

Antifungal Prophylaxis In Hematopoietic Stem Cell Transplant Recipients

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Abstract

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Background. According to immunological deficit the period after hematopoietic stem cell transplantation (HSCT) can be divided in three phases: aplastic phase, phase of acute GVHD, and phase of chronic GVHD. Fungal infections are predominant in first, aplastic phase. Deep neutropenia and implantation of central venous catheter are two major risk factors contributing to infection.

Aim. To retrospectively analyze fungal infections, fungal isolates and to compare success of different antifungal strategies during the first 30 days after HSCT.

Material and methods. During a 7 year period (2000-2007), we have performed 128 HSCT in 120 patients with different hematological diseases. Male: 62 Female: 58. Median age: 34 years. Patients were treated in sterile room, conditioned with HEPA filters, and low microbes diet. Antifungal prophylaxis with Fluconazole 200mg, Itraconazole 200mg, or combination Fluconazole200/Itraconazole 200 (in high-risk patients) was administered from day 0 until day +100.

Results. Patients treated with combination of Fluconazole200/Itraconazol200 have had only few oropharyngeal candidiasis, without signs of invasive fungal infection. There is no statistically significant difference between the prophylaxis with Fluconazole and Itraconazole, ($p=0,302$). *Non-Albicans Candida* is predominantly isolated funga (*Non-Albicans Candida* vs. *Candida Albicans*: 54% vs. 46%). There is no isolation of *Aspergillus* during the first phase after HSCT in our group of patients.

Conclusion. The rising incidence of invasive fungal infections and the currently problematic early diagnosis call for an intensive exploration of new drugs and further developments in diagnosis and treatment of invasive fungal infection.

Introduction

Invasive fungal infections are major risk factor for morbidity and mortality in stem cells transplant recipients. Despite considerable progress in the management of infections complications in hematology patients, fungal infections remain an important issue of morbidity and mortality, mainly after allogeneic stem-cell transplantation. The major advances in the management of invasive fungal infections (IFI), have risen from the understanding of the risk factors for the development of IFI, from the development of new

biological markers of IFI, and also from well-designed therapeutic trials. Fungal infection and especially aspergillosis is nowadays the first case of infections deaths after allogeneic SCT. After introduction of prophylaxis against cytomegalovirus infection, invasive fungal infections are again at the top of the reasons of mortality in transplanted patients. Diagnosis of fungal infection in daily practice is very difficult with standard microbiological methods. As a consequence antifungal prophylaxis is the most important and safe way to decrease frequency and severity of invasive

fungal infections. After allogeneic stem cell transplantation following conventional myeloablative conditioning regimens, the sequence of infections can be divided into three periods:

- **Aplastic phase** - (from day 0 – until day +30) - following the conditioning regimen, until neutrophil recovery from the donated marrow. During this phase infectious complications of stem cell transplanted patient are not very different from those encountered in other profoundly neutropenic patients. This is the beginning of the at – risk period for fungal infections, mainly aspergillosis.
- **Acute GVHD phase** - (from day +30 – until day +100) - the second phase corresponds to the period from initial marrow engraftment to at least the third or fourth month, and is characterized by cell-mediated immune deficiency with decreased number and function of specific and non-specific cytotoxic cells. For many years CMV infection which is mainly due to reactivation, was the greatest problem during this phase.
- **Chronic GVHD phase** - (after day +100) – immune reconstitution is mainly influenced by the presence and severity of chronic GVHD. Most patients have immunoglobulin deficiency (particularly of IgG2). Allogeneic HSCT recipients are particularly vulnerable to encapsulated bacteria (*Streptococcus pneumoniae* and *Haemophilus influenzae*).

Fungal infections are characteristic for the early post-transplant period (aplastic period) were deep neutropenia is dominant immune deficit, due to chemo and/or radiotherapy conditioning regimen. There is a close correlation between degree and duration of neutropenia and severity and frequency of infections in these patients.

The main risk factors for invasive fungal infections are:

- use of broad spectrum antibiotics;
- use of corticosteroids;
- total body irradiation;
- graft versus host disease;
- T-depleted grafts;
- previous invasive fungal infections.

