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ORIGINAL ARTICLE

Efficacy of caspofungin as secondary prophylaxis in patients undergoing allogeneic stem cell transplantation with prior pulmonary and/or systemic fungal infection

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Transplanted patients with a history of invasive fungal infection (IFI) are at high risk of developing relapse and fatal complications. Eighteen patients affected by hematological malignancies and a previous IFI were submitted to allogeneic stem cell transplantation, using Caspofungin as a secondary prophylaxis. Patients had a probable or proven fungal infection and 16 had a pulmonary localization. No side effects were recorded during treatment with Caspofungin. Compared to pre-transplant evaluation, stability or improvement of the previous IFI was observed in 16 of the 18 patients at day 30, in 13 of the 15 evaluable patients at day 180 and in 11 of the 11 evaluable patients at day 360 post transplant. In particular, all the six patients with a proven fungal infection were alive, with a stable or improved IFI after 1 year from transplant. At a maximum follow-up of 31 months, eight patients died for disease progression or transplant-related complications, but only two had evidence of fungal progression. Secondary prophylaxis with Caspofungin may represent a suitable approach to limit IFI relapse or progression, allowing patients with hematological malignancies to adhere to the planned therapeutic program.
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Introduction

Invasive fungal infections (IFI) remain the major cause of death among neutropenic patients receiving chemotherapy for leukemia, or submitted to stem cell transplantation.[1,2] The recent development of more effective, less toxic antifungal drugs and routine early implementation of high-resolution chest computer tomography (CT), leading to prompt intensive antifungal therapy, have improved responses and survival, allowing an increase of antifungal treatments, including secondary antifungal prophylaxis.[3,4] The possibility of reactivation of a previous episode of fungal infection during a following neutropenic phase or in patients undergoing stem cell transplantation is extremely high and is associated with a very poor prognosis.[5] In the particular case of stem cell transplantation, delay of treatment because of previous IFI, mainly due to different classes of *Aspergillus*, may also contribute to leukemia relapse in patients with high-risk disease.[6,7] Very few studies have addressed the role of previous IFI in the feasibility of stem cell transplant, or the secondary prophylaxis with antifungal drugs in preventing recurrence of infection after transplantation.[8,9] However, given the lack of prospective studies, the role of secondary antifungal prophylaxis remains unclear and differently approached by transplant centers.

Caspofungin, the first clinically used echinocandin, is a member of a new class of antifungal antibiotics that inhibit the synthesis of 1,3-β-D-glucan.[10] Its documented clinical efficacy, associated with the lack of interactions with the majority of drugs used during the course of allogeneic stem cell transplantation,[11] may indicate Caspofungin as the best option to be used as a secondary prophylaxis in patients undergoing stem cell transplantation.

In this prospective, multicentric phase-II study of secondary prophylaxis, Caspofungin was given at standard dose to 18 consecutive patients undergoing allogeneic stem cell transplantation. All had a previous probable or proven

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fungal infection and were treated and followed with the same clinical protocol.

Patients and methods

Study population

Between August 2003 and February 2006, 18 patients were submitted to allogeneic stem cell transplantation. Clinical characteristics, including stem cell source and type of transplant are reported in Table 1. Age greater than 18, diagnosis of hematological neoplasia or bone marrow aplasia and stem cell transplant from allogeneic source were the major inclusion criteria. All patients had a prior diagnosis of fungal infection, with residual or absent lesions at lung CT scan or abdominal ultrasound and the absence of clinical signs of fungal infection at the time of enrolment.

Written, informed consent was obtained from all patients.

Drug administration

Caspofungin (70 mg) as a loading dose on the first day, followed by 50 mg daily, was given intravenously (i.v.) from the start of the conditioning regimen until a stable engraftment of $>1 \times 10^9$/l neutrophil cells. Oral Itraconazole at 400 mg/day was given after suspension of Caspofungin, as maintenance therapy.

Table 1 Patient characteristics

Characteristics	*No.*
Sex	
Male	10
Female	8
Age	
Median (range)	40 (19–58)
Diagnosis	
Acute myelogenous leukemia	8
Acute lymphoblastic leukemia	7
Chronic myeloid leukemia	1
Myelodysplastic syndrome	1
Aplastic anemia	1
Type of transplant	
Related	13
Unrelated	5
Source of stem cells	
PBSC	10
Bone marrow	6
Cord blood	1
PBSC + bone marrow	1
Disease status at transplant	
Complete remission	10
Partial responder or progression	7
Engraftment failure post first transplant	1
Conditioning regimen	
Standard	14
Reduced intensity	4

Abbreviation: PBSC = peripheral blood stem cell.

