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ORIGINAL ARTICLE

Incidence and risk factors of post-engraftment invasive fungal disease in adult allogeneic hematopoietic stem cell transplant recipients receiving oral azoles prophylaxis

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Studies that analyze the epidemiology and risk factors for invasive fungal disease (IFD) after engraftment in alloSCT are few in number. This single-center retrospective study included 404 alloSCT adult recipients surviving >40 days who engrafted and were discharged without prior IFD. All patients who received ⩾20 mg/day of prednisone were assigned to primary oral prophylaxis (itraconazole or low-dose voriconazole). The primary end point was the cumulative incidence (CI) of probable/proven IFD using the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria. The independent prognostic factors after multivariate analyses were used to construct a post-engraftment IFD risk score. The 1-year CI of IFD was 11%. The non-relapse mortality was 40% in those developing IFD and 16% in those who did not. The intent-to-treat analysis showed that 17% of patients abandoned the assigned prophylaxis. Age >40 years, ⩾1 previous SCT, pre-engraftment neutropenia >15 days, extensive chronic GVHD and CMV reactivation were independent risk factors. The post-engraftment IFD score stratified patients into low risk (0–1 factor, CI 0.7%), intermediate risk (2 factors, CI 9.9%) and high risk (3–5 factors, CI 24.7%) ($P < 0.0001$). The antifungal prophylaxis strategy failed to prevent post-engraftment IFD in 11% of alloSCT. Our risk score could be useful to implement risk-adapted strategies using antifungal prophylaxis after engraftment.

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INTRODUCTION

Invasive fungal disease (IFD), and particularly invasive aspergillosis, is a challenging cause of morbidity and mortality in patients undergoing alloSCT.[1,2] Many studies, mainly from single-institution retrospective series, have analyzed the incidence and risk factors of these infections after alloSCT,[1,3–5] reporting a variable incidence ranging from 1 to 20%.[6,7] It is well established that severe neutropenia induced by conditioning regimens is associated with an increased risk of developing IFD after alloSCT, and it is generally accepted that antifungal prophylaxis should be administered in the pre-engraftment period. However, despite the knowledge of several post-engraftment risk factors associated with late IFD (for example, corticosteroid therapy and GVHD), considered as a guide for risk-adapted antifungal prophylaxis, the usefulness of such prophylaxis beyond the alloSCT engraftment is still controversial.[8–10] There are a few studies specifically analyzing the epidemiology and risk factors for IFD after engraftment in alloSCT adult recipients[3–5,11] and the potential impact of antifungal prophylaxis in this setting.

This study aims (1) to retrospectively analyze the incidence, outcome and risk factors of IFD after myeloid engraftment in a large series of consecutive adult patients submitted to alloSCT in a single institution, (2) to assess the impact of primary antifungal prophylaxis with oral drugs (itraconazole and low-dose voriconazole) on occurrence of post-engraftment IFD and (3) to construct a scoring system that could be useful to improve risk-adapted prophylaxis strategies with antifungal agents for patients discharged after engraftment.

MATERIALS AND METHODS

Study design and patient selection

From January 2001 to March 2013, 615 consecutive unselected patients with hematological malignancies underwent an alloSCT in the adult Transplant Unit of the Hospital Universitari i Politècnic La Fe. The clinical records of all patients were reviewed to assess their eligibility for this retrospective study. Patients were eligible if they fulfilled all the following criteria: (1) stable myeloid engraftment with neutrophil recovery, defined as more than 3 days with an absolute neutrophil count of above 0.5×10^9/L with full-donor chimerism; (2) patients who survived more than 40 days after alloSCT and were discharged from hospital (outpatient management) and able to receive oral antifungal prophylaxis protocol; and (3) patients without previous suspicion or diagnosis of IFD during the pre-transplant or early post-transplant period (that is, all patients discharged with secondary antifungal prophylaxis or antifungal therapy were not eligible). All patients who were alive at the time of our study provided informed consent according to institutional guidelines. This retrospective study was approved by the Research Ethics Board of the institution according to the Declaration of Helsinki (study number 2013/0036).

Source of progenitors, preparative regimens and GVHD prophylaxis

Patients included in the study were transplanted from a sibling or unrelated donor (cord blood (UCBT), bone marrow or peripheral blood)

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according to donor availability and alloSCT timing. Donor–recipient matching was based on HLA typing. Early disease stage at alloSCT was defined as chronic myeloid leukemia in the chronic phase, acute leukemia in the first or second CR, myelodysplastic syndrome untreated or in CR and lymphoma in CR. Preparative regimens comprised myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) modalities. The conventional preparative regimen consisted of BU plus CY until 2007, and thereafter oral or i.v. BU plus fludarabine (FLU). Patients receiving an alloSCT from an unrelated donor were given additional thiotepa (TT) and lymphoglobulin or thymoglobulin (ATG). Patients older than 55 years or with an expected high mortality using MAC regimens (for example, previous HSCT or severe co-morbidities) were selected for RIC regimen with lower doses of BU plus FLU or FLU plus melphalan. From 2007, some patients received UCBT using RIC with BUFLUTT and ATG.