Despite geographical and center-to-center variations, *Candida* and *Aspergillus* infections are the leading causes of invasive fungal infections, but also *Cryptococcus*, *Mucor*, *Trichosporon*, *Zygomycetes* and *Fusarium species* are seen. Despite different mechanisms of acquisition, they share many risk factors that

are common to many hematological patients, especially transplanted patients.

In healthy individuals certain *Candida species* belongs to the normal flora of skin and mucosal surfaces. These commensal yeasts can be detected in up to 70% of the healthy population (1). As commensals, *Candida species* are harmless, however, if the balance of the normal flora is disrupted or the immune defenses are compromised, these fungi can outgrow the mucosal flora and cause symptoms of disease. Two main types of infections can be observed: superficial and invasive candidiasis. In more severe cases *Candida species* may enter the bloodstream (candidaemia) and penetrate almost all organs of the body. Invasive candidiasis includes:

- acute or chronic haematogenously disseminated candidiasis;
- infection of single or multiple deep organs, either by haematogenous seeding or by direct inoculation.

The clinical aspects of candidaemia are extremely variable. Patients present with fever without organ-specific manifestations, or a wide spectrum of symptoms and signs, including fulminant sepsis. The incidence of invasive fungal infections due to *Candida* is approximately 10-15% in stem cells transplant recipients (2). Transient candidaemia can occur from any source but most frequently follows intravascular catheter infection with prompt resolution following the removal of the catheter. At the time of documented candidaemia, manifestations of systemic and invasive metastatic candidiasis may be present, although frequently when the latter is evident, blood cultures may become negative. Only 50% of patients with disseminated candidiasis have positive blood cultures, although not all patients with candidaemia have the same risk of dissemination (3). Patients with neutropenia have a slightly higher rate of visceral and cutaneous dissemination.

Aspergillus species is the leading cause of deaths due to infections after allogeneic transplant and remains a major complication in the curses of leukemia treatment. As more and more patients with lymphoproliferative diseases are intensively treated with chemotherapy, monoclonal antibodies, and allogeneic stem cell transplantation, it is likely that the incidence of infections with *Aspergillus* will increase in the future. After allogeneic HSCT, reported incidence varies from 0-20%, after autologous HSCT the incidence is 10% (4). *Aspergillus*, a mold, is an exogenous acquired pathogen that usually gains entry by inhala-

tion into nasal passages and the respiratory tract. The most frequent aspergillus infections are sinobronchial aspergillosis, aspergillus pneumonia, CNS aspergillosis and systemic aspergillosis. The mortality rate of CNS aspergillosis is almost 100%.

Rapid detection and identification at the species level is essential for adequate treatment. Automated blood culture system is routinely used as a diagnostic tool. However, in many cases blood cultures fail to detect yeasts (in up to 65%) or need prolonged incubation times or terminal sub-culturing of negative blood culture samples before yeast growth can be detected. This may be due to the fact that many blood culture media are not optimal for fungal growth, or to the presence of antifungal drugs in the blood. Special media for detection of fungal growth are available. A number of alternative, non-culture based methods have been used for rapid detection of fungaemia: PCR, Nucleic Acid Sequenced-Based Amplification (NASBA), CRP, Serum procalcitonin (accurate in distinguishing between candidaemia and bacteraemia), ELISA for Candida antigen, Galactomannan test, ELISA for Candida antibody, detection of beta1,3-D glucan. Obviously, the diagnosis of invasive fungal infection is a problem for the clinicians and the microbiologists too. As a consequence, antifungal prophylaxis is the most important and safe way to decrease frequency and severity of invasive fungal infections. Despite considerable progress in development of supportive measures, introducing of growth factors, powerful antifungal agents, laminar air flow, or HEPA (high-efficiency particulate air) filter conditioning, invasive fungal infections are still the most difficult to manage infections.

There are four classes of drugs used for prophylaxis and therapy of IFIs. These include: polyenes (various formulations of Amphotericin B), nucleosid analogues, azoles, and echinocandins. Several of these drugs have already established roles and others have shown promise in treatment of HCT patients.

