Table 1. Characteristics of recipients of cord blood or bone marrow from unrelated donors in 484 patients with acute myeloid leukemia and 336 patients with acute lymphoblastic leukemia

	Acute myeloid leukemia			Acute lymphoblastic leukemia		
Characteristic	**U-CBT**	**U-BMT**	***P***	**U-CBT**	**U-BMT**	***P***
No. of transplantations	173	311		114	222	
Median patient age at transplantation, y (range)	38 (16-69)	38 (16-60)	.61	34 (16-58)	32 (16-59)	.29
Patient sex, n (%)						
Male	80 (46)	194 (62)	< .001	52 (46)	137 (62)	.005
Female	93 (54)	117 (38)		62 (54)	85 (38)	
Sex matching, n (%)			< .001			.002
Matched	83 (48)	216 (69)		52 (46)	145 (65)	
Male to female	44 (25)	57 (18)		35 (31)	42 (19)	
Female to male	46 (27)	37 (12)		27 (24)	35 (16)	
Unknown	0 (0)	1 (0)		0 (0)	0 (0)	
Disease classification						
AML (French-American-British)			.045			
M0	17 (10)	26 (8)				
M1	30 (17)	38 (12)				
M2	52 (30)	88 (28)				
M3	4 (2)	25 (8)				
M4	27 (16)	55 (18)				
M5	23 (13)	41 (13)				
M6	3 (2)	18 (6)				
M7	2 (1)	5 (2)				
Others/unknown	15 (9)	15 (5)				
Cytogenetics			.042			
Favorable*	19 (11)	66 (21)				
Normal	74 (43)	116 (37)				
Other	57 (33)	95 (31)				
Unknown	23 (13)	34 (11)				
ALL cytogenetics						.022
t(9;22)				43 (38)	52 (23)	
t(4;11)				2 (2)	3 (1)	
Others				22 (19)	51 (23)	
Normal				27 (24)	85 (38)	
Unknown				20 (18)	31 (14)	
Disease status			.003			.33
First CR	50 (29)	130 (42)		63 (55)	130 (59)	
Second or after CR	39 (23)	82 (26)		21 (18)	48 (22)	
Relapse/induction failure	81 (47)	95 (31)		30 (26)	42 (19)	
Unknown	3 (2)	4 (1)		0 (0)	2 (1)	
HLA matching†						
0 mismatched loci	12 (7)			8 (7)		
1 mismatched locus	35 (20)			25 (22)		
2 mismatched loci	126 (73)			81 (71)		
ABO matching			< .001			< .001
Matched	59 (34)	185 (59)		37 (32)	128 (58)	
Minor mismatch	48 (28)	57 (18)		30 (26)	48 (22)	
Major mismatch	37 (21)	59 (19)		24 (21)	41 (18)	
Bidirectional	28 (16)	8 (3)		23 (20)	3 (1)	
Unknown	1 (1)	2 (1)		0 (0)	2 (1)	
Nucleated cells infused per 10^7/kg, median (range)	2.44 (1.65-5.49)	26.3 (2.10-58.8)	< .001	2.48 (1.51-4.06)	28.2 (2.30-79.0)	< .001
Preparative regimen			< .001			.38
CY + TBI	43 (25)	142 (46)		42 (37)	92 (41)	
CY + CA + TBI	62 (36)	41 (13)		31 (27)	53 (24)	
CY + BU + TBI	7 (4)	36 (12)		3 (3)	5 (2)	
Other TBI regimen	42 (24)	33 (11)		34 (30)	54 (24)	
BU + CY	18 (10)	55 (18)		4 (4)	12 (5)	
Other non-TBI regimen	1 (1)	4 (1)		0 (0)	6 (3)	
GVHD prophylaxis			< .001			< .001
Cyclosporine A + sMTX	103 (60)	131 (42)		65 (57)	100 (45)	
Cyclosporine A ± other	20 (12)	4 (1)		6 (5)	3 (1)	
Tacrolimus + sMTX	34 (20)	168 (54)		26 (23)	106 (48)	
Tacrolimus ± other	15 (9)	5 (2)		16 (14)	11 (5)	
Others	1 (1)	3 (1)		1 (1)	2 (1)	

U-CBT, indicates unrelated cord blood transplantation; U-BMT, unrelated bone marrow transplantation; CR, complete remission; HLA, human leukocyte antigen; CY, cyclophosphamide; CA, cytarabine; BU, oral busulfan; TBI, total body irradiation; and sMTX, short-term methotrexate.

*Favorable abnormal karyotypes are defined as t(8;21), inv16,or t(15;17).

†Number of mismatches was counted among HLA-A, -B (low-resolution typing), and DRB1 (high-resolution typing).

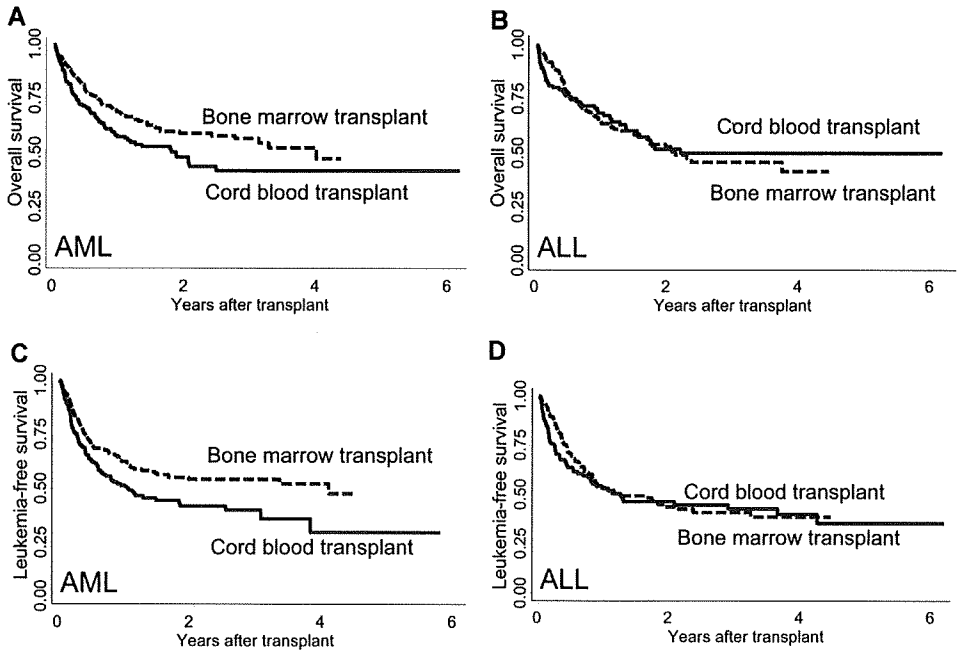

Figure 1. Adjusted OS and LFS of recipients with AML or ALL of CB or BM from unrelated donors. For patients with AML, adjusted probabilities of (A) OS (CB vs BM = 48% vs 59% at 2 years, $P = .010$) and (C) LFS (CB vs BM = 42% vs 54% at 2 years, $P = .004$) were both lower in CB recipients. For patients with ALL, the adjusted probabilities of (B) OS (CB vs BM = 52% vs 53% at 2 years, $P = .99$) and (D) LFS (CB vs BM = 46% vs 44% at 2 years, $P = .41$) were similar between CB recipients and BM recipients.

short-term methotrexate (CB vs BM = 80% vs 96% in AML patients, and CB vs BM = 80% vs 93% in ALL patients) were used preferentially in BM recipients. The median follow-up period for survivors was 1.9 years (range, 0.1-6.2 years) for CB recipients and 1.4 years (range, 0.3-4.5 years) for BM recipients.

Outcome

OS. For patients with AML, the unadjusted probabilities of OS were lower for CB recipients at 1 year (51% vs 69%) and 2 years (43% vs 60%) compared with BM recipients ($P < .001$). For patients with ALL, there were no significant differences between the 2 groups (CB vs BM = 66% vs 66% at 1 year, 49% vs 57% at 2 years, $P = .40$).

Among patients with AML, the use of CB remained a significant risk factor for overall mortality after adjustment for other factors (HR = 1.5; 95% confidence interval [CI], 1.0-2.0; $P = .028$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for overall mortality on multivariate analysis (HR = 1.1; 95% CI, 0.7-1.6; $P = .78$). The adjusted probability of OS was significantly lower for CB recipients (57% vs 69% at 1 year, and 48% vs 59% at 2 years, $P = .010$; Figure 1A) compared with BM recipients for patients with AML, whereas the adjusted probability of OS was similar (69% vs 64% at 1 year, and 52% vs 53% at 2 years, $P = .99$; Figure 1B) between the groups for patients with ALL.

Results of the subgroup analyses showed that the difference in survival among AML patients was prominent in patients demonstrating 1CR at transplantation (RR = 2.9, 95% CI = 1.4-6.2, $P = .005$; Table 3).

LFS. For patients with AML, the unadjusted probabilities of LFS were significantly lower for CB recipients at 1 year (43% vs 62%) and 2 years (36% vs 54%) compared with BM recipients ($P < .001$). For patients with ALL, the unadjusted probabilities of LFS were lower with marginal significance for CB recipients at 1 year (52% vs 58%) and 2 years (45% vs 51%) compared with BM recipients ($P = .06$).

Among patients with AML, the use of CB remained as a significant risk factor for treatment failure (ie, relapse or death) after adjustment for other factors (HR = 1.5; 95% CI, 1.1-2.0; $P = .012$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for treatment failure by multivariate analysis (HR = 1.2; 95% CI, 0.9-1.8; $P = .28$). The adjusted probability of LFS was significantly lower for CB recipients (51% vs 62% at 1 year, and 42% vs 54% at 2 years, $P = .004$; Figure 1C) compared with BM recipients for patients with AML, whereas the adjusted probability of LFS was similar (53% vs 53% at 1 year, and 46% vs 44% at 2 years, $P = .41$; Figure 1D) between the groups for patients with ALL.

Relapse

On univariate analyses, the cumulative incidence of relapse was higher for CB recipients with marginal significance in both AML (27% vs 20% at 1 year, and 31% vs 24% at 2 years) and ALL (27% vs 19% at 1 year, and 31% vs 24% at 2 years) ($P = .067$, and .085, respectively; Figure 2A,B).

On multivariate analyses adjusted by other factors, there was no significantly higher risk of relapse for CB recipients with either AML (RR = 1.2, 95% CI = 0.8-1.9, $P = .38$) or ALL (RR = 1.4, 95% CI = 0.8-2.4, $P = .19$; Table 2).

TRM

For patients with AML, the unadjusted cumulative incidence of TRM was significantly higher for CB recipients at 1 year (30% vs 19%) and 2 years (33% vs 22%) compared with those for BM recipients ($P = .004$; Figure 2C). For patients with ALL, the

Table 2. Results of multivariate analysis of outcomes in 173 recipients of cord blood and 311 recipients of bone marrow with acute myeloid leukemia, and 114 recipients of cord blood and 222 recipients of bone marrow with acute lymphoblastic leukemia

	Acute myeloid leukemia		Acute lymphoblastic leukemia	
Outcome	RR (95% CI)	P	RR (95% CI)	P
Overall survival*				
BM	1.00		1.00	
CB	1.45 (1.04-2.01)	.028	1.06 (0.71-1.57)	.78
Leukemia-free survival†				
BM	1.00		1.00	
CB	1.48 (1.09-2.01)	.012	1.22 (0.85-1.76)	.28
Relapse‡				
BM	1.00		1.00	
CB	1.21 (0.79-1.87)	.38	1.42 (0.84-2.41)	.19
TRM§				
BM	1.00		1.00	
CB	1.47 (0.95-2.28)	.085	1.01 (0.59-1.73)	.98
Neutrophil recovery‖				
BM	1.00		1.00	
CB	0.41 (0.33-0.51)	< .001	0.37 (0.29-0.48)	< .001
Platelet recovery¶				
BM	1.00		1.00	
CB	0.34 (0.27-0.44)	< .001	0.43 (0.33-0.56)	< .001
Acute GVHD#				
BM	1.00		1.00	
CB	0.80 (0.56-1.15)	.23	0.61 (0.39-0.95)	.028
Chronic GVHD**				
BM	1.00		1.00	
CB	0.94 (0.63-1.42)	.79	1.08 (0.66-1.77)	.77
Chronic GVHD, extensive type††				
BM	1.00		1.00	
CB	0.36 (0.18-0.72)	.004	0.58 (0.28-1.20)	.14

RR indicates relative risk; CI, confidence interval; BM, bone marrow; CB, cord blood; and GVHD, graft-versus-host disease.

