Table 4. Univariate and multivariate analyses of factors influencing NRM among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	n	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.21 (0.63-2.34)	.57	—		101	0.79 (0.48-1.30)	.35	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	1.25 (0.41-3.81)	.53	—		54	0.62 (0.29-1.38)	.17	—	
Other	42	0.87 (0.34-2.26)	.78	—		74	1.08 (0.65-1.81)	.76	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.77 (0.16-3.17)	.73	—		11	1.03 (0.25-4.30)	.63	1.11 (0.27-4.64)	.57
Other (no t(9;22))	96	0.92 (0.47-1.81)	.81	—		112	1.47 (0.94-2.29)	.09	1.67 (1.06-2.64)	.03
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.85 (0.86-3.97)	.12	—		192	1.01 (0.62-1.65)	.96	—	
High	108	2.82 (1.09-7.31)	.03	—		94	1.03 (0.50-2.10)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	3.90 (2.09-7.25)	< .0001	3.90 (2.09-7.25)	< .0001	50	1.26 (0.72-2.20)		.42	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	1.64 (0.64-4.18)	.30	—		139	1.69 (1.10-2.60)	.02	1.69 (1.10-2.61)	.02
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.75 (0.94-3.28)	.08	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.64 (0.87-3.11)	.13	—		308	0.31 (0.08-1.25)	.10	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	1.07 (0.42-2.72)	.89	—		165	1.90 (1.21-2.99)	.01	1.98 (1.26-3.13)	.003
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.63 (0.25-1.61)	.34	—		319	0.67 (0.25-1.85)	.44	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.66 (0.65-3.80)	.29	—		160	1.33 (0.86-2.05)	.52	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

In conclusion, comparable survival rates were observed between adult Ph$^-$ ALL patients who underwent related and unrelated allo-SCTs in CR1, although relapse rates, incidences of NRM, and risk factors for transplantation outcomes were different between them. Better outcomes could be achieved by performing allo-SCT at an appropriate timing and HLA compatibility according to donor type.

Table 5. Causes of death among patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
	n	%	n	%	P
Relapse	44	44	32	26	.01
Infection	12	12	23	19	.20
Organ failure	12	12	17	14	.83
GVHD	9	8.9	16	13	.40
Interstitial pneumonia	5	5.0	15	12	.06
Hemorrhage	3	3.0	6	5.0	.52
Graft failure	2	2.0	3	2.5	1.0
ARDS	1	1.0	3	2.5	.63
Other	8	7.9	6	5.0	.42
Unknown	5	5.0	0	0.0	.02
Total	101	100	121	100	

ARDS indicates acute respiratory distress syndrome.

Acknowledgments

The authors thank all of the staff of the participating institutions of the Japan Society for Hematopoietic Cell Transplantation and the Japan Donor Marrow Program as well as Dr T. Kawase for the data collection of JMDP.

This work was supported in part by the Japan Leukemia Research Fund (S.N.) and in part by the Ministry of Health, Labor and Welfare of Japan (Grant-in-Aid) (K.M.).

Authorship

Contribution: S.N., Y.I., and K.M. designed the research and wrote the manuscript; S.N. and Y.I. performed the statistical analysis and interpreted the data; H.S., M. Kurokawa, H.I., H.O., T.F., Y.O., N.K., M. Kasai., T.M., K.I., T.Y., M.O., and

K.M. provided the patient data; and K.K., Y.M., R.S., and Y.A. collected the patient data.

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

Correspondence: Satoshi Nishiwaki, Department of Hematology, Japanese Red Cross Nagoya First Hospital, 3-35 Michishita-cho, Nakamura-ku, Nagoya, Aichi 453-8511, Japan; e-mail: n-3104@tf7.so-net.ne.jp.