The aim of this study was to evaluate the frequency of the fungal infections and the success of different antifungal strategies used for prophylaxis in patients treated with hematopoietic stem-cell transplantation in the Republic of Macedonia.

Material and methods

The study was designed as retrospective. The analyzed patients were treated with hematopoietic stem cells transplantation at the University Hematology clinic-Skopje from September 2000 to September

2007. We have performed 128 high-dose chemotherapy and stem-cells transplantations in 120 patients with different hematological diseases: Acute myeloblastic leukaemia 59 (49,2%), Acute lymphoblastic leukaemia 6 (5%), Chronic myeloid leukaemia 6 (5%), Chronic lymphocytic leukaemia 1 (0,8%), Non Hodgkin Lymphoma 15 (12,5%), Hodgkin disease 13 (10,8%), Multiple myeloma 18 (15%), Severe aplastic anaemia 1 (0,8%), Primary myelofibrosis 1 (0,8%).

As a source of hemopoietic stem cells were used peripheral stem cells in 100 and bone marrow in 28 procedures. Median number of infused CD34+ cells was: $3,04 \times 10^6$ /kg. Allogenic: 35 from HLA identical sibling; Autologous: 93 HSCT. Gender: Male: 62 Female: 58. Median age: 34 years (12-64 years). Patients were treated in sterile room, conditioned with HEPA filters, and low microbes diet. In every patient, blood culture, urine culture, central venous smear and upper and lower respiratory tract smear was obtained 3 times a week. The cultures were analyzed at the Institute of Microbiology and Parasitology, Medical faculty of Skopje, using standard microbiological methods for fungal detection. As antifungal prophylaxis were administered orally Caps. Fluconazole 200 mg per day, or Sol. Itraconazole 200 mg, or combinations with Fluconazole 200/Itraconazole 200 (in high-risk patients) from day 0 until day +100. For Gram-negative bacteria prophylaxis patients received Ciprofloxacin 500 mg/daily divided in two doses. We have retrospectively analyzed fungal infections; fungal isolates and we made a comparative study between different antifungal strategies during the first 30 day after transplantation.

Results

Clinical manifestation of infection were present in 60 (50%) patients. Mucositis was the most frequent infective complication in our group of transplanted patients. Soor was presented in 18 patients. Serious

Table 1: Clinical manifestation of infections.

Infection	No. of patients	%
Mucositis	60	50
Pneumonia	6	5
Neutropenic enterocolitis	12	10
Soor	18	15
Central venous catheter	12	10
Cistitis	6	5
Sepsis	6	5

infection with signs of sepsis was present in 6 patients, and pneumonia in 6 patients. Twelve patients were with central venous catheter infection (2 of them with tunnel infection). During a period of deep neutropenia (from day +5 until day +12) in 12 patients neutropenic enterocolitis was present (Table 1).

Oropharyngeal candidiasis was the most frequent fungal infection, present in 18 (15%) patients. Pneumonia with fungal isolates was present in 3 (2.5%) patients, sepsis with fungaemia in 3 patients (2.5%), and in 4 (3%) patients we had fungal isolates from central venous catheter. In all cases with fungal isolates the central venous catheter was either changed or removed (Table 2).

Table 2: Clinical manifestation of fungal infection.

Fungal infection	No. of patients	%
Soor	18	15
Pneumonia	3	2,5
Sepsis	3	2,5
Central venous catheter	4	3

Gram positive cocci are predominantly isolated microorganisms from all sites (64.3%), *Gram negative bacteria* (19.6%), and *Fungal isolates* (16.1%) (Table 3).

Table 3: The most frequent microorganisms isolated from blood culture, urine culture, central venous catheter, upper and lower respiratory tract smears.

