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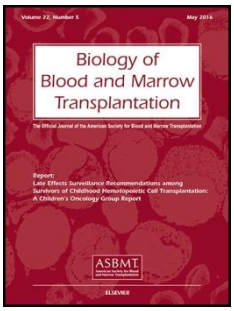

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Long-lasting protective effect of posaconazole prophylaxis in patients with acute myeloid leukemia receiving allogeneic hematopoietic stem cell transplantation

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HIGHLIGHTS

We analysed invasive fungal infections (IFI) after HSCT in 229 AML patients who received antifungal prophylaxis with posaconazole or conventional azoles, during induction/salvage chemotherapy (ISC)

1-year cumulative incidence of IFI post-HSCT was 14% and 1% in patients who received posaconazole (group A) or conventional azoles (group B) (p=0.012).

Multivariate analysis identified the use of alternative donors, prophylaxis with conventional azoles and reduced intensity conditionings as independent risk factors for the development of IFI after HSCT.

Posaconazole prophylaxis during ISC may significantly reduce the fungal burden, thereby limiting the development of overt infection in later phases of the disease including the post-HSCT period.

Abstract
Patients with acute myeloid leukaemia (AML) during induction

chemotherapy and those who receive allogeneic hematopoietic stem cell transplantation (HSCT) are at higher risk of invasive fungal infections (IFI). In the present study we investigated whether the risk of IFI in AML patients receiving HSCT, might be affected by the antifungal prophylaxis with posaconazole administered during the induction/salvage chemotherapy treatment (ISCT).
Between August 2001 and April 2015, 130 patients with AML received itraconazole/fluconazole (group A) and 99 received posaconazole (group B) as antifungal prophylaxis after ISC at 7 Italian Centers while all patients received fluconazole as antifungal prophylaxis after HSCT. Median duration of antifungal prophylaxis after ISCT was significantly longer for patients in group A as compared with group B (24 days vs 20 days, p=0.019). One-year cumulative incidence of proven/probable IFI post-HSCT was 14% and 1% in group A and group B respectively (p=0.012). Fungal free survival and overall survival at 1 year post-HSCT were 66% and 70% in group A, and 75% and 77% in group B (p= 0.139 and p=0.302). Multivariate logistic analysis identified the use of alternative donors (MUD: OR, 3.25; haploidentica/PMRD:OR, 3.19), antifungal prophylaxis with itraconazole/fluconazole (OR, 3.82) and reduced intensity conditionings (OR, 4.92) as independent risk factors for the development of IFI after HSCT.
In summary, the present study suggests that the protective effects of posaconazole during ISC for AML patients may have long-lasting benefits and eventually contribute to reduce the risk of IFI when patients undergo allogeneic HSCT.

Keywords: antifungal prophylaxis, acute myeloid leukemia, invasive fungal infections

INTRODUCTION

Antifungal prophylaxis with mold-active agents has become a widely accepted strategy for patients with acute myeloid leukemia (AML) and hematopoietic stem cell transplantation (HSCT) recipients according to the recommendations of the most recent international guidelines (1-4). Posaconazole was shown effective in reducing the incidence of invasive fungal infections (IFI) in patients with AML as compared with conventional azoles. In a large prospective randomised trial, Cornely *et al* showed a significant reduction of proven and probable IFI in patients with AML and myelodysplastic syndrome (MDS) who received posaconazole (9% vs 1%) which translated into better overall survival (5). In the light of these findings, many “real-life” studies were conducted and substantially confirmed the efficacy of posaconazole (6-9). A consistent proportion of AML patients are also candidates for allogeneic HSCT as part of their treatment plan after induction/consolidation chemotherapy. In this study, we hypothesized that the efficacy of antifungal prophylaxis with posaconazole during induction of AML patients may

have had a favourable impact on the incidence of IFI after the allograft. We retrospectively analysed the incidence of IFI after HSCT in a group of AML patients who received antifungal prophylaxis with either posaconazole or conventional azoles, namely itraconazole/fluconazole, during induction/salvage chemotherapy.