*For overall survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

†For leukemia-free survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

‡For relapse, other significant variables for AML were more advanced disease status at conditioning, donor-recipient ABO major mismatch, chromosome abnormality other than favorable abnormalities, and cyclophosphamide and total body irradiation or busulfan and cyclophosphamide conditioning regimen; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and cyclophosphamide and total body irradiation conditioning.

§For TRM, other significant variables for AML were patient age more than 45 years at transplantation, second or after complete remission disease status, more advanced disease status, and chromosome abnormality other than favorable abnormalities; other significant variables for ALL were patient age more than 45 years at transplantation, more advanced disease status at conditioning, and conditioning other than cyclophosphamide and total body irradiation.

‖For neutrophil recovery, other significant variables for AML were second or after complete remission disease status and more advanced disease status; other significant variables for ALL were more advanced disease status at conditioning and cyclosporine-based GVHD prophylaxis.

¶For platelet recovery; other significant variables for AML were second or after complete remission disease status, more advanced disease status, female donor to male recipient donor-recipient sex mismatch, and tacrolimus-based GVHD prophylaxis; other significant variables for ALL were more advanced disease status at conditioning and conditioning other than cyclophosphamide and total body irradiation.

#For acute GVHD, no other significant variables were identified for both AML and ALL.

**For chronic GVHD, other significant variables for AML were more advanced disease status and conditioning other than cyclophosphamide and total body irradiation or busulfan and cyclophosphamide; there were no other significant variables identified for ALL.

††For extensive chronic GVHD, there were no other significant variables identified for AML; another significant variable for ALL was patient male sex.

cumulative incidence of TRM was similar between the 2 groups (CB vs BM = 21% vs 23% at 1 year, 24% vs 25% at 2 years, $P = .83$; Figure 2D).

On multivariate analyses adjusted by other factors, the risk for TRM was higher for CB recipients compared with that for BM recipients among patients with AML (RR = 1.5, 95% CI = 1.0-2.3, $P = .085$; Table 2) with marginal significance. For patients with ALL, the risk for TRM was similar between CB and BM recipients (RR = 1.0, 95% CI = 0.6-1.7, $P = .98$).

Cause of death

Recurrence of the primary disease was the leading cause of death in each group (CB vs BM = 37% vs 33% in patients with AML and 36% vs 41% in patients with ALL). The following causes were infection and organ failure in all groups (Table 4).

Other outcomes of transplantation

Neutrophil and platelet recovery. The unadjusted cumulative incidence of neutrophil recovery or platelet recovery at day 100 was significantly lower in CB recipients for both AML (77% vs 94%) and ALL (80% vs 97%) compared with that among BM recipients ($P < .001$ for both). On multivariate analyses, neutrophil recovery was significantly lower among CB recipients for both AML (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$) and ALL (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$; Table 2).

Table 3. Results of multivariate analysis of overall survival according to disease status at transplantation

	First complete remission			Second or after complete remission			More advanced		
Overall survival	n	RR (95% CI)	P	n	RR (95% CI)	P	n	RR (95% CI)	P
AML									
UBMT	130	1.00		82	1.00		95	1.00	
UCBT	50	2.92 (1.38-6.18)	.005	39	1.24 (0.51-3.04)	.63	81	1.29 (0.84-1.98)	.25
ALL									
UBMT	130	1.00		48	1.00		42	1.00	
UCBT	63	1.60 (0.84-3.05)	.16	21	0.62 (0.22-1.74)	.36	30	0.80 (0.38-1.69)	.57

RR indicates relative risk; CI, confidence interval; UBMT, unrelated bone marrow transplantation; and UCBT, unrelated cord blood transplantation.

The unadjusted cumulative incidence of platelet recovery greater than 50 000/μL at 4 months was significantly lower among CB recipients for both AML (59% vs 85%) and ALL (61% vs 83%) compared with that of BM recipients ($P < .001$ for both). The difference was also significant on multivariate analyses for both AML (RR = 0.3, 95% CI = 0.3-0.4, $P < .001$) and ALL (RR = 0.4, 95% CI = 0.3-0.6, $P < .001$; Table 2).

Acute GVHD. The unadjusted cumulative incidence of grade 2 to 4 acute GVHD was lower among CB recipients compared with that among BM recipients (32% vs 35% in AML, 28% vs 42% in ALL); the difference was significant in patients with ALL ($P = .39$ in AML, $P = .008$ in ALL). The difference was also significant on multivariate analyses in ALL (RR = 0.6, 95% CI = 0.4-1.0, $P = .028$). There was no significant difference in patients with AML (RR = 0.8, 95% CI = 0.6-1.2, $P = .23$; Table 2).

Chronic GVHD. The unadjusted cumulative incidence of chronic GVHD at 1 year after transplantation did not significantly differ between CB recipients and BM recipients in both AML (28% vs 32%, $P = .46$) and ALL (27% vs 30%, $P = .50$). The cumulative incidence of extensive-type chronic GVHD was significantly lower among CB recipients compared with that among BM recipients in both AML (8% vs 20%, $P < .001$) and ALL (10% vs 17%, $P = .034$). On multivariate analyses, the risk of developing chronic GVHD was similar in CB recipients and BM recipients in both AML (RR = 0.9, 95% CI = 0.6-1.4, $P = .79$) and ALL (RR = 1.1, 95% CI = 0.7-1.8, $P = .77$). The risk of developing extensive chronic GVHD was lower in CB recipients compared with BM recipients (RR = 0.4, 95% CI = 0.2-0.7, $P = .004$ in AML, and RR = 0.6, 95% CI, 0.3-1.2, $P = .14$ in ALL) and was significantly different in patients with AML (Table 2).

Discussion

The objective of our study was to investigate the outcomes of HLA-A, -B, low-resolution, and -DRB1 high-resolution 0 to 2 mismatched single-unit unrelated CBT in adult patients with acute leukemia compared with those of HLA-A, -B, -C, and -DRB1 (8 of 8) allele-matched unrelated BMT. Although AML and ALL are different diseases, previous comparisons of unrelated BMT and

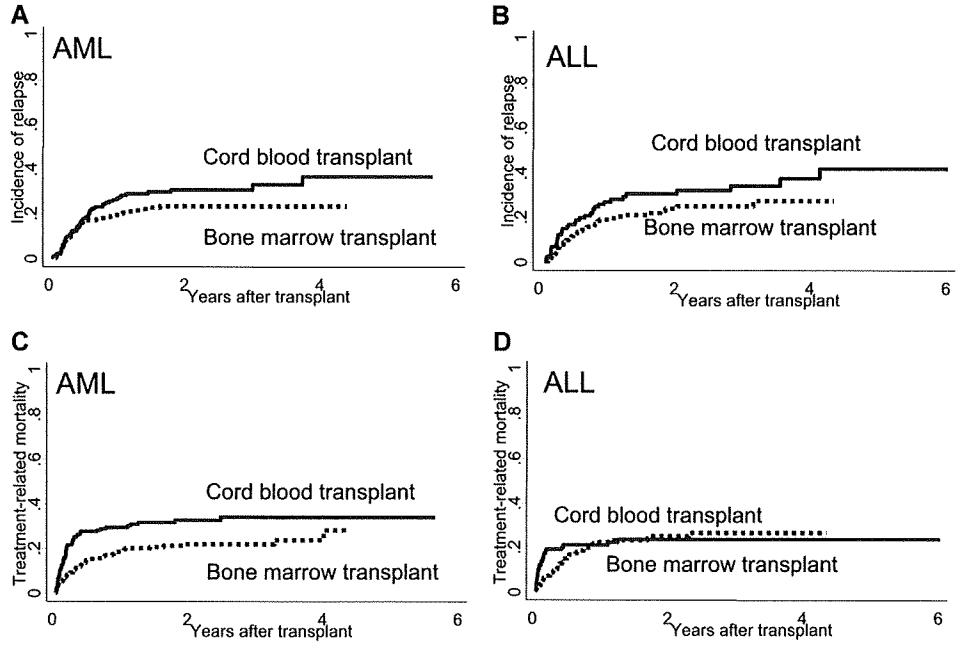

Figure 2. Cumulative incidence of relapse or TRM of recipients of CB or BM among patients with AML or ALL. For patients with AML, the cumulative incidence of (A) relapse (CB vs BM = 31% vs 24% at 2 years, $P = .068$) and (C) TRM (CB vs BM = 33% vs 22% at 2 years, $P = .004$) was higher in CB recipients. For patients with ALL, the cumulative incidence of relapse (B) was higher in CB recipients with marginal significance (CB vs BM = 31% vs 24% at 2 years, $P = .085$), but the incidence of TRM (D) was similar in CB and BM recipients (CB vs BM = 24% vs 25% at 2 years, $P = .83$).

Table 4. Causes of death after transplantation of unrelated cord blood or unrelated bone marrow among patients with acute myeloid leukemia or acute lymphoblastic leukemia

	Acute myeloid leukemia		Acute lymphoblastic leukemia	
Cause of death	UCBT	UBMT	UCBT	UBMT
Recurrence of disease	35 (37)	34 (33)	18 (36)	34 (41)
Graft failure/rejection	3 (3)	4 (4)	0 (0)	3 (4)
Graft-versus-host disease	6 (6)	7 (7)	3 (6)	5 (6)
Infection	22 (23)	19 (18)	13 (26)	11 (13)
Idiopathic pneumonia	4 (4)	4 (4)	2 (4)	6 (7)
Organ failure	17 (18)	17 (16)	8 (16)	10 (12)
Secondary cancer	0 (0)	1 (1)	0 (0)	0 (0)
Other causes	5 (5)	5 (5)	2 (4)	4 (5)
Unknown/data missing	2 (2)	13 (13)	4 (8)	10 (12)
Total	94 (100)	104 (100)	50 (100)	83 (100)

Data are presented as n (%).

UCBT indicates unrelated cord blood transplantation; and UBMT, unrelated bone marrow transplantation.

unrelated CBT did not separate these 2 diseases. Our report is the first to show the result of disease-specific analyses with a sufficient number of patients.

For AML patients, the recipients of CB were more likely to have advanced leukemia at the time of transplantation, as reported previously, suggesting that CB was used as an alternative stem cell source in the later phase of unrelated donor searches, especially in adults.[11,12,14] A larger proportion of CB recipients with ALL had the Philadelphia chromosome abnormality, which correlates with highly aggressive ALL and usually requires urgent transplantation, in which CB has an advantage over BM.[21]

Different outcomes of mortality were found between AML and ALL in a controlled comparison using multivariate analyses. Whereas significantly lower OS and LFS rates were observed in CB recipients with AML, rates of overall mortality and treatment failure were similar between CB and BM recipients with ALL. The relapse rate was not different between CBT and BMT in patients with both AML and ALL, which was consistent with previous reports.[11-13] In adult patients with ALL, a previous report showed no difference in the outcome of related compared with unrelated BM or peripheral blood transplantation in 1CR.[22] Favorable disease status at transplantation could be a more important factor affecting outcome rather than the type of stem cell source or donor type in patients with ALL. It is notable that TRM in HLA allele-matched unrelated BM recipients with AML was quite low in our study. This is probably associated with the low incidence of acute and chronic GVHD in the Japanese population, which is thought to be the result of genetic homogeneity.[23-26] Among patients with AML, although the difference was not statistically significant, a higher trend of TRM observed in CB recipients might be associated with higher overall and TRM rates in CB recipients. Reasons for higher TRM could include the graft source and delayed neutrophil recovery. Better supportive care is required after CBT for patients going through a prolonged neutropenic period. Development of better graft engineering or better conditioning regimens would help to decrease the TRM rate in CB recipients. Because relapse was the major cause of death in all groups, any attempt to decrease TRM should preserve the antileukemia effect to improve OS and LFS. Another reason for the higher TRM could be a higher risk patient population, higher risk for both disease status and comorbid conditions, requiring rapid transplantation. Searching for unrelated donors earlier and providing transplantation earlier in the disease course could help to decrease TRM in CB recipients.

Neutrophil and platelet recovery was slower in CB recipients with either AML or ALL, consistent with the results of previous reports.[11,12,27] Multiple studies have reported lower incidence of acute GVHD in CB recipients.[8-10,12,13] In our study, particularly in patients with ALL, the risk of developing grade 2 to 4 acute GVHD in CB recipients was lower compared with BM recipients, which was reported to be lower compared with the incidence reported from Western countries.[23-25] The risk of developing chronic GVHD was similar between CB and BM recipient with either disease, but the risk of developing extensive-type chronic GVHD was lower in CB recipients; the difference was significant in patients with AML. It is notable that there was no increase in the incidence of acute or chronic GVHD in CB recipients among patients with either AML or ALL, despite HLA disparity.

