisse: Die untersuchten Patientengruppen waren in den demographischen Parametern und Diagnosestellungen vergleichbar. Ein Fall einer möglichen IFI wurde in der Voriconazole- und keiner in der Micafungin Gruppe diagnostiziert. Drei Fälle fungalen Kolonisation wurden in Micafungin Gruppe festgestellt und zwei in der Voricanazole Gruppe. In allen diagnostizierten Fällen war der Rachen der Kolonisationsort.

Fazit: Beide Medikamente waren vergleichbar effizient für die Prophylaxe fungaler Infektionen bei HSCT Patienten. Keine Fälle von Durchbruchsinfektionen wurden detektiert.

Abstract

Background: Invasive fungal infections (IFI) are the cause of significant morbidity and mortality in patients undergoing hematopoietic stem cell transplantation (HSCT). Early diagnosis of IFI is challenging; and a delay in antifungal therapy may increase mortality. Therefore, prophylactic use of several anti-fungal agents was recommended. The aim of the study was to compare the efficiency of Micafungin and Voriconazole for prophylaxis of fungal infections in a retrospective analysis study.

Methods: Of the patients undergoing allogeneic HSCT due to hematologic malignancies in our institution between 2011 and 2014, thirty patients receiving Micafungin for antifungal prophylaxis were compared to thirty patients receiving Voriconazole. Primary endpoint was the occurrence of the breakthrough IFI during antifungal treatment. Secondary endpoints were fungal colonizations, β-D-Glucan levels, Galactomannan (GM) levels, and Voriconazole plasma levels.

Results: Two groups of patients were comparable in their demographic parameters and diagnosis distribution. One case of the possible IFI was diagnosed in the Voriconazole group and non in the Micafungin group. Two cases of fungal colonization were diagnosed in the Micafungin group and two cases in the Voriconazole group. In all cases the oropharynx was the colonization place.

Conclusions: Both antifungals were equally efficient in the prophylaxis of fungal infections in HSCT patients. No cases of breakthrough infections were detected.

Inhaltsverzeichnis

Glossar und Abkürzungen

ALL - Acute lymphocytic leukemia
AMB - Amphotericin B
AML - Acute myeloid leukemia
BAL - Bronchoalveolar lavage
BG – 1.3-β-D-glucans
BW - body weight
CML - Chronic Myelogenous Leukemia
CNS - Central nervous system
CT - Computer tomography
CVC - Central venous catheters
ECIL - European Conference on Infections in Leukemia
EORTC-MSG - European Organization for Research and Treatment of Cancer and Infectious Diseases Mycoses Study Group
GM – Galactomannan
GVHD - Graft-versus-host disease
HSCT - Hematopoietic stem cell transplantation
i.v. – Intravenous
IFI – Invasive fungal infections
IL – Interleukin
MAC - Myeloablative conditioning
MDS - Myelodysplastic syndrome
MM - Multiple Myeloma
MRI - Magnetic resonance imaging
MUD - Matched unrelated donor
NASBA - nucleic acid sequence based amplification
PCR - Polymerase chain reaction
RIC - Reduced intensity conditioning
TLRs - Toll-like receptors
UCB - Umbilical cord blood

Introduction

Invasive fungal infections (IFI) are major complications of the stem cell transplantation therapy associated with significant morbidity and mortality (1,2). Hematopoietic stem cell transplantation (HSCT) is method of choice for the treatment of the hematologic patients with malignancies. Based on site of collection stem cells are collected from:

1) Bone marrow
2) Peripheral blood
3) Umbilical cord blood

Depending on the origin of bone marrow cells used for transplantation HST can be:

- Allogeneic HSCT is the transfer of marrow from a donor to another person.
- Autologous HSCT is the use of the patients own marrow to reestablish hematopoietic cell function.

HSCT requires the use of high-dose chemotherapy or radiotherapy to inactivate recipient's diseased bone marrow. This aggressive therapy is effective in destroying cancer cells, but as side effect, it also carries the risk of severe damage to the liver, lungs, heart and other major organs. Patients with comorbidities are at higher risk of developing side effects.

The complications associated with HSCT include early and late effects.

Early-onset problems include:

- Infections
- Mucositis
- Hemorrhagic cystitis
- Graft-versus-host disease (GVHD)
- Graft failure
- Pulmonary complications
- Hepatic veno-occlusive disease

- Thrombotic microangiopathy

Late-onset problems include:

- Infections
- Chronic GVHD
- Ocular effects
- Endocrine effects
- Pulmonary effects
- Musculoskeletal effects
- Neurologic effects
- Immune effects

Invasive fungal infections

The incidence of invasive fungal infections varies according to the literature between 7.7% and 10.3% (3-5). Although fungal infections can be caused by several types of fungi, *Candida* and *Aspergillus species* are dominant pathogens in patients after HSCT (5,6). Use of the invasive procedures, particularly central venous catheters (CVC), are risk factors for invasive Candidiasis. Prolonged neutropenia related to intensive treatment in patients undergoing HSCT represents the main risk factor for invasive fungal infections (7-9). Invasive candidiasis in hematological patients is primarily caused by *Candida albicans*.

The invasive pulmonary aspergillosis is most frequently observed in neutropenic patients (80-90%). Although recent evidence suggest that incidence has at least stabilized, aspergillus infection is still common (7,10).

Recent studies have demonstrated that HSCT patients carrying specific haplotypes of ”innate immunity genes” such as interleukin (IL10), and toll-like receptors (TLRs) are at higher risk of developing invasive aspergillosis (11,12).

Diagnosis of fungal infections.

Fungal infections are diagnosed based on histological and/or cultural findings from tissue biopsies or positive cultures from normally sterile body fluids. However, these criteria may not be used at early stage of infection. Usually, antifungal therapy in patients with prolonged neutropenia is started when antibiotic-refractory fever persists or pulmonary infiltrates are proven by X-ray and/or computed tomography (CT).

For diagnosis of systemic fungal infections, assessment of the following is included:

- clinical signs and symptoms
- mycological examination by microscopy
- cultural as well as non-cultural techniques
- imaging procedures
- endoscopic methods
- cytology and/or histology of biopsy material

1. Clinical signs and symptoms.

Symptoms of fungal infection are unspecific. Presence of the fungal infection should be suspected if unexplained fever persists in patients treated with broad-spectrum antibiotic. Clinical symptoms of bacterial and fungal infections cannot be differentiated.

2. Mycological examination by microscopy.

Microscopy can be used for differential diagnosis of causes of infection. Microscopy supported by immunohistochemically examination helps in determination of the etiology of infection.

3. Cultural as well as non-cultural techniques.

The best detection of fungal infection can be ensured by cultivation in at least two aerobic/anaerobic blood culture bottles with 10ml of blood each. Sputum or broncho alveolar lavage (BAL) fluid tests positive for yeasts should be considered as

colonization until invasive disease is proven. Selective differentiation agars (e.g. CHROM agar) are helpful for faster identification of the most common *Candida species*.

However, cell culture based tests are time consuming and reliance on them could lead to delay in diagnosing of infections and increase mortality. Biochemical tests based on detection of the components of the fungal cell wall components being increasingly used for early diagnosis of IFI.

Testing for galactomannan (GM) antigen against aspergillus has shown positive predictive value 93% and negative predictive value 95% (13). The GM assay may be useful for early diagnosis of invasive infection, as the test delivered positive results in average six days before clinical suspicion of a fungal infection.

Serological test for the detection 1.3-β-D-glucans (BG) has been established for diagnosis of candidiasis, aspergillosis and cryptococcosis. Recently, it has been reported to identify IFIs caused by Candida spp., Fusarium spp., and Acremonium spp. (14).

Table 1. Major limitations of non-culture tests used for diagnosis of invasive fungal infections. Adopted with modifications from Micell MH. et al. Semin Respir Crit Care Med 2015;35:650-661 (15).

Major caveats	Tests	
	1,3 β-D-glucan (BDG)	Galactomannan (GM)
Cross-reactivity	Pneumocystis jiroveci, Coccidioides immitis, Fusarium sp., Histoplasma capsulatum, Candida sp., Acremonium, Trichsporon sp., Sporothrix schenckii, Saccharomyces cerevisae, Aspergillus sp.	Aspergillus sp., Fusarium sp., Paecilomyces sp., Penicillum sp., Acremonium sp., Alternaria sp., Wangiella dermatidis, Histoplasma capsulatum, Blastomyces dermatitidis, Cryptococcus neoformans, Emmonsia sp.
False positive results	Semi-synthetic β-lactam antibiotics Hemodialysis or hemofiltration with cellulose membranes Bloodstream infections with bacteria (e.g. Pseudomonas aeruginosa)	Semi-synthetic β-lactam antibiotics Severe mucositis or severe gastrointestinal graft vs host disease Blood products collected using Fresenius Kabi bags Multiple myeloma (IgG type) Plasmalyte used in BAL

However, these tests have their limitations since their basis is the detection of a chromogenic reaction substrate of the immunologic interaction. The limitations of the tests are summarized in the Table 1.

4. Polymerase chain reaction (PCR) in combination with other non-cultural tests such as antigen detection is a promising tool and display high sensitivity and specificity. High specificity is achieved by detection of fungal specific gene sequences. Moreover, PCR results were reported to become negative in patients successfully treated with antifungal medicines and remain positive in patients non-responders to the anti-fungal therapy (16). Thus, PCR tests could be used to monitor the success of therapy.

5. Imaging procedures.

At early stage, high-resolution computed tomography or thin-section CT are helpful to detect typical lung infiltrates. Pulmonary aspergillosis is often the complication of the Aspergilla infection. The infiltrates of pulmonary aspergillosis consist of small nodules surrounded by halo (ground-glass opacification) and are localized close to vessels. In patients with neutropenia, who are at high-risk of infection, high-resolution CT is a technique of choice for primary diagnosis.

Hepatosplenic candidiasis can be the result of chronic disseminated candidiasis. The specific image by ultrasound is a lesion with a hyperechoic center and a hypoechoic rim (‘bull’s-eye sign’, size 5-20 mm).

