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ORIGINAL ARTICLE

Risk factors for invasive aspergillosis and related mortality in recipients of allogeneic SCT from alternative donors: an analysis of 306 patients

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Invasive aspergillosis (IA) is a serious complication in patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT), particularly from donors other than HLA-identical sibling. All 306 patients who underwent alternative donor HSCT between 01 January 1999 and 31 December 2006 were studied. Late IA was defined as occurring $\geqslant$40 days after HSCT. The median follow-up was 284 days (range, 1–2709). Donors were matched unrelated ($n=185$), mismatched related ($n=69$), mismatched unrelated ($n=35$) and unrelated cord blood ($n=17$). According to European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria, 2 patients already had IA at HSCT, 23 had early IA and 20 had late IA (IA incidence 15%). Eight patients had proven and 37 probable IA. Multivariate analyses showed that significant predictors of IA were delayed neutrophil engraftment, extensive chronic GVHD (cGVHD), secondary neutropenia and relapse after transplant. Early IA was associated with active malignancy at HSCT, CMV reactivation and delayed lymphocyte engraftment. Late IA was predicted by cGVHD, steroid therapy, secondary neutropenia and relapse after HSCT. IA-related mortality among IA patients was 67% and was influenced by use of antithymocyte globulin, steroids, higher levels of creatinine, and lower levels of IgA and platelets. The outcome of IA depends on the severity of immunodeficiency and the status of the underlying disease.

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Introduction

Invasive aspergillosis (IA) is a severe and frequent infectious complication in allogeneic haematopoietic stem cell transplant (HSCT) recipients, leading to considerable morbidity and mortality.[1–3] Not long ago, the treatment of haematological diseases with allogeneic HSCT was limited by the scarcity of HLA-matched related donors (less than 30%).[4] Thus, the use of alternative sources of haematopoietic stem cells, from cord blood, mismatched related or unrelated donors, has been an important advance. Transplants from alternative donors are increasingly being performed worldwide. Alternative transplant recipients remain severely immunocompromised for a long period of time, due to late engraftment (particularly when cord blood is the source of stem cells) and the frequent occurrence of severe GVHD and its consequent treatment. Usually, in studies focusing on IA, alternative transplants are described together with other allogeneic transplants, even though the incidence rate of IA may vary considerably, from 7.3% in HLA-identical sibling[5] to 27% in alternative HSCT recipients.[6] Once IA develops, mortality is high, ranging from 70% to more than 90%.[1–3,5,7,8]

The aim of this study was to determine the incidence of IA in a population of patients undergoing HSCT from alternative donors, to describe clinical presentations and to identify risk factors influencing both the development and the outcome of IA.

Materials and methods

Data were obtained from prospectively collected sources (BMT registry database) when available and integrated with retrospective chart reviews when necessary, in a joint venture between the HSCT Unit and the Division of Infectious Diseases of the San Martino University Hospital in Genoa, Italy.

Procedures

Transplantation was performed according to institutional protocols. Patients received either myeloablative or reduced-intensity conditioning regimens according to

disease phase, age or comorbidities. The most frequent myeloablative conditioning regimens were either TBI (1200 cGy) and CY (120 mg/kg in 2 days) or a thiotepa-based regimen (thiotepa 15 mg/kg and CY 120–150 mg/kg). Reduced-intensity conditioning included lower-dose thiotepa (10 mg/kg) or fludarabine combined with CY. Standard GVHD prophylaxis consisted of CsA and MTX either at a normal or low dose. Cord blood recipients received mycophenolate mofetil (MMF) 15 mg/kg b.i.d. from day +1 to +28, instead of MTX. Anti-thymocyte globulin (ATG) (Thymoglobulin; Merieux, Lyon, France) at 6–15 mg/kg was administered as part of the conditioning regimens. Diagnosis and clinical grading of acute and chronic GVHD (cGVHD) were performed according to established criteria.[9,10] First- and second-line therapy of acute GVHD included prednisone and ATG, as described elsewhere.[11] Prednisone at a dose higher than 2 mg/kg was defined as high-dose steroid therapy.

All patients were cared for in single rooms with positive pressure and high-efficiency particulate (HEPA)-filtered air, and received antibacterial prophylaxis with ciprofloxacin until engraftment. Fluconazole was used as standard antifungal prophylaxis from the start of conditioning until 80 days after transplantation. Patients with a prior history of mould infection (proven, probable or possible) or with IA at time of HSCT received mould-active agents, such as amphotericin B or more recently, voriconazole. Monitoring and treatment of CMV have been described elsewhere.[12,13] From 2005, EBV reactivation was monitored by quantitative real-time PCR. Viral DNA levels exceeding 1000 genome equivalents per ml were regarded as reactivation.[14] In the event of fever lasting 72–96 h without an identified source, empirical therapy with amphotericin B, either deoxycholate or a lipid formulation, was started and continued until neutrophil recovery. Chest X-ray, computed tomography (CT) scans or both, together with physical examination and blood cultures, were performed as clinically indicated to identify the source of infection. All specimens (sputum, broncoalveolar fluid, blood and so on) were submitted for bacterial and fungal cultures.