Prior fungal infection definitions and treatment

All patients suffered a previous episode of fungal infection, which was graded as probable or proven, according to laboratory and clinical criteria, as described in standardized guidelines.[12] Briefly, invasive mold infections were considered proven if histopathologic examination or culture of sterile tissue revealed the organism. Invasive mold infections were considered probable in patients who had both clinical criteria and at least one microbiologic criterion, defined as growth of an organism from respiratory secretions (sputum, bronchoalveolar lavage fluid and so on), or two positive galactomannan enzyme immunoassays. Growth of any *Candida* species from blood culture in patients with signs and symptoms of infection was considered evidence of proven candidemia. A diagnosis of organ involvement required documentation of Candida by both histologic examination and culture of sterile tissue.

Of the 18 patients, a probable and proven fungal infection was diagnosed in 12 and 6 cases, respectively. Distribution of infection site, type of organism and delay between fungal infection and transplant, according to the classification of previous fungal infection, was reported in Table 2. Eleven patients with probable and five with proven infection had a pulmonary localization; of these five, two cases had isolated lung localization, while in the remaining three cases, lung and skin were the infection site in two patients and lung and paranasal sinus in one. Liver, spleen and kidney were the infection sites in the remaining case with proven infection. *Aspergillus* and *Candida* were the cause of infection in five and one patient of the proven group, respectively. Treatment of the previous IFI included liposomal amphotericin-B in 66%, Caspofungin in 12%, voriconazole in 16% and any combination of the above drugs in 6% of the treated cases. Median time between fungal infection and stem cell transplant was significantly longer ($P = 0.02$) in the group with proven infection (10.5; range 6.7–21.2), than the group with probable infection (2.7; range 0.6–24.6).

Table 2 Prior fungal infections

	Probable	*Proven*
Patients	12	6
Site of infection		
Lung	11	5
Skin	0	2
Liver	0	1
Spleen	0	1
Kidney	0	1
Paranasal sinuses	1	1
Etiology		
Aspergillus species	—	
Aspergillus flavus	—	3
Aspergillus fumigatus	—	1
Aspergillus versicolor	—	1
Candida species	—	
Candida tropicalis	—	1
Time between fungal infection and transplant		
<6 months	9	0
6–12 months	1	3
>12 months	2	3

Transplantation protocols and evaluation criteria
Patients undergoing allogeneic stem cell transplantation were conditioned according to their underlying disease and treated according to the policy of each Center participating in the study, including graft-versus-host disease (GVHD) prophylaxis and the choice of antibiotic therapy. Antimicrobial prophylaxis consisted of Acyclovir, Ciprofloxacin or Levofloxacin at standard dose; if febrile neutropenia developed, prophylaxis was discontinued and replaced with broad-spectrum i.v. antibiotics. All patients underwent daily clinical examination for the recurrence of fungal infection and all were prospectively monitored for galactomannan blood antigenemia (Platelia *Aspergillus*, Biorad) two times a week during the neutropenic phase and once a week thereafter. CT scan or ultrasound were performed according to clinical indications, or at each follow-up indicated in the study. Clinical follow-up was conducted until the end of the study, or until death.

The success of secondary prophylaxis was defined as the absence of documented relapse of the fungal infection and the absence of new proven, probable or possible IFI. Previous IFI were considered stable if no detectable modification of fungal lesions by CT scan or ultrasound together with the absence of clinical signs of infection was observed, compared to pre-transplant evaluation. Lesions were considered improved if the absence of any clinical sign of infection was accompanied by a reduction of the size of previous IFI, while they were considered progressed if an increase of the previous lesion was demonstrated, regardless of the presence of clinical evidence of fungal infection.

Caspofungin side effects were monitored by daily clinical examination and biological tests including blood count, biochemistry and liver function at least three times a week during hospitalization and then once a week during the rest of follow-up. In the case of a serious adverse event, Caspofungin was stopped and replaced by a different i.v. antifungal drug. Data analysis was carried out at day $+30$ from stem cell reinfusion and at 6 and 12 months of follow-up.

Statistical analysis
Continuous variables are expressed as median and range and categorical variables as absolute values. Median time between fungal infection and stem cell transplant, as well as Caspofungin administration between probable and proven groups was compared by Mann–Whitney test. Overall survival was estimated by the Kaplan–Meier product-limit method. Transplant-related mortality (TRM) was defined as death due to causes unrelated to the underlying disease and calculated using cumulative incidence estimates.[13] Significance was defined at the $P<0.05$ level. The statistical analyses were performed using SAS software version 8 (SAS Institute Inc., Cary, NC, USA).

