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Original Article

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Risks and outcomes of invasive fungal infections in pediatric allogeneic hematopoietic stem cell transplant recipients receiving fluconazole prophylaxis: a multicenter cohort study by the Turkish Pediatric Bone Marrow Transplantation Study Group

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Abstract

Invasive fungal infections (IFIs) are a major cause of infection-related morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Data from pediatric settings are scarce. To determine the incidence, risk factors and outcomes of IFIs in a 180-day period post-transplantation, 408 pediatric patients who underwent allogeneic HSCT were retrospectively analyzed. The study included only proven and probable IFIs. The cumulative incidences of IFI were 2.7%, 5.0%, and 6.5% at 30, 100, and 180 days post-transplantation, respectively. According to the multivariate analysis, the factors associated with increased IFI risk in the 180-day period post-HSCT were previous HSCT history (hazard ratio [HR], 4.57; 95% confidence interval [CI] 1.42–14.71; $P = .011$), use of anti-thymocyte globulin (ATG) (HR, 2.94; 95% CI 1.27–6.80; $P = .012$), grade III–IV acute graft-versus-host-disease (GVHD) (HR, 2.91; 95% CI 1.24–6.80; $P = .014$) and late or no lymphocyte engraftment (HR, 2.71; 95% CI 1.30–5.62; $P = .007$). CMV reactivation was marginally associated with an increased risk of IFI development (HR, 1.91; 95% CI 0.97–3.74; $P = .063$). IFI-related mortality was 1.5%, and case fatality rate was 27.0%.The close monitoring of IFIs in pediatric patients with severe acute GVHD who receive ATG during conditioning is critical to reduce morbidity and mortality after allogeneic HSCT, particularly among those with prior HSCT and no or late lymphocyte engraftment.

Key words: allogeneic hematopoietic stem cell transplantation, invasive fungal infections, risk factors, children.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has been an increasingly utilized therapeutic approach for patients with hematologic disorders, malignant disorders, immunodeficiencies, and metabolic disorders. Invasive fungal infection (IFI) after HSCT is a major cause of infectious morbidity and mortality due to the therapies that are used to prevent and treat graft-versus-host-disease (GVHD), and post-transplant leukopenia.[1–3] Despite the recent progress in the diagnosis of IFIs in immunocompromised hosts, which include the galactomannan (GM) antigen enzyme-linked immunosorbent assay and polymerase chain reaction tests for fungal DNA, the diagnosis of this condition remains very difficult, particularly in the early phase of transplantation and for patients with critical status, and this condition may not be discovered until autopsy.[4–8] Due to the poor outcomes of IFI among patients who undergo allogeneic HSCT, identification of the risk factors and prognostic factors is very important for the development of tailored prevention and treatment approaches.

Most of the data for the incidence of IFI among pediatric allogeneic HSCT recipients have originated from cohorts that included both pediatric and adult patients. Studies that focus on pediatric patients are mainly from single-institution series, are limited, and use relatively small cohorts.[9–16] Based on previous reports, the documented IFI incidence among pediatric patients who have undergone allogeneic HSCT has been reported to range from 8 to 24% with a case fatality rate of 37–90%.[9–12,14–16] The incidence peaks in both the pre- and post-engraftment periods.[17] Since the 1990s, after the initiation of the use of prophylactic fluconazole in bone marrow transplantation, a significant reduction in the incidence of IFI was observed, especially among IFIs due to yeasts. However, IFI due to moulds remain a severe problem after transplantation.[18,19] The fungal agents that are primarily responsible for IFIs are *Aspergillus* spp., *Candida* spp., and mucormycosis.[17]

Previously identified risk factors for IFI include age at transplantation, diagnosis, transplantation type, stem cell source, prolonged and profound neutropenia, lymphocytopenia, high-dose steroids, severe acute and chronic graft-versus-host disease (a- and c-GVHD, respectively), and fungal colonization prior to transplantation.[9–11,14,15,17,20]

First 180 days following HSCT is very critical for fungal infections because the immunological functions are not fully recovered and GVHD is a frequent problem. We designed a retrospective multicenter study to determine the frequency of IFI, to identify the risk factors, and to analyze the influence of IFI on non-relapse mortality (NRM) and overall survival (OS) during the first 180-days, in pediatric transplant recipients receiving fluconazole prophylaxis.

