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High Incidence of Invasive Aspergillosis Associated with Intestinal Graft-versus-Host Disease following Nonmyeloablative Transplantation

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ABSTRACT

Invasive aspergillosis (IA) remains a major complication following allogeneic hematopoietic stem cell transplant (HSCT). In contrast to conventional HSCT, few investigators have examined risk factors of IA associated with nonmyeloablative (NMA) regimens characterized by outpatient administration, immunosuppression rather than cytoreduction, and short duration of neutropenia posttransplant. We report our results on a cohort of 125 patients treated homogenously who received a 6/6 matched sibling NMA HSCT designed to be performed on an outpatient basis. Conditioning regimen included fludarabine (30 mg/m^2 × 5 days) and cyclophosphamide (300 mg/m^2 × 5 days) followed by reinfusion of a minimum of 4 × 10^6 CD34$^+$ cells/kg. Acute graft-versus-host disease (aGVHD) prophylaxis consisted of tacrolimus and mycophenolate mofetil (MMF). Overall, 13 patients developed IA (5 proved, 6 probable, 2 possible) 44-791 days (median 229) after NMA HSCT, with a risk of 7% at 1, 11% at 2, and 15% at 3 years. Patients who suffered from IA had poorer overall survival (crude hazard ratio 2.3; 95% confidence interval [CI] 1.0-5.4; P = .045). Intestinal aGVHD or chronic GVHD (cGVHD) was significantly associated with IA at 1 (27% versus 3%, P = .003), 2 (27% versus 8%, P = .01), and 3 years (37% versus 10%, P = .005). The use of daclizumab was also significantly associated with IA at 3 years (47% versus 12%, P = .02). Age, sex, diagnosis, previous autologous transplant, duration of neutropenia, occurrence of cytomegalovirus viremia, duration of steroids or MMF intake, aGVHD, cGVHD, and cumulative number of days spent in hospital were not associated with IA. After multivariate analysis, intestinal GVHD remained the only statistically significant risk factor for IA at 1 (P = .003), 2 (P = .01), and 3 years (P = .005). We conclude that in NMA HSCT, the risk of IA increases over time and is significantly associated with intestinal GVHD. Because there is currently no surrogate in vitro markers of immunocompetence following NMA HSCT, this clinical finding is of particular importance to identify a population at higher risk who should be targeted for antimold prophylaxis.

KEY WORDS

Invasive • Aspergillosis • Nonmyeloablative • Transplant • Intestinal

INTRODUCTION

Invasive aspergillosis (IA) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT), occurring in 10%-15% of patients with approximately a 50% mortality rate despite appropriate antifungal therapy [1]. This high case fatality ratio is explained by the inability to improve the immunosuppression status associated with the underlying hematologic disease or therapy-associated complications. The use of intensive cytoreductive conditioning regimens, histoincompatibility, unrelated donors, T cell depletion (TCD) prolonged neutropenia, cytomegalovirus (CMV) disease, acute and extensive chronic graft-versus-host disease (aGVHD, cGVHD), as well

as care in a room without efficient air filtration have been reported as independent risk factors for IA in conventional allogeneic HSCT [2-6].

Over the last decade, there has been a significant change in conditioning regimens used prior to allogeneic HSCT. Nonmyeloablative (NMA) preparations have become increasingly used to reduce immediate posttransplant complications and broaden access to older patients and candidates with comorbidities who would not otherwise be eligible for conventional HSCT [7-12]. In contrast to conventional conditioning regimens using high-dose chemo- and/or radiation therapy, NMA regimens use immunosuppressive agents such as low-dose irradiation, fludarabine, and antithymocyte globulin (ATG) resulting in significantly less mucositis, shorter duration of cytopenias, and decreased transfusion requirements. Consequently, several transplant teams are now performing NMA on an outpatient basis, a major change in paradigm because allogeneic transplant recipients are no longer confined to a highly protective environment but sent back to their own community [13-15].

Despite the use of NMA conditioning regimens, the promise of lower risks of aGVHD and cGVHD, potentially because of a less intense cytokine storm, has not always materialized, ranging from 30% to 50% and 50% to 70%, respectively [7,16-18]. Such variations reflect heterogeneity of both transplant recipients (particularly age at transplant, a well-known risk factor for GVHD) and GVHD prophylaxis regimens. Consequently, most allogeneic NMA HSCT recipients need immunosuppressors for a prolonged period of time after transplant and become at risk for life-threatening fungal infection such as IA.