Definitions

IA was defined as proven or probable according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria.[15] The day of diagnosis of IA was defined as the day on which the second positive diagnostic test was performed. When IA was diagnosed at autopsy, the day of diagnosis was defined as the day of first clinical suspicion of IA. If IA was never suspected during life, the day of diagnosis was the day of death. IA was defined as an early infection when it occurred less than 40 days after HSCT, and as a late infection if it occurred more than 40 days after HSCT. The cut-off of 40 days has been used in previous studies and was chosen to reflect the immediate post-engraftment period.[1,5] Cases classified as disseminated IA had more than one organ involved, along with characteristic clinical and radiological evidence. An active underlying haematological disease at transplant was defined by the presence of blast cells in the peripheral blood or by persistent disease refractory to chemotherapy.

Deaths in patients with IA were classified as due to, or associated with, IA. IA was regarded as the primary cause of death when the patient died because of progressive failure of the organ in which IA was first found in the absence of other comorbidities, excluding GVHD. A patient was considered to have died with IA if there was no evidence of resolution of IA at time of death, that is, the EORTC/MSG diagnostic criteria were still present, but there was another concomitant cause.

From 1996, the Platelia *Aspergillus* test for detecting galactomannan in blood was routinely used in our hospital. Patients were sampled twice weekly during hospital stay or more often if clinically indicated. Until 2005, a cut-off level of 1 was used. Afterwards, the cut-off level was decreased to 0.7 in a single sample or 0.5 in two consecutive samples.

Risk factors

Baseline and after-transplant variables were analysed as possible risk factors for the development of overall, early and late IA. Baseline variables were age, sex, type and status of the underlying disease at HSCT, donor type, year of transplantation, conditioning regimen, use of ATG and GVHD prophylaxis. After-transplant variables were antifungal prophylaxis, acute and chronic GVHD, neutrophil engraftment (defined as an absolute granulocyte count above 0.5×10^9 per litre for 3 consecutive days), lymphocyte engraftment (defined as a lymphocyte count above 0.3×10^9 per litre for 3 consecutive days), presence of secondary neutropenia, steroid therapy, CMV and EBV reactivation and leukaemia relapse after HSCT.

For the analysis of factors associated with mortality among patients with IA, the same aforementioned baseline and after-transplant variables were included, together with additional variables available at the time of diagnosis, including year of IA diagnosis, time of IA onset with respect to time of transplant (early or late), IA dissemination, steroid therapy, neutropenia, lymphopenia, status of the underlying disease (relapse), serum levels of immunoglobulin A (IgA) and G (IgG), platelet count, serum levels of C-reactive protein, and serum cholinesterase and creatinine levels.

Statistical analyses

Data were analysed with NCSS (2006; Kaysville, UT, USA) and SPSS (version 13.0 for Windows) statistical packages. Comparisons were carried out using the χ^2-test for categorical variables and the non-parametric Mann–Whitney test for continuous data.

Risk factors for development of IA and for mortality among patients with IA were identified by univariate and multivariate Cox regression models. Age, time to neutrophil and lymphocyte engraftments, and platelet count ($\times 10^4$ per litre) were evaluated as continuous variables. Cholinesterase, C-reactive protein, creatinine, IgA and IgG concentrations were stratified according to respective median values. Development of cGVHD, secondary neutropenia and relapse of the underlying malignancy after HSCT were regarded as time-dependent covariates.

Survival after IA was estimated with Kaplan–Meier curves. Confidence limits for all models were calculated assuming normality of parameters. *P*-values were calculated with the likelihood ratio test. Associations in 2×2 tables were analysed with Fisher's exact tests, when applicable. Two-sided *P*-values of $\leqslant 0.05$ were considered significant. Factors with univariate *P*-values of $\leqslant 0.1$ were retained in multivariate regression models.

Results

All 306 patients who underwent HSCT from alternative donors between 1 January 1999 and 31 December 2006 were included in the study, with a median follow-up of 284 days after HSCT (range, 1–2709 days). Patient characteristics, including antifungal prophylactic regimens, are shown in Table 1. Most patients underwent transplantation because of acute leukaemia and 37% of them were transplanted with an active underlying disease. Donors were matched unrelated ($n = 185$), mismatched related ($n = 69$), mismatched unrelated ($n = 35$) and unrelated cord blood ($n = 17$).

Incidence and timing

The incidence of IA was 15% (45 of 306). Two cases were diagnosed at the time of transplant, 20 cases were diagnosed by day 40 after HSCT and 23 were diagnosed thereafter. The median follow-up in these patients was 171 days (range, 5–2078). Excluding the two patients who already had IA at the time of transplant, the median time to diagnosis was 45 days (range, 4–841). In the 20 cases of early IA, the median time to diagnosis was 19.5 days (range, 4–40), whereas in the 23 cases of late IA the median time to diagnosis was 151 days (range, 42–841). The annual rate of proven or probable IA in the years 1999–2006 was 25, 14, 13, 11, 9, 14, 18 and 13%, respectively; these values did not show statistically significant variation.