For differences in outcomes between AML and ALL, one possibility is a difference of treatment before conditioning therapy. Most AML patients received a more intense treatment for induction and consolidation therapy compared with that for ALL. There was no adjustment made for previous treatment, and this could be the reason for higher mortality in CBT, which requires a longer time for neutrophil recovery. Another possible cause of the difference in outcomes is the difference in conditioning regimens. Preparative regimens were similar between CB and BM recipients among ALL patients. However, in patients with AML, the proportion of standard regimens, such as cyclophosphamide and TBI or busulfan and cyclophosphamide, was smaller among CB recipients. These differences in the distribution of preparative regimens were also seen in a previous report.[11] Although the final model was adjusted for conditioning regimens, we cannot rule out the possibility of an effect that larger CB recipients received additional or different chemotherapeutic agents compared with BM recipients among patients with AML. Although the difference was small, the median age of CB recipients with AML was 4 years older than CB recipients with ALL (median age, 38 vs 34 years, $P = .021$), which might have affected the higher mortality rate among CB recipients with AML. It is also possible that some unknown biologic aspects have contributed to these differences, and this would require further evaluation in future studies.

Further subgroup analyses indicated that the superiority of HLA allele-matched BM versus CB for OS was mostly found in patients with AML showing 1CR at conditioning. However, because of the limited numbers of patients in these subgroup analyses and the possibility of an unidentified bias in stem cell source selection, our findings should be verified by further analysis in a larger population.

In conclusion, we found different outcomes between patients with AML and ALL, indicating the importance of disease-specific analyses in alternative donor studies. HLA-A, -B low-resolution, and -DRB1 high-resolution 0 to 2 mismatched single-unit CB is a favorable alternative stem cell source for patients without a suitable related or 8 of 8 matched unrelated BM donor. In the absence of a suitable donor, unrelated CBT should be planned promptly to transplant the patient while in a better disease status and better clinical condition. For patients with AML, decreasing mortality, especially in the early phase of transplantation, is required to improve the outcome for CB recipients.

Acknowledgments

The authors thank all the staff members of the collaborating institutes of the Japan Cord Blood Bank Network and Japan Marrow Donor Program for their assistance and cooperation and

Dr Takakazu Kawase for validating human leukocyte antigen data of the Japan Marrow Donor Program.

This work was supported by a Research Grant for Tissue Engineering (H17-014), a Research Grant for Allergic Disease and Immunology (H20-015), and a Research Grant for Cancer (H19-1) from the Japanese Ministry of Health, Labor, and Welfare.

Authorship

Contribution: Y.A. and R.S. designed the study and wrote the paper; Y.A. analyzed results and made the figures; S. Kato and Y.M. designed the research; T.-N.I., H.A., and M. Takanashi reviewed and cleaned the Japan Cord Blood Bank Network data and reviewed the results; S. Taniguchi, S. Takahashi, S. Kai, H.S., Y. Kouzai, M.K., and T.F. submitted and cleaned the data; and S.O., M. Tsuchida, K.K., Y.M., and Y. Kodera reviewed and cleaned the Japan Marrow Donor Program data and reviewed the results.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of members from the Japan Marrow Donor Program and the Japan Cord Blood Bank Network can be found in the Supplemental Appendix (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Correspondence: Yoshiko Atsuta, Department of Hematopoietic Stem Cell Transplantation Data Management, Nagoya University School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku Nagoya, 461-0047 Japan; e-mail: y-atsuta@med.nagoya-u.ac.jp.

References

1. Szydlo R, Goldman JM, Klein JP, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol. 1997;15:1767-1777.
2. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood. 1998;92:3515-3520.
3. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med. 1998;338:962-968.
4. Wagner JE, Rosenthal J, Sweetman R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. Blood. 1996;88:795-802.
5. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med. 1996;335:157-166.
6. Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord-blood transplantation from related and unrelated donors: Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med. 1997;337:373-381.
7. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med. 1998;339:1565-1577.
8. Rocha V, Wagner JE Jr, Sobocinski KA, et al. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling: Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. N Engl J Med. 2000;342:1846-1854.
9. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood. 2001;97:2962-2971.
10. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. Lancet. 2007;369:1947-1954.
11. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004;351:2265-2275.
12. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med. 2004;351:2276-2285.
13. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood. 2004;104:3813-3820.
14. Sanz MA. Cord-blood transplantation in patients with leukemia: a real alternative for adults. N Engl J Med. 2004;351:2328-2330.
15. Kodera Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. Bone Marrow Transplant. 1999;24:995-1003.
16. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15:825-828.
17. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695-706.
18. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16:1141-1154.
19. Fine JP, Gray RJ. A proportional hazards model for subdistribution of a competing risk. J Am Stat Assoc. 1999;94:456-509.
20. Hsieh FY, Lavori PW. Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. Control Clin Trials. 2000;21:552-560.
21. Barker JN, Krepski TP, DeFor TE, Davies SM, Wagner JE, Weisdorf DJ. Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow. Biol Blood Marrow Transplant. 2002;8:257-260.
22. Kiehl MG, Kraut L, Schwerdtfeger R, et al. Outcome of allogeneic hematopoietic stem-cell transplantation in adult patients with acute lymphoblastic leukemia: no difference in related compared with unrelated transplant in first complete remission. J Clin Oncol. 2004;22:2816-2825.
23. Morishima Y, Morishita Y, Tanimoto M, et al. Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings; possible role of genetic homogeneity. The Nagoya Bone Marrow Transplantation Group. Blood. 1989;74:2252-2256.
24. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor: Japan Marrow Donor Program. N Engl J Med. 1998;339:1177-1185.
25. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002;99:4200-4206.
26. Atsuta Y, Suzuki R, Yamamoto K, et al. Risk and prognostic factors for Japanese patients with chronic graft-versus-host disease after bone marrow transplantation. Bone Marrow Transplant. 2006;37:289-296.
27. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood. 2007;109:1322-1330.

Severe events in donors after allogeneic hematopoietic stem cell donation

Joerg Halter,[1] Yoshihisa Kodera,[2] Alvaro Urbano Ispizua,[3] Hildegard T. Greinix,[4] Norbert Schmitz,[5] Geneviève Favre,[1] Helen Baldomero,[6] Dietger Niederwieser,[7] Jane F. Apperley,[8] and Alois Gratwohl[1] for the European Group for Blood and Marrow Transplantation (EBMT) activity survey office

[1]Hematology, Department of Medicine, University Hospital Basel, Basel, Switzerland; [2]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [3]Department of Hematology, Virgen del Rocio, Sevilla, Spain; [4]Department of Internal Medicine I/BMT, Medical University of Vienna, Austria; [5]Department of Hematology and Stem Cell Transplantation, Asklepios Klinik St. Georg, Hamburg, Germany; [6]EBMT activity survey office, Hematology, University Hospital Basel, Switzerland; [7]Department of Hematology and Oncology, University of Leipzig, Leipzig, Germany, and [8]Department of Haematology, Hammersmith Hospital, DuCane Road, London, UK

ABSTRACT

Background

The risk for donors of allogeneic hematopoietic stem cells transplants is generally considered negligible. Scattered reports of severe complications and a recent controversy on hematopoietic malignancies after granulocyte colony-stimulating factor administration have challenged this opinion.

Design and Methods

Three hundred and thirty-eight allogeneic transplant teams from 35 primarily European countries were asked to report numbers of fatalities, severe adverse events and hematologic malignancies occurring among their hematopoietic stem cell donors.

Results

Two hundred and sixty-two of the 338 teams (77.5%) responded to a first survey (1993-2002) and 169 of the 262 responder teams (65%) to a second survey (2003-2005). They had performed a total of 51,024 first allogeneic hematopoietic stem cell transplantations, of which 27,770 were bone marrow and 23,254 peripheral blood. They observed five donor fatalities, one after a bone marrow donation and four after peripheral blood donation (incidence 0.98 per 10,000 donations; 95% CI 0.32-2.29), 37 severe adverse events (7.25/10,000; 95% CI 5.11-9.99), of which 12 in bone marrow donors (4.32/10,000; 95% CI 2.24-7.75) and 25 in peripheral blood donors (10.76/10,000; 95% CI 6.97-15.85; $p<0.05$) and 20 hematologic malignancies (3.92/10,000; 95% CI 2.39-6.05), of which 8 after donating bone marrow and 12 after donating peripheral blood stem cells. The observed incidence rate of hematologic malignancies did not exceed the expected incidence in an age- and sex-adjusted general population.

Conclusions

Hematopoietic stem cell donation is associated with a small but definite risk of fatalities and serious adverse events. True incidences might be higher, due to potential underreporting by study design. A continuous, standardized donor follow-up is needed to define donor risk groups and to monitor intermediate and long-term sequelae.

Key words: hematopoietic stem cell donation, adverse event, hematologic malignancies, donor fatality.

Citation: Halter J, Kodera Y, Urbano Ispizua A, Greinix HT, Schmitz N, Favre G, Baldomero H, Niederwieser D, Apperley JF, and Gratwohl A, for the European Group for Blood and Marrow Transplantation (EBMT) activity survey office. Severe events in donors after allogeneic hematopoietic stem cell donation. Haematologica 2009; 94:94-101. doi: 10.3324/haematol.13668

Funding: this work was supported in part by the European Leukemia Net LSH-2002-2.2.0-3, by a grant from the Swiss National Research Foundation, 3200B0-118176, the Swiss Cancer League, the Regional Cancer League and the Horton Foundation. EBMT is supported by grants from the corporate members: Amgen Europe GmbH, F. Hoffmann-La Roche, Gilead Sciences, Miltenyl Biotec GmbH, Schering-Plough International Inc., Celegene International SARL, Chugai Sanofi - Aventis SNC, Fresenius Biotech GmbH, Gambro BCT, Genzyme, Pfizer, Berlex AG (Schering AG Germany), Therakos, Bristol Myers Squibb, Novartis, Cephalon, Laboratoires Pierre Fabre and GE Healthcare.

Manuscript received July 15, 2008. Revised version arrived September 8, 2008. Manuscript accepted September 16, 2008.

Correspondence: Joerg Halter, MD, Hematology Department of Medicine, University Hospital Basel, CH-4031 Basel, Switzerland. E-mail: jhalter@uhbs.ch

The online version of this article contains a supplementary appendix.

Introduction

Over the last two decades, allogeneic hematopoietic stem cell transplantation (HSCT) has become an established therapy and the numbers of such procedures increase year by year.[1] HSCT is still associated with significant morbidity and mortality for the patients. These risks are well defined. In contrast to the situation for the recipients, hematopoietic stem cell donation is considered a relatively safe procedure for the donor[2,3] and life-threatening complications are deemed exceedingly rare.

Detailed information on the risks associated with harvesting hematopoietic stem cells comes from prospective, randomized studies comparing bone marrow (BM) and peripheral blood (PB) donations and from unrelated donor registry reports. Both procedures are accompanied by inconvenience for the donor. Adverse events before, during and after donation are frequent but most of them are transient, self-limited and without long-term consequences.[4] Careful donor selection and evaluation have become prerequisites and have been recommended for many years.[5,6]

Sporadic reports of severe or even life-threatening adverse events have been published. These reports define potential areas of risk, such as death, vascular events, bleeding, splenic rupture, triggering of inflammatory disease, transient respiratory disturbances, acute lung injury or sickle cell crises as well as hematologic malignancies but give no estimate of the magnitude of the risk.[7-12] Nevertheless, they document a potential hazard for the donor, which appears to be small but real. Concerns regarding the safety of stem cell donation were recently increased by the debate on potential long-term adverse effects of granulocyte colony-stimulating factor (G-CSF), which is required to mobilize PB stem cells. Experimental data and observational reports raised concern about an elevated risk of hematologic malignancies after G-CSF administration.[13,14] All these data were based on small series of donors;[7,8,15,16] long-term studies or collaborative surveys are still lacking. Careful observation and monitoring of at least 2,000 donors for a minimum of 10 years after G-CSF administration has been postulated to define sufficiently the risk of a hematopoietic malignancy in this group.[17]

Based on the need for such data, the European Group for Blood and Marrow Transplantation (EBMT) attempted to gather information on severe events in donors on a large scale, making use of its activity survey's infrastructure. The data of this survey are reported in adherence with the guidelines of the STROBE statement.[18]

Design and Methods

Study design and participating teams

This is a retrospective analysis of data collected in the EBMT activity survey network. Since 1990, all EBMT members and affiliated teams have been asked to report the numbers of patients undergoing a first HSCT in their centers and provide information on the indication, donor type and stem cell source. In 2003 all 338 teams performing allogeneic HSCT in 30 European and five affiliated countries, outlined in the appendix, were asked to report events occurring in donors; 262 (78%) replied. The 262 teams responding to the 2003 survey were recontacted in 2006, informed about the preliminary data of the first survey and asked again to report events in their donors. One hundred and sixty-nine of these teams responded to the second survey (65%), hence 50% of the initial cohort.