Acute GVHD prophylaxis comprised cyclosporine plus methotrexate in adult siblings and unrelated identical donors alloSCT matched-related donors, except in some cases with positive CD34+ cell selection in which cyclosporine plus prednisone was used. UCBT prophylaxis consisted of cyclosporine plus prednisone or cyclosporine plus mycophenolate. Acute GVHD and chronic GVHD were graded according to criteria published elsewhere.[12,13] CMV monitoring and prophylaxis was performed according to previously reported protocols.[14]

Risk stratification, monitoring and diagnosis of IFD

After myeloid engraftment and hospital discharge, monitoring tests using serial *Aspergillus* galactomannan antigen (AGA) were performed every 7–15 days in patients who were receiving $\geqslant 20$ mg/day of prednisone (or other corticosteroid equivalent dosage). Serial AGA was performed every 7–15 days from discharge until day +100 after all unrelated alloSCT patients. For recipients of HLA-id sibling donor with < 20 mg/day of prednisone intake, AGA monitoring was not mandatory. A computer tomography scan was available for the entire study period.

Diagnosis and classification of IFD was performed according to the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) revised definitions of 2008.[15]

When fungal specimens were isolated, susceptibility testing was not performed to identify resistance to antifungal agents.

Prophylactic management and treatment of IFD after myeloid engraftment

After myeloid engraftment and hospital discharge, according to the institutional strategy of IFD prophylaxis, recipients of an HLA-id sibling donors with < 20 mg/day of prednisone intake (or other corticosteroid equivalent dosage) did not receive any type of prophylaxis against molds (fluconazole 100–400 mg/day was allowed). All patients who were receiving $\geqslant 20$ mg/day of prednisone (or other corticosteroid equivalent dosage) were assigned to a primary prophylaxis using an oral triazole (itraconazole or voriconazole). In addition, recipients of cells from an unrelated donor were assigned to a primary prophylaxis using an oral triazole from engraftment/discharge until day +100, irrespectively of the concomitant dose of prednisone. The oral triazole prophylaxis consisted of itraconazole capsules (200–400 mg/daily) from 2001 to 2004, or low-dose voriconazole (200 mg/daily until 2009 or 100 mg/twice a day from 2010). The dose of voriconazole for prophylaxis was selected in an attempt to prevent toxicity and interactions. Neither voriconazole nor itraconazole plasma concentration monitoring was performed. Oral prophylaxis was stopped when the corticosteroid dose was decreased below 20 mg/day. Oral prophylaxis was also withdrawn when limiting toxicity at the physician's discretion appeared or when antifungal treatment was indicated due to suspicion of or documented IFD.

Patients with previous documented IFD during the neutropenic period before engraftment were discharged with secondary prophylaxis or long-term antifungal therapy and therefore were excluded from the study.

Once IFD was suspected, antifungal therapy was started using monotherapy, with caspofungin (50 mg once a day) or liposomal amphotericin (3 mg/kg once a day). When diagnosis of probable or proven IFD was made, the combination of two antifungal agents (usually containing voriconazole 4 mg/kg i.v. twice a day) was instituted at physician discretion. The clinical course of IFD was monitored using the standard clinical, radiological and microbiological tests, when available.

In all patients, G-CSF was given to maintain neutrophil counts above 1.2×10^9/L.

Data collection and prognostic factors

Data were prospectively and retrospectively collected and registered in a specific form. Twenty-one patient and transplant characteristics were examined to establish their relationship with the occurrence of IFD. Demographic data and transplant characteristics included age, sex, underlying disease, disease stage, prior autologous or allogeneic HSCT, degree of HLA mismatch, type of donor, source of stem cells, stem-cell manipulation (T-cell total or partial depletion), conditioning regimen, acute GVHD prophylaxis, type of antifungal primary prophylaxis after engraftment, ABO incompatibility, donor sex, patient and donor CMV status, development of acute or chronic GVHD, CMV infection, pre-engraftment neutropenia duration and dose of corticosteroids during the first year after alloSCT. Clinical records, serial serum AGA, computerized tomography scans and other imaging tests, antifungal and immunosuppressive therapies, as well as microbiological isolates, were also reviewed.

Study definitions and end points

The primary end point of the study was to assess the 1-year cumulative incidence (CI) of and risk factors for development of post-engraftment IFD (probable and proven) in patients submitted to alloSCT.

