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Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients

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Summary:

In order to analyze the incidence and risk factors for invasive fungal infection (IFI) after allogeneic BMT, 142 consecutive adult BMT recipients (131 sibling donors, 11 unrelated donors) transplanted in 1989–1993 were retrospectively analyzed. There were 21 cases with definite or probable IFI (incidence 15%) (*Aspergillus*, 15; *Candida*, four; *Fusarium*, one; *Absidia*, one). The median time to the diagnosis of IFI was 136 days after BMT (range 6–466 days). Only 14% of the IFIs were found during the neutropenic period post-BMT. Of the pretransplant characteristics, hematological disease (MDS *vs* other) ($P = 0.001$) and unrelated donor ($P = 0.01$) were risk factors for IFI. Acute GVHD grade III–IV ($P = 0.03$) and extensive chronic GVHD ($P = 0.0002$) were also found to be significant risk factors. Only three patients with IFI (14%) became long-term survivors. Invasive fungal infections tended to develop late after BMT, were usually caused by *Aspergillus* sp., and were strongly associated with GVHD and its treatment. Better prophylaxis and treatment of IFI are needed. More effective prophylaxis for GVHD might decrease the risk of IFI after allogeneic BMT.

Keywords: invasive fungal infection; BMT; GVHD; risk factors; incidence

Invasive fungal infections (IFI) mainly caused by *Candida* and *Aspergillus* species constitute a major problem after allogeneic BMT. Invasive *Candida* infections have been reported in 10–15% of patients,[1–4] and the incidence of *Aspergillus* infections has varied between 3 and 7% in recent series.[2,5–7] The high risk of IFI after BMT is due to several factors including neutropenia before engraftment, disruption of mucosal barriers by cytotoxics and radiotherapy, use of broad-spectrum antimicrobial agents, and immunosuppressive effects of prophylaxis and treatment of GVHD. In addition, GVHD itself has a prominent immunosuppressive effect and may delay immunologic reconstitution after BMT.[8,9]

The therapy of IFI after BMT is often unsuccessful, and the mortality ranges from 60 to 95%.[4,5] Knowledge of the epidemiology and risk factors for IFI is important in identifying subsets of BMT recipients for clinical trials investigating the effects of novel preventive measures, as well as clinical decision-making on the management of patients after allogeneic BMT.

In most previous studies, the role of GVHD and especially the effects of regimens used for GVHD prophylaxis or treatment, have not been evaluated as risk factors for IFI. Due to the increasing age of BMT recipients, wider use of unrelated donors, modifications in pre- and post-transplant immunosuppression and antifungal prophylaxis, the incidence of fungal infections, as well as the spectrum of causative agents, may change. We therefore investigated the incidence and risk factors for IFI and especially the effects of acute and chronic GVHD and their treatment with steroids and antithymocyte globulin, on the risk of IFI in allogeneic BMT recipients transplanted between 1989 and 1993.

Patients and methods

Patients

One hundred and forty-two adult patients received their first allogeneic BMT (131 HLA-identical sibling donors, 11 unrelated donors) in the Department of Medicine, Helsinki University Central Hospital between 1989 and 1993. All patient charts were screened for relevant baseline data, post-transplant course, occurrence and treatment of GVHD, as well as invasive fungal infections and their management. The patients had a median follow-up of 26 months post-BMT (range 1–82 months). The main pretransplant characteristics are presented in Table 1.

Transplant protocol

The conditioning regimen consisted of TBI (12 Gy in six fractions over 5 days with lungs shielded not to receive more than 10 Gy) combined with CY (60 mg/kg/day for 2 days) (TBI-CY), or BU (4 mg/kg/day for 4 days) plus CY (60 mg/kg/day for 2 days) (BU-CY). The patients with aplastic anemia were conditioned with CY only (50 mg/kg/day for 4 days). GVHD prophylaxis consisted of CsA for a year with a short course of MTX (15 mg/m^2 on day +1, 10 mg/m^2 on day +3, +6, +11) (CsA-Mtx) or CsA with a short course of MTX plus methylprednisolone (MP) (0.5 mg/kg/day from day +14, 1 mg/kg/day from day +21 for 2