To date, it remains unclear whether risk factors for IA are the same in NMA and conventional allogeneic HSCT. In the present study, we describe the incidence, clinical characteristics, and outcomes of IA in a large cohort of 125 patients who received NMA HSCT on an outpatient basis.

PATIENTS AND METHODS

Transplant Eligibility Criteria

NMA HSCT eligibility criteria included age ≤65 years, a recognized indication for allogeneic transplant according to our transplant program, and a 6/6 HLA-compatible sibling donor. Mismatched or unrelated transplant candidates were excluded from this study. Eligible patients were offered treatment on this protocol either because they were unfit to undergo standard myeloablative transplant or because of age >55 years. Patients with newly diagnosed multiple myeloma were also invited to participate in a sequential therapy protocol (tandem) consisting of autologous transplantation followed by NMA transplant. Written informed consent was obtained from all patients.

Conditioning Regimen and aGVHD Prophylaxis

Our reduced intensity conditioning (RIC) regimen was designed to be performed as an outpatient procedure at Hôpital Maisonneuve-Rosemont (HMR), a 725-bed tertiary care center affiliated to the University of Montreal, Quebec, Canada. The conditioning regimen consisted of fludarabine 30 mg/m^2 intravenously (i.v.) and cyclophosphamide 300 mg/m^2 i.v. daily from day −8 to day −4, given from Monday to Friday in our ambulatory care facility. Tacrolimus was started at 3 mg twice a day orally on day −8 to obtain therapeutic levels (10-12 μg/L) by day 0. Therapeutic levels were maintained until day +50 and then tapered by day +100 (high-risk patients) or day +180 (standard-risk patients) according to estimated risk of relapse. Patients with chronic myelogenous leukemia (CML) in chronic phase, acute myelogenous leukemia (AML) in first complete remission, acute lymphoblastic leukemia (ALL) in first complete remission, or refractory anemia with or without ring sideroblasts were considered standard risk, whereas all other diseases were classified as high risk. Donors received granulocyte-colony stimulating factor (G-CSF) 5 μg/kg subcutaneously twice a day for 9 doses, and were collected by daily apheresis to reach a target of ≥4×10^6 CD34$^+$ cells/kg. Allogeneic stem cells were infused on day 0 after being stored at 4°C overnight, and no attempt was made to limit numbers of CD34$^+$ cells infused. Mycophenolate mofetil (MMF), used instead of methotrexate to avoid mucositis, was initiated at 1000 mg twice a day orally 24 hours after the last infusion of stem cells and discontinued without tapering on day +50. MMF was not weight adjusted and levels were not measured. aGVHD and cGVHD were graded according to clinical presentation with appropriate biopsies using previously reported criteria [19-21].

All recipients were followed in the outpatient clinic by the same medical and nursing staff thrice weekly until neutropenia resolved and every 1-2 weeks thereafter. Red blood cells (RBC) or platelet transfusions were given when hemoglobin was ≤85 g/L or platelet count ≤15×10^9/L. Chimerism studies were performed using short variable tandem repeats by polymerase chain reaction (PCR) assay (GenePrint STR Systems, Promega, Madison, WI) in both lymphocytes and neutrophils every 2 weeks for the first 8 weeks, then monthly for 2 months, and every 3 months thereafter.

Antimicrobial Prophylaxis

Trimethoprim/sulfamethoxazole (TMP/SMX) 1 double strength tablet twice a day was given on weekend days for *Pneumocystis jirovecii* prophylaxis starting day −3 until 1 month after discontinuation of all immunosuppression. Patients seropositive for herpes

simplex received acyclovir 200 mg three times a day orally from day −8 until day +21. CMV seropositive recipients or recipients transplanted from a CMV-seropositive donor were followed using a preemptive approach of weekly quantitative PCR (Cobas Amplicor CMV Monitor, Roche Diagnostics, Branchburg, NJ) from day +14 until day +100 [22]. Upon detection of CMV viremia, patients were promptly treated with intravenous ganciclovir 5 mg/kg twice a day for a minimum of 4 weeks and until weekly negative CMV PCR were obtained over 2 weeks. No patient had previously had IA and no antifungal prophylaxis was administered. G-CSF was only used in neutropenic patients with fever or documented infection and discontinued as soon as neutrophils reached 1.5×10^9/L. Patients developing aGVHD and cGVHD received TMP/SMX twice a day until immunosuppression was completely discontinued. Immunoglobulins (i.v.) (0.5 mg/kg every 28 days) were not routinely used after transplant but given to patients with bronchiolitis obliterans or in presence of hypogammaglobulinemia and recurrent respiratory infections.