References

1. Mattison RJ, Larson RA. Role of allogeneic hematopoietic cell transplantation in adults with acute lymphoblastic leukemia. *Curr Opin Oncol.* 2009;21(6):601-608.
2. Yanada M, Naoe T, Iida H, et al. Myeloablative allogeneic hematopoietic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: significant roles of total body irradiation and chronic graft-versus-host disease. *Bone Marrow Transplant.* 2005; 36(10):867-872.
3. Goldstone AH, Richards SM, Lazarus HM, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood.* 2008; 111(4):1827-1833.
4. Marks DI, Perez WS, He W, et al. Unrelated donor transplants in adults with Philadelphia-negative acute lymphoblastic leukemia in first complete remission. *Blood.* 2008;112(2):426-434.
5. 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(14):2816-2825.
6. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15(6):825-828.
7. Sullivan KM, Shulman HM, Storb R, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood.* 1981; 57(2):267-276.
8. Takeuchi J, Kyo T, Naito K, et al. Induction therapy by frequent administration of doxorubicin with four other drugs, followed by intensive consolidation and maintenance therapy for adult acute lymphoblastic leukemia: the JALSG-ALL93 study. *Leukemia.* 2002;16(7):1259-1266.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations *J Am Stat Assoc.* 1958;53:457-481.
10. Peto R, Peto J. Asymptotically efficient rank invariant test procedures. *J R Stat Soc A.* 1972; 135:185-207.
11. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999;18(6):695-706.
12. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. *Bone Marrow Transplant.* 2007;40(4):381-387.
13. Cox DR. Regression models and life tables. *J R Stat Soc B.* 1972;34:187-220.
14. Yakoub-Agha I, Mesnil F, Kuentz M, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. *J Clin Oncol.* 2006; 24(36):5695-5702.
15. Eapen M, Logan BR, Confer DL, et al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biol Blood Marrow Transplant.* 2007;13(12):1461-1468.
16. Oh H, Loberiza FR Jr, Zhang MJ, et al. Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood.* 2005;105(4): 1408-1416.
17. Baker KS, Loberiza FR Jr, Yu H, et al. Outcome of ethnic minorities with acute or chronic leukemia treated with hematopoietic stem-cell transplantation in the United States. *J Clin Oncol.* 2005; 23(28):7032-7042.
18. Lee S, Cho BS, Kim SY, et al. Allogeneic stem cell transplantation in first complete remission enhances graft-versus-leukemia effect in adults with acute lymphoblastic leukemia: antileukemic activity of chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2007;13(9):1083-1094.
19. Cornelissen JJ, Carston M, Kollman C, et al. Unrelated marrow transplantation for adult patients with poor-risk acute lymphoblastic leukemia: strong graft-versus-leukemia effect and risk factors determining outcome. *Blood.* 2001;97(6): 1572-1577.
20. Weiner RS, Bortin MM, Gale RP, et al. Interstitial pneumonitis after bone marrow transplantation: assessment of risk factors. *Ann Intern Med.* 1986; 104(2):168-175.
21. Socie G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation: Late Effects Working Committee of the International Bone Marrow Transplant Registry. *N Engl J Med.* 1999;341(1): 14-21.
22. Santo Tomas LH, Loberiza FR Jr, Klein JP, et al. Risk factors for bronchiolitis obliterans in allogeneic hematopoietic stem-cell transplantation for leukemia. *Chest.* 2005;128(1):153-161.
23. Alessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001; 28(5):425-434.
24. Sullivan KM, Shulman HM. Chronic graft-versus-host disease, obliterative bronchiolitis, and graft-versus-leukemia effect: case histories. *Transplant Proc.* 1989;21(3)[suppl 1]:51-62.
25. Camus P, Fanton A, Bonniaud P, et al. Interstitial lung disease induced by drugs and radiation. *Respiration.* 2004;71(4):301-326.
26. Bacigalupo A, Lamparelli T, Gualandi F, et al. Allogeneic hemopoietic stem cell transplants for patients with relapsed acute leukemia: long-term outcome. *Bone Marrow Transplant.* 2007;39(6): 341-346.
27. Fung HC, Stein A, Slovak M, et al. A long-term follow-up report on allogeneic stem cell transplantation for patients with primary refractory acute myelogenous leukemia: impact of cytogenetic characteristics on transplantation outcome. *Biol Blood Marrow Transplant.* 2003;9(12):766-771.
28. Forman SJ, Schmidt GM, Nademanee AP, et al. Allogeneic bone marrow transplantation as therapy for primary induction failure for patients with acute leukemia. *J Clin Oncol.* 1991;9(9): 1570-1574.
29. Mehta J. Hematopoietic stem cell transplantation from unrelated donors for acute leukemia. *Blood.* 2008;112:447-448.