Strain	Number	%
<i>Staphylococcus coagulaza neg.</i>	23	21,5%
<i>Streptococcus pneumoniae</i>	19	17,7%
<i>Enterococcus</i>	14	13,0%
<i>Pseudomonas aeruginosa</i>	12	11,2%
<i>Non albicans Candida</i>	11	10,5%
<i>Staphylococcus aureus MRSA</i>	7	6,5%
<i>Staphylococcus aureus</i>	6	5,6%
<i>Candida albicans</i>	6	5,6%
<i>Escherichia coli</i>	4	3,7%
<i>Acinetobacter species</i>	4	3,7%
<i>Klebsiela aerogenes</i>	1	1,0%

The most frequent place of fungal isolation is lower respiratory tract. Fifteen isolates (34.8%) were isolated: *Candida Albicans* in 5 patients (11.6%); *Non Albicans Candida* in 10 patients (23.2%).

Results from different strategies of antifungal prophylaxis:

- From 120 patients, Fluconazole 200 mg/day was used as antifungal prophylaxis in 90 patients (75%).

Table 4. Microorganisms isolated from sputum.

Strain	Number	%
<i>Staphylococcus coagulaza negativ</i>	2	4,6%
<i>Staphylococcus aureus</i>	2	4,6%
<i>Streptococcus pneumoniae</i>	17	39,5%
<i>Pseudomonas aeruginosa</i>	4	9,3%
<i>Acinetobacter species</i>	2	4,6%
<i>Klebsiela aerogenes</i>	1	2,3%
<i>Candida albicans</i>	5	11,6%
<i>Non-Albicans Candida</i>	10	23,2%

There were 13 fungal isolates: *Candida albicans* - 6; *Candida species* - 7.

- Itraconazole 200 mg/day was used in 18 patients (15%). In these patients 3 isolates were isolated: *Candida albicans* - 1; *Candida species* - 2.

- Because of high risk for invasive fungal infection (previous invasive fungal infection and deep immunosuppressive protocol) combination of two antifungal drugs was used as antifungal prophylaxis in 12 patients (10%): Itraconazole 200 mg/day + Fluconazole 200 mg/day. In this group we had only 1 isolate of *Candida Albicans* from sputum.

There were no isolates of *Aspergillus*.

Infection related mortality in our group of patients was 2.5% (3 patients), due to mixed bacterial and fungal infection. The infections were fatale in 2 patients with Acute Myeloblastic Leukemia (AML) (1 treated with allogeneic transplantation, and 1 with autologous transplantation), and in 1 patient with Severe Aplastic Anemia (SAA) (treated with allogeneic transplantation).

First patient with AML died in sepsis. From blood culture *Stenotrophomonas maltophilia* and *Non Albicans Candida* were isolated. He had received Fluconazole as prophylaxis. Second patient with AML died with pneumonia. From sputum and blood *Pseudomonas aeruginosa* and *Non albicans Candida* were isolated. He had received Itraconazole as antifungal prophylaxis. Third patient with SAA died in sepsis. From blood culture *Streptococcus pneumoniae* and *Candida albicans* were isolated. He had received combination of antifungal drugs: Fluconazole + Itraconazole as antifungal prophylaxis.

In 6 patients (5%) fungal infections were proven using invasive clinical and microbiological procedures. The most frequent fungal infection was oropharyngeal candidiasis in 18 (15%) patients. Fungal isolates are third common isolates with 16.1% from total isolated

microorganisms. Non-Albicans *Candida* are predominantly isolated fungi (Non-Albicans versus *Candida albicans* (56%:44%).

Patients treated in active disease (34 patients) have statistically significant more frequent infection, opposite to patient treated in complete remission (86 patients) ($p=0,0013$; $X^2 = 10,38$).

There is no statistically significant differences between three different antifungal prophylactic regimens ($p=0,302$; $X^2 = 2,39$).

Discussion

The two most difficult challenges facing the clinician caring for hematopoietic stem cell transplant (HSCT) recipients are graft-versus-host disease (GVHD) and infective complications. Once *cytomegalovirus* (CMV) was the main infectious threat, but today, invasive fungal infections (IFI) are the major cause of infectious morbidity and mortality after allogeneic HSCT. The change is due not only to a diminution in serious CMV disease or the introduction of new drugs and adoption of new prophylactic or preemptive strategies, but also to an increase in IFI rates, changes in fungal epidemiology, and lack of progress in fungal therapeutic approaches (5).