The first survey covered the years 1993-2002, corresponding to a 10-year period starting from the first allogeneic PB HSCT,[19] while the second survey covered the years 2003 to 2005.

Responding and non-responding teams did not differ with respect to years of practising HSCT, numbers of allogeneic HSCT or World Bank category of the team's country of origin.[1]

Transplant numbers

The 262 teams responding to the first survey performed a total of 39,210 first allogeneic HSCT of which 24,099 used BM (77% from related donors) and 15,111 PB (80% from related donors) during this first period. These transplants correspond to 78% of the total of 50,580 reported first allogeneic HSCT during that time period within the EBMT activity survey. The fact that the responding teams performed 77% of BM-HSCT and 78% of PB-HSCT during that period is another indication that the distribution of the two harvest procedures between teams reporting to the donor survey and those not responding must have been similar.

The 169 of 262 (65%) teams responding to the second survey (2003-2005) treated a total of 11,814 patients with a first allogeneic HSCT during the second time period, of whom 3,671 underwent BM HSCT (48% of them from related donors) and 8,143 PB HSCT (49% of them from related donors). This corresponds to 50% of the total of 23,417 allogeneic HSCT reported during the same time period within the EBMT activity survey.

In total, the present analysis covers 51,024 first allogeneic hematopoietic stem cell donations, of which 27,770 were BM (73% from related donors) and 23,254 PB (67% from related donors). This corresponds to 69% of the 73,997 first allogeneic HSCT reported between 1993 and 2005 to the EBMT activity survey.

Because fewer teams responded to the questionnaire covering the period from 2003 to 2005, when more PB HSCT were performed in general,[19] the present analysis is based on significantly more BM harvests (72%) than PB harvests (65%); furthermore, the observation time span was longer for BM donors than for PB donors. Thus, the observation time was 200,786 person-years for BM donors and 99,875 person-years for PB donors.

Questions and definitions

The questionnaire included questions about the presence of a policy for active donor follow-up, about the numbers of serious adverse events (SAE) or donor fatal-

ities and about the development of hematologic malignancies in donors. Teams were also asked to report whether they felt confident about their data or not and whether they were willing to provide additional information. SAE were defined and restricted to any cardiovascular event or splenic rupture occurring within 30 days of donation and necessitating hospitalization. Fatality was defined as any death within 30 days of donation. Hematologic malignancies were defined as any hematologic malignancy (myeloid or lymphoid) which occurred at any time post-donation and was not present at the time of the initial assessment of the donor. The teams that reported events and agreed to provide more information were contacted again by e-mail, telephone or written letter to obtain the information. All teams were guaranteed strict confidentiality. All replies were sent to a defined mail or fax address with restricted access.

Statistics

Incidence of events and the approximate relative risks of donation were calculated as the incidence of donor events per 10,000 first allogeneic transplants. This approach was based on the assumption that each first transplant came from a different individual donor. It did not take into account that about 15% of all patients received more than one transplant, either because of rejection or relapse or within the framework of a planned double transplant program. Detailed information on this aspect was not available from the survey data. Since there were more donations than first transplants, the true incidence of events per donation is probably slightly lower than those reported here.

The results from BM and PB donors were compared using Fisher's exact test and the χ^2 test (Instant Biostatistics version 3.0, GraphPad Software Inc. San Diego, CA, USA).

The incidences of hematologic malignancies in BM and PB donors were compared by calculating the respective incidence per 10,000 person-years of follow-up. Calculated incidence rates were compared with age-specific (crude) incidence rates in the general white population of leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma and myeloma, obtained from the US Surveillance, Epidemiology, and End Results (SEER) Program.[20] Because the true age and sex distribution of our donor population is not known, information on the gender and age of donors was obtained from the EBMT ProMISe (Project Manager Internet Server) database which contains data from 72,548 donors who donated during the period 1993-2005: 57% were male and 43% were female. For the same period, the age of 19,503 donors was registered. The median age of related BM donors was 32.5 years, of unrelated BM donors 35.9 years, of related PB donors 43.7 years and of unrelated PB donors 34.6 years. Hence, unrelated BM and PB donors had the same age distribution, while related BM donors were significantly younger ($p<0.001$) than related PB donors (Figure 1) and 30.2% of all BM donors were 20 years or younger compared to 6.5% of PB donors. These results were compared with the corresponding age groups in the SEER program.

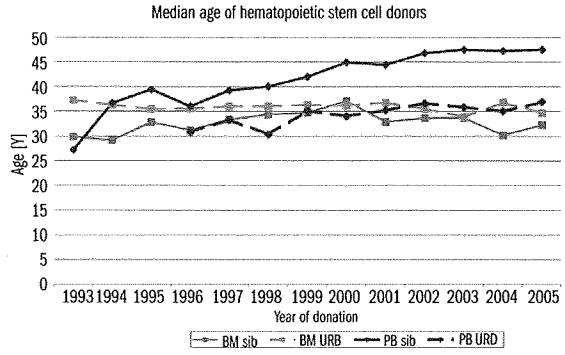

Figure 1. Median age of donors donating from 1993 - 2005 registered in the EBMT ProMISe database (n=19,503) by donor type and stem cell source. Peripheral blood stem cell-transplants from matched unrelated donors started in 1996 only. BM sib = sibling bone marrow donor; BM URD = unrelated bone marrow donor; PB sib = sibling donor of peripheral blood stem cells; PB URD = unrelated donor of peripheral blood stem cells.

Results

Follow-up policies

Of the 262 responding teams in 2003, 146 (55.7%) reported having an active donor follow-up system, 104 (39.7%) indicated that they did not have an active donor follow-up and 12 (4.6%) did not answer this question. The proportion of teams with an active donor follow-up system (60.4%) was slightly higher amongst the 169 teams responding to the second survey in 2006 (34.3% without follow-up, 5.3% with no reply). Donor follow-up in most centers was linked with the follow-up of the recipient and, therefore, ceased with the patient's death. Despite the limited formal donor follow-up, 244 teams (93%) of the first survey and 157 teams (93%) of the second survey responded that they felt confident about their data.

Donor fatalities

A total of five deaths, one in a BM donor and four in PB donors were reported (Table 1), which corresponds to an incidence of 0.98 per 10,000 first transplants (0.32-2.29/10,000 95% confidence interval [CI]) with a wide overlap of the 95% CI between BM (0.36; 0.01-2.01/10,000 95% CI) and PB (1.72; 0.05-4.40/10,000 95% CI) donations. All fatalities occurred in males between 27 and 67 years of age. All were related family donors. Of these five deaths, one (number 5) was due to an error during the donation procedure because of confusion of two infusion solutions. One donor (number 1) died from pulmonary embolism 15 days after BM harvest. He complained of pain in both legs. After two consultations during the first week after donation the diagnosis of deep venous thrombosis in both legs and in the vena cava inferior accompanied by pulmonary embolism was made on day 7. He died from massive pulmonary embolism 1 week later. A relationship with the harvest procedure is probable. Hereditary antithrombin III deficiency was later diagnosed within the family. It is possible, but unconfirmed, that the donor also suffered from

this deficiency. A third donor (number 2) developed a subarachnoid hematoma 1 day after the donation and died on day 29 from it. A minimal platelet count of 82×10^9/L after apheresis together with the patient's concurrent treatment with aspirin because of coronary heart disease might have contributed to the event. In two donors, the relationship between stem cell harvest and death remains unclear. Both died from cardiac arrest within 2 weeks after donation.

Severe adverse events

There were a total of 37 SAE, 12 in BM and 25 in PB donors as outlined in Table 2. The incidence was, therefore, 7.25 per 10,000 first transplants (95% CI 5.11-9.99) with significantly fewer SAE among the BM donors (4.32 per 10,000 first transplants; 95% CI 2.24-7.55) than among the PB donors (10.76 per 10,000 first transplants; 95% CI 6.97-15.85) ($p<0.05$). The types of events differed between the two groups. Cardiac events consisted of four cardiac arrests in the BM donor group and two myocardial infarctions in the PB donor group. Three of the former occurred during anesthesia monitoring in the operating room. Pulmonary embolism and deep venous thrombosis were more frequent in PB donors. One of

Table 1. Characteristics of donors who died within 30 days after stem cell donation.

Donor number	Age (years)	Sex	Mode of harvest	Mobilization	Number of harvest days	Died on day	Donor-recipient relationship	Cause of death
1	38	Male	BM	n.a.	1	15	Related	Massive pulmonary embolism after diagnosis of deep vein thrombosis and pulmonary embolism on day 7. Antithrombin III deficiency was later diagnosed in the family but was unknown at the time of donation
2	67	Male	PB	G-CSF	2	29	Related	Subarachnoid hematoma on day 1. Died on day 29.
3	43	Male	PB	G-CSF	2	15	Related	Cardiac arrest (no autopsy). Risk factors: arterial hypertension, heavy smoker
4	52	Male	PB	G-CSF	2	17	Related	Cardiac arrest Risk factor: smoker
5	27	Male	PB	G-CSF	1	0	Related	Cardiac arrest after human error (see text). Resuscitation unsuccessful

Table 2. Severe adverse events among 51,024 stem cell donations.

Stem cell source	Bone marrow		Peripheral blood	
event	N.	Comment	N.	Comment
Cardiovascular				
Myocardial infarction			2	
Cardiac arrest	4	All during or shortly after harvest		
Supraventricular arrhythmia			1	Probably related to catheter. Needed transesophageal stimulation
Severe hypertension	2	Former normotensive donors	1	Required treatment for 1 month post-donation in a former normotensive donor
Thromboembolic				
PE/DVT			7	Between day –2 and day 30 of harvest. Three events occurred before day 0
Stroke	1	Due to HIT antibodies		
Pulmonary complications				
TRALI			1	Due to priming the cell separator with erythrocyte concentrates (pediatric donor)
Lung edema	1	At the end of anesthesia after two donations within 1 month. Needed mechanical ventilation for 24h.		
Hemorrhage				
Subdural hematoma			1	Day 21 after donation
Unspecified	1	Recovered after transfusion of four units of red blood cells	1	Hemorrhage from femoral artery after insertion of central venous catheter
Seizures			1	Due to severe electrolyte disorder during apheresis
Splenic rupture			5	
Unspecified	3		5	
Total	12		25	

PE/DVT: pulmonary edema/deep vein thrombosis; HIT: heparin-iduced thrombocytopenia; TRALI: transfusion-related acute lung injury.

the thrombo-embolic events was due to heparin-induced thrombocytopenia (HIT). HIT was also associated with a stroke in an unrelated BM donor. Other SAE, such as splenic rupture, transfusion-related acute lung injury (TRALI), local hemorrhage or catheter-related infections were mainly procedure-related, as indicated in Table 2.

Three pediatric donors with severe adverse events were reported, two BM (cardiac arrest, lung edema) and one PB donor (TRALI). Because age was not available for all donors reported we were not able to calculate the incidence of SAE for pediatric donors separately.

Hematologic malignancies

Overall 20 hematologic malignancies were reported, 8 among BM donors and 12 amongst PB donors (Table 3). Neoplasms of both myeloid and lymphoid origin occurred with a wide range of latency from donation to diagnosis in donors of any age at the time of donation.

The incidence rates for developing a hematologic malignancy were 0.40 per 10,000 person-years for BM and 1.20 per 10,000 person-years for PB donation.

An exact comparison with the general population was not possible because of missing individual information on age and sex of the donors.

As reported above, sibling donor age was significantly higher for PB donors and increased over time (Figure 1). This higher age of PB donors might be a factor accounting for the higher incidence of SAE and the higher incidence rate of late hematologic malignancies in PB donors compared to BM donors. The observed incidence rate in both BM and PB donors was compared with that in the general population using the expected age-specific (crude) SEER incidence rates for hematologic malignancies (leukemia, lymphoma and myeloma) for both sexes.[20] The expected rates ranged from 0.9/10,000 individuals for the age group from 20 to 24 years old up to 6.3/10,000 for the age group from 55 to 59 years old with values of 1.3-1.6/10,000 for the age group from 30 to 39 years old and 1.6-2-2.8/10,000 for the age group from 35 to 45 years old. Considering these data, the observed incidence rates of hematologic malignancies in donors in our survey were not significantly different from the expected range.

Table 3. Hematologic malignancies observed in 51,024 stem cell donors.