Methods

Study population

We retrospectively reviewed the medical records of 408 pediatric patients (age ≤18 years at the time of HSCT)

who had no prior IFI histories and who underwent allogeneic HSCT in 13 different centers in Turkey from January 1 to December 31, 2014. For patients who received more than one transplant, only the last transplant was included. All patients received 10–12 mg/kg/day fluconazole up to at least day +70 depending on the transplant center's policy. Oral prophylaxis was discontinued in cases of toxicity at the physician's discretion or when antifungal treatment was indicated due to suspicion of or documented IFI. For patients with high IFI risk, voriconazole, liposomal amphotericin-B (L-AmB), or caspofungin was used as antifungal prophylaxis. High IFI risk included grade II-IV aGVHD and prednisolone treatment with a minimal dosage of 1 mg/kg/day for more than 1 week. These "high-risk" criteria were strictly followed by all centers. During the transplantations, routine anti-infective prophylaxis with quinolones and acyclovir were provided, and trimethoprim-sulfamethoxazole was given for *Pneumocystis jiroveci* prophylaxis after myeloid engraftment was achieved. All patients were screened weekly for cytomegalovirus (CMV), and ganciclovir was initiated preemptively upon the detection of CMV DNA by polymerase chain reaction (PCR). All patients stayed in single rooms with positive pressure and high-efficiency particulate air (HEPA) filters. Empirical anti-pseudomonal and broad-spectrum antibiotic therapy with or without a glycopeptide combination was immediately initiated in cases of febrile neutropenia depending on the transplant center's policy. GM analysis was performed once a week in cases of clinical suspicion of fungal infection in 4 centers, and twice a week during the neutropenic period in others. GM was interpreted to be positive for values >0.8 ng/mL once or >0.5 ng/ml in two consecutive samples for all centers. High-resolution lung computed tomography was performed in cases of clinical suspicion. In case of unexplained fever with negative bacterial cultures during neutropenia >96 hours, empirical antifungal therapy was initiated with L-AmB, voriconazole or caspofungin, depending on the transplant center's policy. Once IFI was suspected, antifungal therapy was initiated as a monotherapy with L-AmB (3 mg/kg, IV, once a day) or voriconazole (4–8 mg/kg, IV, twice a day, after loading dose in the first day) in the presence of positive GM tests and/or radiologically suspicious *Aspergillus* infection, or caspofungin (50 mg/m^2, IV, once a day following 70 mg/m^2 loading dose for patients older than 3 months of age) was initiated when an invasive yeast infection was suspected. These are the doses of antifungals that are used at all centers. Combination therapy was occasionally used at the physician's discretion. The voriconazole blood levels could not be monitored. Monitoring of the clinical course of IFI was performed according to the standard clinical, radiological, and microbiological tests when available. No patient underwent an autopsy. The study was approved separately by the local institutional review boards for all participating centers.

GVHD prophylaxis and treatment

Standard protocol, including cyclosporine (dosage based on plasma levels of 100–250 ng/ml) and short-term, low-dose methotrexate (10 mg/m^2 on days +1, +3 and +6 after HSCT), were used for GVHD prophylaxis for 6 months (the duration was shorter for patients with leukemia). Rabbit anti-thymocyte globulin (Fresenius) was also used for the recipients of unrelated donor transplants and those who were high-risk for GVHD due to high transfusion rates before HSCT. Umbilical cord blood transplantation prophylaxis consisted of cyclosporine plus prednisone (1 mg/kg/day until day +28 after HSCT) or mycophenolate mofetil (1200 mg/m^2/day). The grading of GVHD was based on Glucksberg's criteria.[21] For grade I aGVHD and limited cGVHD, local corticosteroid therapy was used. When aGVHD of grades II–IV or extended cGVHD were established, prednisone at a dose of 1–2 mg/kg/day was initiated. In cases of steroid-refractory GVHD, different treatment approaches were used depending on the transplant center's policy including mesenchymal stroma cell infusion, extracorporeal photopheresis, mycophenolate mofetil, and etanercept.

Definitions

IFIs were defined according to the revised definitions of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria.[22] Proven and probable cases were included for analysis. Proven IFI required histopathological findings from biopsied tissues or culture of sterile tissue. Probable IFI was considered when patients met both the clinical criteria and exhibited mycological evidence, such as cytological test, microscopy, and culture results that indicated a fungus or the detection of GM.

Neutrophil engraftment was defined by the first day of an absolute neutrophil count (ANC) $>500/\mu$l for 3 consecutive days. Platelet engraftment was defined as the first day of 3 consecutive platelet counts $>20 \times 10^9$/l over a period of at least 7 days in the absence of platelet transfusion for at least 7 days before that date. Lymphocyte engraftment was defined as the first day of an absolute lymphocyte count $>300/\mu$l for 3 consecutive days. Late neutrophil and lymphocyte engraftment were defined as engraftment occurring after day +28.