A double peak in the occurrence of IFIs was noted many years ago. The first peak occurs during the first month after HSCT (the pre-engraftment period). *Candida*, a yeast, is a part of the endogenous flora of patients and historically has been the most common fungal pathogen during the pre-engraftment period. Systemic invasion by colonizing *Candida* organisms takes place with bacterial suppression by antibiotics, mucosal injury by intensive conditioning regimens and loss of second-line host defenses (phagocytosis compromised by neutropenia, and cell-mediated immunity suppressed by the immunosuppressive regimen and GVHD) (6). *Aspergillus*, a mold, is an exogenously acquired pathogen that usually gains entry by inhalation into nasal passages and the respiratory tract. *Aspergillus* represents a distant second pathogen during the preengraftment period (7). The second peak occurs in the post-engraftment period, mainly during the second and third months. GVHD and the use of corticosteroids are the most important risk factors. *Aspergillus* is the predominant pathogen in this second peak; *Candida* and other mold pathogens account for a minority of the other IFIs. Other risk factors for IFIs have been identified, such as use of more intensive conditioning regimens, prior aspergillosis, CMV infec-

tion, use of T-cell depletion of the stem cell-graft, use of cord blood as the source of stem-cells, and HLA mismatching of donor and recipient. In recent years, a number of changes have been noted in rates of infection, types of pathogens, and time course. *Candida* infections have dramatically decreased with the adoption of fluconazole prophylaxis during the preengraftment period. Hematopoietic growth factors and the use of peripheral blood to optimize the CD34+ cell content of the stem-cell graft have shortened the time to engraftment. Reduced intensity nonmyeloablative conditioning regimens have reduced the duration of neutropenia and the degree of damage to the mucosa of the gastrointestinal tract. These changes in transplantation practice have all combined to greatly reduce the risk for IFI (as well as bacterial infections) during the preengraftment period. In contrast, several changes in transplantation practice have combined to increase the risk for IFIs in the postengraftment period. The increasing use of alternate allogeneic donors, including matched-unrelated donors, mismatched family donors, and cord blood, and reliance of more potent immunosuppressive regimens to suppress GVHD have increased the risk for IFI after engraftment. The higher rates of chronic GVHD after peripheral blood allografts have been accompanied by more IFIs, along with other infectious complications. Today more and more IFIs occur after day +100, extending the vulnerability period.

For autologous HCT recipients, certain events before transplantation increase the patient's risk. Prior IFI is associated with an almost guaranteed exacerbation during subsequent HCT. Potent purine analogues also increase the susceptibility for IFI by producing a profound, long-lasting deficiency of cell-mediated immunity. Events during the transplantation procedure, including CD34+ cell selection of the stem-cell graft and use of steroids during the peritransplantation period, increase the susceptibility of autologous HCT recipients for IFI in the postengraftment period.

Amphotericin B. Amphotericin B has the widest spectrum of activity against fungi, with activity against most human fungal pathogens, including most *Candida* and *Aspergillus* species. Amphotericin B was introduced 50 years ago; its efficacy was not tested in controlled trials, but for many years it was the only antifungal treatment option (8,9). Toxicity is frequent with Amphotericin B. Infusional reactions include fever and rigor that occurs in approximately half of the treated patients and, less frequently, hypotension, wheezing, hypoxia, and rash. Older age and rapid infusions are factors associated with a greater propensity for infusional reactions. Nephrotoxicity is a major

limitation of the clinical usefulness of Amphotericin B. The risk for nephrotoxicity varies in different series. Risk factors include the mean daily dose, the duration of the treatment course, chronic renal disease, and the use of concomitant nephrotoxins, such as cyclosporine and aminoglycosides. Other toxicities include anemia, electrolyte wasting from renal tubules, hepatic dysfunction, seizures, and anorexia. The high rates of nephrotoxicity of amphotericin B in allogeneic HSCT patients receiving calcineurin inhibitors have severely limited the ability of transplant clinicians to use amphotericin B in the prophylaxis of IFIs. Lipid formulations of amphotericin B were developed to provide a less toxic formulation of amphotericin B. Three lipid formulations have been licensed: amphotericin B lipid complex (ABLC), amphotericin B in colloidal dispersion (ABCD), and liposomal amphotericin B (Ambisome) (8, 10-15). These agents have the same antifungal spectrum of activity as amphotericin B deoxycholate.