Results

Safety of Caspofungin treatment
The 18 patients received Caspofungin for a median of 28 days (range 14–45). No differences in treatment duration were observed among the probable and proven groups. One patient discontinued prophylaxis because of severe veno-occlusive disease. No side effects were observed during Caspofungin administration.

Hematological reconstitution and transplant complications
Three patients died before neutrophil reconstitution. Median time of neutrophil above 0.5×10^9/l and platelets above 50×10^9/l was 18 (range 11–27) and 16 (range 11–22) days, respectively. Grade I/II acute GVHD (aGVHD) was diagnosed in four (22%) patients. At the time of the last follow-up, four patients were receiving treatment for moderate chronic GVHD. Five patients had one or more episodes of cytomegalovirus infection detected by antigenemia or PCR and received treatment with either Gancyclovir or Foscavir.

Infectious episodes during transplant
Of the 18 patients, 15 developed a first fever episode during neutropenic phase, classified as 'unknown origin' (FUO) in 10, 'clinically documented' in 2 (lung and soft tissues) and 'microbiologically proven' in 3: blood (*Escherichia coli*) in 2; blood/urine (*Staphylococcus Epidermidis* and *Pseudomonas Aeruginosa*) in 1. A second fever episode developed in six patients, classified as FUO in two, clinically documented in two patients (colecistis and lung) and microbiologically proven in two patients: blood (*S. epidermidis* and *Enterococcus faecalis*) in one patient and blood/lung (*Klebsiella* and *S. aureous*) in one. No other antifungal agents than Caspofungin was utilized after transplantation and during follow-up, until patients were in the protocol.

Response of previous fungal infection
The effect of transplant procedure on previous fungal infection was evaluated at days 30, 180 and 360 from stem cell reinfusion, comparing to the pre-transplant status (Table 3). Of the 18 patients evaluable at day 30, 4 were considered stable, 12 improved and 2 progressed. In particular, of the six patients with proven fungal infection, five were considered improved and one with a stable IFI. Fifteen patients were evaluable at day 180 because three deaths occurred before day 30. Two patients were considered stable and 11 still improved at day 180, while 2 patients had their previous IFI progressed. Eleven patients were evaluable at 1 year of follow-up, because of three additional deaths observed within 180 days and one patient with a follow-up ahead of time. No patient showed signs of previous IFI progression; in particular, two patients were stable and nine improved. Of the six evaluable patients with proven IFI, one was stable and five improved at day 180, while two were stable and four improved at day 360.

Efficacy of the transplant procedure and cause of deaths
At 31 months of follow-up, the probability of survival of the 18 patients submitted to allogeneic SCT with a previous IFI is 45%. Three patients died due to leukemia relapse or progression; five patients died due to transplant-related complications with evidence of fungal infection in two

Table 3 Evaluation of previous fungal infection during follow-up

	Probable	*Proven*
Day + 30 (n = 18)		
Stable	3	1
Improved	7	5
Progressed	2	—
Day + 180 (n = 15)		
Stable	1	1
Improved	6	5
Progressed	2	—
Day + 365 (n = 12)		
Stable	0	2
Improved	5	4
Progressed	—	—
Not reached	1	—

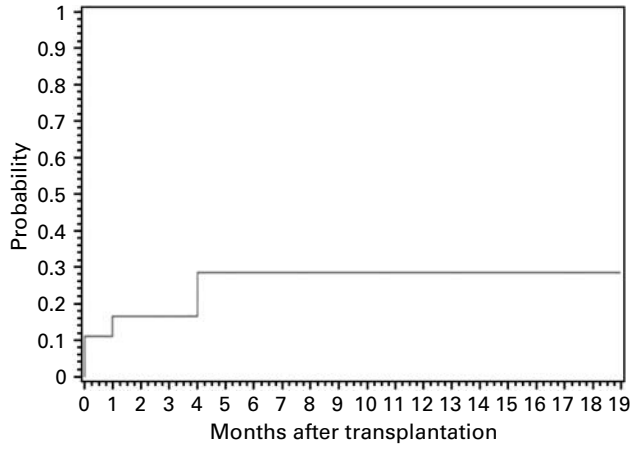

Figure 1 Cumulative incidence of TRM in the 18 patients submitted to allogeneic stem cell transplantation.