Secondary end points were type of IFD (according to microbiological isolates, site of infection and EORTC criteria) and incidence of IFD at 6 and 12 months. Furthermore, the dose-limiting toxicities (DLTs) of voriconazole, itraconazole or fluconazole prophylaxis (defined as dose adjustment or withdrawal due to related toxicity) and the occurrence of breakthrough IFD (defined as development of probable/proven IFD during primary prophylaxis administration) were also assessed.

Response of IFD therapy was evaluated at the end of antifungal treatment. Resolved IFD was defined as clinical improvement and radiological recovery along with the absence of positive microbiologic results or progressive decline in mycological surrogate biomarkers. Treatment failure was defined as death due to direct consequences of the IFD. Patients still presenting signs or symptoms of IFD when dying by another cause were considered not evaluable.

The non-relapse mortality (NRM) rate after alloSCT was also assessed (all deaths occurring before relapse). All patients were followed until death or until the end of the observation period on October 2014.

Statistical methods

Analysis was based on an intent-to-treat principle. The Chi-square test was used to analyze differences in the distribution of variables between patient subsets. Unadjusted time-to-event analyses were calculated from the date of alloSCT. The probabilities of IFD and NRM were estimated by the CI method (for marginal probability) in order to take competing risks into account, and were compared by the Gray test.[16] In the analysis of CI of IFD, death, secondary graft failure and relapse were considered as a competing cause of failure. The characteristics selected for inclusion in the multivariate analysis were those for which there was some indication of a significant association in the univariate analysis ($P < 0.1$). Multivariate analysis was performed using the Fine and Gray model for CI.[17] The significant remaining variables ($P < 0.05$) in the multivariate analysis were used to construct a scoring system to classify the patients into groups according to their risk of IFD. The patient follow-up information was updated in October 2013, and the median follow-up in survivors was 22 months (range, 2–154 months). All *P*-values reported are two-sided. Computations were performed using the R software package (version 2.12.2), available on https://cran.r-project.org/bin/windows/base/old/2.12.2.

RESULTS

Patients and transplant characteristics

Over the period of the study a total of 615 unselected consecutive adult patients underwent an alloSCT. In all, 25 patients (4%) had primary graft failure, 29 (5%) died before day +40, 45 (7%) survived more than 40 days but died before discharge and 112 (21%) were discharged after alloSCT with ambulatory antifungal therapy or secondary antifungal prophylaxis due to suspicion or diagnosis of IFD before SCT or during the first SCT hospitalization. The remaining 404 patients (66%) who fulfilled the eligibility criteria were included in the analysis. Table 1 shows the main

Table 1. Main patient and transplant characteristics according to the occurrence of probable/proven IFD

Characteristic	*Total number of patients*	*IFD*	*No IFD*	P-*value*[a]
	n	n *(%)*	n *(%)*	
Overall	404	57 (14)	347 (86)	
Sex				
Male	232 (57)	35 (61)	197 (57)	0.61
Female	172 (43)	22 (39)	150 (43)	
Age, years				
⩽40	197 (49)	15 (26)	182 (52)	0.0004
>40	207 (51)	42 (74)	165 (48)	
Underlying disease				
ALL	91 (23)	12 (21)	79 (23)	0.79
AML/MDS	192 (48)	25 (44)	167 (48)	
NHL/HL	55 (14)	10 (18)	45 (13)	
Other	66 (16)	10 (18)	56 (16)	
Disease stage				
Early	263 (65)	36 (63)	227 (65)	0.61
Advanced	118 (29)	19 (33)	99 (29)	
Other	23 (6)	2 (4)	21 (6)	
Donor/receptor CMV serology				
Positive/positive or Negative/negative	216 (54)	28 (49)	188 (55)	0.50
Negative/positive or Positive/negative	183 (46)	29 (51)	154 (45)	
Source of SCT				
Bone marrow	10 (2)	2 (4)	8 (2)	0.02
Cord blood	144 (36)	29 (51)	115 (33)	
Peripheral blood	250 (62)	26 (46)	224 (65)	
Previous SCT				
None	325 (80)	40 (70)	285 (82)	0.05
One or more	79 (20)	17 (30)	62 (18)	
Type of transplant				
HLA identical sibling	232 (57)	23 (40)	209 (60)	0.008
Other	172 (43)	34 (60)	138 (40)	
HLA mismatch				
None	256 (63)	28 (49)	228 (66)	0.02
⩾1	148 (37)	29 (51)	119 (34)	
Modality of conditioning regimen				
MAC	269 (67)	37 (65)	232 (67)	0.89
RIC	135 (33)	20 (35)	115 (33)	
FLU containing regimen				
Yes	302 (75)	44 (77)	258 (74)	0.77
No	102 (25)	13 (23)	89 (26)	
ATG containing regimen				
Yes	178 (44)	34 (60)	144 (41)	0.1
No	226 (56)	23 (40)	203 (58)	
aGvHD prophylaxis				
Cya-methotrexate	215 (53)	26 (46)	189 (54)	0.66
Cya-prednisone	142 (35)	23 (16)	119 (34)	
Cya-mycophenolate	40 (10)	7 (12)	33 (10)	
Other	7 (3)	1 (2)	6 (2)	
Type of primary prophylaxis				
No/fluconazole	41 (10)	2 (4)	39 (11)	0.18
Itraconazole	102 (25)	14 (25)	88 (25)	
Voriconazole	261 (65)	41 (72)	220 (63)	