The IFI date was recorded as the date on which the first diagnostic test was positive. Patients who were diagnosed with IFI before HSCT and/or given anti-mold prophylactic

agents were not included in the study. IFI-related mortality was defined as death attributed to a direct consequence of probable or proven IFI such as respiratory failure due to fungal pneumonia or a direct complication of IFI (e.g., bleeding or sepsis with unresolved IFI) in patients who underwent allogeneic HSCT. Case fatality rate was defined as death in patients who were diagnosed as probable or proven IFI. Non-relapse mortality (NRM) was defined as mortality attributable to all deaths before relapse or progression of primary disease.

Statistical analysis

The primary end point of the study was to determine the 180-day cumulative incidence of probable and proven IFI in pediatric allogeneic HSCT recipients. The possible risk factors for IFI were retrospectively analyzed using Cox proportional hazard models. The factors that were analyzed included age at transplantation, sex, diagnosis type, donor type, conditioning type (myeloablative vs nonmyeloablative), conditioning regimen (total body irradiation [TBI] vs chemo-based), CMV reactivation, aGVHD grade III–IV, cGVHD, corticosteroid use (>1 mg/kg/day for 10 or more days), late PNL engraftment, late lymphocyte engraftment, use of anti-thymocyte globulin (ATG), fludarabine, and melphalan, transplant number and seasonal variation. Kaplan–Meier estimates were computed for survival, and a stratified log rank test was used to compare these groups. Factors with p values <0.2 in the univariate analysis were included in the multivariate analysis. The results were expressed as hazards ratios (HRs) and their corresponding 95% confidence intervals (CIs). An HR >1 denotes an unfavorable effect for the occurrence of IFI. The incidences of IFI and TRM were estimated by the cumulative incidence function method. The 180-day cumulative incidence of IFI was calculated while accounting for the competing risk of death from causes other than IFI. Deaths due to relapsed or refractory disease were treated as competing risks in the estimates of the cumulative incidence of TRM. For categorical variables, the χ^2 statistic or Fisher exact test were used to establish differences in their distributions between the subgroups. *P* values <0.05 were considered statistically significant. The data were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and the XLSTAT version 2017.2 statistical packages.

Results

Clinical characteristics of the study population

Four-hundred and eight patients from 13 centers were included in this study. The median number of transplants per center was 23 (range, 6–84). The clinical and transplant characteristics of the patients are summarized in Table 1. The median age at HSCT was 7.1 years (range, 0.1–18.0 years). A total of 62.0% of the patients (n = 253) underwent transplantation for nonmalignant disease, and the other 38.0% had malignant disease (n = 155). The donors used included 250 matched (9/10 or 10/10) sibling/family donors (61.3%), 119 unrelated donors (29.1%), 37 haploidentical family donors (9.1%), and 2 unrelated cord blood (0.5%) donors. Ten patients underwent transplantations twice.

Incidence, classification, and timing of IFI

In the 180-day period after HSCT, overall, 26 cases of IFI were recorded without the presence of any competing risks; these cases included 10 cases of probable and 16 cases of proven IFI, and the lungs were the most frequent site of infection. Among the 26 cases, 61.6% were males. The median time to onset of IFI was 39.5 (range, 3–156) days after HSCT with 11 (42.3%) episodes that were diagnosed before day 30 (day 0–30, n = 11; day 31–60, n = 6; day 61–100, n = 3; day 101–180, n = 6) with an overall IFI rate at 100 patient-days at a risk of 4% (Table 2). In 8/16 proven episodes, fungi were isolated from blood cultures (7 without and 1 with deep organ involvement). Deep tissue involvement was shown by 4 paranasal, 2 skin, 1open lung and 1 open brain biopsies. All cases with probable IFI had both lower respiratory tract infections with CT signs of fungal infection such as a halo sign, air-crescent sign or cavity, and GM positivity. Invasive aspergillosis was the most common infection (14 cases, 53.8%) followed by invasive candidiasis (9 cases, 34.6%), mucormycosis (2 cases, 7.7%) and fusariosis (1 case, 3.9%) (Table 3). The median times after allogeneic HSCT to the onset of IFI were 57 days (range, 15–150 days) for proven mould infections and 68.5 days (range, 3–156 days) for proven yeast infections (P = .347). The cumulative incidence of probable and proven IFI in the study population was 6.5% (95% CI 4.5–9.5) (Fig. 1). At least one competing risk was present in 63 cases (15.5%). The rate of IFI at each transplantation center ranged from 0% to 17.4%. The 180-day cumulative incidence of IFI was 6.1% (95% CI 3.6–10.4) in the centers in which allogeneic transplantation occurred more than 50 times during study period and 6.8% (95% CI 4.0–11.5) in the centers in which allogeneic HSCT occurred equal or less than 50 times (Gray test, P > .05). The cumulative incidence of IFI was statistically higher in centers that performed GM monitoring twice a week than in those that monitored once a week (9.8% [95% CI 6.2–15.7] vs 4.1% [95% CI 2.2–7.6], respectively) (Gray test, P = .019).