Patients and Methods

Eligibility Criteria

The study was conducted at seven Divisions of Hematology at tertiary care centres or university hospitals in Italy. Between August 2001 and April 2015, adult patients with AML who received antifungal prophylaxis with itraconazole (200 mg BID), fluconazole (200 mg BID) or posaconazole oral suspension (200 mg TID) during up front induction chemotherapy or salvage treatments and who then proceeded to allogeneic HSCT were included in the study. Antifungal prophylaxis started on the first day of chemotherapy or 1-2 days later and continued until neutrophil count was higher than 500×10^9 /L or until proven or suspected diagnosis of IFI, whichever occurred first. When possible, posaconazole was administered with fatty food or nutritional supplements and without proton pump inhibitors if gastric hyperacid symptoms were not present. Patients who developed proven or probable IFI during induction or salvage chemotherapies or at any time before allogeneic HSCT were excluded from the analysis. Further exclusion criteria were a time interval from diagnosis of AML to HSCT longer than 365 days and/or antifungal prophylaxis after HSCT other than fluconazole. The study was approved by the Ethics Committees of each participating Centre.

Patient monitoring

Throughout the treatment plan, patients with febrile neutropenia underwent similar diagnostic work-up at all participating centers which included urine and blood cultures, and chest X-ray. Empiric broad spectrum antibiotic treatment was invariably started on the first day of neutropenic fever. A chest CT scan was scheduled for persistent unexplained fever, or at the onset of any clinical signs or symptoms at the discretion of the attending physician. When radiological chest abnormalities were detected without evidence of any microbiologically documented infection, bronchoscopy with bronchoalveolar lavage was scheduled for

microbiological testing and galactomannan antigen detection whenever possible. Moreover, abdominal ultrasound or other CT scans (i.e. sinus or brain CT) were scheduled according to patient symptoms. Aspergillus galactomannan antigen was tested on serum samples twice-a-week by using the double-sandwich ELISA Platelia Aspergillus. Additional blood, sputum, or other relevant samples were cultured from potential sites of infection when clinically indicated.

Study design

This study was retrospective and non interventional. Primary endpoint was the occurrence of proven and probable IFI within 180 days from HSCT. Invasive fungal infections were classified according to the 2008 EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study Group) criteria (10**).** Secondary endpoints included the use of empirical antifungal therapies and clinical outcome. Mortality was considered attributable to IFI (IFI attributable mortality) when patients died within 12 weeks from the onset of fever with microbiological, histological, or clinical evidence of active IFI, and other potential causes of death could be ruled out by the attending physician. All causes of death within 12 weeks were recorded (overall mortality).

Statistical analysis

Categorical variables were expressed as proportions and continuous variables as the median with the respective range. Comparisons were performed with the Chi-square and the Mann-Whitney test for categorical and continuous variables respectively. Probabilities of overall survival (OS) were estimated by the Kaplan-Meier method, using the log-rank test for univariate comparisons (11). Cumulative incidence of IFI was estimated by the Gray test (12) where relapse/death without IFI were considered competing events. Risk factors for the development of IFI were then investigated by the uni/multivariate competing risks regression model and the Fine-Gray test. Finally, the odds of an IFI occurrence (dependent categorical variable) was tested by the uni/multivariate binary logistic regression model, considering as risk factors the previously reported covariates (independent categorical variables). Probability of fungal-free survival (FFS) was estimated using the Kaplan-Meier product limit estimate. FFS was a survival estimate and the events were either death or IFI (probable or proven). Patients who did not experience an event were censored at the time of last follow-up. All reported p-values are two-