Bone Marrow Transplantation (2010), 1–11

www.nature.com/bmt

ORIGINAL ARTICLE

Disease stage stratified effects of cell dose in unrelated BMT for hematological malignancies: a report from Japan marrow donor program

Y Inamoto[1], K Miyamura[2], S Okamoto[3], H Akiyama[4], H Iida[5], T Eto[6], Y Morishima[7], K Kawa[8], A Kikuchi[9], Y Nagatoshi[10], J Tanaka[11], T Ashida[12], M Hirokawa[13], M Tsuchida[14] and S Mori[15] for the Japan Marrow Donor Program

[1]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [2]*Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [3]*Department of Medicine, Keio University School of Medicine, Tokyo, Japan;* [4]*Hematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan;* [5]*Department of Hematology, Meitetsu Hospital, Nagoya, Japan;* [6]*Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan;* [7]*Department of Hematology and Cell Therapy, Aichi Cancer Center, Nagoya, Japan;* [8]*Department of Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan;* [9]*Department of Pediatrics, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan;* [10]*Department of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan;* [11]*Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan;* [12]*Division of Hematology, Department of Internal Medicine, Kinki University School of Medicine, Osaka, Japan;* [13]*Akita University Hospital, Clinical Oncology Center, Akita, Japan;* [14]*Department of Pediatrics, Ibaragi Children's Hospital, Ibaragi, Japan and* [15]*Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan*

Cell dose is one of the major factors that can be manipulated in unrelated BMT. However, regarding disease-stage-stratified effects of cell dose, data are limited. We analyzed the registry data from 3559 patients with acute leukemia, CML and myelodysplastic syndrome who received T-cell replete unrelated BMT through the Japan Marrow Donor Program. Adjusted effects of cell dose were evaluated for various outcomes separately according to disease stages and children or adults. Acute GVHD and nonrelapse mortality were not affected by cell dose. Among children, a cell dose lower than 3.0×10^8/kg was associated with lower engraftment rates in advanced-stage diseases. Among adults, a cell dose of 3.4×10^8/kg or higher was associated with lower relapse rates and better survival rates only in early-stage diseases, whereas cell dose below 2.3×10^8/kg was associated with lower engraftment rates in advanced-stage diseases. In conclusion, effects of cell dose may differ among disease stages. A cell dose of 3.4×10^8/kg or higher is recommended only for adults with early-stage diseases. With the number of patients available for analysis in this study, we could not show any significant benefits associated with 4.6×10^8/kg or higher in children.

Bone Marrow Transplantation advance online publication, 8 November 2010; doi:10.1038/bmt.2010.281

Keywords: allogeneic; cell dose; disease stage; unrelated

Correspondence: Dr Y Inamoto, Clinical Research Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, D5-290, PO Box 19024, Seattle, WA 98109-1024, USA.
E-mail: yinamoto@js3.so-net.ne.jp
Received 29 March 2010; revised 21 June 2010; accepted 12 August 2010

Introduction

Allogeneic hematopoietic cell transplantation has been established as a curative therapy for hematological malignancies.[1,2] Because of the better understanding of the significance of HLA allele compatibility and the advances in supportive care, the results of BMT from unrelated donors are improving.[3–5]

Cell dose is one of the major factors that can be manipulated by physicians and affect transplant outcomes.[6–8] Historically, its importance for engraftment and hematological recovery has been documented in patients with aplastic anemia.[9,10] Several subsequent studies showed that cell dose was also associated with better survival due to decreased nonrelapse mortality (NRM) in hematological malignancies. However, other important factors, such as patient age, disease, conditioning, GVHD prophylaxis, ABO compatibility, donor characteristics and HLA matching, also affect the transplant outcome.[11,12] Therefore, the actual effect of cell dose should be confirmed after adjustment for all of these factors with a sufficient number of patients.

On the other hand, the GVL effect may work differently according to disease stages. Rocha *et al.*[13] showed that cell dose was associated with decreased relapse rates in AML in first CR, whereas no significant associations between cell dose and relapse rates were observed in other studies, including various diseases.[7,8,11] These conflicting results suggested that the cell dose effect is worth analyzing separately according to disease stages.

In this report, we examined adjusted effects of cell dose on various transplant outcomes according to disease stages and children or adults using the detailed registry data of 3559 patients who received T-cell replete unrelated BMT through the Japan Marrow Donor Program.

Patients and methods

Patients

The data set consisted of 5071 unrelated BMTs facilitated by the Japan Marrow Donor Program between 1993 and 2005. Of these 5071 patients, 3559 with AML, ALL, CML and myelodysplastic syndrome who received their first T-cell replete myeloablative transplantation with GVHD prophylaxis containing calcineurin inhibitor without antithymocyte globulin were selected for this study. The patients and donors were all Japanese. Informed consent for this registry study was obtained from patients and donors in accordance with the declaration of Helsinki. This study was approved by the data management committees of Japan Marrow Donor Program.