Table 1. Clinical characteristics of study patients ($n = 408$).

Patient characteristics	*n*	%
Age (years, median, range)	7.13 (0.1–18.0)	
Sex		
Male	252	61.8
Female	156	37.2
Underlying disease		
Malignant	155	38.0
Acute leukemia	118	28.9
Lymphoma	29	7.1
MDS/CML/JMML	8	2.0
Nonmalignant	253	62.0
Hemoglobinopathy	92	22.5
Primary immunodeficiency	65	15.9
Bone marrow failure syndromes	52	12.7
Inborn errors	44	10.9
Previous HSCT		
None	398	97.5
One or more	10	2.5
Source of SCT		
Cord blood	32	7.8
Others	376	92.2
Donor		
Related	287	70.3
Unrelated	121	29.7
Conditioning type		
None	4	0.9
Myeloablative	357	87.5
Reduced intensity	47	11.6
Conditioning regimen		
TBI-based	43	10.5
Chemotherapy-based	365	89.5
Fludarabine containing regimen		
Yes	169	39.2
No	248	60.8
Melphalan containing regimen		
Yes	48	11.8
No	360	88.2
ATG containing regimen		
Yes	233	57.1
No	175	42.9
CMV reactivation		
Yes	127	31.1
No	281	68.9
GVHD prophylaxis		
None	21	5.2
CsA+MTX	222	54.4
Others	165	40.4
aGVHD		
No or Grade I–II	353	86.5
Grade III–IV	55	13.5
cGVHD*		
No or limited	337	94.7
Extensive	19	5.3

*Patients who died before day +100 were not included. ATG, anti-thymocyte globulin; CML, chronic myeloid leukemia; CMV, cytomegalovirus; CsA, cyclosporine A; GVHD, graft-versus-host disease; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; MTX, methotraxate; TBI, total body irradiation.

Risk factors for developing IFI

Donor type, prior transplant history, conditioning regimen including ATG and/or fludarabine, late lymphocyte engraftment, severe aGVHD, CMV reactivation and high-dose steroids have all been documented as predisposing conditions in univariate analysis (Table 4). Multivariate analysis identified the presence of a previous HSCT history (HR, 4.57; 95% CI 1.42–14.71; $P = .011$), the use of ATG (HR, 2.94; 95% CI 1.27–6.80; $P = .012$), a GVHD grade of III–IV (HR, 2.91; 95% CI 1.24–6.80; $P = .014$), and late lymphocyte engraftment (HR, 2.71; 95% CI 1.30–5.62; $P = .007$) as risk factors for the development of IFI (Table 4). CMV reactivation was marginally associated with an increased risk of IFI development (HR, 1.91; 95% CI 0.97–3.74; $P = .063$).

Outcome of IFI and survival

Among all 408 patients, 77 (18.9%) died within 180 days after HSCT, and fungal infections accounted for 1.5% of these cases (6/408) (IFI-related mortality). Of the seven patients who were diagnosed with IFI and died after day +180, six (85.7%) had active fungal disease at the time of death and active fungal disease was the main cause of death. The overall mortality (case fatality) rate for all types of IFI was 27.0% (7/26) at 180 days, including 40.0% (5/16) for invasive mould infections and 20.0% (2/10) for invasive yeast infections. Patients with IFI appeared to have a lower cumulative survival rate at day +180 than those without IFI, but the difference did not reach statistical significance (73.1% [95% CI 55.7–90.5] vs 81.6% [95% CI 77.6–85.6], log rank test $P = .343$). The 100-day follow-up after IFI diagnosis coincided with more than 180 days after transplantation in two patients (their IFI diagnoses days were at day +150 and +155). The mortality rate at 100 days from the diagnosis of IFI was 27.0% (7/26).

By the end of the study, a total of 63 patients had died without suffering a relapse or experiencing disease progression following transplantation (NRM). The cumulative NRM incidence was 16.2% (95% CI 13.0–20.2). Patients who developed IFIs tended to experience higher NRM; however, the differences among the groups did not reach statistical significance (26.9% [95% CI 14.3–50.7] vs 15.5% [95% CI 12.3–19.6%], $P = .163$).

Discussion

This multicenter retrospective study conducted in a large cohort of pediatric patients who underwent allogeneic HSCT and used primary prophylaxis with fluconazole at discharge

Table 2. Incidence of invasive fungal infections due to different periods in 408 patients who underwent allogeneic hematopoietic stem cell transplantation.