Abbreviations: ATG = lymphoglobulin or thymoglobulin; IFD = invasive fungal disease; MDS = myelodysplastic syndrome; NHL = non-Hodgkin lymphoma; HL = Hodgkin lymphoma; MAC = myeloablative conditioning; RIC = reduced intensity conditioning; FLU = fludarabine. [a]Chi-square comparison.

eligible patient and transplant characteristics. Briefly, 232 patients (57%) were male, and the median recipient age was 41 years (range, 14–66 years). The most frequent underlying diseases were AML (43%), ALL (23%) and chronic lymphoproliferative syndromes (14%). In 65% of patients, alloSCT was performed at an early stage of the disease. Previous HSCT was reported in 84 (21%) patients (63 autologous and 21 allogeneic). The majority of patients received alloSCT from HLA identical sibling donor 232 (57%), while 144 (36%) patients underwent an UCBT. Two hundred and sixty-nine (67%) patients received MAC, and the remaining 135 (33%) were RIC alloSCT. The conditioning regimen contained FLU in 302 (75%) patients and ATG in 177 (44%) patients.

Primary antifungal prophylaxis

The distribution of patients according to the primary antifungal prophylaxis after discharge is as follows: no prophylaxis or fluconazole 41 patients (10%), itraconazole 102 patients (25%) and voriconazole 261 patients (65%). Eighteen patients that needed an oral triazole received fluconazole, while thirty-four patients received voriconazole or itraconazole instead of fluconazole (overall, 52 (13%) of deviations according to the institutional guidelines).

Incidence, classification and outcome of post-engraftment IFD

The overall CI of IFD at 6, 12, 24 and 60 months was 8, 11, 13 and 15%, respectively (Figure 1). A total of 57 IFD episodes were documented (47 probable and 10 proven), with lungs as the most frequent site of infection, and *Aspergillus* spp. as the most frequent isolate (Table 2). The short-term outcomes of IFD episodes were resolved in 53% of patients, 16% of deaths due to IFD and 30% of deaths due to other cause but with active IFD. The pathogens directly causing death by IFD were *Aspergillus spp*. (4 patients), *A. fumigatus* (3), *A. flavus* (1) and *Criptococcus spp*. (1). The NRM at 6 and 12 months among patients who developed IFD was 12 and 40%, respectively, while patients without IFD had 9 and 16%, respectively ($P < 0.0001$).

Efficacy of primary prophylaxis for IFD

The 1-year CI of IFD was 5% in the no prophylaxis/fluconazole group, 14% in the itraconazole group and 16% in the low-dose voriconazole group. The 1-year CI of IFD in unrelated donor recipients receiving ⩾20 mg/day of prednisone was 19% under itraconazole prophylaxis and 16% under low-dose voriconazole. The 1-year CI of IFD in HLA-id sibling donor recipients receiving ⩾20 mg/day of prednisone was 5% under itraconazole prophylaxis and 10% under low-dose voriconazole.

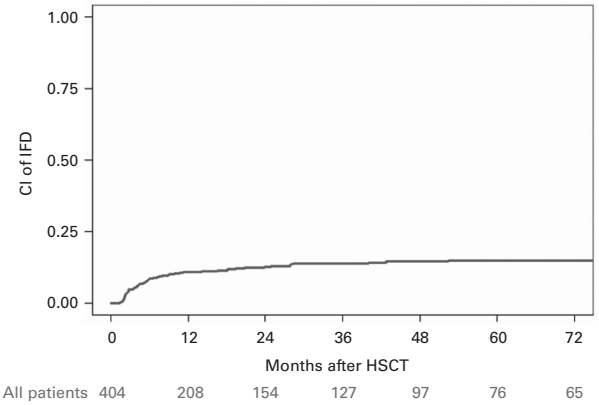

Figure 1. Cumulative incidence of post-engraftment IFD after alloSCT in the entire cohort.