sided and were accepted as statistically significant if <0.05. Potential risk factors for the occurrence of IFI were considered age (>50 vs. ≤50 years), gender, disease phase (induction vs salvage), prophylaxis during chemotherapy treatment (Itraconazole/fluconazole vs posaconazole), duration of neutropenia following chemotherapy treatment, days from diagnosis to HSCT, disease status at HSCT, type of donor (matched sibling vs matched unrelated donor [MUD] vs haploidentical/partially matched related donor [PMRD]), graft source (bone marror [BM] vs peripheral blood stem cell [PBSC] vs umbilical cord blood [UCB]), conditioning regimen (myeloablative vs reduced intensity [RIC]), use of antithymocyte globulin [ATG] and time to neutrophil engraftment after HSCT. Relations between baseline characteristics, including the use of antifungal prophylaxis during induction/salvage treatment, and IFI were tested in univariate analysis by ANOVA and $\chi 2$ test. Data were analyzed as of June 2016 by R 3.2.3 (R Foundation for Statistical Computing, Vienna-A, http://www.R-project.org).

RESULTS

Patient characteristics

Overall 284 AML patients who received up front induction or salvage chemotherapy were screened. Ninety (32%) underwent antifungal prophylaxis with itraconazole, 80 (28%) with fluconazole and 114 (40%) with posaconazole. Seventeen patients in the itraconazole/fluconazole group (Group A) and 5 in the posaconazole group (Group B) who developed probable or proven IFI during induction/salvage chemotherapy were excluded from the analysis. Of the remaining 262 patients who proceeded to allogeneic HSCT, 229 (87%) received post-transplant antifungal prophylaxis with fluconazole and were eligible for data analysis while 33 patients were excluded as they received different antifungal prophylaxes (**Figure 1).** The two treatment groups were similar for age, gender, induction/salvage chemotherapy treatments, and median time to neutrophil recovery after chemotherapy **(Table 1**). Similarly, consolidation chemotherapy treatments, including antracycline and cytarabine in the majority of the patients (162/229, 71%), were equally distributed between group A (No.89) and group B (No.73) (p= 0.470); purine analogues were administered to 41 patients (group A, No.28; group B, No.13), while 26 patients received other consolidation treatments. By contrast, most patients (84%) who received itraconazole/fluconazole were treated between 2001 and 2010 and 65% had received chemotherapy as salvage treatment whereas, after 2010,

most patients (60%) had received posaconazole and 82% induction chemotherapy. Median duration of antifungal prophylaxis was significantly longer in the itraconazole/fluconazole group (24 days vs 20 days, p=0.019) while median duration of empirical antifungal therapy (EAF) was similar in both groups **(Table 1).** Patients in the itraconazole/fluconazole group were empirically treated with lipid formulation of AmB (No. 15), echinocandins (No.6), mold-active azoles (No.4) and with combination therapy (No.1) while in the posaconazole group patients were administered liposomal AmB (No.10) and echinocandins (No. 5).

Patient and transplant characteristics

Table 2 shows HSCT characteristics and clinical outcomes by antifungal prophylaxis; patients are divided into group A (itraconazole/fluconazole) and group B (posaconazole). Median time from the start of chemotherapy (induction/salvage treatment) to HSCT was significantly longer in group B as compared with group A (152 days vs 123 days, p= 0.002). More patients in group A had advanced disease (CR2-CR3/relapse) at transplant while more patients in group B received HSCT from alternative donors. Though marginally significant the difference, in group A and B respectively, 55 and 46 patients were grafted from a matched unrelated donor, 7 and 10 patients from a haploidentical donor, 1 and 4 patients from one antigen mismatched related donor. More patients in group B received peripheral blood stem cells while the only 9 umbilical cord blood transplants were in group A. Myeloablative conditionings were employed in 76% and 91% of the patients in group A and B respectively. Median time to neutrophil engraftment was not significantly different between the two groups. Graft failure was reported in 3 patients in group A and 1 in group B.