Neutropenic enterocolitis is most common of gastrointestinal infection (up 50%) in neutropenia, which is can be visualized by CT and particularly by magnetic resonance imaging (MRI) scanning.

Cerebral aspergillosis is the most infectious complication of the central nervous system (CNS) in patients undergoing HSCT. A cerebral CT scan is best method for rapid diagnosis of CNS infection.

6. Endoscopic methods

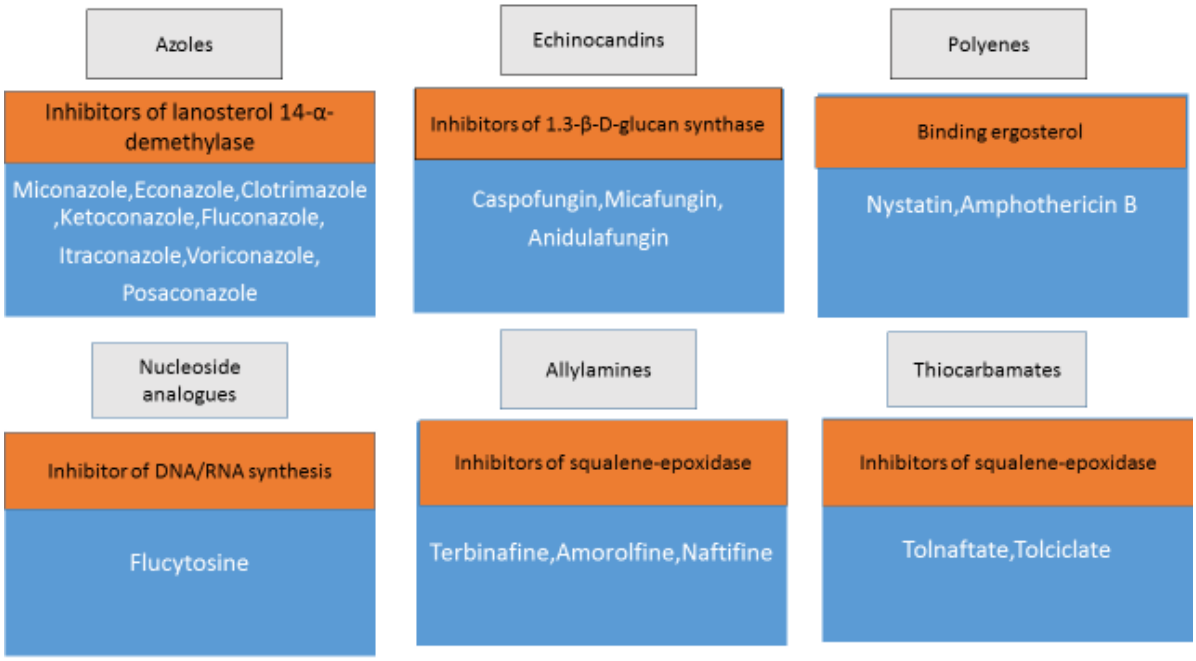
Bronchoscopy and BAL is a useful initial diagnostic tool in the evaluation of pulmonary complications after HCST (17). The BAL in combination with other

diagnostic approaches has the highest diagnostic yields.

Antifungal drugs. Classification.

Currently, variety of substances are available as anti-fungal agents. Chemical classification of the anti-fungal drugs is demonstrated on the scheme 1.

Scheme 1. Chemical classification of the anti-fungal agents.



Adapted from Spampinato C. and Leonardi D. Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Res Int. 2013; 2013:204237.

Azoles

Azoles inhibit the activity of the lanosterol 14-α-demethylase. Although the enzyme is present in mammals, it is of highest importance in fungi, where it is involved in the ergosterol biosynthesis. Ergosterol is the largest sterol component of the fungal cell membrane. Inhibition of ergosterol synthesis leads to disruption of cell membrane,

inhibition of its functions and inhibits fungal growth. The family of azoles consist of imidazoles (miconazole, econazole, clotrimazole, and ketoconazole) and triazoles (fluconazole, itraconazole, voriconazole and posaconazole). Voriconazole is the latest agent, second-generation, synthetic triazole derivative of fluconazole. Many azoles are effective for the treatment and prophylaxis of invasive fungal infections.

Echinocandins

Echinocandins inhibit the activity of 1.3-β-D-Glucan synthase. This inhibition leads to inhibition of beta-glucan synthesis, a component of the cell wall resulting in impaired structural integrity of fungal cell walls and vulnerability to osmotic lysis. Three agents from this group (caspofungin, micafungin, and anidulafungin) have fungicidal activity against most species of *Candida* and approved for the treatment of esophageal and invasive candidiasis, including candidemia.

Micafungin is an echinocandin antifungal agent that inhibits the synthesis of 1. 3 – ß – D - glucan, an essential cell wall component of fungi. It distributes well into tissues lung, liver and spleen. Micafungin is metabolized in the liver and utilized in an inactive form into bile and urine (18,19). Moderate accumulation of Micafungin has been reported after 14 to 21 days of repeated daily i.v. administration (18).

Polyenes

The polyenes bind ergosterol and form pore-like structure, which dramatically increases cell membrane permeability. The alteration of cellular permeability leads to the leakage of K^+ ions, electrolyte disbalance and finally fungal death. To polyenes belong nystatin and amphotericin B (both isolated from *Streptomyces* spp.).

In a meta-analysis Micafungin was more effective for antifungal prophylaxis of broad spectrum of Candida spp. than other drugs und suppressed invasive Aspergillosis spp.(20). Currently, Micafungin is used for the treatment of invasive candidiasis, oesophageal candidiasis. Voriconazole is one of the most effective drugs for the targeted treatment of Aspergillus spp. infections (21). Micafungin has been granted a European license for use in prophylaxis of invasive fungal infection in patients

undergoing HSCT.

Prophylactic treatment vs empiric treatment.

In 2005, several groups created the European Conference on Infections in Leukemia (ECIL). The aim of ECIL is to create and update the recommendations on management of infections in HSCT patients. IFIs are one of the main topics and is addressed in three parts:

- antifungal prophylaxis in high-risk hematology patients
- empirical antifungal therapy
- treatment of invasive Candida and Aspergillus infections

Table 3. Antifungal prophylaxis in patients undergoing HSCT according to guidelines ECIL 3 (22)

Antifungal drug	Grading	Comments
Leukemia patients, induction chemotherapy Fluconazole (50–400 mg/day)	CI	Azoles should not be used empirically in case of previous azole prophylaxis. Combined with a mould-directed diagnostic approach for centers not having HEPA-filtered rooms and/or having a high baseline incidence of mould infections
Itraconazole oral solution (2.5 mg/kg b.i.d.)	CI	May be limited by drug interactions and/or patient tolerability Azoles should not be used empirically in case of prior azole prophylaxis It is recommended to monitor serum drug concentrations
Posaconazole (200 mg t.i.d.)	AI	Azoles should not be used empirically in case of previous azole prophylaxis It is recommended to monitor serum drug concentrations

Echinocandins IV	Insufficient data	
Polyenes IV	CI	Includes low doses of conventional ampho B and lipid formulations
Aerosolized liposomal ampho B combined with oral fluconazole	***BI***	***The ECIL recommendation for aerosolized amphotericin B deoxycholate is DI***
<u>Allogeneic HSCT recipients, initial neutropenic phase</u> Fluconazole (400 mg q.d. i.v. or oral)	AI	Azoles should not be used empirically in case of previous azole prophylaxis Combined with a mould-directed diagnostic approach for centers not having HEPA-filtered rooms and/or having a high baseline incidence of mould infections
Itraconazole (200 mg i.v. followed by oral solution 200 mg b.i.d.)	BI	May be limited by drug interactions and/or patient tolerability Azoles should not be used empirically in case of previous azole prophylaxis It is recommended to monitor serum drug concentrations
Posaconazole	No data	
Voriconazole (200 mg b.i.d. oral)	***Provisional AI***	***Grading pending the publication of the full paper***
Micafungin (50 mg q.d. i.v.)	CI	
Polyenes i.v	CI	Includes low doses of conventional ampho B and lipid formulations
Aerosolized liposomal ampho B combined with oral fluconazole	***BII***	***The ECIL recommendation for aerosolized ampho B deoxycholate is DI***
<u>Allogeneic HSCT recipients, GVHD phase</u> Fluconazole (400 mg q.d. i.v. or oral)	CI	Azoles should not be used empirically in case of previous azole prophylaxis

Itraconazole (200 mg i.v. followed by oral solution 200 mg b.i.d.)	BI	May be limited by drug interactions and/or patient tolerability Azoles should not be used empirically in case of prior azole prophylaxis It is recommended to monitor serum drug concentrations
Posaconazole	AI	Azoles should not be used empirically in case of previous azole prophylaxis. It is recommended to monitor serum drug concentrations
Voriconazole (200 mg b.i.d. oral) Provisional	***AI***	***Grading pending the publication of the full paper***
Echinocandins i.v.	Insuffici ent data	
Polyenes i.v.	CI	Includes low doses of conventional ampho B and lipid formulations
Aerosolized liposomal ampho B combined with oral fluconazole	Insuffici ent data	

Adapted from Maertens J 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 May; 46(5):709

Antifungal prophylaxis.

Primary prophylaxis

Primary prophylaxis is a prevention of the occurrence of infection in the patient at risk. The ECIL 3 Working group has summarized the literature published since release of ECIL 2 guidelines and updated its recommendations. Primary prophylaxis recommendations are provided separately for the HSCT patients and for leukemia patients receiving chemotherapy. For the HSCT patients, it is proposed to provide phase-specific guidelines, summarized in the Table 3.