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Table 1 Pretransplant characteristics of the transplant patients

Gender		
Male	68	(48)
Female	74	(52)
Age (years)		
Mean	39	
Range	16–53	
Hematological disease		
AML	53[a]	(37)
CML	36[b]	(25)
ALL	22[c]	(15)
MM	14	(10)
MDS	10	(7)
Other	7	(6)
Donor		
Sibling	131	(92)
MUD	11	(8)
CMV serology		
Positive[d]	111	(78)
Negative	31	(22)
Conditioning regimen[e]		
TBI-CY	86	(61)
BU-CY	53	(37)
GVHD prophylaxis		
Mtx-CsA	74	(52)
Mtx-CsA-MP	68	(48)

Frequencies (%) are presented in parenthesis.
AML = acute myeloid leukemia; CML = chronic myelogenous leukemia; ALL = acute lymphoblastic leukemia; MM = multiple myeloma; MDS = myelodysplastic syndrome; MUD = matched unrelated donor; CMV = cytomegalovirus; TBI-CY = total body irradiation plus cyclophosphamide; BU-CY = busulphan plus cyclophosphamide; GVHD = graft-versus-host disease; Mtx-CsA = methotrexate plus cyclosporine; MP = methylprednisolone.
[a]40 1 CR, 13 >1 CR.
[b]28 CP, 8 >CP.
[c]20 1 CR, 2 >1 CR.
[d]CMV antibodies >10 EIU.
[e]Three patients with aplastic anemia were conditioned with cyclophosphamide only.

weeks, then halved approximately at 2-week intervals, and discontinued by day +110) (CsA-MTX-MP). All patients with a matched unrelated donor (MUD) received CsA-MTX-MP prophylaxis. In addition, four out of 11 patients with unrelated donor received antithymocyte globulin (ATG) (20 mg/kg/day for 3 days) as part of their conditioning regimen. No T cell-depleted grafts were used.

All patients were cared for in single rooms with HEPA filters until recovery from neutropenia ($>1 \times 10^9$/l). Antimicrobial prophylaxis included oral acyclovir (1600 mg/day for 5 weeks) and oral sulfamethoxazole (1600 mg/day) plus trimethoprim (320 mg/day) until recovery from neutropenia and three times a week thereafter until 6 months post-BMT. Patients with allergy to sulphonamides received intravenous pentamidine during hospitalization and monthly inhalations thereafter. Patients who received an allograft from a matched unrelated donor received ciprofloxacin 750 twice a day orally or 200 mg twice a day intravenously instead of sulfamethoxazole-trimethoprim until engraftment. All patients received amphotericin B (AmB) tablets or miconazole gel during the neutropenic period after BMT. No systemic antifungal prophylaxis was given. Systemic antifungal agents, primarily intravenous AmB, were given during neutropenia for fever unresponsive to empirical antibiotics (dose of AmB 0.5 mg/kg/day) and in patients with known or suspected fungal infections.

Diagnosis and treatment of GVHD

Acute GVHD (aGVHD) was graded from 0–IV according to established criteria.[10] Chronic GVHD (cGVHD) was graded as absent, limited or extensive.[11] Patients who survived for >100 days after BMT were considered evaluable for cGVHD. aGVHD was treated with high-dose methylprednisolone (MP) (initial dose 10 mg/kg/day halved every 3 days three times) with a few exceptions where a lower initial dosage of 1–5 mg/kg/day was used. In patients refractory to high-dose MP, ATG was used for at least 7–10 days depending on the treatment response. The therapy of cGVHD was mainly MP, sometimes with CsA. A few patients with extensive cGVHD were also treated with low-dose (1 Gy) total lymphoid irradiation.