Impact of azoles prophylaxis on post-HSCT fungal infections

Overall cumulative incidence of proven-probable IFI at 6 months and 1 year post-HSCT for the entire study cohort was 8% and 10% respectively. Cumulative incidences of IFI (proven or probable) were 13% at 6 months and 14% at 1 year in group A, and 2% and 4% in group B respectively (p=0.012) **(Figure 2)**. Median time of onset of the 18 IFI in

group A was day 35 as compared with day 209 in group B (p= 0.195). Seven IFI in the group A and 1 IFI in group B occurred before engraftment. All mould infections were caused by Aspergillus spp, while yeast infections were caused by C. albicans (No.1) and Torulopsis glabrata (No.1). Six month and 1-year FFS rates were similar: 71% and 66% for group A and 84% and 75% for group B respectively (p= 0.139) **(Figure 3).** Likewise, six months and 1-year OS rates were not significantly different between group A, 79% and 70%, and group B, 87% and 77%, in respectively (p= 0.302).

Risk factors for proven and probable IFIs

Table 3 illustrates risk factors for proven and probable IFI in HSCT recipients. By multivariate analysis, the use of alternative donors (MUD: OR, 3.25; CI 95%,1.13-9.39; haploidentica/PMRD:OR, 3.19; CI 95%, 0.70-14.48), previous antifungal prophylaxis with itraconazole/fluconazole (OR, 3.82; CI 95% 1.25-11.67) and a reduced intensity conditioning (OR, 4.92; CI 95% 1.95-12.39) were independent risk factors for the development of IFI after HSCT. Patients prepared for HSCT with a reduced intensity conditioning were however significantly older (median age 60 years) and mostly received itraconazole/fluconazole prophylaxis (31/40, 77%).

The potential synergistic effect of antifungal prophylaxis and empirical antifungal treatment (EAT) following induction/salvage chemotherapy on the occurrence of IFI post-HSCT was also investigated. In the whole study cohort, cumulative incidence of proven/probable IFI at 1 year post-HSCT for patients who received or did not receive EAT was 8% and 14% respectively (p=0.226). In a patient subgroup analysis of group A, itraconazole/fluconazole did not show any cumulative effect with EAT on post-HSCT incidence of IFI: 13% versus 15% respectively in patients who received and did not receive EAT (p=0.774). However, in group B, posaconazole and EAT showed a marginal statistical significance with a cumulative incidence of IFI of 2% and 13% respectively for patients who received or did not receive EAT (p=0.057).

Finally, the cumulative incidence of developing IFI was investigated by the Fine and Gray competing risk regression model to identify the effect of prognostic factors on the cumulative incidence function for competing risks data. Multivariate analysis showed that main determinants associated with a higher risk of developing IFI were the use of

alternative donors (MUD/haploidentical/PMRD vs sibling: SDHR 2.40; 95%CI 1.41-4.09), antifungal prophylaxis with itraconazole/fluconazole prior to HSCT (SDHR 3.52; 95%CI 1.19-10.39) and reduced-intensity conditionings (SDHR 3.80; 95%CI 1.72-8.41).

Transplant clinical outcomes

After a median follow-up of 63 months (range 5-160 months) after HSCT, 129 patients were alive, 66 in group A and 63 in group B. Overall, 64 patients in group A died, 38 with progressive disease and 26 because of transplant-related complications, while 36 patients in group B died, 28 with progressive disease and 8 of transplant-related complications. Five of the 18 patients (28%) with IFI in group A and 1 of the 4 patients (25%) with IFI in group B died of fungal infection. Kaplan-Meier estimate of OS at 5 years after HSCT was 55% for the entire study population, and it was significantly worse for patients who developed IFI after HSCT (9% vs 60%, $p<0.001$) and for patients who underwent HSCT after salvage chemotherapy (34% vs 63%, $p<0.001$).

DISCUSSION

It is widely assumed that induction and salvage chemotherapy represent the treatment phases with the highest risk of developing IFI for patients with AML. Thus, antifungal prophylaxis with mold-active agents is of upmost importance (13). A consistent number of studies have documented that posaconazole is highly effective in preventing IFI in AML. We then hypothesized that this protective effect may have translated into a long lasting benefit also in later phases of the disease including the post-transplant period. To address this issue, a study cohort of 229 adult AML patients were divided into two groups. Prior to HSCT, Group A received conventional azoles (flluconazole/itraconazole) while group B received posaconazole during induction/salvage chemotherapy. Both groups received prophylactic conventional azoles after HSCT.