Secondary prophylaxis

The aim of secondary prophylaxis is to prevent the relapse of a previous fungal infection or the onset of another IFI. The measures of secondary prophylaxis are performed during at-risk period such as a prolonged neutropenic phase or after allogeneic HSCT. There is no standard approach in this setting. Potential of the secondary IFI prophylaxis was investigated with Voriconazole on 45 allogeneic HSCT recipients with a previous history of IFI (23). Only 3 patients developed an IFI during the Voriconazole treatment. Based on the results of the study, Voriconazole was graded AI for secondary IFI prophylaxis in HSCT patients. It was considered that there were not enough data for efficacy and safety of Micafungin, therefore Micafungin was granted level of recommendation CI. In several studies the efficiency of Micafungin for prophylaxis of IFI has been investigated since then. In a retrospective study, Micafungin was superior to Itraconazole and Fluconazole in preventing IFI (24). A multicenter, randomized open-label study has demonstrated that Micafungin was as efficient as Itraconazole in prevention of IFI in HSCT patients with neutropenia (25). Thus, evidences are accumulating which suggest that Micafungin could be as efficient as Voriconazole in preventing IFI in HSCT patients.

Empirical antifungal treatment in neutropenic patients

Empirical therapy aims to treat IFI in patients if: 1) they are at risk to develop IFI; 2) neutropenic patients; 3) have persistent fever despite 4-7 days of broad-spectrum antibacterial therapy, or have relapsing fever (26).

In the large retrospective analysis study, Micafungin showed comparable efficiency with Caspofungin as empirical anti-fungal therapy (27). In-hospital mortality and incidence of IFI were not significantly different between two groups. In a prospective study on 277 patients, Micafungin was efficient as a therapy for both empirical and targeted therapy for IFIs (28). On the basis of these two studies, ECIL 3 recommended Micafungin for empirical treatment of IFI as graded BII.

Antifungal therapeutic management of invasive Aspergillus and Candida infections

Aspergillosis.

Two studies tested Caspofungin for treatment of invasive aspergillosis in non-HSCT (29) and in HCST patients (30). The studies used the standard treatment scheme of Caspofungin (70mg on day 1 followed by 50mg/day). The success rate in non-HSCT patients was 33% (20/61) and in HSCT patients was 42% (10/24). ECIL 2 graded Caspofungin to CII as first-line therapy of invasive aspergillosis.

Candidiasis.

The ECIL 2 recommended Caspofungin for treatment of invasive candidiasis. The double-blind study compared two doses of Caspofungin (70 mg on day 1 followed by 50 versus 150 mg/day) in 204 patients with proven invasive candidiasis.

There was no difference in the efficacy of Caspofungin high or low doses (31).

The approach to breakthrough invasive fungal infections

Resistance to fluconazole in the range of 7-33% cases of breakthrough invasive candidiasis has been reported in the studies investigating the susceptibility of fungal species isolated from blood cultures (32,33). Later, breakthrough infections caused by Candida strains (such as Candida parapsilosis, Candida guilliermondii, Candida albeit) resistant to Echinocandin therapy have been reported (34).

Arendrup et al. showed that in 30-35% of blood cultures Candida isolates were resistant to Itraconazole and Posaconazole, while 15% were also resistant to Voriconazole (35).

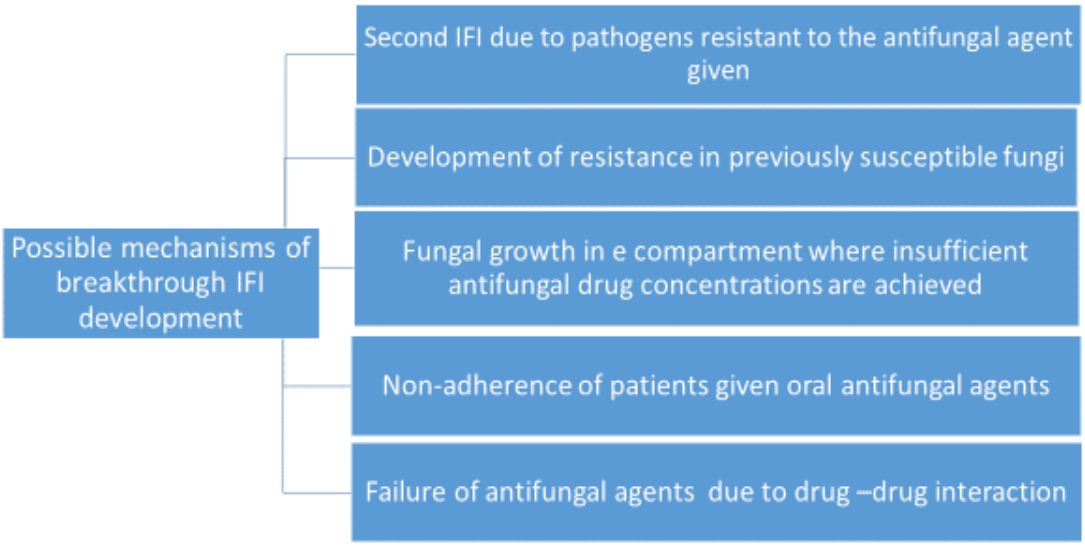
Azoles are the drugs of choice for treatment of invasive infections. However, multi-azole-resistant Aspergillus spp. has been reported (36).

Breakthrough IFI in patients receiving systemic antifungal treatment may have

different causes.

Several factors related to the host, the type of the fungus and others could facilitate breakthrough infections. Most important ones are summarized on the scheme 2.

Scheme 2. Possible mechanisms of breakthrough IFI development



Adapted from Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. Mycoses. 2014 Nov; 57(11):645.

Breakthrough IFI in patients receiving Echinocandin therapy

If in the patients receiving Echinocandin therapy (Anidulafungin, Caspofungin or Micafungin), a blood culture positive for yeast has been detected, a possible cause could be the less susceptible or resistant strains of yeasts (37). In these cases a switch to a broad-spectrum azole or liposomal AmB should be considered.

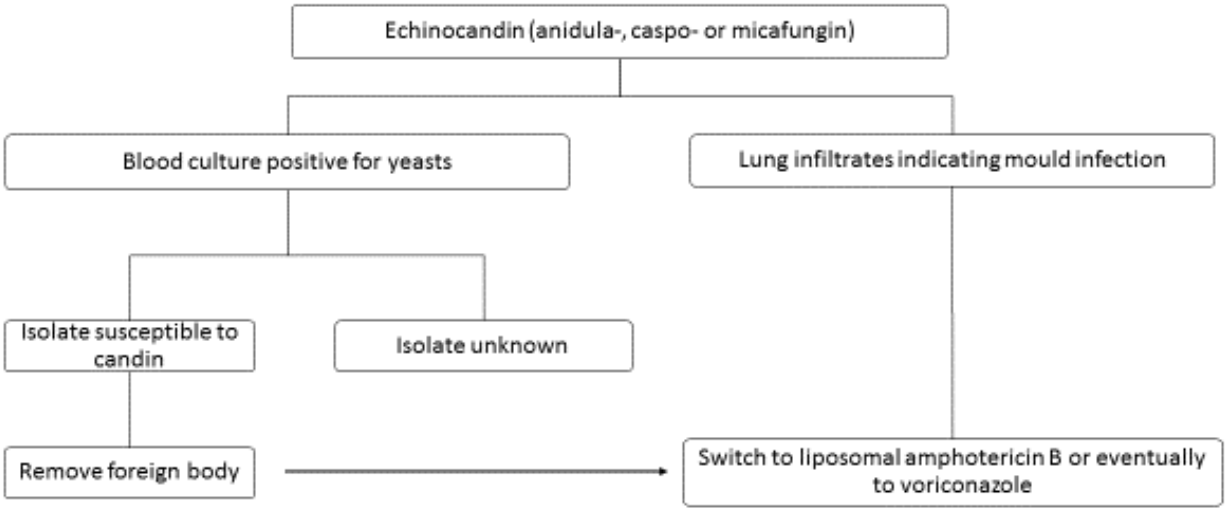


Fig. 1. Clinical approach to breakthrough fungal infections in echinocandin-treated patients. Adopted from Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. Mycoses. 2014 Nov; 57(11):645.

Breakthrough IFI in patients receiving systemic Fluconazole treatment

Breakthrough candidemia in patients receiving full-dose Fluconazole may be assumed due to Candida krusei or Candida glabrata. In such cases it is recommended to switch to Echinocandin or to Amphotericin (38).

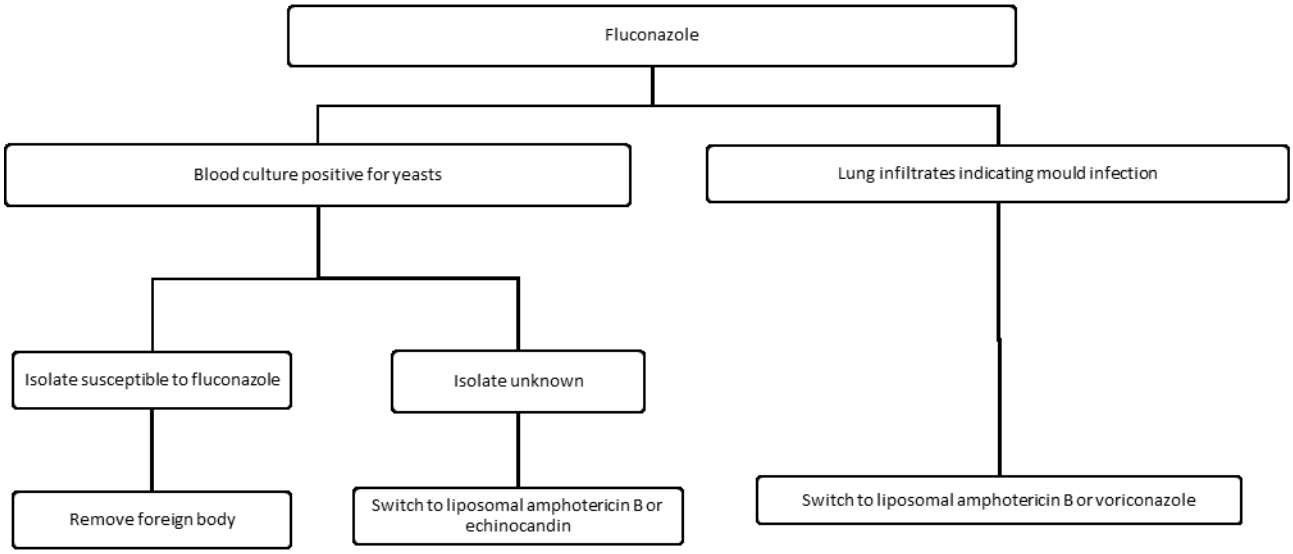


Fig. 2. Clinical approach to breakthrough fungal infections in fluconazole-treated patients. Adopted from Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. Mycoses. 2014 Nov; 57(11):645.