Azoles. There are several azoles licensed for clinical use, and they differ by molecular structure according to specific side chains that lead to differences in pharmacologic properties, toxicity profiles and spectra of activity.

Fluconazol. The effectiveness of fluconazole prophylaxis in reduction of *Candida* infections has shaped the fungal therapy map for a decade. Currently, there is clear evidence (level A, I) that fluconazole prophylaxis is of proven benefit in the primary prophylaxis at a daily dose of 400 mg in recipients of allogeneic bone marrow or hematopoietic stem cell transplants. Two placebo-controlled studies involving allogeneic transplant recipients demonstrate the prophylactic efficacy of fluconazole 400 mg/day in terms of preventing a documented invasive fungal infection and the attributable mortality (16). Most *Candida* species are highly susceptible to fluconazol, however several species are not reliably controlled by fluconazol. The standard dose of fluconazol in the treatment of *Candida* mucosal infections is generally 100-200 mg/day. For candidemia caused by susceptible *Candida* species, doses of 400 mg/day are recommended, with the exception of *C. glabrata* species for which higher doses of up to 800 mg/day may be necessary. Fluconazol has a favorable safety profile compared with other antifungal agents and is well tolerated, with few severe toxicities. Fluconazol is highly active against *Candida* isolates, there is also activity against *Cryptococcus* species, *Histoplasmosis* and *Coccidioidomycosis*, randomized trials and case-controlled studies have shown fluconazole to be highly effective as therapy of systemic *Candida* infections, with response and survival rates comparable to those of Amphotericin B

(17, 18). Although concerns were raised nearly a decade ago that emergence of fluconazole resistance would mitigate the effectiveness of candida prophylaxis that fear has fortunately largely remained unrealized. Scattered report of several outbreaks of candida infection have been noted in HCT patients receiving fluconazole because of resistant candida species (*C. crusei*, and *C. glabrata*) and also because of fluconazole-sensitive species (*C. parapsilosis*) yet these have been largely isolated (19). Shifts to *Non-Albicans candida* species have occurred, but the net effect of fluconazole has been enormous reduction in the more prevalent *C. albicans* and *C. tropicalis* species; this net decrease to date has overshadowed the smaller increase in the *Non-Albicans* species.

Although *Candida* infections have decreased, *Aspergillus* infections have relentlessly increased in frequency. *Aspergillus* species once infected only 4%

Table 5: Fluconazole resistance in different *Candida* strains (19).

Candida strains	Patients	%
<i>C. albicans</i>	885	5,3
<i>C. lipolytica</i>	22	100
<i>C. guilliermondi</i>	9	100
<i>C. krusei</i>	80	100
<i>C. tropicalis</i>	100	30
<i>C. glabrata</i>	398	99

5% of allogeneic HCT recipients, but today rates of 12%-15% are regularly being reported, and even higher rates have been observed in some centers and in some subgroups of high-risk patients (20, 21). Although it was hoped that nonmyeloablative allogeneic transplants would be associated with fewer infections, a decrease in IFIs has not been realized in several reported series. In part, the high rate of IFI after nonmyeloablative allogeneic HSCT isolated to the more aggressive tapering of immunosuppressive therapy in many such regimens that is designed to maximize the potential for graft-versus-tumor effects. Infections from other mold pathogens have also increased including *Zygomycetes* and *Fusarium* species, but still these are relatively infrequent. Infections due to *Scedosporium* species have remained infrequent.