Treatment of GVHD

Briefly, patients with grade I aGVHD were treated with topical steroids. All patients with a diagnosis of grade II-IV aGVHD received methylprednisolone 1 mg/kg i.v. twice a day for 8 doses; responders were then treated with methylprednisolone 0.9 mg/kg i.v. twice a day for 8 doses, then 0.8 mg/kg i.v. twice a day for 8 additional doses, then switched to prednisone 1.4 mg/kg daily for 1 week, after which prednisone was subsequently tapered to 0.6 mg/kg over 3 weeks (weeks 1-4). Prednisone was then decreased to 0.6 mg/kg every other day over 3 weeks (weeks 5-7) and tapered completely over 3 more weeks (weeks 8-10). Tacrolimus blood levels were maintained between 10 and 15 μg/L for 4 weeks, then tapered progressively until day +180. Patients not responding or progressing after 8 doses of methylprednisolone and those with grades III-IV at presentation received MMF 15 mg/kg i.v. twice a day, whereas patients unresponsive/refractory to steroids were given daclizumab 1 mg/kg i.v. × 5 doses [23]. Patients improving following MMF or daclizumab received prednisone for a total of 8 weeks; MMF and tacrolimus were both given for 4 months, after which MMF was tapered over 4 weeks. Tacrolimus was subsequently stopped 4 weeks later. Progressing/relapsing patients at taper were retreated with i.v. methylprednisolone, tacrolimus, MMF, and daclizumab as previously reported [23,24].

Patients with extensive cGVHD were all treated with systemic therapy. De novo and quiescent cGVHD were treated with prednisone 1 mg/kg daily for 6-8 weeks; responsive patients were then switched to prednisone 1 mg/kg every other day and treated for a minimum of 4 months, followed by a taper of 10 mg/kg/week until complete discontinuation.

Unresponsive/relapsing patients received cyclosporine A (CSA) 10 mg/kg/day (or tacrolimus 0.1 mg/kg/day) every other day in addition to prednisone for a minimum of 6 months; calcineurin inhibitors were then tapered progressively over 4-6 weeks 1 month after discontinuation of prednisone. Progressive cGVHD was treated with both CSA 10 mg/kg/day and prednisone 1 mg/kg/day for 4 weeks; patients improving were then switched to alternate therapy for 9 months, after which prednisone was first tapered over 10 weeks, followed by CSA over 10 weeks. In addition, unresponsive/progressing patients were treated with MMF 15 mg/kg by mouth twice a day. Daclizumab 1 mg/kg × 5 doses was used in patients with cGVHD unresponsive to the triple combination of prednisone, a calcineurin inhibitor, and MMF.

Diagnosis of Invasive Aspergillosis

IA was defined according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [25]. Proven disease required histopathologic or microbiologic documentation of disease in tissues, whereas probable infection was defined by identification of fungus from culture of bronchoalveolar lavage (BAL) fluid in a patient with 1 major (typical new infiltrates on CT imaging) or 2 minor clinical criteria (symptoms, signs on physical examination, or nonspecific infiltrate on CT imaging). IA was considered possible if *Aspergillus* sp. was isolated from BAL in a patient with only 1 minor clinical criterion. The day on which the first positive diagnostic test was performed was considered the day of diagnosis. Because the galactomannan assay was not routinely nor prospectively used in our institution during the study period, this data was not included in our analysis.

Statistical Analysis

The risk of developing IA over time was calculated using Kaplan-Meier plots, in which day 0 was the day of NMA HSCT and the end point was the day of diagnosis of IA, with censoring at the date of last follow-up or death. Risk factors associated with IA were identified in univariate and multivariate Cox regression models. Results are expressed as crude (CHR) and adjusted hazard ratios (AHR) with their 95% confidence intervals (CI). Models were built up sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance or altered the hazard ratios of variables already in the model.