Period (days after HSCT*)	No. of patients followed in each period	Patient-days at risk	Total episodes (%)	Rate/100 patient-days at risk
Overall	408	67 863	26	0.04
0–30	408	11 859	11	0.09
31–60	373	22 171	6	0.03
61–100	353	35 005	3	0.01
101–180	337	59 638	6	0.01

*HSCT, hematopoietic stem cell transplantation.

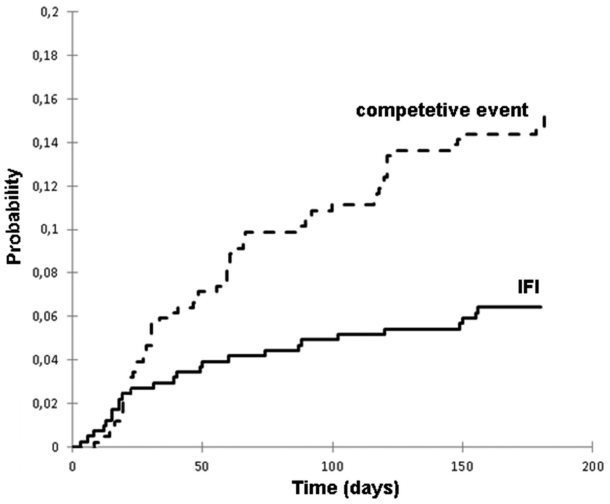

Figure 1. Cumulative incidence curve for IFI in study population.

Table 3. Characteristics of IFI episodes.

Type of IFI*	No. (%)
Probable	10 (38.5)
Proven	16 (61.5)
Site of infection	
Lungs	12 (46.1)
Blood stream	7 (27.0)
Rhinosinusal	5 (19.1)
Brain	1 (3.9)
Skin	1 (3.9)
Fungal pathogen	
Aspergillus spp.	14 (53.8)
Aspergillus fumigatus	3
Aspergillus flavus	1
Aspergillus spp. (positive galactomannan+CT findings such as halo sign)	10
Candida spp.	9 (34.6)
Candida albicans	4
Candida kruzei	2
Candida parapsilosis	2
Candida tropicalis	1
Other	3 (11.6)
Mucorales	2
Fusarium spp.	1

*IFI, invasive fungal infection.

revealed an overall incidence of probable or proven IFI of 6.4% in the 180-day period after allogeneic HSCT. Of these IFIs, 53.8% were caused by invasive aspergillosis, 34.6% were caused by *Candida* spp., and other fungal agents were relatively uncommon. We may have underestimated the true infection rate in this study because we excluded possible cases from the analysis, and our follow-up period was shorter than those of other published studies. The incidence of IFI was highest during the pre-engraftment period (day 0–30) and declined over time (Table 2). This observation has been confirmed by others.[10,14,17] More than 70% of survival at 100 days of IFI diagnosis is much higher than the previous reports for the patients who underwent allogeneic HSCT.[9–13] This study also showed that frequent GM screening may increase the chance of diagnosing IFI.

The IFI frequency according to the donor type was 4.5% for related donors and 10.7% for unrelated donors. These results are partly comparable to those reported in Italian surveys and from the Transplant-Associated Infections Surveillance Network (TRANSNET).[17,23,24] While a retrospective analysis from the Gaslini Children Hospital revealed that the overall incidences of IFIs were 6% and 13% for allogeneic HSCT from matched-related and -unrelated donors, retrospectively,[17] in another Italian survey (the Gruppo Italiano Trapianto Midolla Osseo-GITMO), these values were 4.6% and 11.8%, respectively.[23] A prospective national survey from the United States (TRANSNET) that included both pediatric and adult allogeneic graft recipients reported a 12-month cumulative incidence of 5.8 cases per 100 transplants for matched-related allogeneic and 7.7 cases per 100 transplants for matched-unrelated allogeneic HSCTs.[24]

The observation regarding the timing of the IFI in our study is not entirely consistent with the results of prior studies, some of which were small and so might not fully

Table 4. Risk factors for development of IFI: univariate and multivariate analysis.