Donor number	Age	Sex	Relationship	Mode of harvest	Mode of mobilization	Number of donations	Diagnosis	Interval between donation and diagnosis	Treatment	Outcome	Duration of follow-up
06	20	F	Unrelated	BM	–	1	AML M2	1y6m	Allogeneic HSCT	Alive in CR	2y
07	n.r.	F	Syngenic twin	BM	–	1	AML M1	12y	n.r.	n.r.	n.r.
08	n.r.	M	Sibling	BM	–	1	T-ALL	12y2m	n.r.	Died	n.a.
09	1	M	Sibling	BM	–	1	B-ALL	10m	Chemotherapy	Alive in CR	6y
10	n.r.	n.r.	Sibling	BM	–	1	NHL low grade (follicular)	6y	Radiotherapy	Alive in CR	n.r.
11	53	M	Sibling	BM	–	1	DLBCL	4m	Chemo-/ radiotherapy	Died from lymphoma	8y
12	n.r.	n.r.	n.r.	BM	–	n.r.	Lymphoma	n.r.	n.r.	n.r.	n.r.
13	57	F	Sibling	BM	–	1	Nasopharyngeal plasmacytoma	7m	Radiation, surgical resection	Alive in CR	10y
14	34	F	Sibling	PB	G-CSF	1x2	AML	2y8m	Chemotherapy/ allogeneic HSCT	Alive in CR	1y
15	38	F	Sibling	PB	G-CSF	1x2	ALL	1y5m	Chemotherapy	Died in induction	na.
16	47	F	Sibling	PB	G-CSF	1	MPN	4y3m	n.r.	n.r.	n.r.
17	n.r.	n.r.	Sibling	PB	n.r.	n.r.	CLL (familial?)	several years	n.r.	n.r.	n.r.
18	25	n.r.	Sibling	PB	n.r.	1	NHL low grade	9m	Chlorambucil	Alive in CR	3y3m
19	45	F	Sibling	PB	G-CSF	1(BM)*	NHL low grade	7y3m	Chemotherapy	Alive in CR	9m
20	41	M	Sibling	PB	G-CSF	1x2	DLBCL	4y3m	Chemotherapy	Alive in CR	4y
21	28	M	Sibling	PB	G-CSF	1	HD	1y	Chemotherapy	Alive	2y
22	68	M	Sibling	PB	G-CSF	1	Splenic maginal zone lymphoma	7y	none	Alive	1y
23	n.r.	n.r.	n.r.	PB	n.r.	n.r.	Malignancy not specified	n.r.	n.r.	n.r.	n.r.
24	n.r.	n.r.	n.r.	PB	n.r.	n.r.	Malignancy not specified	n.r.	n.r.	n.r.	n.r.
25	n.r.	n.r.	n.r.	PB	n.r.	n.r.	Malignancy not specified	n.r.	n.r.	n.r.	n.r.

**No apheresis due to intolerance after completion of G-CSF mobilization, donor finally underwent BM harvest. AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; NHL: non-Hodgkin's lymphoma; DLBCL: diffuse large B-cell lymphoma; MPN: myelo-proliferative neoplasm; CLL: chronic lymphocytic leukemia; HD: Hodgkin's disease; CR: complete remission.*

Discussion

This report illustrates the quantitative and qualitative aspects of severe events in donors of hematopoietic stem cells for allogeneic HSCT. It adds to the detailed information on minor and transient side effects of the harvest procedure. Despite several limitations due to the retrospective nature of the present survey, we estimate that about 1 in 10,000 donors had a fatal complication, about 1 in 1,500 donors had a severe complication leading to hospitalization and at least 1 in 3,000 developed a hematologic malignancy. The risk of death was not different between BM or PB donors, but there was a two-fold higher risk of SAE (1 in 1,000) after PB donation than after BM donation (1 in 2,500). Having focused on cardiovascular events and splenic rupture we cannot exclude that other important SAE were missed. Furthermore, given the retrospective nature of the survey and the lack of donor follow-up in some centers, underreporting must be assumed and *true* incidences are likely to be higher. Prospective studies which include all SAE are needed to define the risk more precisely and to enable the identification of potential risk factors. Most of the reported events occurred in related donors. The data do not allow definition of the relative impact of age, donor type (related/unrelated) or the harvest procedure on the events reported. However the higher average age of related PB donors as an important imbalance between the different groups of donors must be kept in mind when interpreting the data. Hematologic malignancies were observed after both BM and PB donation with incidence rates within the expected ranges for an age and sex-adjusted general population. Again, for the same reasons as stated above, underreporting is likely and *true* incidences may be higher.

These data contradict in part observations from carefully conducted surveys of data in unrelated donor registries. In a nation-wide prospective survey of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) no death within 30 days after donation was reported among 2,784 donors [*Y Kodera, personal communication*]. Likewise, no harvest-related death was reported in a survey conducted by the National Marrow Donor Program (NMDP) among 5,165 donors and the German Deutsche Knochenmarkspender Datei (DKMS) registry among 10,949 donors [*D. Confer and A. Schmidt, personal communications, presented at the EBMT meeting 2007*]. None of these reports covered a number of donations comparable to that in our survey. Moreover, these data came from unrelated donors and no comparable data are available for related donors. These discrepancies between the registry data and the data reported here may explain why stem cell harvesting in healthy donors has been deemed absolutely safe. The scattered publications of fatal complications and SAE in donors do, however, fit with our report. There are literature reports of nine deaths among stem cell donors, six in BM-donors (two of which occurred before the donation procedure could be done) and three in PB-donors.[7] An internal company report (which remains unpublished but was made available to Y. Kodera; *personal communication*) revealed seven additional deaths of donors of both genders between 1998 and 2001 from all over the world. Just one of them is listed in our survey. Hence, an estimate of one fatal event in 10,000 donations is likely to reflect the reality. For obvious reasons eligibility criteria for donor clearance might have been less strict for related donors than for unrelated donors, among whom no donation-related deaths have been reported so far.

The five fatal events observed in our survey had different causes. All affected donors were adult, related donors. Fatal outcomes have been reported in both male and female donors[7] (*Y. Kodera, personal communication*). The fact that in our survey all the donors who died were males is, therefore, most likely to be by chance even if 57% of more than 72,000 donors registered to the EBMT ProMISe database from 1993-2005 were males. An unequivocal relationship to the donation can be established for one of the five deaths. Human error remains a risk factor, even if people are well organized and highly trained. In a second donor it is very likely that BM donation contributed to a fatal pulmonary embolism. Surgery is a well known risk factor for venous thromboembolism and the congenital antithrombin III deficiency which was diagnosed in the family after the donor's death and which the donor, may, therefore, have had could have been a co-factor. The moderate decrease of the platelet count after apheresis together with concomitant use of aspirin may have contributed to the subarachnoid hemorrhage that led to the death of a third donor. For the other two cases only a temporal relationship with the donation exists, as death occurred within 30 days after the donation. A causal link with donation cannot be excluded. Non-fatal myocardial infarcts were also reported.

The 37 reported SAE reflect the known risks of both procedures and reveal new findings. Splenic enlargement and splenic rupture are well-known complications in healthy PB donors. Three of the five cases in our series have already been published.[9,10,21] Four donors had a cardiac arrest during or shortly after BM harvest. This is a well-known, rare complication of anesthesia. The incidence of 1.44 events per 10,000 BM donations in our survey is compatible with the results of two large recent studies in which the risk of anesthesia contributing to cardiac arrest was 1.37 and 1.1 per 10,000 episodes of anesthesia.[22,23]

Thromboembolic events apart from catheter-associated thrombosis[16] were not reported in previous studies on G-CSF-mobilized donations. Seven cases were noted in this survey (incidence 3 in 10,000). Three occurred before stem cell harvest and the mobilization had to be stopped prematurely. Activation of the coagulation system during G-CSF mobilization has been repeatedly demonstrated.[15] The two cases of myocardial infarction in the PB group might reflect the pro-inflammatory effect of G-CSF on unstable atherosclerotic plaques. This fits with the report from a series of patients with severe coronary artery disease undergoing stem cell mobilization. Angina pectoris was precipitated during mobilization in almost 90% of cases.[24]

Of special interest is the question of hematologic malignancies after stem cell donation.[12] G-CSF has been

described to induce genetic alterations in mononuclear cells of normal donors. These effects were transient and their impact is not clear yet.[13] Recent reports about a doubling of the risk of acute myeloid leukemia or myelodysplastic syndrome in patients treated for breast cancer[25] as well as a few new cases in healthy donors[14] initiated a controversial debate about the risk of G-CSF. These observations must be set in the context of the known predisposition for hematologic malignancies within families.[26] That family members have an at least doubled incidence of hematologic malignancies is widely accepted and a case of acute leukemia found in a donor on the day of BM donation underlines this risk.[27]

Twenty hematologic malignancies were reported in this survey. They occurred with a latency of a few months to more than 10 years after the donation. Malignancies of myeloid and lymphoid origin were seen, with no relation to the type of hematologic malignancy in the recipient, i.e. donors donating for siblings with myeloid neoplasias developed lymphoma and vice versa (*data not shown*). Only one of the surviving recipients developed donor-type leukemia or lymphoma during the follow-up, a rare but well-known event.[28] Hematologic neoplasias developed in both BM and PB stem-cell donors. In both groups the incidence rates were below the age-specific crude incidence rates for a normal population. Bearing in mind that even a slightly higher rate than the age-specific incidence rate could be expected,[26] underreporting of hematologic neoplasias in our survey is likely.

Information on related donors – who comprised the majority of donors in our survey – was highly dependent on survival of the recipient. Underreporting of data from donors whose recipients died, loss of follow-up of surviving recipients and donors, poor contact between donors and recipients and physicians not asking for donor health data might explain the fact that no excess incidence compared to that in a general population was observed. Since the overall survival for BM and PB recipients transplanted in responding centers from 1993-2005 was not significantly different (*data not shown*), the higher incidence rate of hematologic malignancies in PB donors is most likely to be explained by the fact that PB donors were older than BM donors, but a reporting bias or an effect of the method used for harvesting cannot be excluded.

There are additional limitations to this study. It was a retrospective analysis which relied on the team members' capacity for remembering such events. Only about half of the responding centers had a policy of active donor follow-up, which was rather heterogeneous. Considering patients' survival and its presumed impact on the quality of donor follow-up, we might have missed reliable long-term donor follow-up data for half of the related donor population. Only a selected group of teams reported the exact number of donors, their gender and age distribution was unknown and data on some of the SAE and hematologic malignancies were incomplete. Nevertheless, the large majority of centers felt confident about the data reported. In any case, the *true* incidence would be higher.

What are the consequences of this report? SAE and donor fatalities are likely to continue. With the increasing age of the recipients of HSCT, the number of older family donors with co-morbidities will increase. Efforts to improve training, safety and quality control systems by implementing the Joint Accreditation Committee-ISCT & EBMT (JACIE)[29] accreditation process (*www.jacie.org*) will further safeguard against errors but cannot prevent all of them. Harvest centers need to know about potential complications, need to inform donors about their risks and establish policies for insurance cover for donors and their families in the case of an event. Rules for standardized donor follow-up should be established by the international transplant and donor community, which would probably be best conducted within the framework of a global organization, such as the World Marrow Donor Association (*www.worldmarrow.org*), EBMT (*www.ebmt.org*), Center for International Blood and Marrow Transplant Research (*www.cibmtr.org*) or World Wide Group for Blood and Marrow Transplantation (*www.wbmt.org*). Even more importantly, rules and regulations covering legal aspects of events related to donation procedures must be established in order to protect the staff working at harvest centers.

This report demonstrates that SAE, including fatal events and hematologic malignancies do occur during follow-up in healthy donors. The incidence of these events can be estimated; it is small but real, in BM as well as in PB donors. Related PB donors are older than other donors and more frequently suffer severe adverse events during donation. Hematologic malignancies occur in both BM and PB donors. The estimation of the true incidence rates is limited by incomplete donor follow-up and significant underreporting is likely.

Donors must be informed about the potential risks of making a donation. Systematic follow-up is already well established for HSCT recipients. Such a follow-up should be extended to donors and should cover established mobilizing agents as well as new agents to come.[5,6,30]

Appendix

The co-operation of all participating teams and their staff (listed in the Online Appendix), at the EBMT Coordination Office Barcelona; EBMT Central Registry Office London, EBMT Data Office Paris, the Austrian Registry (ASCTR), the Czech Registry, the French Registry (SFGM-TC), the German Registry (DRST), the Italian Registry (GITMO), the Dutch Registry, the Spanish Registry (GETH), the Swiss Registry (STABMT), the Turkish Registry and the British Registry (BSBMT) is greatly appreciated. The authors also thank M. Stern for excellent statistical assistance, S. Stöckli for secretarial assistance, as well as L. John for technical assistance with data management.

Authorship and Disclosures

AG, JH and YK designed the current analysis. HB, GG and JH were primarily responsible for data collection.

AG and JH were primarily responsible for drafting the paper. All authors contributed to data collection, data analysis and interpretation and to the final version of the manuscript.

AG is member of a Novartis advisory committee and gets research support from AMGEN, Roche, Novartis, BMS and Pfizer. All other authors declared that they have no conflicts of interest.