Table 1. *Characteristics of 125 Patients Who Underwent Outpatient NMA Allogeneic HSCT*

	Number of Patients N = 125 (%)
Age (years)	
20-44	**11 (9)**
45-49	**24 (19)**
50-54	**32 (26)**
55-59	**28 (22)**
≥60	**30 (24)**
Sex (M/F)	**70 (56)/55 (44)**
Indication for NMA HSCT	
Multiple myeloma	**56 (45)**
Non-Hodgkin lymphoma	**34 (27)**
Other hematologic malignancies	**35 (28)**
Previous autologous transplant	**88 (70)**
Severe neutropenia following NMA HSCT*	**19 (15)**
Cytomegalovirus viremia†	**44 (35)**
Grades II-IV aGVHD	**8 (6)**
cGVHD	**95 (76)**
Intestinal GVHD‡	**21 (17)**
Duration of MMF intake (days)	
30-59 days	**66 (53)**
≥60 days	**59 (47)**
Use of steroids	**101 (81)**
0 day	**24 (19)**
1-59 days	**11 (9)**
≥60 days	**90 (72)**
Use of daclizumab	**8 (6)**
Need for hospital admission†	**83 (66)**

NMA indicates nonmyeloablative; HSCT, hematopoietic stem cell transplant; GVHD, graft-versus-host disease; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

*Severe neutropenia defined as neutrophils $<0.1 \times 10^9$/L within 30 days of conditioning regimen.

†At any time following NMA HSCT.

‡Intestinal GVHD was defined as aGVHD or cGVHD with intestinal involvement.

RESULTS

Between July 2000 and June 2006, 125 consecutive patients underwent outpatient NMA HSCT at HMR. Median follow-up period of our cohort is 2 years, and ranges from 70 days to 5.7 years. Patients' characteristics are shown in Table 1. Median age at transplantation was 53 years (range: 20-65) and the most common indication was multiple myeloma (45% of all patients). Previous autologous HSCT was done in 88 (70%) patients, either as part of an upfront tandem procedure (n = 36) or as dictated by the patients' medical condition (n = 52). Engraftment occurred in all patients but 4 (2 with CML and 2 with chronic myelomonocytic leukemia). Severe neutropenia ($<0.1 \times 10^9$/L) following transplant occurred in only 19 (15%) patients and was of short duration (median 3 days, range: 1-4).

Overall, CMV viremia was detected in 44 (35%) patients, a median of 7 weeks post-NMA HSCT. Of these, 41 had viremia within 6 months of NMA HSCT. aGVHD developed in only 14 patients (11%): 6 had grade I, 6 grade II, and 2 grade III aGVHD. In sharp contrast, extensive cGVHD was identified in 95 patients (76%). Only 5 patients suffered from both aGVHD and cGVHD. Intestinal involvement (by either aGVHD or cGVHD) occurred in 21 patients (17%). In 20 patients, intestinal GVHD occurred within 6 months of NMA HSCT (range: 36-154 days; median 128), and in 1 patient 23 months posttransplant. MMF administration ranged from 36 to 1704 days, with 47% of patients receiving at least 60 days. Steroids were required for most (81%) patients for a mean duration of 15 months (median 10 months). Daclizumab was administered in 8 patients (6%), all with severe intestinal GVHD.

Although our NMA protocol was designed to be performed on an outpatient basis, hospitalization at any given time between stem cell infusion and the last follow-up visit was required for 83 (66%) patients. The most common causes of first hospitalization following transplant included complications related to aGVHD or cGVHD (n = 16; 20%), pneumonia, or other bacterial infections (n = 15; 19%), varicella-zoster infections (n = 6; 7%), febrile neutropenia (n = 6; 7%), relapse of initial hematologic malignancy (n = 6; 7%), CMV disease (n = 3; 4%), and IA (n = 3; 4%). The cumulative number of days spent in the hospital at any time following NMA HSCT ranged from 2 to 258 (median 18 days).