Predictor	Patients number	IFI		Univariate analysis	Multivariate analysis		
		n	%	*P*-value	HR	95% CI	*P*-value
Age at HSCT				.711			
<10 years	268	18	6.7				
≥10 years	139	8	5.8				
Gender				.570			
Male	156	10	6.4				
Female	252	16	6.3				
Indication				.822			
Malignant	155	8	5.2				
Non-malignant	253	18	7.1				
Donor				.013	1.50	0.60-3.74	.950
Related	287	13	4.5				
Unrelated	121	13	10.7				
Prior HSCT history				<.001	4.57	1.42-14.71	.011
Yes	10	3	30.0				
No	398	23	5.8				
Conditioning				.772			
None	4	0	0.0				
MAC	357	23	6.4				
RIC	47	3	6.4				
Stem cell source				.580			
CB	32	3	9.4				
Others	376	23	6.1				
ATG in conditioning				.002	2.94	1.27-6.80	.012
Yes	175	6	3.4				
No	233	20	8.6				
TBI in conditioning				.432			
Yes	43	2	4.7				
No	365	24	6.6				
Melphalan in conditioning	48	6	12.5	.063	2.58	0.87-7.70	.111
Yes	360	20	5.6				
No							
Fludarabine in conditioning							
Yes	160	15	9.4	.020	1.26	0.78-3.15	.771
No	248	11	4.4				
Late PNL engraftment				.084	1.92	0.58-6.33	.384
Yes	58	7	12.1				
No	350	19	5.4				
Late lymphocyte engraftment							
Yes	113	13	11.5	<.001	2.71	1.30-5.62	.007
No	295	13	4.4				
GVHD prophylaxis				.691			
None	20	1	4.8				
CyA+MTX	222	24	10.8				
Others	165	16	9.7				
aGVHD				.001	2.91	1.24-6.80	.014
No or Grade I-II	353	17	4.8				
Grade III-IV	55	9	16.4				

Table 4. (*Continued*).

Predictor	Patients number	IFI		Univariate analysis	Multivariate analysis		
		n	%	*P*-value	HR	95% CI	*P*-value
cGVHD*				.352			
No or limited	337	21	6.2				
Extensive	19	2	10.5				
CMV reactivation				.001	1.91	0.97-3.74	.063
Yes	127	13	10.2				
No	281	13	4.6				
Corticosteroid				.031	2.02	0.67-6.10	.349
Yes	154	15	9.7	0.904			
No	253	11	4.3				
Season	111	6	5.4				
Spring	109	7	6.5				
Summer	100	6	6.0				
Fall	88	7	8.0				
Winter							

*Patients who died before day +100 were not included. CB, cord blood; CI, confidence interval; CyA, cyclosporine; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplantation; IFI, invasive fungal infections; MAC, myeloablative conditioning;; MTX, methotrexate; PNL, polymorphonucleated leukocyte; RIC, reduced intensity conditioning.

characterize IFI epidemiology.[9–11,14,23,25] In our patients, the median time from the date of HSCT to the onset of IFI was 39.5 days, with 42.3% of episodes occurring before day 30 and 23.1% occurring between day 101 and 180. While Hovi et al.[9] reported a bimodal peak of IFI in children who underwent allogeneic HSCT, only 8.8% of episodes occurred during the 100 days after transplantation in this Japanese data set.[11] In a prospective survey of IFI in children with cancer, the median interval between transplantation and IFI was 34 days, and most of the episodes (54%) occurred before day 30.[25] This observation has been noted in other reports.[10,14,23]

The incidence of IFI among centers ranged from 0% to 17.4% in this study. In multicenter studies, despite the high numbers of included patients, a potential center effect could represent a limit of the study due to the different clinical care habits. To evaluate the possible role of different centers on the incidence of IFI, we divided the centers into two groups according to their allogeneic transplant activity during the study period (>50 and ≤50 allogeneic HSCTs). Univariate analysis did not identify transplant activity as a risk factor for the development of IFI.

Concerning the multivariate analysis of the risk factors for the development of IFI, we identified associations with several important immunologically based risk factors, most of which have biological plausibility for contribution to IFI risk, and some have been identified as associated with IFI risk in previous literature.[9–12,14] Our study confirmed that severe aGVHD (grade III–IV), which is required for severe immune suppressive medication, such as high-dose corticosteroids and monoclonal antibodies that affect and cause a combined (T- and B-cell) profound and prolonged disruption of immunity, is an independent risk factor. Another factor associated with IFI is the lack of or late lymphocyte engraftment, which is present in the majority of IFI episodes that are observed in nonneutropenic patients. Additionally, our data suggest that ATG use in the conditioning regimen could increase the risk of IFI as confirmed by several reports.[25,26] Further studies are needed to reveal the correlation between ATG and the development of IFI after allogeneic HSCT. Our study also demonstrated that previously undergoing HSCT is a significant risk factor for the development of IFI, which likely depends on immune defects resulting from previous transplantations.