References

1. Gratwohl A, Baldomero H, Schwendener A, Gratwohl M, Apperley J, Niederwieser D, et al. Predictability of hematopoietic stem cell transplantation rates. Hematologica 2007;92: 1679-86.
2. Anderlini P, Chan FA , Champlin RE, Körbling M, Strom SS. Long-term follow-up of normal peripheral blood progenitor cell donors treated with filgrastim: no evidence of increased risk of leukemia development. Bone Marrow Transplant 2002;30:661-3.
3. Cavallaro AM, Lilleby K, Majolino I, Storb R, Appelbaum FR, Rowley SD, et al. Three to six year follow-up of normal donors who received recombinant human granulocyte colony-stimulating factor. Bone Marrow Transplant 2000;25:85-9.
4. Favre G, Beksac M, Bacigalupo A, Ruutu T, Nagler A, Gluckman E, et al. Differences between graft product and donor side effects following bone marrow or stem cell donation. Bone Marrow Transplant 2003;32:873-80.
5. Anderlini P, Korbling M, Dale D, Gratwohl A, Schmitz N, Stroncek D, et al. Allogeneic blood stem cell transplantation: considerations for donors. Blood 1997;90:903-8.
6. Niederwieser D, Gentilini C, Hegenbart U, Lange T, Moosmann P, Ponisch W, et al. Transmission of donor illness by stem cell transplantation: should screening be different in older donors? Bone Marrow Transplant 2004;34:657-65.
7. Horowitz MM, Confer DL. Evaluation of hematopoietic stem cell donors. Hematology 2005:469-75.
8. Buckner CD, Clift RA, Sanders JE, Stewart P, Bensinger WI, Doney KC, et al. Marrow harvesting from normal donors. Blood 1984;64:630-4.
9. Falzetti F, Aversa F, Minelli O, Tabilio A. Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor. Lancet 1999; 353:555.
10. Nuamah NM, Goker H, Kilic YA, Dagmoura H, Cakmak A. Spontaneous splenic rupture in a healthy allogeneic donor of peripheral-blood stem cell following the administration of granulocyte colony-stimulating factor (G-CSF). A case report and review of the literature. Haematologica 2006;91(5 Suppl):ECR08.
11. Yoshida I, Matsuo K, Teshima T, Hashimoto D, Tanimoto Y, Harada M, et al. Transient respiratory disturbance by granulocyte-colony-stimulating factor administration in healthy donors of allogeneic peripheral blood progenitor cell transplantation. Transfusion 2006;46:186-92.
12. Makita K, Ohta K, Mugitani A, Hagihara K, Ohta T, Yamane T, et al. Acute myelogenous leukemia in a donor after granulocyte colony-stimulating factor-primed peripheral blood stem cell harvest. Bone Marrow Transplant 2004;33:661-5.
13. Anderlini P, Champlin RE. Biologic and molecular effects of granulocyte colony-stimulating factor in healthy individuals: recent findings and current challenges. Blood 2008;111:1767-72.
14. Bennett CL, Evens AM, Andritsos LA, Balasubramanian L, Mai M, Fisher MJ, et al. Haematological malignancies developing in previously healthy individuals who received haematopoietic growth factors: report from the Research on Adverse Drug Events and Reports (RADAR) project. Br J Haematol 2006;135:642-50.
15. Confer DL. Hematopoietic stem cell donors. In: Blume KG, Forman SJ, Appelbaum F, eds. Thomas' Hematopoietic Cell Transplantation. 3rd edition. Blackwell Publishing 2004. p. 538-49.
16. Anderlini P, Rizzo JD, Nugent ML, Schmitz N, Champlin RE, Horowitz MM. Peripheral blood stem cell donation: an analysis from the International Bone Marrow Transplant Registry (IBMTR) and European Group for Blood and Marrow Transplant (EBMT) databases. Bone Marrow Transplant 2001;27:689-92.
17. Hasenclever D, Sextro M. Safety of alloPBSCT donors: biometrical considerations on monitoring long term risks. Bone Marrow Transplant 1996; 17(Suppl 2):S28-30.
18. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Ann Intern Med 2007;147:573-7.
19. Gratwohl A, Baldomero H, Schmid O, Horisberger B, Bargetzi M, Urbano-Ispizua A. Change in stem cell source for hematopoietic stem cell transplantation (HSCT) in Europe: a report of the EBMT activity survey 2003. Bone Marrow Transplant 2005;36:575-90.
20. Surveillance, Epidemiology, and End Results (SEER) Program SEER*Stat Database: Incidence - SEER 13 Regs Limited-Use, Nov 2006 Sub (1992-2004), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2007, based on the November 2006 submission. Available from: www.seer.cancer.gov.
21. Balaguer H, Galmes A, Ventayol G, Bargay J, Besalduch J. Splenic rupture after granulocyte-colony-stimulating factor mobilization in a peripheral blood progenitor cell donor. Transfusion 2004;44:1260-1.
22. Newland MC, Ellis SJ, Lydiatt CA, Peters KR, Tinker JH, Romberger DJ, et al. Anesthetic-related cardiac arrest and its mortality: a report covering 72,959 anesthetics over 10 years from a US teaching hospital. Anesthesiology 2002;97:108-15.
23. Biboulet P, Aubas P, Dubourdieu J, Rubenovitch J, Capdevila X, d'Athis F. Fatal and non fatal cardiac arrests related to anesthesia. Can J Anaesth 2001;48:326-32.
24. Sinha S, Poh KK, Sodano D, Flanagan J, Ouilette C, Kearney M, et al. Safety and efficacy of peripheral blood progenitor cell mobilization and collection in patients with advanced coronary heart disease. J Clin Apher 2006; 21:116-20.
25. Hershman D, Neugut AI, Jacobson JS, Wang J, Tsai WY, McBride R, et al. Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst 2007;99:196-205.
26. Wang SS, Slager SL, Brennan P, Holly EA, De Sanjose S, Bernstein L, et al. Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007;109: 3479-88.
27. Niederwieser DW, Appelbaum FR, Gastl G, Gersdorf E, Meister B, Geissler D, et al. Inadvertent transmission of a donor's acute myeloid leukemia in bone marrow transplantation for chronic myelocytic leukemia. N Engl J Med 1990;322:1794-6.
28. Hertenstein B, Hambach L, Bacigalupo A, Schmitz N, McCann S, Slavin S, et al. Development of leukemia in donor cells after allogeneic stem cell transplantation-a survey of the European Group for Blood and Marrow Transplantation (EBMT). Haematologica 2005;90:969-75.
29. Samson D, Slaper-Cortenbach I, Pamphilon D, McGrath E, McDonald F, Urbano Ispizua A. Current status of JACIE accreditation in Europe: a special report from the Joint Accreditation Committee of the ISCT and the EBMT (JACIE). Bone Marrow Transplant 2007;39:133-41.
30. Cashen AF, Lazarus HM, Devine SM. Mobilizing stem cells from normal donors: is it possible to improve upon G-CSF? Bone Marrow Transplant 2007;39:577-88.

Bone Marrow Transplantation (2009), 1–7

www.nature.com/bmt

ORIGINAL ARTICLE

Pharmacokinetics of CsA during the switch from continuous intravenous infusion to oral administration after allogeneic hematopoietic stem cell transplantation

S Kimura[1], K Oshima[1], S Okuda[1], K Sato, M Sato, K Terasako, H Nakasone, S Kako, R Yamazaki, Y Tanaka, A Tanihara, T Higuchi, J Nishida and Y Kanda

Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

We investigated the serial changes in the blood CsA concentration during the switch from continuous intravenous infusion to twice-daily oral administration in allogeneic hematopoietic stem cell transplant recipients ($n = 12$). The microemulsion form of CsA, Neoral, was started at twice the last dose in intravenous infusion in two equally divided doses. The area under the concentration–time curve during oral administration (AUC_{PO}) was significantly higher than the AUC during intravenous infusion (AUC_{IV}) (median 7508 vs 6705 ng/ml × h, $P = 0.050$). The median bioavailability of Neoral, defined as ($AUC_{PO}/DOSE_{PO}$) divided by ($AUC_{IV}/DOSE_{IV}$), was 0.685 (range, 0.45–1.04). Concomitant administration of oral voriconazole ($n = 4$) significantly increased the bioavailability of Neoral (median 0.87 vs 0.54, $P = 0.017$), probably due to the inhibition of gut CYP3A4 by voriconazole. Although the conversion from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio may be better in patients taking oral voriconazole. To obtain a similar AUC, the target trough concentrations during twice-daily oral administration should be halved compared with the target concentration during continuous infusion.

Bone Marrow Transplantation advance online publication, 9 November 2009; doi:10.1038/bmt.2009.316

Keywords: CsA; pharmacokinetics; bioavailability; drug interaction

Introduction

CsA is the most widely used immunosuppressive agent for the prophylaxis of GVHD after allogeneic hematopoietic stem cell transplantation (HSCT). It is usually administered by intravenous infusion for at least several weeks after allogeneic HSCT because of the damage done to the oral and gastrointestinal mucosa by the conditioning regimen. However, the dose, target blood level, and schedule of administration vary among protocols and have not been optimized.[1] It has been shown that the blood concentration of CsA affects the incidences of acute GVHD and adverse events,[2] and an increase in the target blood concentration from 300 to 500 ng/ml in the continuous infusion of CsA significantly decreased the incidence of acute GVHD.[3] On the basis of these results, we are currently administering CsA by continuous infusion with target concentrations of 500 ng/ml for standard-risk patients and 300 ng/ml in high-risk patients. When patients can tolerate oral intake, CsA is switched from intravenous to oral administration at a dose ratio of 1:2. Neoral, a microemulsion formulation of CsA, has improved bioavailability and is the most commonly used oral product.[4] However, the appropriateness of this conversion rate has been inconsistent among earlier studies.[5,6] Parquet *et al.* reported that doubling the last intravenous dose provided the best therapeutic range concentration, whereas the concentration/dose ratio was similar in intravenous administration and oral administration and thus, 1:1 conversion seemed appropriate in the McGuire's study. In addition, no data are available regarding the detailed pharmacokinetics in allogeneic HSCT recipients. Therefore, in this study, we investigated the serial changes in the CsA blood concentration during the switch from intravenous to oral administration and assessed the bioavailability of Neoral.

Patients and methods

Patients

Patients who underwent allogeneic HSCT with GVHD prophylaxis consisting of the continuous infusion of CsA and short-term MTX were included. This single-center prospective study was approved by the Institutional Review Board of Jichi Medical University, and each patient provided their written informed consent to be enrolled in the study.

Correspondence: Dr Y Kanda, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama-city, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp
[1]These authors contributed equally to this work.
Received 22 June 2009; revised 17 September 2009; accepted 29 September 2009

Transplantation procedure

The conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) and TBI (2 Gy twice daily for 3 days) ($n=8$). Patients with severe aplastic anemia ($n=3$) were prepared with fludarabine, cyclophosphamide, and anti-thymoglobulin with or without a low dose of TBI at 2 Gy.[7] A reduced-intensity regimen with fludarabine and melphalan was used for a 58-year-old patient with acute lymphoblastic leukemia ($n=1$). GVHD prophylaxis consisted of the continuous infusion of CsA with a starting dose of 3 mg/kg/day and short-term MTX (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11 in HSCT from a donor other than an HLA-matched sibling). The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in standard-risk patients ($n=9$) or 250 and 350 ng/ml in high-risk patients ($n=3$) according to the disease status.[3] Acute GVHD was graded as described earlier.[8] Prophylaxis against bacterial, fungal, and Pneumocystis jiroveci infection consisted of levofloxacin, fluconazole (FLCZ), and sulfamethoxazole/trimethoprim (ST) or inhalation of pentamidine. In three patients, micafungin (MCFG) was used instead of FLCZ because of persistent fever despite broad-spectrum antibiotic therapy, development of Candidemia, and high risk for invasive aspergillosis, respectively. As prophylaxis against herpes simplex virus infection, acyclovir (ACV) was given from days −7 to 35, followed by a long-term low-dose administration of ACV for varicella zoster reactivation.[9] Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia.[10]

Study schedule

When patients were able to tolerate oral intake, CsA was switched from continuous infusion to oral administration. Intravenous infusion was stopped just before the first oral administration. The initial dose of Neoral was twice the last daily dose of continuous infusion, and was given in two equally divided doses based on the reported bioavailability of Neoral of about 0.4 (40%) in allogeneic HSCT recipients.[5] On the last day of the continuous infusion of CsA (day −1), the serum CsA concentration was measured at 9:00, 15:00, and 21:00. After the patient was switched to Neoral, the CsA concentration was measured just before (C_0), and 1 (C_1), 2 (C_2), 3 (C_3), 4 (C_4), 6 (C_6), and 12 (C_{12}) hours after the oral administration of Neoral on the first day (day 0) and between day 3 and day 5. The CsA concentration was measured using the CYCLO-Trac SP-whole blood kit (DiaSorin, Inc., Stillwater, MN, USA).[11] In brief, 200 µl of whole blood sample was mixed with 800 µl of methanol and centrifuged at 1600 *g* for 5 min. The methanolic supernatant (50 µl in duplicate) was mixed with 100 µl of ^{125}I-ligand and 1 ml of anti-CYCLO-Trac Immune Sep (pre-mixed mouse monoclonal antibody, donkey anti-mouse serum, and normal mouse serum). After centrifuging, the ligand was discarded by decanting and the amount of radioactivity of the pellet was determined. Data were analyzed by logit-log reduction. The standard curve was obtained using the CsA standard sera provided in the kit. The intra-assay coefficient of variance was <15%. The inter-assay coefficient of variance was <14%. The limit of detection was 4.0 ng/ml. The results of this assay showed good correlation with those obtained by high-performance liquid chromatography ($r=0.98$).