Our study showed that 1-year cumulative incidence of proven/probable IFI after HSCT was significantly lower in group B (4%) as compared with group A (14%). The incidence of IFI in group B was superimposable to that reported by Cornely et al. (2%) (5) and Pagano et al (2.7%) (6) with posaconazole prophylaxis in AML patients, and by

Winston et al in HSCT recipients (7.5%) respectively (14). Multivariate analysis confirmed that patients receiving conventional azoles had a four-fold greater risk of IFI after HSCT as compared with those who received posaconazole during induction/salvage treatments. These findings suggest that posaconazole prophylaxis prior to HSCT may significantly reduce the fungal burden, thereby limiting the development of overt infection in the post-transplant follow up. Furthermore, we also observed a possible synergistic effect of posaconazole with EAT during induction/salvage chemotherapy. This finding may reinforce the hypothesis that a better control of fungal growth during the phases of higher risk of developing IFI may also effectively contribute to lower it during subsequent treatments including HSCT.

Interestingly, we reported a late onset of IFI (median day +209 post-HSCT) in group B even though the very low number of events (No=4) may have influenced our findings. However, the reduced incidence of IFI in group B was not associated with better clinical outcome after HSCT. This undoubtedly may have been partly due to potential confounding factors including the more frequent use of alternative donors (in two thirds of the patients) in group B. Most importantly, overall, our study showed that the occurrence of IFI remains an adverse variable for the clinical outcome of allogeneic HSCT. Therefore, efforts to maximize preventive measures of fungal infections appear of paramount importance.

Limitations of our study should be acknowledged. Given its retrospective nature, some heterogeneities between the two patient groups were inevitable. The year of AML diagnosis significantly differed over the 14-year study period. Sixty percent of the patients in group B were enrolled during the past 5 years (2011-2015) while the vast majority (84%) of the patients (84%) in group A were diagnosed between 2001 and 2010. This difference may have been clinically relevant given the recent remarkable improvements in supportive care and diagnostic options including non-invasive serological tests combined with high resolution CT scans. More patients in group A were evaluated after salvage chemotherapy as compared with group B (35% vs 18%, p=0.005). The potential effect of disease phase on the risk of IFI after HSCT was however excluded by univariate analysis.

In conclusion, the present study showed that the effects of antifungal prophylaxis with posaconazole during induction/salvage chemotherapy in AML patients may lead to long-lasting benefits including a reduced risk of IFI in patients who proceed to allogeneic HSCT. Prospective randomized large trials are warranted to confirm our findings.

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Figure Legends

Figure 1. Flow diagram outlining patients' enrollment into the study protocol

Figure 2. Cumulative incidence of IFI in AML patients submitted to allogeneic HSCT after receiving fluconazole/itraconazole (dashed line) or posaconazole (solid line) prophylaxis during induction/salvage chemotherapy (p=0.012)

Figure 3. Fungal-free survival of AML patients submitted to allogeneic HSCT after receiving fluconazole/itraconazole (dashed line) or posaconazole (solid line) prophylaxis during induction/salvage chemotherapy (p= 0.139)

Table 1. Demographic and clinical characteristics of 229 patients with AML receiving either fluconazole/itraconazole or posaconazole as antifungal prophylaxis during induction/salvage chemotherapy treatment