Overall, 8 patients were classified as having proven IA and 37 as having probable IA. In only one of the eight cases of proven IA, the diagnosis had been obtained before death by cultures repeatedly positive for *A. flavus* from the right knee synovial fluid. All other proven cases were diagnosed at autopsy. Before death, most patients were classified as having probable and possible IA (two and four patients, respectively), whereas in one patient the fungal disease had never been suspected.

Clinical presentation

Sites of infection were the lungs in almost all patients (43 of 45), whereas 2 patients had central nervous system (CNS) infection. All proven cases had pulmonary infection, four had additional CNS disease and one had additional joint infection. Of the 37 cases with probable IA, 35 patients (95%) had respiratory symptoms, and 9 patients had signs or symptoms suggesting other organ involvement as well (CNS $n = 4$, skin $n = 2$, eye $n = 1$, pericardium $n = 1$, oral cavity $n = 1$).

Clinical symptoms included fever (34 of 45, 75%), cough (12 of 45, 27%), dyspnoea (12 of 45, 27%) and chest pain

Table 1 Characteristics of all HSCT recipients from alternative donors, including those with IA

Variable	n = *306*
Age, median (range), years	36 (16–64)
Male sex	174 (57)
Year of HSCT	
1999–2001 (median per year; range)	98 (34; 27–37)
2002–2003 (median per year; range)	79 (40; 35–44)
2004–2006 (median per year; range)	129 (45; 36–48)
Underlying disease	
Acute leukaemia	144 (47)
CML/MF	83 (27)
MM/CLL	36 (12)
Myelodysplastic syndrome	28 (9)
Severe aplastic anaemia	15 (5)
Phase of disease	
First remission	94 (31)
CR $\geqslant$2nd	99 (32)
Relapse	111 (37)
Donor type	
Matched unrelated	185 (60)
Mismatched related	69 (23)
Mismatched unrelated	35 (11)
Cord blood	17 (6)
Conditioning	
Myeloablative	218 (71)
Reduced intensity	88 (29)
ATG doses	
No	17 (6)
$\leqslant$2 doses	165 (54)
>2 doses	124 (40)
GVHD prophylaxis	
CsA + MMF	26 (9)
CsA + MTX low dose	148 (48)
CsA + MTX	132 (43)
Antifungal prophylaxis	
Fluconazole	124 (41)
Fluconazole/Amphotericin B[a]	129 (42)
Amphotericin B	41 (13)
Voriconazole	12 (4)
Neutrophil engraftment, median day (range)	22 (10–119)
Lymphocyte engraftment, median day (range)	39 (10–141)
Acute GVHD	
Grade 0–I	205 (67)
Grade II	78 (25)
Grade III–IV	23 (8)
Chronic GVHD, n = 217	
No	74 (34)
Limited	98 (45)
Extensive	45 (21)
Steroids	
0	84 (28)
$\leqslant$2 mg/kg	188 (61)
>2 mg/kg	34 (11)
CMV reactivation	166 (54)
EBV reactivation, tested in 223 patients	64 (29)
Secondary neutropenia	54 (18)
Relapse after HSCT	85 (28)

Abbreviations: ATG = anti-thymocyte globulin; HSCT = haematopoietic stem cell transplantation; MF = myelofibrosis; MM = multiple myeloma; MMF = mycophenolate mofetil.
Data are number (%) of patients, unless otherwise indicated.
[a]Fluconazole followed by amphotericin B as empirical therapy in febrile neutropenia.

(9 of 45, 20%). Only one patient presented with haemoptysis (2%). In IA with CNS involvement, altered consciousness to coma, psychotic crisis, paresis and diplopic vision were the main symptoms observed. Three patients had neither fever nor other clinical signs or symptoms and the diagnosis of probable IA was triggered by positive galactomannan antigenemia and confirmed by the presence of multiple pulmonary nodules on CT scan. Radiological pulmonary lesions were detectable in 42 of 45 patients (93%), including nodules (8 of 42, 19%), air-crescent sign or cavitations (10 of 42, 24%) and wedge-shaped consolidations (4 of 42, 10%). Notably, the halo sign was not found. Microbiological confirmation of probable IA was obtained by a positive galactomannan test in 30 of 37 (81%) patients (29 in blood and 1 from the pericardial fluid). Four patients (11%) had a positive sputum culture (two *A. flavus* and two *Aspergillus* spp) and three patients (8%) had both a positive serum galactomannan test and positive culture (one *A. flavus*, one *A. terreus* and one *Aspergillus* spp).