In our study, CMV reactivation was marginally associated with an increased risk of IFI development. Post-transplant CMV reactivation has been reported to be a risk factor for IFI in some studies, which likely depends on the immune suppressive agents that are used to prevent and treat GVHD.[27–30] CMV is associated with the development of IFI in both the early and late post-transplant phases.[31] Although the mechanism is unclear, IFI development could be due to other variables such as corticosteroids.[32]

Examination of the mortality rates of patients with IFI who underwent allogeneic HSCT between several studies is difficult due to the use of different definitions. Thus, we collected data from previous studies under one title with collective definitions and attempted to compare them with our data (Table 5). In our experience, 1.5% of all allogeneic graft recipients died from IFI, which accounted for 7.8%

Table 5. Incidence and outcome of IFI in several pediatric studies including patients who underwent allogeneic HSCT.

	Number of patients	Probable-proven IFI incidence		Overall mortality		Case fatality rate		IFI-related mortality		Post-HSCT deaths due to IFI	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Hovi et al.[9]	148 (including autologous HSCT)	18	12.2	63/148	42.6	15/18	83.4	12/148	8.1	12/63	19.1
Dvorak et al.[10]	115	15	13.1	43/115	37.0	11/15	73.4	7/115	6.1	7/43	16.3
Kobayashi et al.[11]	149	12 (including possible IFI)	8.1	n.a	n.a	10/12	83.4	7/149	4.7	n.a	n.a
Hol et al.[12]	209	25	12.0	72/209	34.5	15/25	60.0	12/209	5.8	12/72	16.7
Simms-Waldrip et al.[13]	318	47	14.8	142/318	44.7	37/47	78.8	25/318	7.9	25/142	17.6
Present study	408	26	6.4	77/408	18.9	7/26	27.0	6/408	1.5	6/77	7.8

HSCT, hematopoietic stem cell transplantation; IFI, invasive fungal infections; n.a, not available.

of the post-transplant deaths, which in turn was very low compared to earlier studies.[9–13] Overall survival rate was 60.0% in patients with proven or probable invasive mould infection in this study. It is much higher than reported in the literature for pediatric patients by Burgos et al.[33] and is nearly on par with the report by Wattier et al.[34], which included not only the patients who underwent alloHSCT but also other high risk populations. This result was probably related to the use of new and more active antifungal drugs and/or earlier diagnosis and treatment.

Notably, our study has some limitations, which include its retrospective nature, short follow-up period, and underestimation of the true incidence of IFI, which may be lower than the actual incidence due the failure to include possible IFI cases. Although centers participating in the study indicated compliance with standard anti-fungal drug doses, drug doses might have been modified at the physician's discretion in some patients who had refractory disease. So this could have modified the risk of subsequent IFI and treatment success. Different protocols in GM monitoring were also another limitation of this work.

In summary, IFIs were rare but serious complications of allogeneic HSCT with, 27.0% case fatality rate. The close monitoring of IFI in pediatric patients with severe aGVHD and who receive ATG during conditioning is critical to reduce morbidity and mortality after allogenic HSCT, particularly among those with prior HSCT histories and no or late lymphocyte engraftment.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Jantunen E, Ruutu P, Niskanenen L et al. Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant*. 1997; 19: 801–808.
2. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mold infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis*. 2002; 34; 909–917.
3. Bow EJ. Invasive fungal infection in haematopoietic stem cell transplant recipients: epidemiology from transplant physician's viewpoint. *Mycopathologia*. 2009; 168: 283–297.
4. Chandrasekar PH, Weinmann A, Shearer C. Autopsy identified infections among bone marrow transplant recipients: a clinicopathologic study of 56 patients. *Bone Marrow Transplant*. 1995; 16: 676–681.
5. Dignani MC. Epidemiology of invasive fungal diseases on the basis of autopsy reports. *F1000 Prime Rep*. 2014; 6: 81.
6. Kontoyiannis DP, Patterson TF. Diagnosis and treatment of invasive fungal infections in the cancer patient: recent progress and ongoing questions. *Clin Infect Dis*. 2014; 59: S356–S359.
7. Chamilos G, Luna M, Lewis RE et al. Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989–2003). *Haematologica*. 2006; 91: 986–989.
8. Hachem R, Sumoza D, Hanna H, Girgawy E, Munsell M, Raad I. Clinical and radiologic predictors of invasive pulmonary aspergillosis in cancer patients: should the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria be revised? *Cancer*. 2006; 106: 1581–1586.
9. Hovi L, Saarinen-Pihkala UM, Vettenranta K, Saxen H. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. *Bone Marrow Transplant*. 2000; 26: 999–1004.
10. Dvorak CC, Steinbach WJ, Brown JM, Agarwal R. Risks and outcomes of invasive fungal infections in pediatric patients undergoing allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant*. 2005; 36: 621–629.
11. Kobayashi R, Kaneda M, Sato T, Suzuki D, Ichikawa M, Ariga T. Evaluation of risk factors for invasive fungal infection after allogeneic stem cell transplantation in pediatric patients. *J Pediatr Hematol Oncol*. 2007; 29: 786–791.