Diagnosis of invasive fungal infections

The diagnosis of invasive fungal infection was based on the identification of fungus from a normally sterile site. This included (1) blood cultures taken from peripheral veins; (2) microscopy and culture of needle aspirates or biopsies from normally sterile sites; or (3) identification of invasive fungal infection by microscopy with or without positive culture at autopsy. The findings with morphological features compatible with filamentous fungus without culture positivity were designated *Aspergillus*. In addition, patients with radiological findings suggestive of fungal infection with positive cultures or microscopy for *Aspergillus* from a specimen taken at bronchoalveolar lavage (BAL) were considered cases of probable invasive aspergillosis. Standard microbiological methods for direct microscopy, culture and identification of fungi were used.[12,13]

Statistical methods

Univariate comparisons between the groups of patients with and without invasive fungal infection were performed with χ^2 test or with Fisher's test (in case of small cell numbers) for dichotomised variables. In case of three or more classes, the Mantel–Haenzel extension test was used. The distribution of continuous variables was first analyzed with Kolmogorov–Smirnov's test and, if found normal, the two-tailed Student's *t*-test for independent samples was used. Thereafter, variables with a P value <0.10 after adjustment for age and sex (which were considered putative determinants) were analyzed with a stepwise forward multiple logistic regression analysis based on the maximum-likelihood method. Two final models are shown; one including aGVHD and the other cGVHD (these variables were not entered simultaneously). P values <0.05 were considered statistically significant. All the analyses were performed with the SPSS for WINDOWS program (SPSS Inc, Chicago, IL, USA).

Results

Fungal infections

Invasive fungal infection (IFI) was found in 21 of the 142 BMT patients (15%). In 17 patients (12%) the diagnosis of IFI was definite, whereas in four patients a diagnosis of probable invasive pulmonary aspergillosis was made on the basis of positive culture or microscopy of a specimen taken at BAL and radiological findings suggestive of fungal infection. Radiological findings included a single pulmonary cavity in one patient and multiple focal round lesions in another. One patient had bilateral pulmonary infiltrates with rapidly progressive neurological symptoms and multiple cerebral lesions on magnetic resonance imaging. The fourth patient had non-specific progressive radiological findings (chest X-ray/computerized tomography). All these four patients with probable invasive aspergillosis died, but autopsy was performed only in one patient after 2 months of amphotericin therapy; the diagnosis of *Aspergillus* infection could not be confirmed.

Aspergillus sp was the causative agent in 15 patients (incidence 11%), Candida sp. in four patients (3%), and *Fusarium* and *Absidia* each in one patient. The hematological disease, GVHD prophylaxis, severity and treatment of GVHD as well as the diagnostic findings in patients with IFI are presented in Table 2.

Fungal infection was diagnosed during life in 17 out of 21 patients (81%); four additional patients were found to have IFI at autopsy. The median time from BMT to the diagnosis of IFI was 136 days (range 6–466 days). Only three cases of IFI (14%) (*Candida*, two; *Absidia*, one) were diagnosed during the neutropenic period after BMT. The cumulative incidence of IFI was 4% at 3 months, 9% at 6 months, and 14% at 12 months after BMT (Figure 1).

All patients diagnosed to have IFI during life were treated with antifungal agents. 19 out of 21 (90%) patients with IFI died. The median survival from diagnosis of IFI was 28 days (range 5–260 days). In 13 patients (68%), IFI was considered the main cause of death. Only three patients (14%) (*Absidia*, one; *Aspergillus*, one; *Candida*, one) were cured of fungal infection.

GVHD and risk of invasive fungal infections

Two patients (Nos 18 and 21 in Table 2) had invasive fungal infections (*Candida* sepsis, locally invasive *Absidia* infection) diagnosed before day +8. Since aGVHD rarely presents so early after BMT, these patients were excluded from the subsequent analysis of GVHD as a risk factor for IFI.

No signs of aGVHD were observed in 78 (55%) patients. In this patient group, five patients (6%) had IFI (*Aspergillus*, two; *Candida*, two; *Fusarium*, one) diagnosed from 17 to 278 days post-transplant. In three patients (Nos 5, 7 and 8 in Table 2), the diagnosis of invasive fungal infection was made after treatment of extensive cGVHD.