Table 2. Characteristics and short-term outcomes of IFD episodes

Type of IFD	N *(%)*
Proven	10 (18)
Probable	47 (82)
Site of infection	
Lungs	44 (77)
Bloodstream	2 (4)
Rhinosinusal	3 (5)
Central nervous system	2 (4)
Skin	2 (4)
Disseminated	3 (5)
Fungal pathogen	
Aspergillus spp.	50 (88)
A. fumigatus	8
A. flavus	4
A. terreus	6
A. niger	4
A. versicolor	1
Aspergillus spp. (Galactomannan +)	27
Candida spp.	2 (4)
C. parapsilosis	1
C. krusei	1
Other	5 (9)
Rhizopus oryzae	2
*Mucor spp. (*Not specified)	1
Acremonium curvularum	1
Criptococcus ssp.	1
Response to therapy	
Resolved	30 (53)
Death due to IFD	9 (16)
Death due to other cause with active IFD	17 (30)

Abbreviation: IFD = invasive fungal disease.

Breakthrough IFD developed in 1 out of 13 patients receiving fluconazole (8%), in 9 out of 102 patients under itraconazole prophylaxis (9%) and in 25 out of 261 patients under low-dose voriconazole (10%). IFD occurred after chemoprophylaxis termination in 1 patient who received fluconazole (8%), in 5 who received itraconazole (5%) and in 16 who received low-dose voriconazole (6%).

Toxicity of risk-adapted primary prophylaxis for IFD

Overall, 68 (17%) of patients presented DLT or discontinued primary prophylaxis by any cause. DLT occurred in 28 patients (11%) under voriconazole prophylaxis, 2 patients (2%) under itraconazole prophylaxis and no patients (0%) under fluconazole ($P = 0.003$). The DLT consisted of hypertransaminasemia in 26 patients (10%), visual hallucinations in 2 patients (1%) from the voriconazole group and gastrointestinal in 2 patients under itraconazole (2%).

In addition, 19 (7%) patients from the voriconazole group discontinued prophylaxis due to concomitant hepatic GVHD. A further 19 patients (5%) discontinued primary prophylaxis because of unconfirmed suspicion of IFD (5 (38%), 7 (7%) and 7 (3%) in the fluconazole, itraconazole and voriconazole group, respectively).

The 1-year CI of IFD among patients discontinuing prophylaxis by any cause was 15, and 10% among patients who did not discontinued ($P = 0.41$). The 1-year CI of IFD among patients discontinuing due to toxicity was 10, and 22% among patients discontinuing due to concomitant hepatic GVHD.

Prognostic factors for post-engraftment IFD

The univariate analyses showed that the following factors were associated with an increased CI of IFD: age >40 years, bone marrow and cord blood SCT, $\geqslant 1$ previous SCT, non HLA-identical sibling SCT, $\geqslant 1$ HLA-mismatch, ATG-containing regimens, >15 days of pre-engraftment neutropenia, grade 2–4 aGVHD, extensive cGVHD, dose of corticosteroids >30 mg/day during the first year, and CMV reactivation until day +180 (Table 3). The following variables remained as independent risk factors in the multivariate analyses: age >40 years (HR, 3.47; $P < 0.0001$), $\geqslant 1$ previous SCT (HR, 2.56; $P = 0.004$), >15 days of pre-engraftment neutropenia (HR, 2.28; $P = 0.02$), extensive cGVHD (HR, 2.35; $P = 0.02$) and CMV reactivation until day +180 (HR, 2.20; $P = 0.009$) (Figure 2).

We assigned 1 point to each independent risk factor to build the post-engraftment IFD score system. Patients were grouped into the following categories: low risk (0–1 points), 143 patients (35%); intermediate risk (2 points), 151 patients (37%); and high risk (3–5 points), 110 patients (27%). The CI of IFD at 12 months for low-, intermediate- and high-risk patients was 0.7% (95% CI 0–2.1%), 9.9% (95% CI 5.2–14.7%) and 24.7% (95% CI 16.6–32.7%), respectively ($P < 0.0001$) (Figure 3).

DISCUSSION

This single institution retrospective study conducted in a large cohort of adult alloSCT recipients shows that in spite of using primary prophylaxis with oral azoles at discharge, the 1-year CI of post-engraftment IFD was high (11%). As expected, the clinical outcome of patients who developed late IFD was dismal (40% of NRM at 1 year). The multivariate analyses showed that factors other than the type of alloSCT and dosage of corticosteroids were independently associated with the risk of post-engraftment IFD. We built a simple prognostic score using five variables, which could be useful to improve our risk-adapted prophylactic strategies in the future.