12. Hol JA, Wolfs TF, Bierings MB et al. Predictors of invasive fungal infection in pediatric allogeneic hematopoietic SCT recipients. *Bone Marrow Transplant*. 2014; 49: 95–101.
13. Simms-Waldrip T, Rosen G, Nielsen-Saines K, Ikeda A, Brown B, Moore T. Invasive fungal infections in pediatric hematopoietic stem cell transplant patients. *Infect Dis (Lond)*. 2015; 47: 218–224.
14. Benjamin DKJ, Miller WC, Bayliff S, Martel L, Alexander KA, Martin PL. Infections diagnosed in the first year after pediatric stem cell transplantation. *Pediatr Infect Dis J*. 2002; 21: 227–234.
15. Barker JN, Hough RE, van Burik JA et al. Serious infections after unrelated donor transplantation in 136 children: impact of stem cell source. *Biol Blood Marrow Transplant*. 2005; 11: 362–370.
16. George B, Mathews V, Viswabandya A et al. Infections in children undergoing allogeneic bone marrow transplantation in India. *Pediatr Transplant*. 2006; 10: 48–54.
17. Castagnola E, Faraci M, Moroni C et al. Invasive mycoses in children receiving hemopoietic SCT. *Bone Marrow Transplant*. 2008; 4: S107–S111.
18. Blyth CC, Hale K, Palasanthiran P, O'Brien T, Bennett MH. Antifungal therapy in infants and children with proven, probable or suspected invasive fungal infections. *Cochrane Database Syst Rev*. 2010; 17: CD006343.
19. Ziakas PD, Kourbeti IS, Mylonakis E. Systemic antifungal prophylaxis after hematopoietic stem cell transplantation: a meta-analysis. *Clin Ther*. 2014; 36: 292–306.
20. Crassard N, Hadden H, Pondarre C et al. Invasive aspergillosis and allogeneic hematopoietic stem cell transplantation in children: a 15-year experience. *Transpl Infect Dis*. 2008; 10: 177–183.
21. Glucksberg H, Storb R, Fefer A et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974; 18: 295–304.
22. De Pauw B, Walsh TJ, Donnelly JP et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008; 46: 1813–1821.
23. Girmenia C, Raiola AA, Piciocchi A et al. Incidence and outcome of invasive fungal diseases after allogeneic stem cell transplantation: a prospective study of the Gruppo Italiano Trapianto Midollo Osseo (GITMO). *Biol Blood Marrow Transplant*. 2014; 20: 872–880.
24. Kontoyiannis DP, Marr KA, Park BJ et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis*. 2010; 50: 1091–1100.
25. Montesinos P, Rodríguez-Veiga R, Boluda B et al. Incidence and risk factors of post-engraftment invasive fungal disease in adult allogeneic hematopoietic stem cell transplant recipients receiving oral azoles prophylaxis. *Bone Marrow Transplant*. 2015; 50: 1465–1472.
26. Omer AK, Ziakas PD, Anagnostou T et al. Risk factors for invasive fungal disease after allogeneic hematopoietic stem cell transplantation: a single center experience. *Biol Blood Marrow Transplant*. 2013; 19: 1190–1196.
27. Castagnola E, Cesaro S, Giacchino M et al. Fungal infections in children with cancer: a prospective, multicenter surveillance study. *Pediatr Infect Dis J*. 2006; 25: 634–639.
28. Mikulska M, Raiola AM, Bruno B et al. Risk factors for invasive aspergillosis and related mortality in recipients of allogeneic SCT from alternative donors: an analysis of 306 patients. *Bone Marrow Transplant*. 2009; 44: 361–370.
29. Zhang P, Jiang EL, Yang DL et al. Risk factors and prognosis of invasive fungal infections in allogeneic stem cell transplantation recipients: a single-institution experience. *Transpl Infect Dis*. 2010; 12: 316–321.
30. Shi JM, Pei XY, Luo Y et al. Invasive fungal infection in allogeneic hematopoietic stem cell transplant recipients: single center experiences of 12 years. *J Zhejiang Univ Sci B*. 2015; 16: 796–804.
31. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood*. 2002; 100: 4358–4366.
32. Grow WB, Moreb JS, Roque D et al. Late onset of invasive aspergillus infection in bone marrow transplant patients at a university hospital. *Bone Marrow Transplant*. 2002; 29: 15–19.
33. Burgos A, Zaoutis TE, Dvorak CC et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics*. 2008; 121: e1286–e1294.
34. Wattier RL, Dvorak CC, Hoffman JA et al. A prospective, international cohort study of invasive mold infections in children. *J Pediatric Infect Dis Soc*. 2015; 4: 313–322.