Breakthrough IFI on Voriconazole therap*y*

Candida glabrata may be the cause of breakthrough IFI in patients receiving Voriconazole therapy. In such cases, potentially, a switch to liposomal Amphotericin B or to an Echinocandin is recommended. In some patients with invasive aspergillosis low blood Voriconazole levels could be the reason of insufficient response (39). Measurement of Voriconazole plasma concentrations can be helpful in such cases.

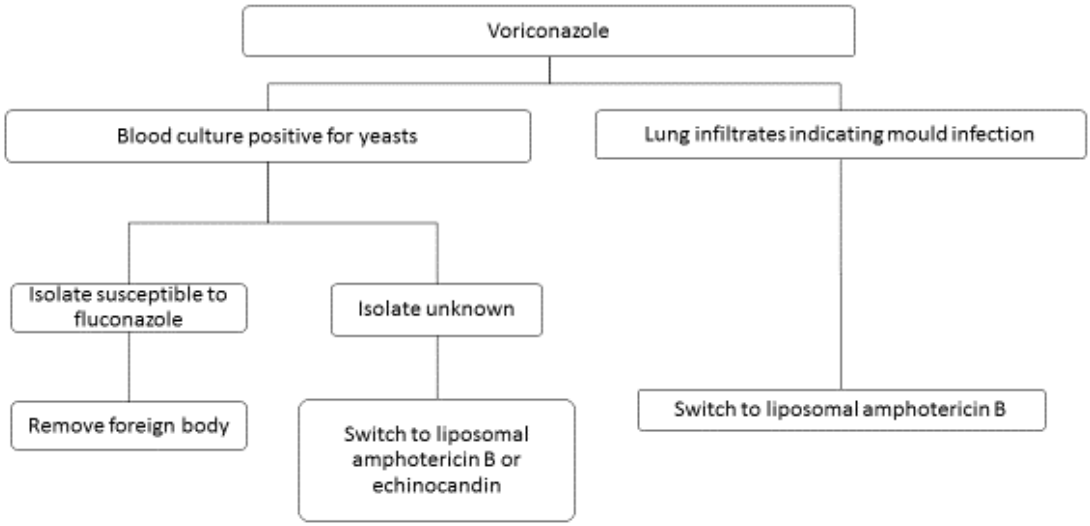


Fig. 3. Clinical approach to breakthrough fungal infections in Voriconazole-treated patients. Adopted from Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. Mycoses. 2014 Nov; 57(11):645.

Aim of study

There is no unambiguous recommendation for the selection of the first line anti-fungal agent in the hematologic patients undergoing HSCT. Moreover, since the publication of ECIL 3 recommendations, several studies were published demonstrating at least non-inferiority of Micafungin vs. azoles. In the currently available ECIL 5 guidelines (officially not published yet, available at http://www.kobe.fr/ecil/program2013.htm), both Voriconazole and Micafungin are granted recommendation level B-I for antifungal prophylaxis in patients with low risk for molds and B-I and C-I, respectively, in patients with high risk for molds. These changes reflect current progress in the efficiency of these medicines in prophylaxis of IFI. Several studies compared efficiency of Micafungin vs different azoles. However, there are no published studies comparing directly Micafungin vs Voriconazole.

The aim of the study was to compare efficiency of Micafungin and Voriconazole for

the prophylaxis of fungal infections in allo-HSCT patients. The design of the study is a retrospective analysis of data of patient treated with either Micafungin or Voriconazole.

Table 5. Recommendations for antifungal prophylaxis in patients undergoing allogeneic HSCT (Update: ECIL 5, 2013)

Antifungal prophylaxis	**Pre-engraftment Low risk for moulds**	**Pre-engraftment High risk for moulds**	**GvHD**
Fluconazole	AI	AIII-against	AIII-against
Itraconazole	BI	BI	BI
Voriconazole	BI	BI	BI
Posaconazole OS/Tablet	BII	BII	AI
Micafingin	BI	CI	CII
Caspofungin/anidulafungin	No data	No data	No data
Liposomal Amphotericin B	CII	CII	CII
Aerosolized amphotericin B plus fluconazole	CIII	BII	No data

Adopted from http://www.kobe.fr/ecil/program2013.htm

Material and Methods

Protocol of the study

We conducted a retrospective comparative analysis in patients in our department who received Micafungin vs Voriconazole for the prophylaxis of IFI.

The patients were adult patients undergoing allogenic-HSCT for the treatment of various high-risk hematological malignancies from January 2011 to December 2014, a total of 60 Patients were identified. The most representative diagnoses are: Acute myeloid leukemia**,** secondary AML, Acute lymphocytic leukemia (ALL), Myelodysplastic syndrome (MDS), Chronic Myelogenous Leukemia (CML), Multiple Myeloma (MM).

The day of the HSCT procedure was considered to be day 0 and the days after and before that, were named to be + and – respectively. The time between HSCT and hospital discharge was referred to as the recovery period.

Antifungal treatment was initiated at the day of HCST (day 0). Patients were consecutively treated with Voriconazole or by Micafungin prophylaxis during the neutropenic phase of allo-HSCT.

The study drug, Micafungin was administrated i.v. at a dose of 50mg/day. Voriconazole was administrated as a tablet taken orally at a dose of 6mg/kg body weight (BW) twice daily followed by 4mg/kg BW twice daily and then adapted according to actual plasma levels. The measured mean plasma concentration of Voriconazole was 2.3 ± 1.7 mg/L**,** which is within the range of its therapeutic concentrations (normal values 1-5.5 mg/l) (40).

Patients were evaluated at baseline, during prophylactic treatment, at the end of treatment at 4 weeks.

Screening for fungal infection

In all patients β-D-glucan in serum was measured from the beginning of study. If fever (≥ 38°C) developed during HSCT, blood culture was performed repeatedly, and if fever persisted beyond 72 h, X-ray followed by chest CT scan was performed.

Efficacy and Safety assessments.

The incidence of proven, probable or possible IFI in the two groups was calculated. Diagnoses were based on the criteria of IFI developed by the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer, National Institute of Allergy and Infectious Diseases Mycoses Study Group (41). The committee chose the terms "proven", "probable", and "possible" to classification criteria of IFI (Table 6).

Three elements form the basis of the definitions: host factors, clinical manifestations and mycological results (Table 6).

Host factors	Clinical factors	mycology
Neutropenia (<500neutrophils/mm³for>10days)	Any of the following new infiltrates on CT imaging: halo sign, air-crescent sign	Culture of mould from
Persistent fever for>96h	Sinunasal infection: suggestive radiological evidence of IFI in sinuses	tissue, aspirate, BAL
Signs and symptoms indicating GvHD	CNS infection: radiological evidence suggesting CNS	or sputum
Prolonged>3 w use of corticosteroids	Disseminated fungal infection: unexplained papular or nodular skin lesions	Fungus seen in tissue or
prior mycosis	Chronic disseminated candidiasis: small, peripheral, targetlike abscesses in liver and/or spleen demonstrated by CT,MRI	sterile body fluid
AIDS		Aspergillus antigen in BAL,
Immunosuppressives		CSF or >2 blood

EORTC-MSG criteria

The EORTC-MSG criteria for the diagnosis of proven, probable or possible IFI are summarized in the Figure 4. The diagnosis if IFI is categorized based on combination of the factors and test results available for the patients.

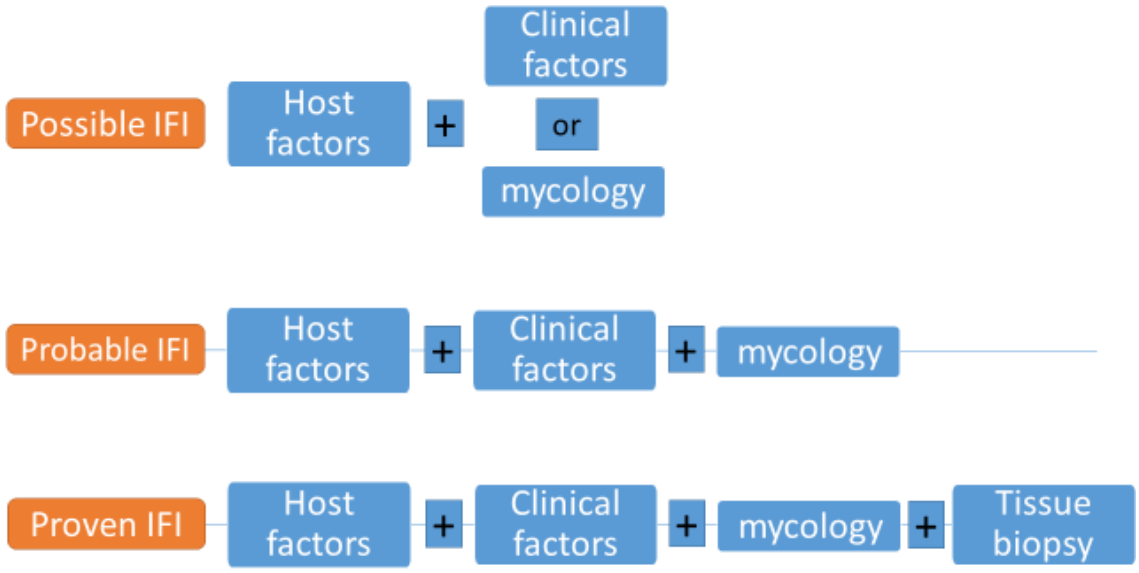


Fig. 4. Summarized representation of the EORTC-MSG criteria for the diagnosis of invasive fungal infections in HSCT patients. Host factors permissive for fungal infections, clinical signs and mycological test results necessary for diagnosis are presented on the table 6.