The incidence of grade I–II aGVHD was 28%, and grade III–IV aGVHD was observed in 17% of the patients. The incidence of IFI was 13% in the patients with grade I–II aGVHD but 35% in the patients with grade III–IV aGVHD (Figure 2). Invasive fungal infection was diagnosed in 13 patients after the diagnosis and treatment of aGVHD from 69 to 466 days post-BMT (*Aspergillus*, 12; *Candida*, one). In five patients (3, 4, 10, 12 and 14 in Table 2), IFI was found after the diagnosis and treatment of extensive cGVHD.

One hundred and twenty-eight patients (90%) survived more than 100 days post-BMT and were thus evaluable for the development of cGVHD. No signs of cGVHD were observed in 71 patients (55%), limited cGVHD was found in 34 patients (27%) and extensive cGVHD in 23 patients (18%). Extensive cGVHD imposed a significant increase in the risk of IFI with an incidence of 39% for late IFIs (diagnosed >100 days from BMT) (Figure 3).

Treatment of GVHD and risk of fungal infection

aGVHD was treated in most patients with high-dose methylprednisolone (MP) (10 mg/kg/day initially). Thirty-two patients received high-dose MP as the only modality of GVHD therapy. In addition, three patients were treated with smaller doses of MP (1–5 mg/kg/day initially). Invasive fungal infection was found after high-dose MP in five patients (*Aspergillus*, 4; *Candida*, 1) giving an incidence of 14% for IFI in this patient group.

ATG was used in 29 patients with aGVHD unresponsive to high-dose MP. Twenty-two patients received one 7–10 day course of ATG, six patients two courses and one patient four courses. IFI was found in 11 patients after the treatment with ATG, giving an incidence of 38%. The fungal infections consisted of 10 cases of *Aspergillus* infection; one patient had *Candida* infection.

Analysis of risk factors for fungal infection

An attempt was made to analyze possible risk factors for the development of IFI (Table 3). In the univariate analysis, type of hematological disease (MDS *vs* other) ($P = 0.001$), risk category of patients (high-risk *vs* standard-risk) ($P = 0.02$), and matched unrelated donor ($P = 0.01$) were significant risk factors. Age, sex, CMV serology, conditioning regimen or type of GVHD prophylaxis were not associated with the risk of IFI. However, the incidence of IFI tended to be lower in the patients with sibling transplants who received MTX-CsA-MP prophylaxis than in those who received MTX-CsA prophylaxis (9 *vs* 16%), although the difference did not reach statistical significance.

No difference in the mean time to engraftment was observed in the patients who later developed IFI and in those who did not. Of the post-transplant characteristics, aGVHD ($P = 0.03$), cGVHD ($P = 0.0002$), and treatment of GVHD ($P = 0.0001$) were risk factors for IFI. The factors with P value <0.10 were entered in the multiple logistic regression analysis models. However, as the risk category of patients is strongly dependent on hematological disease and donor type, it was omitted from the logistic regression analysis. Similarly, the treatment of GVHD is strongly dependent on the severity of GVHD, and therefore treatment of GVHD was omitted from logistic regression analysis. Odds ratios (OR) with 95% confidence intervals (CI) adjusted for age and sex are presented in Table 4. The

Table 2 Clinical and laboratory features of the patients with invasive fungal infection