Risk factors for developing IA

The two patients who were diagnosed with IA at the time of transplant were excluded from the risk factor analyses. The results of univariate and multivariate analyses are detailed in Table 2. In the univariate analysis, the status of the underlying disease was the only baseline variable influencing the risk of IA ($P=0.03$). The presence of active disease increased the risk of IA by almost threefold. Post transplant predictors of IA were the duration of lymphopenia and neutropenia ($P<0.01$ and 0.03, respectively), development of severe acute GVHD (grade II or higher) and chronic extensive GVHD ($P=0.05$ and 0.03, respectively), steroid therapy ($P=0.02$), occurrence of secondary neutropenia ($P<0.01$) and relapse of the underlying haematological disease ($P<0.01$). Duration of neutropenia, chronic extensive GVHD, secondary neutropenia and relapse after HSCT remained significant in the multivariate model.

Additional analyses were performed to clarify factors associated with early and late disease (Table 3). In the

Table 2 Risk factors for developing IA after HSCT from alternative donors (Cox regression model)

Factor	*Invasive aspergillosis*		*Hazard ratio (95% CI)*	P-*value*	
	No	*Yes*		*Univariate*	*Multivariate*
Number of patients	261 (85)	43 (15)			
Age, median (range), years	36 (16–64)	40 (16–64)	1.02 (0.99–1.04)	—	NA
Male sex	151 (58)	23 (53)	0.92 (0.50–1.67)	—	NA
Year of HSCT				—	NA
1999–2001	81 (31)	17 (40)	1.00		
2002–2003	72 (28)	7 (16)	0.50 (0.21–1.19)		
2004–2006	108 (41)	19 (44)	0.94 (0.49–1.81)		
Underlying disease				—	NA
Severe aplastic anaemia	12 (4.5)	2 (5)	1.00		
Acute leukaemia	122 (47)	21 (49)	1.08 (0.25–4.59)		
CML/MF	72 (27.5)	11 (25.5)	0.80 (0.18–3.59)		
MM/CLL	32 (12)	4 (9)	0.82 (0.15–4.45)		
Myelodysplastic syndrome	23 (9)	5 (11.5)	1.26 (0.24–6.50)		
Phase				0.03	—
First CR	85 (32.5)	9 (21)	1.00		
CR $\geqslant$2nd	85 (32.5)	14 (32.5)	1.70 (0.74–3.94)		
Relapse	91 (35)	20 (46.5)	2.88 (1.31–6.36)		
Donor				0.11	—
Matched unrelated	162 (62)	22 (51)	1.00		
Mismatched related	52 (20)	16 (37)	2.03 (1.06–3.86)		
Mismatched unrelated	32 (12)	3 (7)	0.65 (0.20–2.18)		
Cord blood	15 (6)	2 (5)	1.47 (0.35–6.29)		
Conditioning				—	NA
Myeloablative	189 (72)	28 (65)	1.00		
Reduced intensity	72 (28)	15 (35)	1.40 (0.75–2.62)		
ATG				—	NA
No	15 (6)	2 (5)	1.00		
$\leqslant$2 doses	145 (55.5)	18 (42)	1.05 (0.24–4.25)		
$>$2 doses	101 (38.5)	23 (53)	1.66 (0.39–7.05)		
GVHD prophylaxis				—	NA
CsA + MMF	23 (9)	3 (7)	1.00		
CsA + MTX low dose	122 (47)	25 (58)	1.07 (0.32–3.56)		
CsA + MTX	116 (44)	15 (35)	0.66 (0.19–2.29)		

Table 2 Continued

Factor	*Invasive aspergillosis*		*Hazard ratio (95% CI)*	P-*value*	
	No	*Yes*		*Univariate*	*Multivariate*
Antifungal prophylaxis				—	NA
Fluconazole	108 (41)	16 (37)	1.00		
Fluconazole/ Amphotericin B[a]	110 (42)	18 (42)	1.08 (0.55–2.11)		
Amphotericin B	33 (13)	8 (19)	1.66 (0.71–3.87)		
Voriconazole	10 (4)	1 (2)	0.86 (0.11–6.50)		
Days of primary neutropenia	22 (10–100)	23 (12–119)	1.02 (1.00–1.03)	0.03	0.01
Days of lymphopenia	39 (10–141)	43 (17–141)	1.02 (1.01–1.02)	<0.01	—
CMV reactivation[b]	141 (54)	16 (37)	1.12 (0.53–2.37)	—	NA
EBV reactivation[c]	51 (27)	13 (41)	1.64 (0.80–3.39)	—	NA
Acute GVHD[b]				0.05	—
0–I	181 (69)	25 (58)	1.00		
⩾II	80 (31)	18 (42)	1.87 (1.01–3.47)		
Chronic GVHD[b]	193	24		0.03	0.01
No or limited	154 (80)	18 (75)	1.00		
Extensive	39 (20)	6 (25)	3.66 (1.11–12.04)		
Steroids[b]				0.02	—
0	78 (30)	6 (14)	1.00		
⩽2 mg/kg	158 (60.5)	28 (65)	2.07 (0.960–4.48)		
>2 mg/kg	25 (9.5)	9 (21)	3.96 (1.48–10.59)		
Secondary neutropenia[b]	44 (17)	7 (16)	4.61 (1.86–11.41)	<0.01	<0.01
Relapse[b]	68 (26)	7 (16)	5.33 (2.05–13.87)	<0.01	<0.01