Patients are categorized 'possible' IFI, if they have at least 1 criterion from the host factors category but do not exhibit clinical signs or mycological evidences of fungal infection. In the EORTC-MSG consensus paper, it is not recommended to use this category in the clinical trials as the least specific. Due to the design of our study, retrospective analysis study, we decided to use this category. It reflects clinical practice, where the decision to start empirical therapy is based on limited information.

Patients in the category 'probable' IFI have at least one host factor, clinical sign and a positive mycological test. No tissue based evidences are available for these patients.

The 'proven' category belongs to the highest level of certainty in diagnosing of IFI. The patients in this category have at least one host factor permissive for IFI, clinical signs and a positive mycological test. In addition, the presence of fungi in tissue by biopsy or a needle aspirate is established. The demonstration of the infection in the material by cell culture or histology is sufficient.

Results

Patient group and transplant characteristics

From the patients treated in the department from 2011 to 2014, sixty patients were randomly selected (Micafungin, n=30; Voriconazole, n=30) for analysis. Baseline demographic data, disease and transplant characteristics are listed in Table 7. The median duration of therapy was 30 days for patients and was similarly balanced in both study arms for patients subgroups.

Table 7. Main patient group and transplant characteristics.

Patients and transplant Characteristics Total=60	**Micafungin n=30**	**Voriconazole n=30**	**Statistics**	**value**
Patient Age (median) [range]	56 [21-69]	56 [26-79]	t-test	0.920
Patient sex				
Male/female	16/14	22/8	Chi-square	0.188
Disease type			Chi-square	0.507
AML	13	17		
ALL	4	4		
MDS	3	3		
CML	3	0		
NHL	1	1		
Sec. AML	1	0		
MM	0	2		
ETH	1	0		
Myelofibrosis	2	0		

Sezary Syndrom	0	1		
Donor type				
MAC HSCT Allogeneic sibling MAC	8	8	Chi-square	1.000
Allogoeneic MUD MAC	22	10	Chi-square	0.002
Allo UCB SCT	0	1	Chi-square	0.313
Allo MUD RIC	0	7	Chi-square	0.005
Allo sibl RIC	0	3	Chi-square	0,076

MAC: myeloablative conditioning

MUD: matched unrelated donor

UCB: umbilical cord blood

HSCT: hematopoietic stem cell transplantation

RIC: reduced intensity conditioning

Mean age of patients in Micafungin group was 56 years [range 21-69] which was not significantly different from the age of patients in the Voriconazole group 56 [26-79], p=0.920. In the Micafungin group, 16 patients (53.3%) were male, whereas 22 patients (73.3%) were male in the Voriconazole group. However, this difference in male/female patient distribution was not statistically significant (p=0.188). The major indications for HSCT in our cohort were AML and ALL accounting for 17 cases (56.7%) in the Micafungin group and 21 cases (70%) in the Voriconazole group. There were 3 patients per group with MDS.

Sibling donor HSCT was performed in 8 cases in each group. Allogeneic MAC MUD transplant was applied in 22 cases in Micafungin group vs. 10 cases in Voriconazole group (p=0.002). Allogeneic MUD RIC procedure was applied in 7 cases in Voriconazole group and none cases in Micafungin group (p=0.005). RIC procedure

with allogeneic sibling transplant was used in 3 cases in the Voriconazole group and in none cases in the Micafungin group.

Occurrence of bacterial infections

Table 8. Frequency of bacterial colonizations in the patient groups after HSCT.

Bacteria	**Micafungin**	**Voriconazole**	**Chi-square p-value**	**Fisher' test p-value**
Enterococcus	12	16	0,301	0,438
coag. neg. Staphylococci	14	16	0,438	0,606
Escherichia coli	2	4	1	1
Pseudomonas	1	5	0,085	0,195
Stenotrophomonas maltophilia	1	1	1	1
Staphyl.epiderm	0	1	0,076	0,237
ESBL	0	1	0,313	1
Bacillus sp	0	1	0,313	1
Serratia sp	0	1	0,313	1
Klebs.pneum	0	1	0,313	1
Micrococcus sp	0	1	0,313	1

In our study *Enterococcus* and *coag.neg.Staphylococci* were the most frequently detected pathogens in both groups. Enterococcus colonization was observed in 12 (40%) and 16 (53.3%) patients in Micafungin and Voriconazole groups, respectively. Coag. neg. Staphylococci were observed in 14 (46.7%) and 16 (56.3%) patients in Micafungin and Voriconazole groups, respectively.

Cases of *Escherichia coli, Pseudomonas, Stenotrophomonas maltophilia* colonization were observed in both groups.

Staphyl. epiderm, ESBL, Bacillus spp, Serratia spp, Klebs.pneum., Micrococcus spp were only detected in the Voriconazole group.

All patients were tested for bacterial colonization in the pharynx, nose, umbilicus, rectal swab cultures, excrements, urine, vagina or penis. Rectum was the place of the most frequent bacterial colonizations both in the Voriconazole and in the Micafungin group, 40 and 28 cases respectively. In the Voriconazole group, there were more cases than in Micafungin group. Moreover, several different pathogens were detected in the rectal samples. In the umbilicus samples, bacterial colonization was detected in 25 cases in Voriconazole group and 15 cases in Micafungin group. In other localizations, no difference in frequency of bacterial colonizations were detected.

Table 9. Localizations of bacterial colonization in the treatment groups:

Localization	Micafungin	Voriconazole	Chi-square p-value	Fisher' test p-value
Oropharynx	16	21	0.184	0.288
Nose	2	4	0.389	0.671
Umbilicus	15	25	0.006	0.013
Rectum	28	40	0.0002	00002
Penis/Vagina	2/9	11/4	0.598	0.793

Fungal colonizations

Fever is typically the earliest sign of infection during neutropenic episodes in haematologic patients. Because infection can progress rapidly in neutropenic patients, special events are warranted to ensure that infections are detected and treated as soon as possible.

All patients were tested for fungal colonization in the pharynx, nose, umbilicus, rectal swab cultures, excrements, urine, vagina or penis. In the Micafungin group, there were 3 patients with Candida albicans colonization and one patient in the Voriconazole group. No patients in the Micafungin group had other fungi, while in Voriconazole group *Geotrichum capipatum, Candida krusei, Candida spp.* were detected. In one patient in the Voriconazole group, both Geotrichum capitatum and Candida spp. were detected. All cases of fungal colonization were detected in pharynx.

No cases of *Aspergillus* colonization was detected in both groups (Table 10).

Table 10. Frequency of fungal colonizations in the prophylactically treated patient groups after HSCT.

Fungi	**Micafungin**	**Voriconazole**	**Chi-square p-value**	**Fisher' test p-value**
Candida albicans	3	1	0,301	0,612
Candida spp.	0	1	0,313	1,000
Geotrichum capitatum	0	1	0,313	1,000
Candida krusei	0	1	0,313	1,000
Aspergillus	0	0		

Patients with prolonged neutropenia are at high risk for infections complications. The physical examinations should encompasses a careful search for potentially infected sites, including the gastrointestinal tract (oropharynx, esophagus and perineum), skin, lungs, vascular access sites and other biopsy sites.

All patients must be tested for bacterial and fungal colonization in blood culture, in pharynx, in nose, in umbilicus, in rectal swab cultures, in excrements, in urine, in vagina or penis.

Laboratory evaluation is recommended for all febrile neutropenic patients including 2 sets of blood samples for culture for bacteria and fungi (1 from peripheral blood and 1 from each catheter, if present).

Most studies recommend the chest radiography for all febrile neutropenic patients only when respiratory signs or symptoms are present (42).

Characteristics of the patients with detected fungi

In the Micafungin treatment group, colonization of pharynx with Candida albicans was detected in three patients. In all of them, the blood culture tests were negative. Patient 1 had bacterial pneumonia, resulting in fever and pneumonia related changes in the lung CT scan. In the pharynx samples of the patient 3 Candida was found on the day 1 after treatment initiation suggesting that the colonization was existing before start of treatment. Patient 3 developed fever, in his blood culture samples gram positive bacteria were detected.

In the Voriconazole treatment group, three cases of pharynx colonization were detected. In one case colonization was by Candida albicans, in one case – Candida krusei, and in one case Candida spp. and Geotrichum cap. In patient 4, pharynx colonization with Candida albicans was detected on the day 4 after Voriconazole initiation. On the day 20, positive blood culture for Aspergillus and gram-negative

bacteria was found. However, no changes were observed on chest X-ray. Patient 5 had Candida krusei colonization of the pharynx. Colonization with different bacteria was found in his pharynx, anus and penis. He had developed fever, but his X-ray and blood culture tests were negative. Patient 6 had pharynx colonization with two species of fungi: Candida spp. and Geotrichum cap. His blood tests and CT scans were negative. Patient 7 showed weakly positive test for galactomannan. However, CT scan was negative and investigations for fungal colonization delivered negative results. This patient had colonization in multiple organs with several bacteria including Enterococcus, Klebs. pneum., Staphyl. coag.

Patient	Treatment	Days on treatment	Diagnosis	Colonization	Localization	Blood culture	Fever	Marker (β-DG, GM levels)	CT
1	Mica	4	CML	Candida albicans	Pharynx	neg	37.5	neg	No specific
2	Mica	4	DG-B-NHL	Candida albicans	Pharynx	neg	afebr	neg	neg
3	Mica	1	ALL	Candida albicans	Pharynx	neg	38.5	No test	neg
4	Vori	4 20	AML	Candida albicans	Pharynx	Aspergillus	38.5	No test	X-ray: neg
5	Vori	27	MDS	Candida krusei	Pharynx	neg	39	No test	X-ray: neg
6	Vori	11	ALL	Geotric. Capitatum and Candida spp.	Pharynx	neg	37.5	neg	neg
7	Vori	28	Sezary-syndrome	neg			38	GM pos	neg

Table 11. Summary of the findings on the patients with any changes in tests.