Patient No.	*Age/ Diagnosis*	*cGVHD prophylaxis*	*aGVHD grade*	*cGVHD*	*Treatment of GVHD*		*Criteria and time (days) of diagnosis of fungal infection*	*Fungus*
					HD-MP[a]	*ATG*[a]		
1	45/MM	Mtx-CsA	0	no	–	–	Blood culture +17	*Candida krusei*[d]
2	45/ALL	Mtx-CsA	II	no	+(2)	+	Autopsy +187	*Aspergillus fumigatus*[f]
3	34/ALL	Mtx-CsA	II	ext	+	–	BAL, radiology +167[c]	*Aspergillus*[e]
4	39/ALL	Mtx-CsA	IV	ext	+(2)	+(2)	Blood culture +249	*Candida tropicalis*[d]
5	37/CML	Mtx-CsA-MP	0	ext	+	–	Liver biopsy +278	*Torulopsis glabrata*[f]
6	44/MDS	Mtx-CsA-MP	III	NE	+	+	Autopsy +75	*Aspergillus fumigatus*[f]
7	47/MM	Mtx-CsA-MP	0	ext	+	+	Autopsy +226	*Aspergillus*[e]
8	47/MDS	Mtx-CsA-MP	0	ext	+(2)	–	Biopsy of subcutaneous tissue +179	*Aspergillus fumigatus*[f]
9	39/MDS	Mtx-CsA	III	ext	+(2)	+(2)	Lung biopsy +94	*Aspergillus fumigatus*[f]
10[b]	30/CML	Mtx-CsA-MP	III	ext	+(2)	+	Lung biopsy +269	*Aspergillus*[e]
11	42/NHL	Mtx-CsA	I	no	+	–	BAL, radiology +105[c]	*Aspergillus fumigatus*[f]
12	44/MDS	Mtx-CsA	IV	ext	+(2)	+(2)	Autopsy +353	*Aspergillus*[e]
13[b]	38/CML	Mtx-CsA-MP	0	NE	–	–	Blood culture +87	*Fusarium*[d]
14	42/CLL	Mtx-CsA	II	ext	+	+(2)	BAL, radiology +466[c]	*Aspergillus fumigatus*[d]
15[b]	30/AML	Mtx-CsA-MP	III	ext	+	+(4)	CNS biopsy +137	*Aspergillus fumigatus*[f]
16	29/AML	Mtx-CsA	IV	ext	+	+	Liver biopsy +69	*Aspergillus fumigatus*[f]
17[b]	41/CML	Mtx-CsA-MP	III	no	+	+	CSF culture +97 autopsy +108	*Candida albicans*[d] *Aspergillus fumigatus*[f]
18	45/AML	Mtx-CsA	III	ext	+	–	Blood culture +6	*Candida albicans*[d]
19[b]	17/AML	Mtx-CsA-MP	I	no	+	–	BAL, radiology +98 Autopsy +135	*Aspergillus fumigatus*[f]
20	33/AA	Mtx-CsA-MP	0	no	–	–	BAL, radiology +149[c]	*Aspergillus*[e]
21	41/MDS	Mtx-CsA	0	lim	+	–	Skin biopsy +8	*Absidia corymbifera*[f]

BAL = bronchoalveolar lavage; CNS = central nervous system; CSF = cerebrospinal fluid; HD-MP = high-dose methylprednisolone; MP = methylprednisolone; NE = not evaluable (survival <100 days); ext = extensive; lim = limited.
[a]Number of courses in parenthesis.
[b]Matched unrelated donor.
[c]Probable invasive *Aspergillus* infection.
[d]Culture.
[e]Microscopy.
[f]Microscopy and culture.

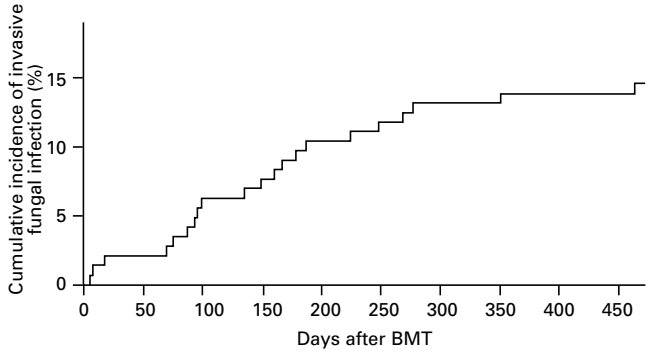

Figure 1 Cumulative incidence of invasive fungal infections after allogeneic BMT.