This large series of 615 patients undergoing alloSCT at a single institution is one of the few studies to specifically analyze the incidence and risk factors of IFD after engraftment. Distinct from other studies, which analyzed the late IFD (that is, occurring beyond day +40), we have analyzed the CI of IFD in patients alive after day +40, with stable engraftment, with no antecedents of IFD, and discharged from their first alloSCT hospitalization. We did so considering that this was the target population for an outpatient primary prophylaxis against IFD, and thus, the best alloSCT subset to identify the risk factors to implement risk-adapted strategies using ambulatory regimens. In fact, the first observation is that only 404 (66%) patients were eligible for the study, as many of them had early fatal events during first hospitalization or were discharged with antifungal therapy or secondary prophylaxis. Of note, our series comprised 36% of UCBT, 21% of patients had prior SCT and almost 50% developed extensive cGHVD. For these reasons, our overall 1-year CI of 11% cannot be compared with other studies reporting late or very-late IFD incidence after alloSCT (ranging from 7 to 10%).[3–5,11] Concerning the type and timing of IFD, we found that, as in previous studies, *Aspergillus* spp. was the most frequent isolate and the lungs were the most common site,[18] and that the CI of IFD increased during the first year with a gradual decline of episodes until 5 years after SCT.

There are several limitations to this study: (1) patients who received a diagnosis of possible IFD (3%, data not shown) were included in the non-IFD group, but some of these may have had IFD, (2) necropsy studies were not systematically performed, leading to a probable underestimation of the CI of proven IFD, (3) although our retrospective analysis was limited to a historical period in which diagnosis procedures, as well as prophylactic and therapeutic management of IFD were homogeneous, we could not analyze changes of environmental exposure to the organisms, which may have had a role in the risk of IFD,[19] and (4) our intent-to-treat analysis in a real-life population including all

Table 3. Risk factors for IFD development: univariate and multivariate analyses

Characteristic	*Total number of patients*	*IFD events*	*6 months CI of IFD*	*12 months CI of IFD*	P-*value univariate analyses*	*Multivariate analysis*	P-*value multivariate analyses*
	n	n *(%)*	*%*	*%*		*Hazard ratio (95% CI)*	
Overall	404	57	8	11			
Age, years							
⩽40	197	15 (8)	4	6	0.0001	3.47 (1.86–6.51)	< 0.0001
>40	207	42 (20)	12	16			
Source of SCT							
Other	154	31 (20)	11	15	0.008		0.6
Peripheral blood	250	26 (10)	6	8			
Previous SCT							
0	325	40 (12)	7	9	0.048	2.56 (1.34–4.89)	0.004
⩾1	79	17 (22)	14	16			
Type of transplant							
HLA-identical sibling	232	23 (10)	6	7	0.005		0.5
Other	172	34 (20)	12	15			
HLA mismatch							
0	256	28 (11)	7	9	0.02		0.7
⩾1	148	29 (20)	11	14			
ATG containing regimen							
Yes	177	33 (19)	11	14	0.0003		0.9
No	227	24 (11)	6	8			
Days of pre-engraftment neutropenia							
⩽15	259	25 (10)	5	7	0.0004	2.28 (1.14–4.57)	0.02
>15	144	32 (22)	13	17			
Acute GvHD							
Grade 0–1	248	27 (11)	6	6	0.01		0.3
Grade 2–4	156	30 (19)	12	17			
Extensive cGvHD[a]							
No	194	16 (8)	5	6	0.0003	2.35 (1.21–4.56)	0.02
Yes	186	39 (21)	12	16			
Corticosteroids during the first year							
⩽30 mg/day	68	4 (6)	4	4	0.03		0.9
>30 mg/day	336	53 (16)	9	12			
CMV reactivation until day 180							
No	263	30 (11)	5	6	0.002	2.22 (1.22–4.04)	0.009
Yes	141	27 (19)	13	18			
Type of prophylaxis							
No/fluconazole	41	2 (5)	2	2	0.17		
Itraconazole	102	14 (14)	7	10			
Voriconazole	261	41 (16)	10	12			

Abbreviations: ATG = lymphoglobulin or thymoglobulin; FLU = fludarabine; HL = Hodgkin lymphoma; IFD = invasive fungal disease; MDS = myelodysplastic syndrome; NHL = non-Hodgkin lymphoma. [a]cGVHD assessed only in evaluable patients (alive after +100 days).

kinds of alloSCT recipients, is not an optimal design for a randomized clinical trial. Protocol deviations were not excluded (as much as 13%), reflecting routine clinical practice, but it limited our conclusions regarding the impact of antifungal prophylaxis.