Transplantation procedure

Patients were conditioned with various regimens determined by each transplant center. The proportions of TBI regimen were assessed from the database. Red cells and/or plasma removal from the graft was performed for ABO-major and/or -minor mismatched transplantation. All grafts were BM because the donation of PBSCs from unrelated donors is not yet approved in Japan. GVHD prophylaxis was categorized into either a CsA-based or tacrolimus-based prophylaxis.

HLA matching

HLA-A, -B and -DRB1 alleles were identified by high-resolution DNA typing as described previously.[3,4] As our previous study showed that a single-allele mismatch at DRB1 locus had no impact on engraftment, acute and chronic GVHD, NRM, relapse and OS in the Japanese population,[4] it was considered as a HLA-matched transplantation in this study.

Definition of disease stage and outcomes

Early stage was defined as the status of the first and second CR of AML and ALL, the first chronic phase of CML and refractory anemia of myelodysplastic syndrome, whereas advanced stage was defined as other status. For cytogenetic categorization, patients were divided into three categories: good risk (AML with t(15;17), inv16 or t(8;21)), intermediate risk (other than good or poor risk) or poor risk (ALL with t(9;22) or t(4;11), CML with additional abnormalities other than t(9;21) or myelodysplastic syndrome with complex or chromosome 7 abnormalities).[14] Engraftment was defined as an ANC of more than 500/μl for 3 consecutive days in the peripheral blood, and analyzed among all patients. Acute GVHD was graded by established criteria.[15] Chronic GVHD was assessed in patients surviving beyond day +100, and was classified as limited or extensive according to the Seattle criteria.[16]

Statistical analysis

Cell dose was defined as harvested total nucleated cell dose. Analysis was performed separately for disease stages, and children or adults. Children were defined as patients who were aged 12 years or younger for two reasons. One reason was because cell dose per patient body wt had a stronger linear correlation with age at these ages. Another reason was because patients aged 12 years or younger were usually treated with children's protocols. To determine the impacts of low and high cell doses on the outcomes in the current practices, cut-off points were set at upper and lower 25% of the cell dose separately in children and adults. Patient characteristics and causes of NRM were tested for associations using the χ^2-test for discrete variables, and the Spearman rank correlation test for continuous variables. Cumulative incidences of NRM, relapse and GVHD were estimated by Gray's method. Relapse was considered as a competing risk in NRM, deaths without relapse as a competing risk in relapse, and deaths without GVHD as a competing risk in GVHD. OS was calculated using the Kaplan–Meier method and *P*-values were calculated using a Log-rank test. Multivariate analyses were performed using logistic regression model for engraftment, the Cox proportional hazard regression model for OS, and the multivariate proportional hazard modeling of subdistribution functions in competing risks for NRM, relapse and GVHD.[17] Variables considered in the analysis were cell dose, patient age (linear), ABO incompatibility (none, major or minor), disease stage (early or advanced), cytogenetics (good, intermediate or poor), the number of HLA-mismatched loci, patient sex, donor sex, female to male transplantation, conditioning (TBI regimen, antithymocyte globulin regimen, and reduced-intensity regimen), GVHD prophylaxis (CsA-based or tacrolimus-based), donor age (linear), year of transplant (categorical) and preceding grades II–IV acute GVHD (only for chronic GVHD analysis). Cell dose was kept in the final model even though it was not statistically significant. All statistical tests were two-sided, and *P*-values less than 0.05 were considered significant. Analysis was performed using STATA (Stata Statistical Software: Release 10.0., Stata Corporation, College Station, TX, USA) and R version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

The number of patients with AML, ALL, CML and myelodysplastic syndrome were 1205 (34%), 1140 (32%), 755 (21%) and 459 (13%), respectively. The median volumes of harvested marrow for child and adult recipients were 426 mL (range, 83–1045) and 850 mL (range, 220–1500), respectively ($P<0.0001$). The median numbers of harvested cells for child and adult recipients were 3.63×10^8/kg (range, 0.58–13.7) and 2.92×10^8/kg (range, 0.16–12.1), respectively ($P<0.0001$). Cut-off points were set at 3.0 and 4.6×10^8/kg for children, and 2.3 and 3.4×10^8/kg for adults. Patient characteristics were summarized in Tables 1 and 2. Recipient age, recipient–donor gender compatibility, recipient body wt, GVHD prophylaxis and the year of transplantation showed statistically significant differences according to cell dose in children. Recipient age, recipient–donor gender compatibility, recipient body wt, ABO mismatch, disease type in early-stage malignancy, GVHD prophylaxis and the year of transplantation showed statistically significant differences according to cell dose in adults.