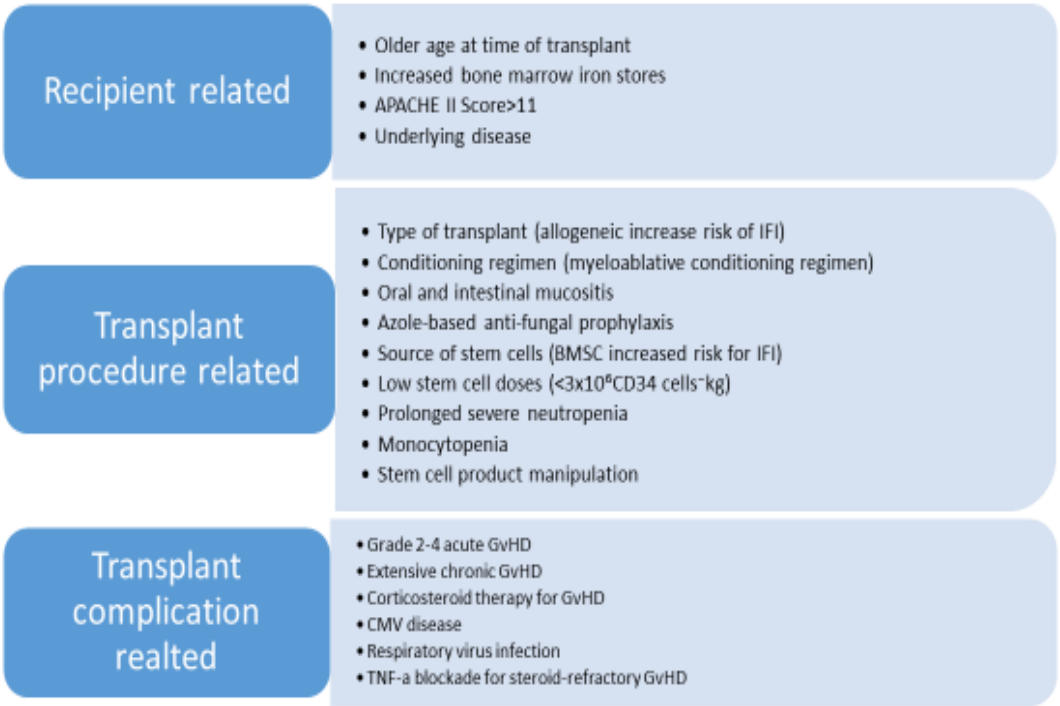
MICA denotes: Micafungin, VORI denotes Voriconazole, GM: Galactomannan test, **β-DG** – β-D-glucan

Discussion

Relevance of the findings

Allogeneic HSCT is an effective and sometimes the only curative therapy for patients with high-risk hematologic diseases. The incidence of IFI among patients undergoing HSCT varies between 3% and 14 % according to different authors (43,44). Invasive fungal infection (IFI) have been associated with high mortality rate in these patients (1,45). Early diagnosis and initiation of antifungal therapy has been shown to improve IFI survival to >80% (46).

Risk factors associated with invasive fungal infection in hematopoietic stem cell transplant recipients



HSCT is a very complex and invasive procedure. Many factors directly related to the procedure such as neutropenia duration, receiving glucocorticoids, donor type, age, underlying disease, graft-versus-host disease (GVHD) could contribute to IFI development (47).

The post-transplant complications are the major risk factors for IFI. They may be categorized according to recipient related, transplant procedure related or transplant complication related.

Concomitant factors; bacterial infections

Historically, gram-negative bacilli from the alimentary tract have been the prominent pathogens in neutropenic patients. Between the 1960s to the mid- 1970s, Escherichia coli, Klebsiella species, and Pseudomonas aeruginosa were the majority of microbiologically documented infections at most cancer centers (48). Since the introduction of extended-spectrum β-lactams, several institutions have experienced a decrease in gram-negative rod bacteremia and an increase in infections due to gram-positive cocci (49).

In our study, bacterial infections were very frequent concomitant factor requiring complex approach in interpretation of the data. Enterococcus and coag. neg. Staphylococci were the most frequently detected pathogens in both groups. Very frequently colonization with several different bacteria was detected. All the investigated organs were colonized with bacteria with most heavily populated gastro-intestinal tract. Fever caused by bacterial infection was a factor interfering with fungal infection diagnosis.

Diagnosis of IFI

Since their introduction as antigen-based or/and molecular assays, the novel more sensitive diagnostic tests became an important part of diagnostic workout of IFI (50). Galactomannan is a component of the cell wall of *Aspergillus spp* that is released into the bloodstream. The diagnostic performance of galactomannan testing with BAL

fluid and/or serum specimens is a promising approach for early and specific diagnosis. But in some case false-positive results may occur, such as co-medication, underlying diseases, host factors (15).

The other diagnostic criteria for IFI diagnosis is a β-D-glucan, a component of cell wall of pathogenic fungi, such as *Candida spp* (51,52). Its presence in the bloodstream correlates with IFI (53). The Fungitell assay (Associates of Cape Cod, Inc.) is a test that reliably detects β-D-glucan (54).

Molecular methods based on amplification and detection of fungal DNA (PCR and NASBA) have the great potential in IFI diagnosis. Detection of the fungal DNA should result in high sensitivity and specificity of these methods. However, these methods are not included in EORTC/MSG criteria mainly due to the lack of standardization and absence of a commercial systems (55).

Radiography has limited usefulness for early diagnosis of IFI. The radiographic appearances of IFI are often non-specific. Infiltrates, segmental or subsegmental consolidation, single or multiple nodules, nodular infiltrates and cavitation can be observed. However, all these changes occur late in the stage of disease. Chest computed tomography (CT) can be helpful in early detection of IFI. The detection of the halo sign is a specific sign of Aspergillosis infection. The halo sign and macronodules are an early indicator of IFI, later progressing to consolidation, infarcts and cavitary disease with time (46,56).

The epidemiologic characteristics of IFI in hematologic patients.

Since introduction of HSCT into clinical praxis, IFI has emerged as a serious complication of this life-saving treatment. Wide use of the anti-fungal treatment and progress in the supportive care of the patients caused a change in the fungal infections causing a disease in patients (Fig. 5).

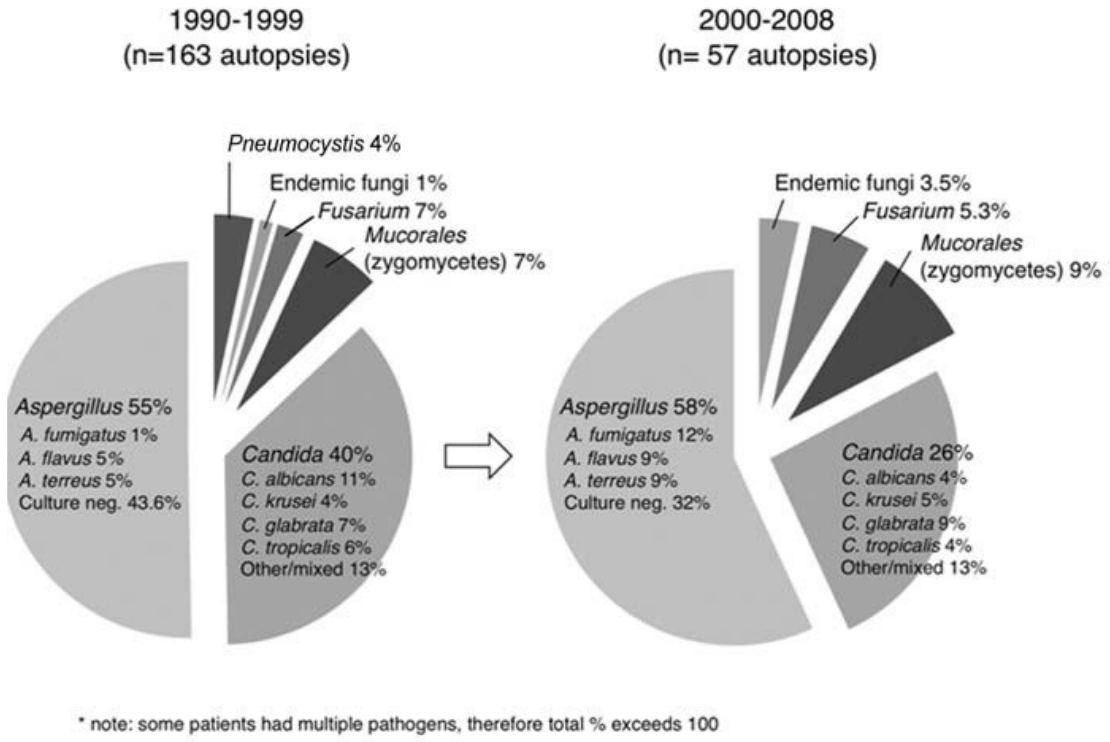


Fig. 5. Change in the frequency and distribution of the IFI causing fungi in HSCT treated patients. Adapted from Leventakos K et al., Fungal infections in leukemia patients: how do we prevent and treat them? Clin Infect Dis. 2010 Feb 1; 50 (3):405

Endogenous microflora of the host is a source of most invasive Candida infections as evidenced by experimental, clinical and molecular-relatedness studies (57-59).

Etiology of fungal infections

In the 1980s candidiasis was the most common cause of IFI (45). The use of the azoles for prophylaxis since 1990s caused a decrease of candida infections (60). Nevertheless, the fluconazole-resistant (*Candida krusei*) and susceptible-dose-dependent (*Candida glabrata*) species of Candida accounted for the majority (>80%)

of candidiasis infections in hematological patients (61,62). Treatment of candidemia with echinocandins did not reduce the overall and Candida-attributable mortality rates. Sipsas NV et al. using echinocandins reduced incidence of C. glabrata and C. krusei, with a parallel increase in the incidence of C. parapsilosis and C. tropicalis (63). These results demonstrate the challenges of finding the optimal approach for management of fungal infection in high-risk patient population. According to the results of the retrospective data analysis, the prompt administration of empirical or preemptive therapy improves outcome of IFI (64). Therefore, since there is no single drug of choice in IFI, the drugs with the broad spectrum anti-mold activity, such as Caspofungin, Voriconazole and lipid formulations of AMB (amphotericin B) are the most attractive choices (65,66). The combination of echinocandins and newer azoles may play an important role in the control of candidiasis epidemiology.