Overall, 13 patients developed IA, with an incidence of 70 of 1000 persons-year at 1 year, 110 of 1000 persons-year at 2 years, and 150 of 1000 persons-year at 3 years. Of our 13 patients with IA, 5 were classified as proven, 6 as probable, and 2 as possible. IA was diagnosed between 44 and 791 (median 229) days after NMA HSCT (Table 2). Six patients with intestinal GVHD experienced IA, 40-102 (median 68) days after diagnosis of intestinal GVHD. In 4 patients, IA occurred while GI GVHD was active and poorly controlled. Two outpatients were diagnosed (2 and 3 months) after GI GVHD clinical manifestations had resolved but while still taking heavy immunosuppression. No case of intestinal IA was observed. The diagnosis was clinically unsuspected and established at autopsy in 1 patient; all other patients received appropriate antifungal therapy for a median of 70 days (range: 5-194). Seven patients died: 6 while on appropriate antifungal therapy for 5 to 189 days, and the other 6 months after completing antifungal therapy.

Survival at 1, 2, and 3 years following NMA HSCT in all 125 patients was 86%, 79%, and 72%, respectively. As shown in Figure 1, patients who suffered from IA had poorer survival (overall CHR 2.3; 95% CI 1.0-5.4; $P = .045$): 77% versus 88% at 1 year, 69% versus 80% at 2 years, and 43% versus 77% at 3 years. Death was considered directly attributable to IA in 2 patients: 1 died before the diagnosis of IA was

Table 2. *Cases of Invasive Aspergillosis following Outpatient NMA Allogeneic HSCT in 125 Patients with Hematologic Malignancy*

	Species	Day after NMA HSCT	Culture or Histopathologic Positive Specimen
Proved			
1	*Aspergillus* sp.	190	Autopsy
2	*A. fumigatus*	229	Autopsy*
3	*A. fumigatus*	239	Autopsy*
4	*A. fumigatus*	497	Autopsy*
5	*A. fumigatus*	793	Transbronchial biopsy
Probable			
6	*Aspergillus* sp.	44	Sputum
7	*A. fumigatus*	154	BAL + sputum
8	*A. fumigatus*	212	BAL
9	*A. fumigatus*	545	Sputum
10	*A. glaucus*	723	BAL
11	*A. fumigatus*	763	Sputum
Possible			
12	*A. fumigatus*	169	Sputum
13	*A. versicolor*	188	BAL

A indicates *Aspergillus*; NMA, nonmyeloablative; HSCT, hematopoietic stem cell transplant; BAL, bronchoalveolar lavage.
*Clinical diagnosis was made before death on bronchoalveolar lavage (positive cultures).

established, whereas the other expired 6 days later. IA was a contributing cause in 4 additional patients who died from the following (days after diagnosis of IA): septic shock and acute respiratory distress syndrome (day +4), CMV pneumonia (day +7), pulmonary plasmocytoma (day +15), and severe refractory cGVHD with multiple organ failure (day +105). Finally, 1 patient died from *Clostridium difficile* colitis and pulmonary mucormycosis 6 months after completing anti-Aspergillus therapy.

We then sought to identify potential risk factors for IA following NMA HSCT and results of univariate analysis are shown in Table 3. Among the 14 patients who presented with aGVHD, 1 did so after developing IA. IA following aGVHD occurred in 1 of 6 patients with grade I and in 2 of 6 patients with grade II GVHD. Although the 1-year risk of IA was significantly higher among patients who developed grades I-IV aGVHD (20% versus 6%, $P = .02$), it was no longer significant when restricting to grades II-IV. Surprisingly, cGVHD was not associated with IA in our cohort of patients.

Among patients developing GVHD, we hypothesized that more profound immunosuppression would further increase the risk of IA. Because intestinal GVHD is usually associated with prolonged hospitalizations and immunosuppression, we elected to study this subset of patients. We indeed found that intestinal GVHD, whether aGVHD (n = 5) or cGVHD (n = 16), was significantly associated with IA at 1 (27% versus 3%; CHR 8.9; 95%CI 2.1-37.4; $P = .003$), 2 (27% versus 8%; CHR 4.6; 95%CI 1.4-15.1; $P = .01$), and 3 (37% versus 10%; CHR 4.7; 95%CI 1.6-14.0; $P = .005$) years. Of note, 1 patient developed acute intestinal GVHD after diagnosis of IA and was therefore considered as not having developed intestinal GVHD for this analysis. Although the incidence of IA was more frequent in patients taking steroids during the study period (8% versus 3% at 1 year, 12% versus 4% at 2 years, 16% versus 4% at 3 years), this observation did not reach statistical significance. Among 100 patients taking steroids, 12 eventually developed IA; the risk was significantly higher among those who received 1-59 days of steroids at 2 (CHR 9.2; 95%CI 1.0-83.4; $P = .05$) and 3 years post-NMA HSCT