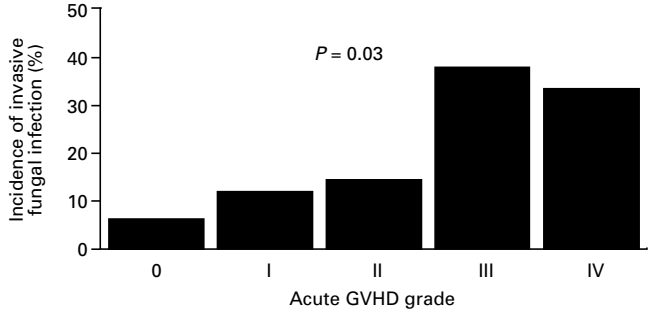

Figure 2 Incidence of invasive fungal infections according to the grade of acute GVHD.

type of hematological disease (MDS *vs* others), donor type (MUD *vs* sibling), aGVHD (grade III–IV *vs* 0–II) and cGVHD (extensive *vs* limited or none) were all statistically significant risk factors for IFI after BMT.

Discussion

This single-center analysis of invasive fungal infections after allogeneic BMT with a long follow-up (median 26 months) revealed a clear predominance of late infections caused by *Aspergillus* sp. In most patients, the IFIs were

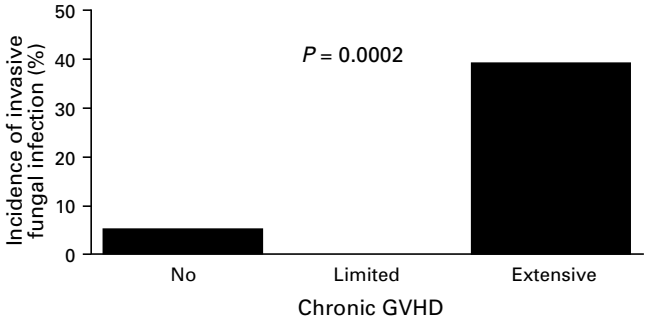

Figure 3 Incidence of late invasive fungal infections (diagnosed >100 days post-transplant) according to the severity of chronic GVHD.

associated with post-transplant immunosuppression caused by aGVHD and/or extensive cGVHD as well as their treatment. These observations of the epidemiology of fungal infections and high-risk patients might be of importance especially in finding preventive measures in the BMT setting.[14–16]

This analysis involved all adult patients who received allogeneic BMT in the Department of Medicine, Helsinki University Central Hospital between 1989 and 1993. The mean patient age was higher than in previously published reports, partly because of the exclusion of pediatric patients and because of the tendency to perform allogeneic BMT in older patients. In regard to the spectrum of underlying diseases, our material is comparable to previous series. A sig-

Table 3 Characteristics of BMT patients with invasive fungal infection and those without fungal infection

Variable	*Fungal infection (n = 21)*	*No fungal infection (n = 121)*	*P value*
Gender (M/F)	9/12	58/63	NS
Age (m ± s.d.)	39 ± 7	38 ± 10	NS
Disease			
AML	4 (19)	49 (40)	0.001
CML	4 (19)	32 (26)	
ALL	3 (14)	19 (16)	
MM	2 (10)	12 (10)	
MDS	5 (24)	5 (4)	
Other	3 (14)	4 (4)	
Risk category of patients[c]			
Standard risk	6 (29)	73 (60)	0.02
High risk	15 (71)	48 (40)	
CMV serology of patients			
Positive	15 (71)	96 (79)	NS
Negative	6 (29)	25 (21)	
donor positive	5 (83)	13 (52)	NS
donor negative	1 (17)	12 (48)	
Donor			
Sibling	16 (76)	115 (95)	0.01
MUD	5 (24)	6 (5)	
Conditioning regimen			
TBI-CY	13 (62)	73 (60)	NS
BU-CY	7 (33)	46 (38)	
CY	1 (5)	2 (2)	
Time to engraftment (neutroph $>0.5 \times 10^9$/l, days) (m ± s.d.)	19 ± 5	19 ± 4	NS
GVHD prophylaxis			
MTX-CsA	12 (57)	62 (51)	NS
MTX-CsA-MP	9 (43)	59 (49)	
Acute GVHD grade[a]			
0	6 (32)	71 (59)	0.03
I–II	5 (26)	35 (29)	
III–IV	8 (42)	15 (12)	
Chronic GVHD[b]			
No	3 (25)	66 (58)	0.0002
Limited	0	34 (30)	
Extensive	9 (75)	13 (12)	
Treatment of GVHD			
No	3 (14)	75 (62)	0.0001
High-dose MP	7 (33)	28 (23)	
High dose MP + ATG	11 (53)	18 (15)	