Abbreviations: ATG = anti-thymocyte globulin; HSCT = haematopoietic stem cell transplantation; MF = myelofibrosis; MM = multiple myeloma; MMF = mycophenolate mofetil; NA = not applicable (not included in multivariate analysis).
Data are number (%) of patients, unless otherwise indicated. *P*-values of more than 0.11 are not shown (–).
[a]Fluconazole followed by amphotericin B as empirical therapy in febrile neutropenia.
[b]Time-dependent variable.
[c]Tested in 223 patients.

Table 3 Risk factors for developing early and late IA after HSCT from alternative donors

Factor	*Number*	*Early IA* (n = *20*)	P-*value*		*Number*	*Late IA* (n = *23*)[a]	P-*value*	
		HR (95% CI)	*Univ*	*Multiv*		*HR (95% CI)*	*Univ*	*Multiv*
Age, median (range), years	37 (18–64)	1.02 (0.98–1.06)	—	NA	40 (16–56)	1.014 (0.98–1.05)	—	NA
Male sex	10	0.73 (0.30–1.74)	—	NA	13	1.13 (0.49–2.58)	—	NA
Year of HSCT			0.15	—			—	NA
1999–2001	7	1.00			10	1.00		
2002–2003	1	0.18 (0.02–1.44)			6	0.61 (0.26–1.94)		
2004–2006	12	1.32 (0.52–3.36)			7	0.63 (0.24–1.65)		
Disease			—	NA			—	NA
Severe aplastic anaemia	1	1.00			1	1.00		
Acute leukaemia	13	1.31 (0.17–9.99)			8	0.83 (0.10–6.67)		
CML/MF	2	0.31 (0.03–3.42)			9	1.23 (0.16–9.73)		
MM/CLL	2	0.79 (0.07–8.68)			2	0.84 (0.08–9.26)		
Myelodysplastic syndrome	2	0.98 (0.09–10.76)			3	1.56 (0.16–14.98)		
Phase			<0.01	<0.01			—	NA
First CR	2	1.00			7	1.00		
CR⩾2nd	4	1.94 (0.36–10.61)			10	1.77 (0.65–4.49)		
Relapse	14	7.24 (1.65–31.90)			6	1.34 (0.45–3.99)		
Donor			—	NA			0.02	—
Matched unrelated	13	1.00			9	1.00		
Mismatched related	4	0.85 (0.28–2.61)			12	3.74 (1.57–8.88)		
Mismatched unrelated	1	0.41 (0.05–3.10)			2	0.98 (0.21–4.55)		
Cord blood	2	1.96 (0.44–8.71)			0	NA		
Conditioning			0.11	—			—	NA
Myeloablative	11	1.00			17	1.00		
Reduced intensity	9	2.03 (0.84–4.91)			6	0.96 (0.38–2.45)		

Table 3 Continued

Factor	*Number*	*Early IA (n = 20)*	P-*value*		*Number*	*Late IA (n = 23)*[a]	P-*value*	
		HR (95% CI)	*Univ*	*Multiv*		*HR (95% CI)*	*Univ*	*Multiv*
ATG			—	NA			0.14	—
No	0				2	1.00		
⩽2 doses	11	1.00			7	0.42 (0.09–2.02)		
>2 doses	9	1.02 (0.42–2.46)			14	1.03 (0.23–4.53)		
GVHD prophylaxis			—	NA			—	NA
CsA + MMF	2	1.00			1	1.00		
CsA + MTX low dose	12	0.98 (0.22–4.38)			13	1.27 (0.16–9.88)		
CsA + MTX	6	0.54 (0.11–2.68)			9	0.85 (0.11–6.79)		
Antifungal prophylaxis			—	NA			—	NA
Fluconazole	10	1.00			6	1.00		
Fluconazole/ Amphotericin B[b]	6	0.56 (0.20–1.53)			12	1.97 (0.74–5.24)		
Amphotericin B	4	1.30 (0.41–4.15)			4	2.24 (0.63–7.95)		
Voriconazole	0	NA			1	2.40 (0.29–19.96)		
Days of primary neutropenia, median (range)	23 (13–119)	1.02 (1.00–1.03)	0.04	—	23 (12–44)	1.01 (0.98–1.056)	—	NA
Days of primary lymphopenia, median (range)	67 (21–141)	1.02 (1.01–1.03)	<0.01	<0.01	35 (17–126)	1.01 (1.00–1.06)	0.15	—
CMV reactivation[c]	4	2.88 (0.90–9.27)	0.08	0.05	12	0.76 (0.33–1.75)	—	NA
EBV reactivation[d]	NA				6/9	1.44 (0.51–4.08)	—	NA
Acute GVHD[c]			0.05	—			0.02	—
0–I	15	1.00			10	1.00		
⩾II	5	1.84 (0.99–3.42)			13	2.60 (1.14–5.93)		
Chronic GVHD[c]		NA					0.03	0.05
No or limited					6	1.00		
Extensive					6	3.66 (1.11–12.04)		
Steroids[c]			—	NA			0.03	0.04
0	5	1.00			1	1.00		
⩽2 mg/kg	11	1.15 (0.58–2.27)			17	7.27 (0.97–54.74)		
>2 mg/kg	4	1.83 (0.66–5.05)			5	16.40 (1.92–140.46)		
Secondary neutropenia[c]	0	NA			7	5.02 (1.98–12.73)	<0.01	<0.01
Relapse[c]	0	NA			7	5.56 (2.11–14.67)	<0.01	<0.01