Localizations

Several studies have demonstrated gastrointestinal tract involvement in disseminated candidiasis (67) and identified of gastrointestinal colonization as a risk factor of candidemia (68). The factors facilitating fungal entrance through gastrointestinal tract could be the disruption of the protective mucocutaneous membrane barrier due to chemotherapy and/or radiotherapy, decreased activity of phagocytic cells in the blood and tissues and lack of an antifungal therapy (69). In the study of Schwetz et al. the high prevalence of orointestinal Candida colonization in patients undergoing HSCT has been shown (70).

Huang et al. in their study comparing the efficacy of micafungin and itraconazole have reported oropharynx as the most frequently colonized by fungi organ (25).

In our study 6 cases of fungal colonization were detected. In all cases oropharynx was the place of colonization, which is in line with the existing literature.

Antifungal compounds

Micafungin was active against both *Candida spp* and *Aspergillus spp.* Kuse et al. showed that Micafungin is non-inferior to liposomal amphotericin B as the first-line treatment of candidemia and invasive candidiasis (71). Historically liposomal amphotericin B has demonstrated efficacy against both Candida and Aspergillus and its administration was the standard of care for febrile neutropenic patients unresponsive to broad-spectrum antibacterial therapy. However, its therapeutic efficacy could be associated by renal toxicity (72). Micafungin showed a better safety profile than liposomal amphotericin B (71). Moreover, Micafungin usage was associated with a low incidence of hepatic and infusion-related events.

Voriconazole showed non-inferiority in comparison to amphotericin B in treatment of candidaemia (73). Additionally, Voriconazole due to its activity against a wide variety of yeasts and molds and good bioavailability could be a first choice therapy for invasive aspergillosis and is a new treatment option in candidiasis and other emerging IFI, such as fusariosis, and refractory IFI (74,75).

Studies comparing echinocandines vs azoles

Micafungin vs Itraconazole

Huang et al. compared the efficacy and safety of Micafungin and Itraconazole in prophylaxis of IFI in patients undergoing HSCT (25). Total 287 adult patients in the age of 18 to 70 years old were enrolled. The 136 patients received Micafungin i.v. at a dose of 50 mg/day. The control drug, Itraconazole was administered to 147 patients, as a solution taken orally at a dose of 5 mg/kg/day. Patients received the therapy during the neutropenic phase of HCST for ≤ 42 days. There were no statistically significant or clinically relevant differences between Micafungin and Itraconazole in the incidence of proven, probable, or suspected IFI occurring during prophylactic

therapy, after therapy, or at any time during the study. A suspected fungal infection was reported in 3.2% of patients in the Micafungin group and in 3.6% patients in Itraconazole group after completion of prophylactic treatment. The colonization with Candida spp was 3-fold higher in the Micafungin than in the Itraconazole group. Most frequently colonization with Candida spp occurred in oropharynx. There were less treatment related adverse events in Micafungin group. The results of this study show evidence for comparable efficacy of Micafungin and Itraconazole in IF prophylaxis and better tolerability of Micafungin over Itraconazole.

Micafungin versus Liposomal amphotericin B

Efficacy of Micafungin in treatment of invasive candidosis was tested in the randomized double-blinded multi-center study (71). 537 patients were enrolled into study and randomized to receive either Micafungin or liposomal Amphotericin B. Patients enrolled into study were those, who has clinical signs of systemic Candida infection and one or more blood culture tests positive for candida. Treatment success was observed in 89.6% patients treated with Micafungin and 89.5% patients treated with Amphotericin B. Less treatment related adverse event were reported for Micafungin. Thus, Micafungin is as efficient as liposomal amphotericin B in treatment of invasive candida infection.

Micafungin versus Fluconazole

In the randomized, double-blind, multicenter clinical trial, van Burik et al. tested safety and efficacy of Micafungin vs. Fluconazole in antifungal prophylaxis in neutropenic patients undergoing HSCT (20). 426 patients were allocated to Micafungin group and 463 patients to the Fluconazole group. The rate of treatment success was higher in patients treated with Micafungin (80%) than in patients treated

with Fluconazole (73.5%). Moreover, Micafungin had better safety profile since less adverse events were detected in this group.

Our observations

We observed 3 cases of orotracheal colonization with *Candida albicans* in the Micafungin group. However, results of blood culture, fungal markers and chest CT were negative suggesting that these patients did not have the invasive fungal infection.

In Voriconazole group, one patient had Candida albicans, one patient had Candida krusei and one patient had multicolonization by Geotric. capitatum and Candida spp. colonization. In two cases, blood tests and CT were negative. In another case, Candida colonization was detected at day 4 after start of treatment and at day 20 a positive blood culture for Aspergillus was found. Subsequently, chest X-ray and investigations for colonization were negative. Thus, findings on this patient did not fulfill 2008 EORTC-MSG criteria for possible or probable IFI. Rather, this was the case for diagnostic- driven pre-emptive therapy.

In Voriconazole group, galactomannan test was positive in one patient. Screening for colonization, blood culture test and CT were negative suggesting very low possibility of the invasive fungal infection. One possible interpretation of the GM test could be as false positive test. The patient did receive a broad spectrum antibiotics against concomitant bacterial infection, which could potentially cross-react with the test (15). On the other hand, in the presence of anti-fungal treatment in case of IFI, the tests would deliver results below detection level enabling only the most sensitive one to detect fungi. Thus, this could be the case for diagnostic- driven pre-emptive therapy.

Limitations of the study

Our study is a retrospective analysis study, and therefore, it has all the limitations of retrospective studies such as absence of randomization, selection bias and misclassification (76). Nevertheless, our study groups were not different in the major demographic parameters suggesting that, despite the retrospective nature of our study, we collected comparable cohorts. Even if there could be some selection bias, it should be acting on both groups since both groups are intervention groups treated in the same department. Additionally, our study includes 30 patients per group, which could be too low to detect minor differences in efficacy of two efficient drugs. Low occurrence of fungal infections in our patient cohort is in line with observations of others demonstrating high efficiency of both substances to treat fungal infections (71,77) and low incidence of IFI upon prophylactic use of these medicines (25,78).

Summary

Both, Micafungin and Voriconazole have demonstrated high efficiency in prophylaxis of invasive fungal infections. There was no significant difference in anti-fungal prophylaxis efficiency between these two medications.

Literature

1. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002;34:909-17.
2. Martino R, Subira M, Rovira M, Solano C, Vazquez L, Sanz GF et al. Invasive fungal infections after allogeneic peripheral blood stem cell transplantation: incidence and risk factors in 395 patients. Br J Haematol 2002;116:475-82.
3. Sun Y, Meng F, Han M, Zhang X, Yu L, Huang H et al. Epidemiology, management, and outcome of invasive fungal disease in patients undergoing hematopoietic stem cell transplantation in China: a multicenter prospective observational study. Biol Blood Marrow Transplant 2015;21:1117-26.
4. Harrison N, Mitterbauer M, Tobudic S, Kalhs P, Rabitsch W, Greinix H et al. Incidence and characteristics of invasive fungal diseases in allogeneic hematopoietic stem cell transplant recipients: a retrospective cohort study. BMC Infect Dis 2015;15:584.
5. Simms-Waldrip T, Rosen G, Nielsen-Saines K, Ikeda A, Brown B, Moore T. Invasive fungal infections in pediatric hematopoietic stem cell transplant patients. Infect Dis (Lond) 2015;47:218-24.
6. Harrison D, Muskett H, Harvey S, Grieve R, Shahin J, Patel K et al. Development and validation of a risk model for identification of non-neutropenic, critically ill adult patients at high risk of invasive Candida infection: the Fungal Infection Risk Evaluation (FIRE) Study. Health Technol Assess 2013;17:1-156.
7. Oz Y, Aslan M, Aksit F, Metintas S, Gunduz E. The effect of clinical characteristics on the performance of galactomannan and PCR for the diagnosis of invasive aspergillosis in febrile neutropenic patients. Mycoses 2016;59:86-92.
8. Loschi M, Thill C, Gray C, David M, Bagatha MF, Chamseddine A et al. Invasive aspergillosis in neutropenic patients during hospital renovation: effectiveness of

mechanical preventive measures in a prospective cohort of 438 patients. Mycopathologia 2015;179:337-45.

9. Sun Y, Huang H, Chen J, Li J, Ma J, Li J et al. Invasive fungal infection in patients receiving chemotherapy for hematological malignancy: a multicenter, prospective, observational study in China. Tumour Biol 2015;36:757-67.

10. Pilaniya V, Gera K, Gothi R, Shah A. Acute invasive pulmonary aspergillosis, shortly after occupational exposure to polluted muddy water, in a previously healthy subject. J Bras Pneumol 2015;41:473-7.

11. Sainz J, Hassan L, Perez E, Romero A, Moratalla A, Lopez-Fernandez E et al. Interleukin-10 promoter polymorphism as risk factor to develop invasive pulmonary aspergillosis. Immunol Lett 2007;109:76-82.

12. Bochud PY, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med 2008;359:1766-77.

13. Maertens J, Verhaegen J, Demuynck H, Brock P, Verhoef G, Vandenberghe P et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive Aspergillosis. J Clin Microbiol 1999;37:3223-8.

14. Yoshida M, Obayashi T, Iwama A, Ito M, Tsunoda S, Suzuki T et al. Detection of plasma (1 --> 3)-beta-D-glucan in patients with Fusarium, Trichosporon, Saccharomyces and Acremonium fungaemias. J Med Vet Mycol 1997;35:371-4.

15. Miceli MH, Maertens J. Role of Non-Culture-Based Tests, with an Emphasis on Galactomannan Testing for the Diagnosis of Invasive Aspergillosis. Semin Respir Crit Care Med 2015;36:650-61.

16. Einsele H, Hebart H, Roller G, Loffler J, Rothenhofer I, Muller CA et al. Detection and identification of fungal pathogens in blood by using molecular probes. J Clin Microbiol 1997;35:1353-60.

17. Hohenadel IA, Kiworr M, Genitsariotis R, Zeidler D, Lorenz J. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broad spectrum antibiotic and antifungal regimen. Thorax 2001;56:115-20.