Table 4 Deaths

Causes	*No. of patients*	*Time of death (day after transplant)*
Cerebral hemorrhage	1	25
Relapse or progression	3	24–184–419
Multiple organ failure	3	61–141–166
Veno-occlusive disease	1	26

patients. TRM of the 18 patients was 28.6% (Figure 1). The cause and the time of death for each of the eight patients are indicated in Table 4.

Discussion

Although IFI remain one of the major causes of death or chemotherapy discontinuation in patients with leukemia or lymphoma, this study demonstrates the feasibility and efficacy of Caspofungin as a secondary prophylaxis in patients undergoing stem cell transplantation at high risk of fungal relapse. Caspofungin represents the first drug from the novel echinocandin class to be introduced into clinical practice and, based on the available studies published, it is generally very well tolerated, including in long treatments of *Aspergillus* infections.[14] Safety and efficacy were recently assessed on a large randomized multicenter trial, showing that Caspofungin was as efficacious and better tolerated than liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia.[15] All but one of our patients surviving to day 30 adhered to the planned protocol or transplant schedule; there were no major side effects and, as already reported, no difficulties in managing cyclosporin levels during GVHD prophylaxis and treatment.[11]

Of the 18, 15 and 11 patients evaluable for previous IFI response on days 30, 180 and 360, respectively, a total of 16, 13 and 11 patients had the previous fungal infection stable or improved, allowing TRM rate to be not different from that reported in other studies, including patients who lack previous IFI.[16,17] Of the eight patients who died in our series, two had evidence of fungal infection; however, since autopsy was performed in a minority of cases, we cannot exclude the presence of fungal infection at the time of death in the remaining patients, although in all of them, signs of infection by clinical and radiological parameters were not present.

All studies assessing the value of secondary prophylaxis during transplant or intensive chemotherapy were limited by small, heterogeneous patient populations and by uncontrolled, retrospective designs.[4] However, there is a general consensus for giving antifungals to patients undergoing a new neutropenic phase or a transplant, supported by better prognosis in patients who have received any secondary prophylaxis, than in patients who have not.[5] The successful use of Caspofungin given to prevent relapse of fungal infection in transplanted patients was reported in 26 patients with a history of probable or proven infection, although in 15% treatment had to be changed to voriconazole, amphotericin B or Ambisome because of clinically manifest fungal infection under Caspofungin prophylaxis.[9] These results were similar to ours and confirm that 85–90% of patients at high risk of developing IFI relapse can be safely submitted to allogeneic stem cell transplantation. The evaluation of secondary prophylaxis in patients with previous history of IFI is a common problem among patients submitted to a stem cell transplant procedure. For the majority of high-risk patients with leukemia, or those with leukemia or other hematological diseases enrolled in clinical trials, stem cell transplant may still represent the only type of treatment, which can guarantee a substantial proportion of cure. Different therapeutic approaches have been proposed to minimize the risk of previous fungal infections: if the lesion is unique and easily accessible, surgery may be effective; however, the risk of surgery, the delay of transplant with the consequent risk of disease progression and the often multiple sites of lung lesions contraindicate the surgical approach.[18,19] The use of reduced-intensity conditioning (RIC) regimens has been shown to reduce short-term TRM and in a recent retrospective study with a relatively large number of patients, RIC showed a reduced risk of invasive Aspergillosis progression after transplant;[20] however,

fungal infections in the long-term follow-up may be less influenced by the use of non-myeloablative regimens, mainly because of the prolonged immune-suppression.[21,22] Immune dysfunction after transplantation, although variable in duration and severity, remains one of the major causes of fungal infection susceptibility. The restoration of immune function is critical and efforts to enhance the immune system by several modalities, including interferon-g-1b, Pentraxin, or adoptive transfer of *Aspergillus*-specific T cells are currently being explored as a treatment for IFI.[23]

On the basis of our results, it is conceivable that the use of an efficacy and safe antifungal compound administered during the transplant procedure may represent an advantageous approach, both because of the very low risk of infection progression and the possibility to fully adhere to the scheduled transplant protocols. The poor outcome of fungal infections in highly immunosuppressed hosts has caused delay or modifications of life-saving chemotherapy or stem cell transplant in patients with previous IFI. Prospective studies assessing the efficacy of new antifungals in secondary prophylaxis in comparison to alternative strategies to reduce the risk of IFI relapse or progression are needed, together with further development of molecular and other non-culture-based diagnostic techniques.

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