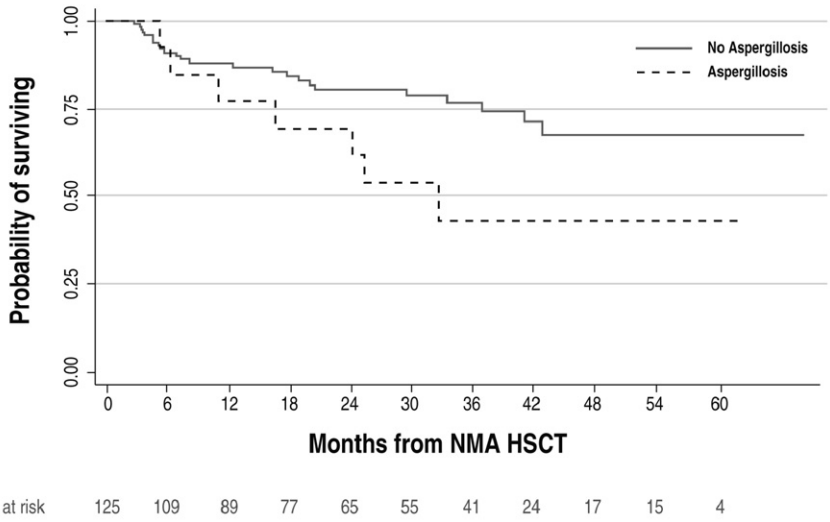

Figure 1. Kaplan-Meier probability of survival following NMA allogeneic HSCT in patients who did or did not develop invasive aspergillosis (log-rank test $P = .045$). NMA, nonmyeloablative; HSCT, hematopoietic stem cell transplant.

Table 3. *Risk Factors for Invasive Aspergillosis among 125 Patients Who Underwent Outpatient NMA Allogeneic HSCT*

	Patients with IA/Total	Risk of IA (%)			Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)*
		1 Year	2 Years	3 Years		
Cohort overall	13/125	7	11	15		
Age at time of HSCT						
<55 years	8/67	9	12	15	1.0	1.0
≥55 years	5/58	5	11	15	0.8 (0.3-2.4)	0.8 (0.2-2.3)
Sex						
Female	7/55	8	17	17	1.0	1.0
Male	6/70	7	7	13	0.7 (0.2-2.0)	0.6 (0.2-1.9)
NMA HSCT indication						
MM	8/56	10	15	18	1.0	1.0
NHL	4/34	9	9	18	0.8 (0.2-2.6)	0.9 (0.3-3.1)
Other	1/35	0	7	7	0.3 (0.03-2.2)	0.4 (0.04-3.7)
Previous autologous HSCT						
No	2/37	0	5	13	1.0	1.0
Yes	11/88	10	14	16	2.1 (0.5-9.5)	1.5 (0.3-7.2)
Duration of neutropenia†						
0-9 days	5/59	4	10	16	1.0	1.0
≥10 days	8/66	11	14	16	1.5 (0.5-4.6)	1.6 (0.5-4.8)
CMV viremia						
No	7/81	5	11	11	1.0	1.0
Yes	6/44	14	13	25	2.0 (0.7-6.0)	1.7 (0.6-5.1)
aGVHD (grades)‡						
0-I	11/117	7	11	13	1.0	1.0
II-IV	2/8	14	14	43	3.5 (0.8-15.8)	2.7 (0.7-9.9)
cGVHD (N = 122)§						
No	1/28	4	5	5	1.0	1.0
Yes	11/94	7	12	16	1.9 (0.3-15.1)	1.2 (0.2-10.2)
Intestinal GVHD‡‖						
No	7/105	3	8	10	1.0	1.0
Yes	6/20	27¶	27¶	37¶	4.7 (1.6-14.0)¶	4.7 (1.6-14.0)¶
Duration of MMF intake						
30-59 days	5/66	10	10	10	1.0	1.0
≥60 days	8/59	5	12	18	1.3 (0.4-3.9)	1.1 (0.3-3.3)
Duration of steroid intake						
0	1/25	3	4	4	1.0	1.0
1-59 days	4/13	30	56#	69#	10.2 (1.1-93.3)#	6.4 (0.6-62.7)
≥60 days	8/87	5	8	13	1.2 (0.1-9.3)	0.7 (0.08-6.1)
Daclizumab						
No	10/117	6	10	12	1.0	1.0
Yes	3/8	30#	30	47#	4.4 (1.2-16.1)#	1.4 (0.3-7.1)
Cumulative hospitalization—days post-HSCT (hazard ratio for each additional day; 95% CI)	NA	1.007 (0.995-1.019)	1.003 (0.991-1.015)	1.004 (0.994-1.015)	1.004 (0.994-1.014)	1.002 (0.990-1.015)