[a]Patients with invasive fungal infection diagnosed before day +8 after BMT excluded.
[b]Patients who survived less than 100 days after BMT and patients who had invasive fungal infection diagnosed before day 100 excluded.
[c]Standard-risk patients: AML 1 CR, ALL 1 CR, CML chronic phase/sibling donor.
High-risk patients: other disease/disease status/unrelated donor.
Frequency (%) in parenthesis.

Table 4 Age- and sex-adjusted odds ratios (OR) with 95% confidence intervals (CI) for the development of invasive fungal infection in BMT recipients according to multiple logistic regression analysis

Variable	*OR*		*CI*		*P value*	
	Model A[a]	*Model B*	*Model A*	*Model B*	*Model A*	*Model B*
Disease (0 = other, 1 = MDS)	11.3	11.1	2.4–53.2	0.9–135.4	0.002	0.06
Donor (0 = sibling, 1 = MUD)	8.0	25.1	1.8–36.8	2.1–307.2	0.007	0.01
Acute GVHD grade (0 = 0–II, 1 = III–IV)	3.9		1.2–12.4		0.02	
Chronic GVHD (0 = no/limited, 1 = extensive)		33.1		5.5–199.9		0.001

[a]Model A involves disease, donor and acute GVHD; in Model B acute GVHD is substituted with chronic GVHD.

nificant number of our patients received a corticosteroid as part of their GVHD prophylaxis in addition to MTX-CsA, in contrast to most published reports. Further, we have used a rather aggressive approach to treat established aGVHD, with almost all patients with aGVHD receiving high-dose MP initially. A significant number of patients with corticosteroid-resistant aGVHD were treated also with ATG.

Aspergillus species were responsible for more than two thirds of IFIs in the present study with an incidence of 11% for definite or probable *Aspergillus* infection. In other studies, the incidence of *Aspergillus* infection has ranged from 3 to 7%.[2,5,7,17] There may be at least three explanations for the high incidence of *Aspergillus* infections observed in this study. First, our study included patients who received their transplants from a matched unrelated donor with a high incidence (45%) of *Aspergillus* infection. The rather aggressive post-transplant immunosuppression may also have been a contributing factor. We also had a long follow-up after BMT. In view of the late appearance of the *Aspergillus* infections observed, the true incidence in BMT recipients is critically dependent on the length of the follow-up.

Invasive *Candida* infection was observed only in 3% of the patients, which is probably the lowest incidence reported in allogeneic BMT recipients. In previous studies, the incidence of *Candida* infections has been 10% or more.[1–4] With this low incidence observed little efficacy would be expected from systemic antifungal prophylaxis with activity against yeasts only. Hence, the local epidemiological situation is important in determining preventive strategies.

Only 14% of the IFIs in the present material were diagnosed during the neutropenic period post-transplant, in contrast to the data of Morrison and co-workers[7] with more than half of non-*Candida* fungal infections detected before engraftment. The median time from BMT to the diagnosis of IFI in our study was more than 4 months, considerably longer than in most previous series.[4,5] On the other hand, Saugier-Veber *et al*[17] reported a mean of 115 days post-BMT for the diagnosis of invasive pulmonary aspergillosis, and several patients with late *Aspergillus* infection were also observed in a recent report from the UK.[18] HEPA filtration may be effective in preventing early infections caused by *Aspergillus*, but is insufficient to prevent late infections.