In spite of using oral itraconazole capsules (in the first cohort) or low-dose voriconazole (in the second cohort) as primary prophylaxis for patients receiving maximum dosage of corticosteroids equal or greater than 20 mg/day, the CI of post-engraftment IFD was high, and was not affected by the type of prophylactic azole. Of note, our CI of IFD was higher than in other studies using full-dose voriconazole,[10,20] posaconazole[7] or once-weekly high dose liposomal amphotericin B[21] for similar patients. It should be noted, however, that oral itraconazole capsules have low bioavailability,[22] and low-dose voriconazole may not reach the therapeutic serum levels.[23] Our data support the view that both regimens should not be considered as optimal prophylaxes in this setting. The low-dose voriconazole regimen was instituted to avoid interactions and hepatic toxicity, but the percentage of patients developing hypertransaminasemia was similar than in other studies using full dosage of voriconazole.[6]

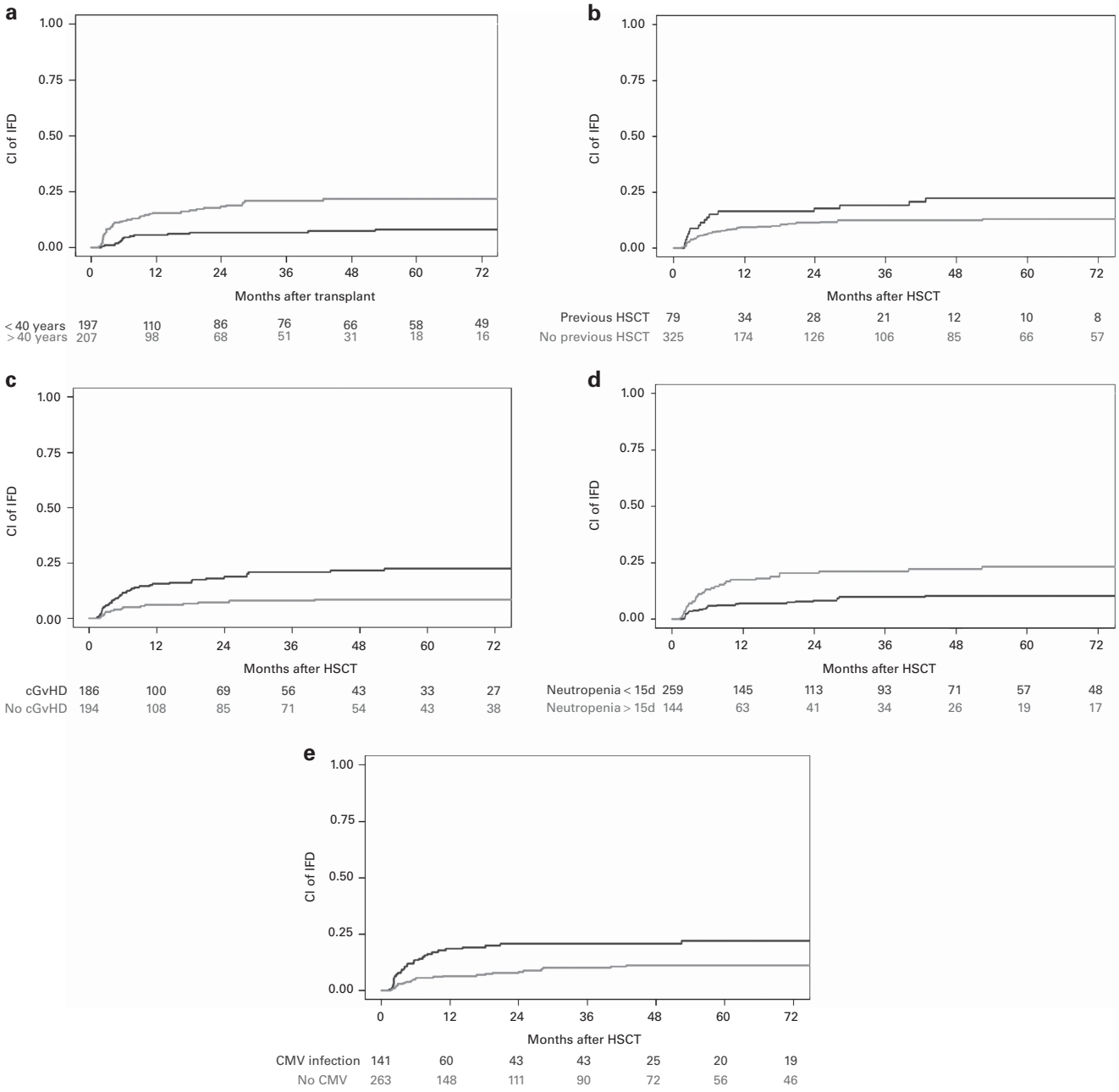

Figure 2. Cumulative incidence of post-engraftment IFD after AlloSCT according to the selected independent risk factors. (**a**) CI according to the age at alloSCT. (**b**) CI according to the number of prior AlloSCT. (**c**) CI in patients developing or not extensive chronic GVHD. (**d**) CI according to pre-engraftment neutropenia duration. (**e**) CI in patients developing or not CMV reactivation in the first 180 days after AlloSCT.