IA indicates invasive aspergillosis; HSCT, hematopoietic stem cell transplantation; MM, multiple myeloma; NHL, Non-Hodgkin lymphoma; GVHD, graft-versus-host disease; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CMV, cytomegalovirus; MMF, mycophenolate mofetil; NA, not applicable.

*Adjusted for presence of intestinal GVHD.

†Neutropenia was defined as neutrophils $<0.5 \times 10^9$/L following NMA transplant.

‡One patient developed acute intestinal GVHD after diagnosis of IA and was therefore considered as not having developed acute intestinal GVHD for these analysis.

§For cGVHD analysis, 3 cases were excluded because of death (n = 2) or development of IA (n = 1) before day +100.

‖Intestinal GVHD was defined as aGVHD or cGVHD with intestinal involvement.

¶$P < .01$.

#$P < .05$.

(CHR 11.4; 95%CI 1.2-104.6; $P = .03$). Daclizumab was given to 8 patients, but 3 of them developed IA. At 1 year posttransplant, patients who had received daclizumab were 5 times more at risk of developing IA (30% versus 6%; CHR 5.0; 95%CI 1.0-24.7; $P = .049$). At 3 years, having received daclizumab was even more significantly associated with the risk of IA (47% versus 12%; CHR 4.4; 95%CI 1.2-16.1; $P = .02$). Age, sex, underlying hematologic diagnosis, previous autologous transplantation, duration of neutropenia following NMA HSCT, occurrence of CMV viremia, duration of MMF intake, and cumulative number of

days spent in the hospital were not associated with IA in univariate analyses.

Following multivariate analysis, intestinal GVHD remained the only statistically significant risk factor for IA at 1, 2, and 3 years as well as for the entire follow-up period. All patients who received daclizumab had intestinal GVHD. Among patients who did not receive daclizumab, intestinal GVHD remained significantly associated with IA (27% versus 10%; CHR 4.0; 95%CI 1.0-15.4; $P = .046$).

DISCUSSION

We report a risk of IA of 7% at 1 year, 11% at 2 years, and 15% at 3 years in a large, homogenously treated cohort of 125 HSCT recipients. Our cohort is the largest reported thus far that specifically sought to identify both incidence and risk factors for IA among recipients of a 6/6 matched sibling NMA transplantation [26-29]. It is characterized by short duration of neutropenia, absence of mucositis, and a very low risk (11%) of grade I-IV aGVHD, which allows transplant to be conducted on an outpatient basis. Of note, a majority of patients (76%) experienced extensive cGVHD requiring immunosuppression; 66% were eventually hospitalized for a median of 18 days, primarily from GVHD-related conditions.

Increasing risk of IA over time in NMA transplant recipients has not been reported before. Previously studied NMA cohorts have either not stated a cumulative risk [29] or published risks at 1 year only [26-28]. Increasing risk over time most likely reflects ongoing T cell function impairment from GVHD and/or immunosuppressive medication [5,6]. This emphasizes the need for longer follow-up in patients who have received NMA transplantation to precisely determine those at risk of IA and the continuity in that risk. Our 7% 1-year risk is lower than the 15% and 11% reported by the Seattle team in 2 cohorts of NMA transplant recipients of 56 (52 HLA-matched related donors; 4 HLA-matched unrelated donors) and 163 (108 HLA-matched related donors; 55 HLA-matched unrelated donors) patients, respectively [26,27]. In contrast, it is higher than what has been observed by the Spanish Transplant Group (4%) in 71 sibling matched recipients [28]. The reasons for such variations remain unclear. Nevertheless, our observation of an increasing risk of IA over time indicates that further studies are needed to better define patients who could benefit from systematic surrogate marker surveillance and preemptive antifungal therapy. Oral antifungal therapy will be particularly challenging in patients with intestinal GVHD owing to poor oral tolerance and potentially erratic drug absorption. Clearly, future preemptive studies will need to address these issues.