During the study, the dose of CsA could be modified at the discretion of each physician. Vital signs and laboratory variables including renal and liver function tests were evaluated on days 0, 3, 7, and 14. Concomitant medications that could potentially interact with CsA were recorded.

Statistical considerations

The area under the concentration–time curve (AUC) (0–12 h) of CsA was calculated by the trapezoidal method. We estimated the bioavailability of Neoral by dividing ($AUC_{PO}/DOSE_{PO}$) by ($AUC_{IV}/DOSE_{IV}$). Toxicities after switching from intravenous to oral administration were evaluated compared with the baseline data on day 0. Renal toxicity was defined as an elevation of the creatinine (Cr) level above × 1.5 the baseline value. Liver dysfunction was defined as an elevation of alanine aminotransferase (ALT) above × 2 the baseline value, or elevation of the total bilirubin (T-bil) level by 2 mg per 100 ml compared with the baseline value. Comparisons were made using the Wilcoxon signed-rank test for continuous variables. The Pearson correlation coefficient was used to analyze the correlation between AUC and the CsA concentration at each measurement point after logarithmic transformation. The effect of concomitant medications on CsA pharmacokinetics was first analyzed by a univariate analysis with the Mann–Whitney *U*-test, and then those with at least borderline significance ($P<0.10$) were subjected to a multivariate analysis using multiple regression modeling. A *P*-value of <0.05 was considered to be significant.

Results

Patients

Between January 2008 and April 2009, 12 patients were enrolled in the study. There were 7 males and 5 females with a median age of 34.5 years (range, 16–58). Underlying diseases included acute myeloblastic leukemia ($n=4$), acute lymphoblastic leukemia ($n=3$), severe aplastic anemia ($n=3$), chronic myelogenous leukemia ($n=1$), and myelodysplastic syndrome ($n=1$). Five patients received bone marrow graft from an unrelated donor, whereas 1 and 6 patients, respectively, received bone marrow and peripheral blood stem cell graft from a related donor. There was an HLA mismatch in three donor-recipient pairs.

Pharmacokinetic analysis

The median duration from transplantation to the switch from intravenous to oral administration was 40 days (range, 27–60). The dose of CsA and the pharmacokinetic parameters during intravenous and oral administration are shown in Table 1. Neoral was started at approximately twice the last dose of intravenous infusion, except that 1 patient (No. 8) received Neoral at the same dose as in intravenous infusion, as the mean CsA concentration on the last day of intravenous infusion was >700 ng/ml.

Table 1 Dose of CsA and pharmacokinetic parameters during the intravenous and oral administration of CsA

Patient no.	*Day −1*			*Day 0*					*Steady state (Days 3–5)*				
	$DOSE_{IV}$ *(mg/day)*	C_{mean} *(ng/ml)*	AUC_{IV} *(ng/ml × h)*	$DOSE_{PO}$ *(mg/day)*	C_{max} *(ng/ml)*	T_{max} *(h)*	C_{min} *(ng/ml)*	AUC_{IV-PO} *(ng/ml × h)*	$DOSE_{PO}$ *(mg/day)*	C_{max} *(ng/ml)*	T_{max} *(h)*	C_{min} *(ng/ml)*	AUC_{PO} *(ng/ml × h)*
1	96	590	7110	200	1300	2	370	9525	160	1400	3	550	10 625
2	140	643	7680	280	1600	3	480	10860	250	1000	2	320	7080
3	130	553	6630	260	2700	3	360	12555	160	1200	2	290	7790
4	173	663	7950	360	1900	2	340	11785	360	2500	1	420	12 420
5	192	677	7920	400	1500	3	240	8685	400	1500	2	280	8355
6	125	577	6780	260	1200	2	360	8300	260	1200	3	360	8450
7	80	527	6330	160	650	0	390	5725	160	800	2	280	6105
8	192	717	8730	200	930	2	360	8100	200	990	4	300	7225
9	240	477	5820	500	1600	3	280	9035	500	2400	2	290	11 265
10	125	357	4350	260	840	2	210	5285	260	880	2	210	5310
11	58	257	3090	120	720	2	130	3375	120	360	4	110	2860
12	77	303	3690	160	1100	2	190	6025	160	1000	1	260	6590

Abbreviations: AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration.

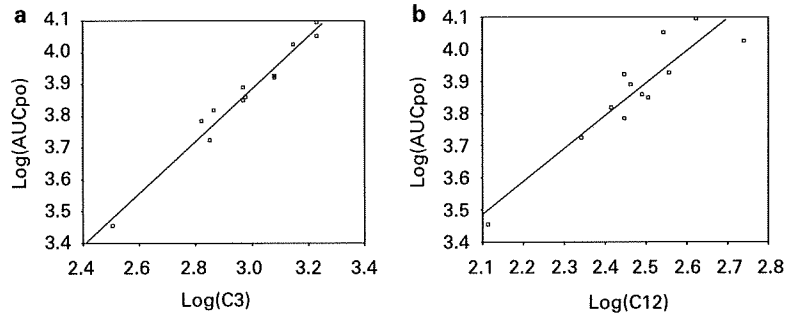

Figure 1 Correlation between the AUC and the CsA peak (**a**: C_3) and trough (**b**: C_{12}) levels.

In three patients (Nos. 1, 2, and 3), the dose of CsA was reduced on day 1 due to the high CsA concentration on day 0 (the day when Neoral was started).

The median AUC value was 6705 ng/ml × h (AUC_{IV}; range, 3090–8730) before the conversion from intravenous to oral administration (day −1), 8493 ng/ml × h (AUC_{IV-PO}; range, 3375–12 555) on day 0, and 7508 ng/ml × h (AUC_{PO}; range, 2860–12420) on days 3–5, respectively. AUC_{PO} was considered to be the AUC of Neoral in the steady state, as AUC_{IV-PO} was affected by the intravenous administration of CsA and at least 3 days are required for the CsA concentration to stabilize after a change in the administration route. As a result, not only AUC_{IV-PO} but also AUC_{PO} was significantly higher than AUC_{IV} ($P = 0.050$), even though the dose of Neoral was reduced in three patients and the conversion ratio was 1:1 in another patient. The median bioavailability of Neoral was 0.685 (range, 0.45–1.04).

Relationship between AUC and the CsA concentration at each measurement point

Although the CsA concentration at each measurement point significantly correlated with AUC_{PO} after logarithmic transformation, the strongest correlation was observed between C_3 and AUC_{PO} (Figure 1a and Table 2, correlation coefficient 0.984, $P<0.001$). The AUC_{PO} could be predicted from the trough concentration (C_0 or C_{12}), which is widely measured in daily practice, by the following formula based on the linear regression model: $\text{Log}(AUC_{PO}) = 1.020 \times \text{Log}(C_{12}) + 1.344$ (Figure 1b). Accordingly, each trough concentration between 50 and 250 ng/ml corresponds to the CsA concentration during the continuous intravenous infusion of CsA with the same AUC, calculated by dividing the predicted AUC by 12, between 99 and 514 ng/ml (Table 3). Thus, when the continuous intravenous administration of CsA with a target concentration of 500 ng/ml was switched to twice-daily oral administration, the target trough level should be about 250 ng/ml to obtain the same AUC. Also, the target blood concentration of 300 ng/ml during continuous infusion corresponds to the target trough concentration at 150 ng/ml during twice-daily oral administration. This estimation was different from that in kidney transplantation by Nakamura *et al.* (Table 3).[12]

Influence of possible confounding factors on the bioavailability of Neoral

With regard to laboratory data, there were no statistically significant correlations between the bioavailability of Neoral and the serum Cr level, ALT level, and T-bil level

Table 2 Correlation coefficients between the AUC and the cyclosporine concentration at each measurement point

	Correlation coefficient	P-*value*	*Conversion formula*
C0	0.869	<0.001	$Log(AUCPO) = 0.846 \times Log(C0) + 1.747$
C1	0.874	<0.001	$Log(AUCPO) = 0.465 \times Log(C1) + 2.539$
C2	0.953	<0.001	$Log(AUC_{PO}) = 0.718 \times Log(C_2) + 1.693$
C3	0.984	<0.001	$Log(AUC_{PO}) = 0.821 \times Log(C_3) + 1.424$
C4	0.918	<0.001	$Log(AUC_{PO}) = 0.876 \times Log(C_4) + 1.319$
C6	0.961	<0.001	$Log(AUC_{PO}) = 1.314 \times Log(C_6) + 0.258$
C12	0.921	<0.001	$Log(AUC_{PO}) = 1.020 \times Log(C_{12}) + 1.344$

Abbreviation: AUC_{PO} = area under the concentration–time curve during oral administration.

Table 3 Target cyclosporine concentration during continuous infusion to obtain a similar AUC during twice-daily oral administration with each target trough concentration

Trough level of CsA during twice-daily oral administration (ng/ml)	*Corresponding CsA concentration during continuous infusion*	
	Nakamura et al.[12]	*Current study*
50	128	99
100	255	202
150	383	305
200	510	409
250	638	514

Abbreviation: AUC = area under the concentration–time curve.

($P = 0.867$, $P = 0.159$, and $P = 0.770$, respectively). Four patients had developed acute GVHD before the change in the route of CsA administration, but all of them had stage 1 skin GVHD that was successfully controlled by topical steroid. None of the patients had gastrointestinal involvement and thus the influence of gut GVHD on the bioavailability of Neoral could not be evaluated.

With regard to drug interactions, the effects of the following drugs on the bioavailability of Neoral were evaluated; antifungal agents including FLCZ, itraconazole (ITCZ), voriconazole (VRCZ), and MCFG, antibacterial agents including ST, vancomycin, fluoroquinolones (FQ), and cefepime, antiviral agents including ACV and ganciclovir (DHPG), and other drugs including amlodipine, sulpiride, gabapentin, and prednisolone (PSL) (Table 4). FLCZ ($n = 3$), ITCZ ($n = 3$), and VRCZ ($n = 4$) were exclusively administered orally. These agents had been started at least 7 days before the change in the route of CsA administration. By the Mann–Whitney *U*-test, VRCZ, FQ, and ST were shown to have significant effects with at least borderline significance ($P = 0.048$, $P = 0.061$, and $P = 0.100$, respectively). Among these, only VRCZ was identified as an independent significant factor by a multivariate analysis ($P = 0.017$). The median bioavailability of Neoral in patients taking VRCZ was 0.87 (range, 0.76–1.04), whereas it was only 0.54 (range, 0.45–0.94) in those without VRCZ.