18. Petraitis V, Petraitiene R, Groll AH, Roussillon K, Hemmings M, Lyman CA et al. Comparative antifungal activities and plasma pharmacokinetics of micafungin (FK463) against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. Antimicrob Agents Chemother 2002;46:1857-69.

19. Denning DW. Echinocandin antifungal drugs. Lancet 2003;362:1142-51.

20. van Burik JA, Ratanatharathorn V, Stepan DE, Miller CB, Lipton JH, Vesole DH 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.

21. Morgand M, Rammaert B, Poiree S, Bougnoux ME, Tran H, Kania R et al. Chronic Invasive Aspergillus Sinusitis and Otitis with Meningeal Extension Successfully Treated with Voriconazole. Antimicrob Agents Chemother 2015;59:7857-61.

22. Maertens J, Marchetti O, Herbrecht R, Cornely OA, Fluckiger U, Frere P 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.

23. Cordonnier C, Rovira M, Maertens J, Olavarria E, Faucher C, Bilger K 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.

24. El-Cheikh J, Crocchiolo R, Vai A, Furst S, Bramanti S, Sarina B et al. Comparison of Three Distinct Prophylactic Agents Against Invasive Fungal Infections in Patients Undergoing Haplo-identical Hematopoietic Stem Cell Transplantation and Post-transplant Cyclophosphamide. Mediterr J Hematol Infect Dis 2015;7:e2015048.

25. Huang X, Chen H, Han M, Zou P, Wu D, Lai Y et al. Multicenter, randomized, open-label study comparing the efficacy and safety of micafungin versus itraconazole for

prophylaxis of invasive fungal infections in patients undergoing hematopoietic stem cell transplant. Biol Blood Marrow Transplant 2012;18:1509-16.

26. Gea-Banacloche J. Evidence-based approach to treatment of febrile neutropenia in hematologic malignancies. Hematology Am Soc Hematol Educ Program 2013;2013:414-22.

27. Shalhoub S, Wang L, Ching A, Husain S, Rotstein C. Micafungin compared with caspofungin for the treatment of febrile episodes in neutropenic patients with hematological malignancies: A retrospective study. Can J Infect Dis Med Microbiol 2014;25:299-304.

28. Tamura K, Urabe A, Yoshida M, Kanamaru A, Kodera Y, Okamoto S et al. Efficacy and safety of micafungin, an echinocandin antifungal agent, on invasive fungal infections in patients with hematological disorders. Leuk Lymphoma 2009;50:92-100.

29. Candoni A, Mestroni R, Damiani D, Tiribelli M, Michelutti A, Silvestri F et al. Caspofungin as first line therapy of pulmonary invasive fungal infections in 32 immunocompromised patients with hematologic malignancies. Eur J Haematol 2005;75:227-33.

30. Jarque I, Tormo M, Bello JL, Rovira M, Batlle M, Julia A et al. Caspofungin for the treatment of invasive fungal disease in hematological patients (ProCAS Study). Med Mycol 2013;51:150-4.

31. Betts RF, Nucci M, Talwar D, Gareca M, Queiroz-Telles F, Bedimo RJ et al. A Multicenter, double-blind trial of a high-dose caspofungin treatment regimen versus a standard caspofungin treatment regimen for adult patients with invasive candidiasis. Clin Infect Dis 2009;48:1676-84.

32. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2008;46:327-60.

33. Mann PA, McNicholas PM, Chau AS, Patel R, Mendrick C, Ullmann AJ et al. Impact of antifungal prophylaxis on colonization and azole susceptibility of Candida species. Antimicrob Agents Chemother 2009;53:5026-34.

34. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. J Clin Microbiol 2010;48:2373-80.

35. Arendrup MC. Update on antifungal resistance in Aspergillus and Candida. Clin Microbiol Infect 2014;20 Suppl 6:42-8.

36. Hamprecht A, Buchheidt D, Vehreschild JJ, Cornely OA, Spiess B, Plum G et al. Azole-resistant invasive aspergillosis in a patient with acute myeloid leukaemia in Germany. Euro Surveill 2012;17:20262.

37. Lewis JS, 2nd, Wiederhold NP, Wickes BL, Patterson TF, Jorgensen JH. Rapid emergence of echinocandin resistance in Candida glabrata resulting in clinical and microbiologic failure. Antimicrob Agents Chemother 2013;57:4559-61.

38. Maschmeyer G, Patterson TF. Our 2014 approach to breakthrough invasive fungal infections. Mycoses 2014;57:645-51.

39. 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.

40. Lewis RE. What is the "therapeutic range" for voriconazole? Clin Infect Dis 2008;46:212-4.

41. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002;34:7-14.

42. Roberts SD, Wells GM, Gandhi NM, York NR, Maron G, Razzouk B et al. Diagnostic value of routine chest radiography in febrile, neutropenic children for early detection of pneumonia and mould infections. Support Care Cancer 2012;20:2589-94.

43. Neofytos D, Treadway S, Ostrander D, Alonso CD, Dierberg KL, Nussenblatt V et al. Epidemiology, outcomes, and mortality predictors of invasive mold infections among transplant recipients: a 10-year, single-center experience. Transpl Infect Dis 2013;15:233-42.

44. Corzo-Leon DE, Satlin MJ, Soave R, Shore TB, Schuetz AN, Jacobs SE et al. Epidemiology and outcomes of invasive fungal infections in allogeneic haematopoietic stem cell transplant recipients in the era of antifungal prophylaxis: a single-centre study with focus on emerging pathogens. Mycoses 2015;58:325-36.

45. Garcia-Vidal C, Upton A, Kirby KA, Marr KA. 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.

46. 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.

47. Camps IR. Risk factors for invasive fungal infections in haematopoietic stem cell transplantation. Int J Antimicrob Agents 2008;32 Suppl 2:S119-23.

48. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 1966;64:328-40.

49. Nesher L, Rolston KV. The current spectrum of infection in cancer patients with chemotherapy related neutropenia. Infection 2014;42:5-13.

50. Bretagne S. Advances and prospects for molecular diagnostics of fungal infections. Curr Infect Dis Rep 2010;12:430-6.

51. De Vlieger G, Lagrou K, Maertens J, Verbeken E, Meersseman W, Van Wijngaerden E. Beta-D-glucan detection as a diagnostic test for invasive aspergillosis in immunocompromised critically ill patients with symptoms of respiratory infection: an autopsy-based study. J Clin Microbiol 2011;49:3783-7.

52. Heyland D, Jiang X, Day AG, Laverdiere M. Serum beta-d-glucan of critically ill patients with suspected ventilator-associated pneumonia: preliminary observations. J Crit Care 2011;26:536.e1-9.

53. Obayashi T, Yoshida M, Mori T, Goto H, Yasuoka A, Iwasaki H et al. Plasma (1-->3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet 1995;345:17-20.

54. Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL. Evaluation of a (1->3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J Clin Microbiol 2005;43:5957-62.

55. Donnelly JP. Polymerase chain reaction for diagnosing invasive aspergillosis: getting closer but still a ways to go. Clin Infect Dis 2006;42:487-9.

56. 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.

57. Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? Clin Infect Dis 2001;33:1959-67.

58. Myerowitz RL. Gastrointestinal and disseminated candidiasis. An experimental model in the immunosuppressed rat. Arch Pathol Lab Med 1981;105:138-43.

59. Klempp-Selb B, Rimek D, Kappe R. Karyotyping of Candida albicans and Candida glabrata from patients with Candida sepsis. Mycoses 2000;43:159-63.

60. Marr KA. The changing spectrum of candidemia in oncology patients: therapeutic implications. Curr Opin Infect Dis 2000;13:615-620.

61. Pagano L, Antinori A, Ammassari A, Mele L, Nosari A, Melillo L et al. Retrospective study of candidemia in patients with hematological malignancies. Clinical features, risk factors and outcome of 76 episodes. Eur J Haematol 1999;63:77-85.

62. Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clin Infect Dis 2009;48:1695-703.

63. Sipsas NV, Lewis RE, Tarrand J, Hachem R, Rolston KV, Raad, II et al. 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.

64. Sipsas NV, Bodey GP, Kontoyiannis DP. Perspectives for the management of febrile neutropenic patients with cancer in the 21st century. Cancer 2005;103:1103-13.

65. Walsh TJ, Groll A, Hiemenz J, Fleming R, Roilides E, Anaissie E. Infections due to emerging and uncommon medically important fungal pathogens. Clin Microbiol Infect 2004;10 Suppl 1:48-66.

66. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D et al. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. National Institute of Allergy and Infectious Diseases Mycoses Study Group. N Engl J Med 1999;340:764-71.

67. Walsh TJ, Merz WG. Pathologic features in the human alimentary tract associated with invasiveness of Candida tropicalis. Am J Clin Pathol 1986;85:498-502.

68. Pittet D, Monod M, Suter PM, Frenk E, Auckenthaler R. Candida colonization and subsequent infections in critically ill surgical patients. Ann Surg 1994;220:751-8.

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

70. Zollner-Schwetz I, Auner HW, Paulitsch A, Buzina W, Staber PB, Ofner-Kopeinig P et al. Oral and intestinal Candida colonization in patients undergoing hematopoietic stem-cell transplantation. J Infect Dis 2008;198:150-3.

71. Kuse ER, Chetchotisakd P, da Cunha CA, Ruhnke M, Barrios C, Raghunadharao D et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. Lancet 2007;369:1519-27.

72. 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.

73. Kullberg BJ, Sobel JD, Ruhnke M, Pappas PG, Viscoli C, Rex JH et al. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidaemia in non-neutropenic patients: a randomised non-inferiority trial. Lancet 2005;366:1435-42.

74. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. Clin Infect Dis 2002;34:563-71.

75. Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. Clin Infect Dis 2003;36:630-7.

76. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719-48.

77. Schwartz S, Reisman A, Troke PF. The efficacy of voriconazole in the treatment of 192 fungal central nervous system infections: a retrospective analysis. Infection 2011;39:201-10.

78. Hicheri Y, Cook G, Cordonnier C. Antifungal prophylaxis in haematology patients: the role of voriconazole. Clin Microbiol Infect 2012;18 Suppl 2:1-15.