Our most significant finding is an association between IA and intestinal GVHD at 1, 2, and 3 years posttransplant, which has not been reported before. Following multivariate analysis, we found that intestinal GVHD was the only significant risk factor for IA, with a risk 9-fold higher at 1 year and 5-fold higher at 2 and 3 years among patients with this complication. Intestinal GVHD is a morbid complication of allogeneic HSCT that may occur early (as part of aGVHD) or later (after day +100, as part of multisystemic involvement of cGVHD) [30,31]. When compared to conventional allogeneic HSCT, NMA HSCT is associated with a lower incidence of aGVHD but a similar rate of cGVHD, as well as a late onset and more severe gut morbidity occurring 6 to 12 months after transplant [32]. The change in presentation of GVHD following NMA transplant underlines the need to revisit the risk factors in this population [33].

The association between IA and intestinal GVHD in our cohort of NMA recipients most likely reflects the heavy immunosuppression encountered in these patients. Patients with intestinal GVHD usually require aggressive treatment including hospitalization, parenteral nutrition, and a combination of immunosuppressors for adequate control of disease. Intestinal GVHD manifestations usually resolve gradually, which in turn, results in prolonged hospitalization and requires the use of several immunosuppressive drugs. Our reported association between IA and the administration of daclizumab in steroid refractory intestinal GVHD patients is in keeping with this hypothesis [23,34-36]. Although survival rates of patients with IA in our cohort are comparable to those observed after conventional [2] or other NMA allogeneic transplant series [26], these characteristics are important to recognize because patients bearing them should be candidates for antimold prophylaxis [37]. Additionally, clinical criteria identifying patients at higher risk of infection may be particularly useful considering the lack of surrogate in vitro markers of immunocompetence. As a corollary, our observation of a higher risk of IA in patients with intestinal GVHD emphasizes that not all patients with cGVHD have the same degree of T cell deficiency, and that subsets of patients should be analyzed separately in the future. Further studies are also needed to decipher the mechanisms of immune deficiency associated with intestinal GVHD and clarify whether intestinal involvement aggravates immune deficiency or this condition requires more immunosuppression.

In contrast to conventional allogeneic HSCT, IA was not associated with duration of neutropenia or CMV viremia in our cohort [2]. Infusion of blood stem cells and the lack of methotrexate (MTX) for GVHD prophylaxis contributed to an extremely short duration of neutropenia (median: 3 days) which was present in only a minority (15%) of patients, possibly

decreasing the risk of IA immediately post transplant. CMV viremia was also managed with a preemptive therapy approach using short course ganciclovir avoiding neutropenia. Similar to studies of risk factors for IA in conventional HSCT, IA was not associated with age, sex, initial diagnosis, previous transplant, and use of MMF [26]. MMF is usually given in combination with calcineurin inhibitors for prevention of aGVHD and with other immunosuppressors (steroids, daclizumab) for treatment of cGVHD, making difficult the definition of its particular contribution to the occurrence of IA [27]. Finally, despite the fact that our cohort of NMA sibling transplants is the largest reported thus far, it remains limited by the few cases of IA observed. Given these limitations in power, it is possible that having intestinal GVHD ultimately represented a composite variable of the several modes of immunosuppression used for its control. A larger multicentric study or meta-analysis might allow the identification of specific components of immunosuppression driving the risk of IA.

In conclusion, despite an apparent overall low risk of IA in our population of NMA HSCT recipients, we report a high 1-year risk of IA of 27% in the subgroup of patients who developed intestinal GVHD. These patients most likely have profound T cell dysfunction resulting from intensive immunosuppressive therapy and GVHD; they should be considered for close monitoring with surrogate markers of invasive mold infections as well as for antimold prophylaxis.

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