Itraconazol. This azoles have activities not only against *Candida* species, and *Cryptococcus* species, *histoplasmosis*, *blastomycosis*, and *coccidioidomycosis*, but also against *Aspergillus* species. Itraconazol is less reliably and less well absorbed by mouth (55% for the solution and much less for the oral

capsules) and has substantial interpatient variability. The intravenous formulation is well tolerated. Itraconazole is an agent suitable for oral (capsules and suspension) and intravenous administration. Its spectrum of action includes *non-albicans Candida species* and molds. Oral itraconazole suspension was studied in a double-blind placebo-controlled trial. The dosage was 2.5 mg/kg twice a day. All patients additionally received nystatin 500 000 IU, 4 times a day. The itraconazole arm was superior to the placebo arm in terms of reducing the rate of fatal candidaemia (1.96% versus 0%). Effective prophylaxis against molds was not documented (22). An open-label analysis of high-risk patients suggested that itraconazole oral suspension 100 mg twice daily was superior to polyenes. Winston et al., 2003 investigated 35 randomized allogeneic bone marrow transplant recipients to receive either 400 mg itraconazole or 400 mg fluconazole. Preliminary results suggest itraconazole prophylaxis confers an advantage in terms of incidence of documented invasive fungal infections (23).

Voriconazol. Voriconazol has the broadest antifungal spectrum of all licensed azole antifungals (24-28). In clinical trials, responses have been noted in aspergillus, *Candida* (including fluconazole-resistant *Candida species*), fusarium, and scedosporium infections. A major gap in its coverage is a lack of activity against zygomycetes. A randomized trial comparing amphotericin B and Voriconazole as first-line therapy of invasive aspergillosis demonstrated voriconazole to be more effective than amphotericin B with high response rate and better overall survival (29). In addition, voriconazole was associated with fewer toxicities and greater tolerance. Amphotericin B has the broadest spectrum of activity of all antifungal agents available. It is in widespread use as an oral suspension (1.5-3 g/d). Apart from liposomal amphotericin B, the use of amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD) would be conceivable (10). Widespread use is unlikely owing to the high cost of liposomal amphotericin B formulations. At present, due to a lack of study data on the efficacy of lipid formulations, no evidence supports the use of these agents for prophylaxis (level C, I) (30).

Newly developed drugs worth mentioning include the new triazoles voriconazole, posaconazole, and ravuconazole, liposomal nystatin, and the new class of echinocandins. Representatives of the latter include caspofungin, micafungin, and anidulafungin, of which caspofungin has been licensed in the United States and the European Union since 2001 for second-line treatment of invasive aspergillosis.

Echinocandins. The mechanism of action of the echinocandins is inhibition of beta-(1,3)-glucan synthase, which leads to interference of the synthesis of the glucan, a major constituent of the fungal cell wall and a unique fungal target. Reduced glucan makes the fungal cell vulnerable to osmotic lysis. Caspofungin is the only licensed member of this family of antifungals. Caspofungin has excellent in vitro activity against *Candida* (including azole-resistant species) and *Aspergillus species* (31-33). In vitro testing studies have raised concerns of lower activities against several *non-Albicans Candida species*, but whether these in vitro findings are clinically important is unclear because the clinical responses for these species seem to be comparable to those with amphotericin B. It was first licensed on the basis of clinical responses noted in patients with invasive aspergillosis who had not responded to amphotericin B, or who were intolerant of amphotericin B (34).

The broad spectrum of action of the oral allylamine terbinafine suggests its suitability for prophylactic use, especially given that allylamines are not used for treating invasive fungal infection. As far as prophylaxis is concerned, except micafungin, these drugs have to date only been studied on an individual case basis, so that there is no evidence-based recommendation for their prophylactic use against systemic fungal infections at present. In addition to safety and efficacy aspects, daily dosage costs will be a decisive factor in determining the feasibility of clinical use for prophylaxis. The time of onset has gradually been pushed to later times. The apparent increase in late onset IFI is impaired attributable to the abrogation of the initial pre-engraftment peak from *Candida*, but it is also attributed to the greater occurrence of chronic GVHD with peripheral blood grafts and to an increase in older patients receiving transplants. These later onset frequently takes place after the patient has left the transplant center, which poses additional challenges for close monitoring and vigilance for longer periods and often at great distances from the transplant center.

We can conclude that the rising incidence of invasive fungal infections and the currently problematic early diagnosis call for the intensive exploration of new drugs and further developments in diagnosis and treatment of invasive fungal infection.

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