Abbreviations: ATG = anti-thymocyte globulin; CI = confidence interval; HR = hazard ratio; HSCT = haematopoietic stem cell transplantation; IA = invasive aspergillosis; MF = myelofibrosis; MM = multiple myeloma; MMF = mycophenolate mofetil; Multiv = multivariate analysis; NA = not applicable; Univ = univariate analysis.
Data are number of patients, unless otherwise indicated.
P-values of more than 0.11 are not shown (–).
[a]For analysis of risk factors for late IA, 235 evaluable patients were included—patients with early IA or with follow-up less than 40 days were excluded.
[b]Fluconazole followed by amphotericin B as empirical therapy in febrile neutropenia.
[c]Cox regression time dependent.
[d]Tested in 223 patients, included for late IA only.

univariate analysis, early IA was more frequent in patients with an active haematological disease at transplant ($P<0.01$), longer duration of primary neutropenia and lymphopenia ($P=0.04$ and <0.01, respectively), and severe acute GVHD ($P=0.05$). Late disease was more frequent in mismatched related donor transplants ($P=0.02$), in patients with severe acute or chronic GVHD ($P=0.02$ and 0.03, respectively), in steroid recipients ($P=0.03$), in patients with secondary neutropenia ($P<0.01$) and in those relapsing after HSCT ($P<0.01$). The multivariate analysis for early disease confirmed the role of an active underlying disease at transplant and longer lymphopenia ($P<0.01$ for both variables). Surprisingly, CMV reactivation, which was not significant in the univariate analysis for early disease ($P=0.08$), was significant in the final multivariate model ($P=0.05$). Factors actively affecting the risk of late IA, which were retained in the final multivariate model, were chronic extensive GVHD, steroid therapy, secondary neutropenia and relapse after HSCT, all with strongly significant *P*-values.

Treatment and outcome

Among all 306 patients, 168 (55%) died after a median follow-up of 284 days post-HSCT (range, 1–2709 days). A total of 26 autopsies were preformed (15%). Among the 168 patients who died, 18 died from IA and a further 12 died with IA. Therefore, the overall mortality rate

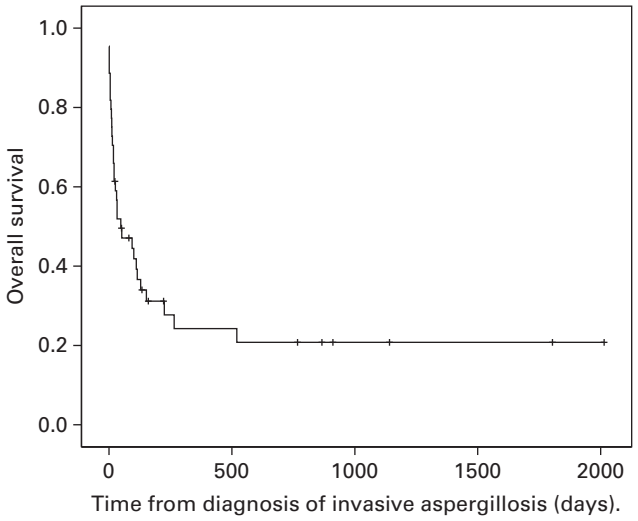

Figure 1 Probability of survival after diagnosis of invasive aspergillosis in 45 recipients of haematopoietic stem cell transplant from alternative donors.

attributable to IA was 10% (30 of 306) and IA was responsible or co-responsible for 18% (30 of 168) of deaths. Among the 138 patients who died from other causes, there were 4 cases of IA that were cured at the time of death.

Among patients with IA, crude overall survival was 24% (11 of 45), whereas the survival rate 30, 100 and 365 days after diagnosis was 59% (95% CI, 43–74), 41% (95% CI, 26–56) and 21% (95% CI, 8–35), respectively (Figure 1). Among the 34 patients with IA who died, 30 died from or with active IA, resulting in IA-related mortality rate of 67% (30 of 45).

Patients with IA received various treatment regimens, mostly in succession. Therefore, we can only provide information regarding the association of first-line treatments with death. For this very reason the variable 'treatment' was not included in the statistical analyses for survival. Survival rates were 7% (1 of 15), 33% (4 of 12) and 38% (6 of 16) with amphotericin B deoxycholate, amphotericin B lipid formulations and voriconazole, respectively. One patient, who died, received itraconazole and one patient was not treated with antifungals, because IA was never suspected before death.