	Antifungal prophylaxis		
	Itraconazole (n= 73) Fluconazole (n= 57)	**Posaconazole (n= 99)**	***P value***
Median age, years (range)	48 (20-69)	49 (17-66)	0.909
Gender			
Male	71	49	0.505
Female	59	50	
Disease phase			
1st induction	84 (65%)	81(82%)	0.005
salvage	46 (35%)	18 (18%)	
Chemotherapy Treatment			
Antracycline-based	102 (78%)	74 (75%)	
Fludarabine-based	10 (8%)	11 (11%)	
High-dose Cytarabine	14 (11%)	12 (12%)	0.276
Other	4 (3%)	2 (2%)	
Median duration of neutropenia, days (range)	20 (9-50)	20 (8-50)	0.513
Median duration of antifungal prophylaxis, days (range)	24 (3-55)	20 (5-60)	0.019
EAF treatment, No. patients	26 (20%)	15 (15%)	0.494
Median duration of EAF treatment, days (range)	12 (3-27)	13 (3-20)	0.787

Abbreviation: EAF treatment, empirical antifungal treatment.

Table 2. Transplant Characteristics and outcomes according to the antifungal prophylaxis received during AML induction/salvage chemotherapy treatment: Group A, itraconazole/fluconazole; Group B, posaconazole

	Group A N= 130	**Group B N= 99**	***p***
Median interval between induction/salvage chemotherapy and HSCT, days (range)	123 (32-368)	152 (22-369)	0.002
Disease phase at transplant			
CR1	85 (66%)	79 (80%)	
CR2-CR3	20 (15%)	8 (8%)	0.056
PIF/relapse	25 (19%)	12 (12%)	
Type of HSCT			
Marched related	67 (52%)	39 (39%)	
Matched unrelated	55 (42%)	46 (47%)	0.057
Mismatched related donor	8 (6%)	14 (14%)	
Graft source			
PBSC	89 (68%)	84 (85%)	0.002
BM	32 (25%)	15 (15%)	
Cord blood	9 (7%)	-	
Preparative regimen			
Myeloablative	99 (76%)	90 (91%)	0.005
Reduced intensity	31 (24%)	9 (9%)	
Use of ATG	58 (45%)	61 (62%)	0.560
Median time to engraftment (GN>0.5 x 10^9/L)	17 (9-56)	15 (4-29)	0.560
IFI			
total	21 (16%)	7 (7%)	
Possible	3 (2%)	3 (3%)	0.043
Probable	14 (11%)	3 (3%)	
proven	4 (3%)	1 (1%)	
Median time from HSCT to IFI, days (range)	35 (7-208)	209 (8-337)	0.195
Fungal species			
Mould	16	4	1.000
Yeast	2	-	
Site of infection			
Lung	13*	3	
CNS	2	-	0.445
Blood	2	-	
Sinus	1	-	
other	-	1	
Outcome			
Alive	66 (51%)	63 (64%)	0.060
Died	64 (49%)	36 (36%)	

*In 1 case combined with CNS involvement

Abbreviation: EAF treatment, empirical antifungal treatment; PBSC, peripheral blood stem cells; BM, bone marrow

Table 3. Uni and multivariate analysis of risk factors for proven/probable IFIs among 229 patients with AML receiving allogeneic HSCT.

Variable	Univariate		Multivariate	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Time interval from chemotherapy to HSCT	0.54 (0.22-1.35)	0.191		
Disease phase of AML				
Salvage vs induction	1.54 (0.61-3.87)	0.358		
Donor type				
MUD vs MSD	3.25 (1.13-9.39)	**0.029**	3.76 (1.25-11.36)	**0.019**
Haplo/PMRD vs MSD	3.19 (0.70-14.48)	0.133	5.52 (1.07-28.38)	**0.041**
Status at HSCT				
Relapse vs CR	1.61 (0.55-4.67)	0.382		
Stem cell source				
PBSC vs BM	0.52 (0.17-1.59)	0.248		
Conditioning				
RIC vs MAC	4.92 (1.95-12.39)	**0.001**	4.16 (1.56-11.09)	**0.004**
Antifungal prophylaxis				
Itra/fluco vs posa	3.82 (1.25-11.67)	**0.019**	3.72 (1.15-12.01)	**0.028**