The question is whether systemic chemoprophylaxis using full-dose oral triazoles could be safe and efficacious for high-risk patients, who frequently have hepatic GVHD, critical drug–drug interactions or insufficient gastrointestinal absorption? Regarding oral voriconazole and posaconazole that may produce liver toxicity and drug interactions, we believe that successful chemoprophylaxis is feasible in the majority of patients given that the transplant physician: (1) distinguishes between drug hepatotoxicity and hepatic GVHD, as well as reinstitute chemoprophylaxis when GVHD caused the liver injury; (2) monitors plasma levels and adjust dosage of drugs interacting with triazoles (for example, cyclosporine); and (3) monitors triazole plasma levels, especially when posaconazole is used. On the other hand, we found that the 1-year CI of post-engraftment IFD in recipients of HLA-id sibling donor with < 20 mg/day of prednisone, who did not receive prophylaxis against molds, was also considerable (5%). We may hypothesize that effective prophylaxis could be valuable also for this subset of patients.[24]

Concerning the univariate analysis of prognostic factors, our findings were consistent with other studies showing that corticosteroid dosage, aGVHD, cGVHD and CMV infection could increase the risk of post-engraftment or late IFD.[1,5] In addition, we found that post-engraftment IFD was associated with several risk factors that have been previously related with early or overall IFD, such as older age, delayed neutrophil engraftment, previous SCT, HLA mismatch and non PBSCT.[3,4] Our data suggest that ATG-containing regimens could increase the risk of IFD, which is in line with other studies reporting *ex vivo* T-cell depletion using

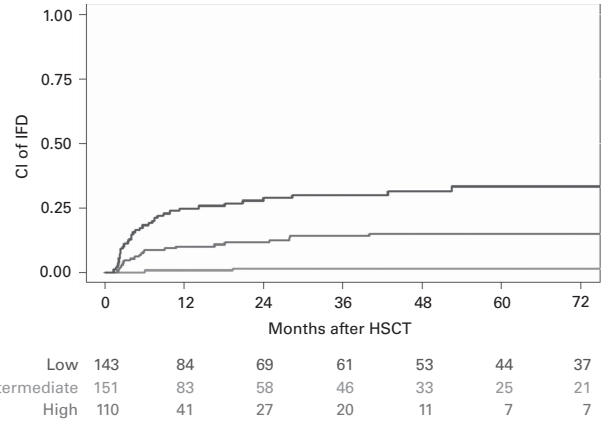

Figure 3. Cumulative incidence of post-engraftment IFD after alloSCT according to the prognostic score system.

alemtuzumab as a risk factor for IFD.[25] Unfortunately, we did not evaluate other potential risk factors such as iron overload and lymphocyte counts in the PB. Another limitation is that, due to the low number of events, we could not analyze the specific risk of developing non-Aspergillus IFD episodes.

We built a score system to predict the risk of post-engraftment IFD, using the five independent risk factors selected in the multivariate analyses. To our knowledge, no specific risk prediction scores for late or post-engraftment IFD have been reported. Our simple prognostic score model classified alloSCT recipients into three groups of roughly one-third in each. Interestingly, the low-risk group showed only 0.7% CI of IFD, while in the high-risk group it was 24.7%, allowing for improved risk-adapted prophylaxis (for example, no antifungal prophylaxis in the low-risk group, and full-dose aggressive prophylaxis in the high-risk setting). It should be noted that the score system is composed of three variables that are available at patient discharge, while CMV reactivation and development of extensive cGVHD could require time to occur. This particularity raises the question on how to implement the score in real time. As some patients would be upgraded on their risk score through the follow-up, they could require more aggressive prophylaxis based on this dynamic assessment. Nevertheless, the post-engraftment IFD score should be validated in an external cohort before its routine clinical use. It should be highlighted that the scoring system cannot be used without taking into account the environmental factors of each institution (for example, high concentrations of dust loaded with fungal spores), as they are probably *per se* independent risk factors for the development of mold IFD.

In summary, in spite of a risk-adapted antifungal prophylaxis after alloSCT, the 1-year CI of post-engraftment IFD was high (11%). Our intent-to-treat analysis showed that oral prophylaxis using itraconazole capsules or low-dose voriconazole failed to prevent episodes of IFD. After identifying five independent risk factors, we developed a risk prediction score for post-engraftment IFD, which could be useful to improve our risk-adapted prophylactic strategies in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

PM, RR-V and MAS conceived the study, analyzed and interpreted the data; PM and RR-V wrote the paper and performed the statistical analyses; BB, DM-C, IC, AL, JS, MJA, FL-C, IN, IL, MS, JP, JM, NC, IJ and GFS reviewed the manuscript and contributed to the final draft.

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