Risk factors for mortality among IA patients

Factors influencing mortality in IA patients who died from or with IA are shown in Table 4. In the univariate analysis, the risk of death was lower if one or two doses of ATG were used ($P<0.01$), and higher in those with relapsed underlying disease ($P<0.01$), lymphopenia ($P=0.02$), thrombocytopenia ($P=0.02$), higher creatinine values ($P=0.04$) and decreased concentrations of cholinesterase ($P<0.01$), IgA ($P<0.01$) and IgG ($P=0.01$), with respect to median values. The multivariate analysis confirmed the benefit of one or two doses of ATG ($P<0.01$) and the effect of lower IgA levels ($P<0.01$), thrombocytopenia ($P<0.01$) and lower creatinine levels ($P=0.01$). Steroid therapy, which did not reach statistical significance in the univariate analysis ($P=0.06$), was significant in the final multivariate model ($P=0.01$).

Discussion

The characteristics of the patients included in this study are peculiar and somewhat different from those reported in other studies. Indeed, we studied only patients receiving allogeneic HSCT from alternative donors, of which 60% were transplants from matched unrelated donors and 34% were from mismatched related or unrelated donors. Approximately 50% of the patients had acute leukaemia and, more importantly, about one-third underwent transplantation with active underlying disease. Probably for these reasons, one-third of patients developed severe acute GVHD, 21% had chronic extensive GVHD and almost 75% received prolonged steroid therapy, often with high dosages. This defines a population of patients who had often received multiple cycles of chemotherapy and therefore at high risk of infectious complications. In this population we found a 15% incidence of IA, which is similar to that previously reported in mixed groups of sibling and unrelated donors.[6,16,17] Given the fact that all but one case of proven IA were demonstrated at autopsy and that the autopsy rate at our centre is low, it is likely that the incidence of IA found in our study is underestimated rather than overestimated. Compared with other studies,[5,6,16,18] we did not find a higher rate of late vs early infections. This may be due to the characteristics of our patients, who frequently had advanced and/or active disease and may have come to our centre already colonized with *Aspergillus* or, possibly, with an existing but undetectable infection. Indeed, two patients, who were excluded from the statistical analysis for predisposing factors, were diagnosed with IA at the time of transplantation, and in those with early IA the median time to diagnosis was quite short (19.5 days), despite patients being transplanted in HEPA-filtered rooms. Of note, the halo sign, thought to be specific to early angioinvasive infections, was not found on CT scans. This may be due to the fact that in the first years of the study, doctors were reluctant to move the patients to the radiology rooms during severe neutropenia. Therefore, patients might have been evaluated too late in the course of the disease.

Considering risk factors, our study confirms the essential role of neutropenia in predisposing patients to IA, as reported by others.[1,5,7] Neutrophils appear to be crucial for protection against IA, as shown by the fact that both delayed neutrophil engraftment and secondary neutropenia were strong predictors of IA. In fact, neutrophils have long been considered a key cell population for host defence against *Aspergillus*, as they are known to have a direct role in the destruction of hyphae and in the prevention of conidia germination.[19,20]

The importance of the status of the underlying disease has emerged from both univariate and multivariate analyses. Patients with advanced disease at transplantation had a sevenfold greater probability of developing early IA. On the other hand, relapse of haematological disease after HSCT and consequent secondary neutropenia were strongly associated with the risk of IA. Traditionally, patients with this condition are excluded from studies of IA after HSCT; therefore, these studies are unable to capture this important information.[21] Because of this, information

Table 4 Risk factors for IA-related mortality in 45 recipients of alternative HSCT with proven or probable IA