Several studies have investigated risk factors for IFI after BMT.[1,3–5] Identified risk factors include recipient CMV seropositivity, higher recipient age, prolonged neutropenia after BMT, donor mismatch and aGVHD. We observed no difference in the mean age or CMV serology of recipients, type of conditioning, GVHD prophylaxis or time to engraftment between the patients with or without IFI.

The type of hematological disease (MDS *vs* other) was an important pre-transplant risk factor for the development of IFI in this analysis. In a report by the EBMT,[19] an incidence of 10% for *Aspergillus* infection was observed in patients transplanted for secondary leukemia or MDS. In our series, half of the patients with MDS (5/10) had IFI after BMT. The number of the patients is, however, too small to draw conclusions and more data are needed.

A high incidence of *Aspergillus* infection (45%) was found in the patients with a matched unrelated donor. A previous study has indicated that donor mismatch is a risk factor for IFI.[3] It is possible that despite improving tissue typing techniques, MUD transplants still carry a higher risk of IFI than do sibling transplants due to more complex immunobiology. Since the number of BMTs from unrelated donors was small in this series, more data is needed to appreciate the risk of IFI in patients with unrelated donors.

O'Donnell and co-workers[20] observed an increase in the incidence of IFI after introduction of methylprednisolone as GVHD prophylaxis. In the present material the incidence of IFI was lower (9 *vs* 16%) in the patients with sibling donor who received triple prophylaxis including methylprednisolone when compared to those who received prophylaxis with MTX-CsA. It is possible that by decreasing the incidence of aGVHD[21] triple prophylaxis may even decrease the morbidity and mortality caused by IFI.

Conflicting results have been reported in regard to aGVHD as a risk factor for IFI.[1,3,4,7,20] In our patients with grade III–IV aGVHD, the risk of IFI was very high. The patients with extensive cGVHD were also at high risk of late infections mostly caused by *Aspergillus* sp., which is in line with the recent observations by Guiot and co-workers.[22]

The type of GVHD treatment has not been analyzed as

a possible risk factor for IFI in most published series. The risk of IFI was 14% in our patients treated with high-dose MP and as high as 38% in the patients treated with ATG. Although it is conceivable that the patients who responded poorly to high-dose methylprednisolone obviously had more severe GVHD and hence more severe immunosuppression, it should be borne in mind that ATG treatment causes profound and prolonged immunosuppression in its own right. Most of our patients were severely immunosuppressed after repeated courses of immunosuppressive drugs and GVHD itself when IFI was diagnosed. This is probably the most likely explanation for the poor response to antifungal therapy observed.

By identifying risk factors for IFI it is possible to define high-risk patients who are likely to benefit from prophylactic measures. Fluconazole prophylaxis has reduced *Candida* infections in BMT recipients.[23–25] However, in the study of Slavin and co-workers, the incidence of yeast infections in the placebo arm was as high as 20%, far higher than observed in the present study. Fluconazole lacks activity against *Aspergillus* sp and may increase the proportion of infections caused by *Candida krusei*.[26] Low-dose amphotericin B (AmB) has reduced the incidence of systemic fungal infections in one study.[27] Also, intravenous liposomal AmB,[28,29] AmB inhalations,[30,31] and intranasal AmB[32] have been tried as prophylactic measures in BMT recipients with inconclusive results. Considering the time pattern of *Aspergillus* infections observed in the present study, antifungal prophylaxis should be continued for several months, and in patients with extensive cGVHD, up to a year after BMT.

In conclusion, most cases of IFI were caused by *Aspergillus* sp, and occurred late after BMT. They were associated with GVHD and its treatment, and were often fatal. High risk for IFI was associated with an unrelated donor, grade III–IV aGVHD or extensive cGVHD. These patients are candidates for prospective studies to discover new methods for preventing invasive fungal infections in allogeneic BMT recipients and they may also be considered candidates for early empirical antifungal treatment. More effective prevention of GVHD may reduce the risk of IFI after BMT.

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