Clinical course after the change in the route of CsA administration

One patient (No. 2) developed liver dysfunction with an elevation of ALT from 28 IU/l at baseline to 300 IU/l 2

Table 4 Clinical and laboratory data at the conversion that could influence the cyclosporine pharmacokinetics

Patient no.	AUC_{IV} / $DOSE_{IV}$	AUC_{PO} / $DOSE_{PO}$	*Bioavailability*	*aGVHD*			*Cr (mg per 100 ml)*	*Liver function*		*Concomitant medications*	
				Grade	*Stage*			*ALT (IU/l)*	*T-bil (mg per 100ml)*	*Antifungal agents*	*Others*
					Skin	*Liver*	*Gut*				
1	74	66	0.89	I	(1	0	0)	1.14	40	0.24	VRCZ 400 mg po	VCM, ST, ACV, PPI
2	55	28	0.51	0	(0	0	0)	0.65	28	0.9	ITCZ 200 mg po	ACV, PPI, FQ
3	47	49	1.04	0	(0	0	0)	0.81	182	0.77	VRCZ 400 mg po	ST, ACV, PPI, amlodipine gabapentin
4	46	35	0.76	I	(1	0	0)	0.98	28	1.06	VRCZ 400 mg po	ST, ACV, PPI, PSL
5	41	21	0.51	0	(0	0	0)	0.89	43	0.33	FLCZ 200 mg po	ACV, PPI
6	54	33	0.61	0	(0	0	0)	0.65	92	0.79	ITCZ 200 mg po	DHPG, PPI, amlodipine
7	79	38	0.48	I	(1	0	0)	0.72	85	0.59	ITCZ 200 mg po	DHPG, PPI, amlodipine
8	45	36	0.8	0	(0	0	0)	0.60	78	0.78	FLCZ 200 mg po	ACV, PPI
9	24	23	0.94	0	(0	0	0)	0.66	96	0.65	MCFG 150 mg iv	CFPM, ACV, PPI, amlodipine
10	35	20	0.57	0	(0	0	0)	1.43	46	0.37	FLCZ 200 mg po	CFPM, ACV, PPI
11	53	24	0.45	I	(1	0	0)	0.84	16	0.53	MCFG 150 mg iv	ACV, PPI, FQ, sulpiride
12	48	41	0.85	0	(0	0	0)	1.19	20	0.55	VRCZ 400 mg po	ACV, PPI

Abbreviations: ACV = acyclovir; ALT = alanine aminotransferase; AUC_{IV} = area under the concentration–time curve (AUC) during continuous infusion; AUC_{PO} = AUC during oral administration; CFPM = cefepime; DHPG = ganciclovir; $DOSE_{IV}$ = dose of CsA during continuous infusion; $DOSE_{PO}$ = dose of CsA during oral administration; FLCZ = fluconazole; FQ = fluoroquinolones; ITCZ = itraconazole; MCFG = micafungin; PPI = proton pump inhibitors; PSL = predonisolone; ST = sulphametoxazole-trimetoprim; VCM = vancomycin; VRCZ = voriconazole.

Table 5 Serial changes in laboratory data and blood pressure after the change in the route of CsA administration

	Mean (minimum–maximum)			
	Serum creatinine (mg per 100 ml)	*ALT (IU/l)*	*Total bilirubin (mg per 100 ml)*	*Blood pressure level (mm Hg)*
Day 0	0.87 (0.60–1.43)	64.4 (16–182)	0.63 (0.24–1.06)	Systolic 130 (114–173) Diastolic 82 (63–103)
Day 3	0.86 (0.32–1.63)	50.1 (10–106)	0.62 (0.27–1.47)	Systolic 124 (109–150) Diastolic 79 (51–103)
Day 7	0.92 (0.69–1.31)	44.6 (10–103)	0.61 (0.30–1.17)	Systolic 122 (109–132) Diastolic 80 (51–103)
Day 14	0.83 (0.67–1.29)	65.8 (10–300)	0.64 (0.27–0.96)	Systolic 121 (113–135) Diastolic 76 (68–89)

Abbreviation: ALT = alanine aminotransferase.

weeks after the conversion. The AUC of CsA was rather lower after conversion, and thus CsA was not considered to be the causative agent of liver dysfunction. Otherwise, no notable changes in laboratory and clinical data were observed (Table 5).

Four patients had developed grade I acute GVHD of the skin before the change in the route of CsA administration. During the 2 weeks after the switch, 3 of the 4 patients had persistent grade I skin GVHD, whereas GVHD was improved in 1 patient. Among the eight patients who did not have acute GVHD at the switch, one patient developed grade I acute GVHD of the skin, which was well controlled by topical steroid, and the other seven patients did not develop acute GVHD during the observation period. No clinically significant changes in vital or biological parameters occurred in the study patients. One patient (No. 9) developed nausea soon after conversion. An excessive increase in the CsA concentration was considered to be the cause of nausea and this symptom was improved after the dose of Neoral was reduced.

Discussion

Neoral is a microemulsion formulation of CsA that has improved bioavailability and reduced variability in pharmacokinetic parameters within and between patients compared with a conventional CsA formulation (Sandimmun).[4] Its bioavailability has been reported to be 0.38 (38%) in healthy volunteers.[13] However, allogeneic HSCT patients have complications that could influence the CsA pharmacokinetics, such as damaged gastrointestinal mucosa and multiple drug interactions. The results of this study showed that the median value of the bioavailability of Neoral was 0.685 (range, 0.45–1.04). Detailed analyses revealed that the oral administration of VRCZ strongly affected the bioavailability of Neoral (0.87 vs 0.54). Therefore, although the switch from intravenous to oral administration of CsA at a ratio of 1:2 seemed to be appropriate in most patients, a lower conversion ratio such as 1:1.1 or 1:1.2 may be better in patients taking oral VRCZ.

The drug interactions between CsA and azole antifungal agents including FLCZ, ITCZ, and VRCZ have been well recognized.[14] Azole antifungal agents are metabolized through the cytochrome P450-3A (CYP3A4) enzyme system, interfere with the metabolism of CsA, and thereby increase the exposure to CsA. Therefore, careful monitoring of the blood CsA concentration is recommended when these agents are added during CsA administration. On the other hand, there are considerable differences among azole antifungals with regard to their ability to inhibit CYP3A4.[14] Interestingly, the concomitant use of oral VRCZ significantly increased the bioavailability of Neoral. We confirmed that VRCZ was started at least 7 days before the switch from intravenous to oral administration of CsA and was continued at the same dose after the switch. Therefore, the drug interaction between CsA and VRCZ seemed to be stronger during oral administration than during the intravenous infusion of CsA. We hypothesized that this stronger interaction can be explained by the presence of the P450 enzyme system in the gastrointestinal mucosa. The CYP3A4 isoenzymes are the most abundant isoforms of CYP and it has been postulated that CsA is also metabolized in the intestine by gut CYP3A4 isoenzymes.[15] The administration of VRCZ might have inhibited the gut metabolism of CsA and increased the bioavailability of CsA. However, a prospective controlled study is required to confirm this hypothesis.

ITCZ, another strong inhibitor of CYP3A4, did not increase the bioavailability of Neoral. As the ratio of $AUC_{IV}/DOSE_{IV}$ was higher not only in patients taking VRCZ but also in patients taking ITCZ compared with other patients (median 47.5, 55, and 41), ITCZ might have inhibited liver CYP3A4 similar to VRCZ, but inhibited gut CYP3A4 less strongly than VRCZ. This might have been affected by the different bioavailable dose of these agents, as the bioavailability of ITCZ is lower than that of VRCZ, in addition to the fact that the dose of ITCZ was lower than that of VRCZ (200 vs 400 mg/day).

With regard to the route of VRCZ, it was exclusively administered orally in this study. Therefore, we could not conclude whether the intravenous administration of VRCZ would similarly affect the bioavailability of CsA. In earlier reports, the extent of drug interaction between CsA and azole antifungals varied according to the route of administration and the dose or kind of antifungal agent. Numerous reports have shown a significant interaction (>84%) between oral FLCZ with a dose of 200 mg/day or greater and oral CsA.[16,17] On the other hand, Osowski *et al.*[18] evaluated the drug interaction between intravenous FLCZ at 400 mg/day and intravenous CsA in HSCT recipients and there was a statistically significant but smaller increase (21%) in the serum CsA concentration.

Mihara *et al.*[19] reported that the mean steady-state whole-blood level of CsA significantly increased after the route of FLCZ administration was switched from intravenous to oral. These data suggest that the drug interaction between CsA and FLCZ was stronger when FLCZ was administered orally. With regard to other azole antifungal agents, not only oral but also intravenous administration of ITCZ significantly affected the blood concentration of CsA.[20–22] Concerning the interaction between VRCZ and CsA, Mori *et al.*[23] reported that the administration of VRCZ to patients receiving CsA resulted in a significant increase in the concentration/dose ratio of CsA, but the route of VRCZ administration did not affect the changes in the concentration/dose ratio. If we consider these findings together, it may be reasonable to suggest that the interaction between azole antifungal agents and CsA is stronger when the antifungals are given orally, but the difference becomes unclear with ITCZ and VRCZ, as the interactions of these agents are stronger than that of FLCZ and can be detected even when they are given intravenously. Therefore, when we interpret pharmacokinetic data of CsA, we must be cautious not only about concomitantly used agents but also the route of administration of both CsA and the other drugs. For example, Parquet *et al.* reported that a ratio of 1:2 in the switch from intravenous to oral administration was appropriate,[5] whereas a 1:1 ratio seemed to be appropriate in the study by McGuire *et al.*[6] In the former study, oral FLCZ was used concomitantly and thus their conclusion was consistent with our data. In the latter study, information on the use of antifungal agents was not described, and thus the data were difficult to interpret.

When we switch the route of CsA administration from continuous infusion to twice-daily oral administration, the target blood concentration should also be changed. Nakamura *et al.*[12] reported that the CsA blood concentration during continuous infusion was estimated to be 2.55 times the trough level during twice-daily oral administration of Neoral to obtain an equal AUC of CsA in kidney transplant patients. In this study, we concluded that the CsA concentration during continuous infusion should be doubled compared with the trough concentration during twice-daily oral administration in allogeneic HSCT recipients. Although the calculation method was different, the conclusion was consistent (mean 2.01) when we applied their methods. Although the reason for the difference between these studies remains unclear, it may have been due to the differences in the use of concomitant drugs or the status of the gastrointestinal tract.

In conclusion, when switching CsA from continuous infusion to oral administration, concomitant medications that could affect the bioavailability of CsA, especially azole antifungal agents, should be taken into account. Although a 1:2 ratio on switching may be appropriate in most patients, a lower conversion ratio is recommended in patients taking oral VRCZ.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported in part by grants from the Ministry of Health, Labor and Welfare of Japan.

References

1 Ruutu T, Niederwieser D, Gratwohl A, Apperley JF. A survey of the prophylaxis and treatment of acute GVHD in Europe: a report of the European Group for Blood and Marrow, Transplantation (EBMT). Chronic Leukaemia Working Party of the EBMT. *Bone Marrow Transplant* 1997; **19**: 759–764.
2 Kanda Y, Hyo R, Yamashita T, Fujimaki K, Oshima K, Onoda M *et al.* Effect of blood cyclosporine concentration on the outcome of hematopoietic stem cell transplantation from an HLA-matched sibling donor. *Am J Hematol* 2006; **81**: 838–844.
3 Oshima K, Kanda Y, Nakasone H, Arai S, Nishimoto N, Sato H *et al.* Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level. *Am J Hematol* 2008; **83**: 226–232.
4 Holt DW, Mueller EA, Kovarik JM, van Bree JB, Kutz K. The pharmacokinetics of Sandimmun Neoral: a new oral formulation of cyclosporine. *Transplant Proc* 1994; **26**: 2935–2939.
5 Parquet N, Reigneau O, Humbert H, Guignard M, Ribaud P, Socié G *et al.* New oral formulation of cyclosporin A (Neoral) pharmacokinetics in allogeneic bone marrow transplant recipients. *Bone Marrow Transplant* 2000; **25**: 965–968.
6 McGuire TR, Honaker M, Lynch JC, Tarantolo SR, Bishop MR, Ketcham MA *et al.* Renal dysfunction associated with cyclosporine (CSA) prophylaxis in HLA matched sibling peripheral blood stem cell transplantation (AlloBSCT): conversion from intravenous CSA to a new oral formulation (Neoral). *Blood* 1999; **94**(Suppl 1): 334A abstr 1492.
7 Okuda S, Terasako K, Oshima K, Sato M, Nakasone H, Kako S *et al.* Fludarabine, cyclophosphamide, anti-thymocyteglobulin, and low-dose total body irradiation conditioning enables 1-HLA-locus-mismatched hematopoietic stem cell transplantation for very severe aplastic anemia without affecting ovarian function. *Am J Hematol* 2009; **84**: 167–169.
8 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
9 Asano-Mori Y, Kanda Y, Oshima K, Kako S, Shinohara A, Nakasone H *et al.* Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. *Am J Hematol* 2008; **83**: 472–476.
10 Kanda Y, Mineishi S, Saito T, Seo S, Saito A, Suenaga K *et al.* Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. *Bone Marrow Transplant* 2001; **27**: 437–444.
11 Wolf BA, Daft MC, Koenig JW, Flye MW, Turk JW, Scott MG. Measurement of cyclosporine concentrations in whole blood: HPLC and radioimmunoassay with a specific monoclonal antibody and 3H- or 125I-labeled ligand compared. *Clin Chem* 1989; **35**: 120–124.
12 Nakamura Y, Takeuchi H, Okuyama K, Akashi T, Jojima Y, Konno O *et al.* Evaluation of appropriate blood level in continuous intravenous infusion from trough concentrations after oral administration based on area under trough level in tacrolimus and cyclosporine therapy. *Transplant Proc* 2005; **37**: 1725–1727.
13 Ku YM, Min DI, Flanigan M. Effect of grapefruit juice on the pharmacokinetics of microemulsion cyclosporine and its