Factor	*Hazard ratio (95% CI)*	P-*value*	
		Univariate	*Multivariate*
Age	1.02 (0.99–1.05)	0.16	—
Male sex	1.41 (0.69–2.91)	—	NA
Year of HSCT		0.16	—
1999–2001	1.00		
2002–2003	0.54 (0.18–1.65)		
2004–2006	0.44 (0.20–0.96)		
Disease		—	NA
Severe aplastic anaemia	1.00		
Acute leukaemia	2.91 (0.38–22.45)		
CML/MF	3.13 (0.39–25.29)		
MM/CLL	1.73 (0.16–19.29)		
Myelodysplastic syndrome	4.32 (0.50–37.23)		
Phase		—	NA
First CR	1.00		
CR$\geqslant$2nd	1.25 (0.43–3.67)		
Relapse	0.97 (0.35–2.71)		
Donor		—	NA
Matched unrelated	1.00		
Mismatched related	1.54 (0.72–3.28)		
Mismatched unrelated	3.32 (0.90–12.23)		
Cord blood	NA		
Conditioning		—	NA
Myeloablative	1.00		
Reduced intensity	0.70 (0.39–1.51)		
ATG		<0.01	<0.01
No	1.00		
$\leqslant$2 doses	0.11 (0.02–0.54)		
$>$2 doses	0.44 (0.10–1.97)		
GVHD prophylaxis		—	NA
CsA + MMF	1.00		
CsA + MTX low dose	1.73 (0.23–13.08)		
CsA + MTX	1.65 (0.21–13.04)		
GVHD at IA	1.26 (0.61–2.61)	—	NA
Steroids		0.06	0.01
0	1.00		
$\leqslant$2 mg/kg	1.07 (0.31–3.67)		
$>$2 mg/kg	2.84 (0.75–10.72)		
Relapse at IA[a]	3.80 (1.58–9.13)	<0.01	—
Neutropenia 30 days before IA	1.03 (0.48–2.23)	—	NA
IgA at IA, above median of 37 mg/100 ml	0.35 (0.16–0.74)	<0.01	<0.01
IgG at IA, above median of 498 mg/100 ml	0.38 (0.18–0.80)	0.01	—
Platelets (1×10^4)	0.97 (0.95–1.00)	0.08	<0.01
Lymphopenia at IA	2.69 (1.19–6.11)	0.02	—
Cholinesterase at IA, above median of 2197 mg/100 ml	0.35 (0.16–0.77)	<0.01	—
CRP at IA, above median of 98 mg/100 ml	1.56 (0.76–3.22)	—	NA
Creatinine above 1.5 mg/100 ml	2.31 (1.05–5.09)	0.04	0.01
Year of IA, 2004–2006	0.51 (0.24–1.08)	0.08	—
Late IA	1.28 (0.61–2.68)	—	NA
Disseminated IA	1.96 (0.95–4.02)	0.07	—

Abbreviations: ATG = anti-thymocyte globulin; CI = confidence interval; CRP = C-reactive protein; HSCT = haematopoietic stem cell transplantation; IA = invasive aspergillosis; MF = myelofibrosis; MM = multiple myeloma; MMF = mycophenolate mofetil; NA = not applicable.
P-values of more than 0.11 are not shown (−).
[a]Cox regression time dependent.

about the natural history of leukaemia, transplantation and aspergillosis is lost. More importantly, survival in HSCT recipients with relapsed disease has improved significantly in the past decade, because of immune interventions, re-transplantation[22] and the advent of tyrosine kinase inhibitors for certain types of leukaemia.[23] These innova-

tions have created an expanding group of patients who live with relapsed disease after HSCT and might need *ad hoc* antifungal interventions, so data on fungal infections in this population are of great interest.

Other factors associated with early and late IA differed. Lymphopenia and CMV infection predisposed patients to early IA, whereas GVHD and its treatment were leading risk factors for late IA. Indeed, cGVHD and steroid therapy resulted in a 3- to 5-fold increase in late IA in our patients.

In our experience, 10% of all HSCT recipients died from or with IA, which accounted for 18% of post transplant deaths. The IA-related mortality rate was 67%. Many studies reported extremely high mortality rates in the 1990s,[3,7] but more recent studies have found slightly improved survival rates apparently related to the use of new and more active antifungal drugs and/or earlier diagnosis and treatment.[2,21] Certainly, the mortality rates in our study were very different from those reported in recent clinical trials of antifungals,[24,25] suggesting that epidemiological data extrapolated from clinical trials inadequately describe the actual situation in the general patient population, because in clinical trials patients are selected according to inclusion and exclusion criteria.

Regarding risk factors for IA-related mortality, as found by others, we confirm the role of the state of immunity in influencing survival.[1,6,8,16,21,26] All the factors found to be significant in our analysis, such as steroid therapy, GVHD, lymphopenia, thrombocytopenia and low immunoglobulin levels, confirm that the ability of the patient's immune system to control and fight fungal infections is inversely related to the dosage of immunosuppressive therapy and directly related to the integrity of the immune system. Similar, although rarely reported in the past, is the effect of lower immunoglobulin concentrations, suggesting that in these patients achieving higher immunoglobulin levels might be important for overcoming infections.[27] The favourable impact on mortality of the inclusion of ATG in the conditioning regimen may be explained by a protective effect on GVHD. Although it did not reach statistical significance, disseminated IA seemed to increase the risk of death, as reported by others.[28]

In conclusion, the risk of IA in patients undergoing an alternative HSCT is high, both immediately after transplant and during the post-engraftment phase. Aggressive monitoring and/or mould-active prophylaxis during particular risk periods might be a solution, depending on statistical and clinical considerations. As the population of alternative HSCT recipient is expanding, due to more frequent cord blood transplantations and the increased age of patients eligible for such a procedure, the knowledge of specific risk factors influencing IA risk and mortality in this group of patients is of crucial importance.

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Conflict of interest

CV has acted as a speaker and served on advisory boards for Pfizer, Merck, Gilead, Novartis and Schering-Plough. He has received grants from Gilead and Abbott. All other authors declare no conflict of interest.

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