Table 1 Patient characteristics in children

Characteristic	*Cell dose*						P
	<3.0 × 10⁸/kg (n = 140)		*3.0–4.6 × 10⁸/kg (n = 248)*		*≥4.6 × 10⁸/kg (n = 128)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	
Recipient age, years							
Median	9		8		5		<0.001
Range	0–12		0–12		0–12		
Donor age, years							
Median	35		34		32		0.20
Range	21–50		20–50		20–50		
Sex (recipient/donor)							
Male/male	33	24	71	29	47	37	0.001
Female/female	41	29	65	26	23	18	
Male/female	50	36	58	23	25	20	
Female/male	16	11	54	22	33	26	
Recipient body wt, kg							
Median	27		25		17		<0.001
Range	5–72		5–49		4–44		
ABO mismatch							
Match	96	69	154	62	66	52	0.063
Major mismatch	29	21	55	22	37	29	
Minor mismatch	15	11	39	16	25	20	
Disease							
Early-stage malignancy							0.50
AML	18	20	53	30	23	26	
ALL	62	68	107	60	52	58	
CML	7	8	14	8	10	11	
MDS	4	4	4	2	4	4	
Advanced-stage malignancy							0.51
AML	10	20	18	26	9	23	
ALL	28	57	37	53	18	46	
CML	4	8	1	1	2	5	
MDS	7	14	14	20	10	26	
Cytogenetics							
Good risk	4	3	17	7	8	6	0.55
Intermediate risk	110	79	189	76	98	77	
Poor risk	18	13	25	10	17	13	
Not available	8	6	17	7	5	4	
Conditioning							
TBI regimen	122	87	209	84	102	80	0.25
Non-TBI regimen	18	13	39	16	26	20	
GVHD prophylaxis							
Cyclosporin-based	44	31	100	40	71	55	<0.001
Tacrolimus-based	96	69	148	60	57	45	
No. of HLA mismatch by DNA typing							
0	95	68	190	77	90	70	0.39
1 locus	40	29	52	21	33	26	
2 or more loci	5	4	6	2	5	4	
Year of transplantation							
1993–1996	18	13	44	18	31	24	0.009
1997–2000	39	28	67	27	50	39	
2001–2003	54	39	87	35	32	25	
2004–2005	29	21	50	20	15	12	

Abbreviation: MDS = myelodysplastic syndrome.

Engraftment

Engraftment was achieved in 500 of 516 (97%) child patients and 2882 of 3043 (95%) adult patients. Multivariate analysis showed that $<3.0 \times 10^8$/kg was associated with lower engraftment rates in children with advanced-stage diseases (odds ratio, 0.15; 95% confidence interval (CI), 0.03–0.74; $P = 0.02$) and $<2.3 \times 10^8$/kg was associated with lower engraftment rates in adults with advanced-stage diseases (odds ratio, 0.60; 95% CI, 0.37–0.97; $P = 0.039$).

Table 2 Patient characteristics in adults

Characteristic	*Cell dose*						P
	<2.3 × 10⁸/kg (n = 755)		*2.3–3.4 × 10⁸/kg (n = 1519)*		*≥3.4 × 10⁸/kg (n = 769)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	
Recipient age, years							
Median	34		34		32		0.0076
Range	13–65		13–66		13–62		
Donor age, years							
Median	34		34		34		0.42
Range	20–51		20–68		20–51		
Sex (recipient/donor)							
Male/male	309	41	666	44	336	44	<0.001
Female/female	179	24	287	19	132	17	
Male/female	188	25	253	17	91	12	
Female/male	79	10	313	21	210	27	
Recipient body wt, kg							
Median	61		59		55		<0.001
Range	29–120		25–112		23–90		
ABO mismatch							
Match	401	53	800	53	355	46	<0.001
Major mismatch	191	25	417	27	271	35	
Minor mismatch	163	22	302	20	143	19	
Disease							
Early-stage malignancy							0.002
AML	187	40	347	37	149	32	
ALL	148	31	281	30	155	33	
CML	89	19	248	26	135	29	
MDS	48	10	62	7	34	7	
Advanced-stage malignancy							0.83
AML	104	37	189	33	98	33	
ALL	62	22	129	22	61	21	
CML	59	21	124	21	62	21	
MDS	58	20	139	24	75	25	
Cytogenetics							
Good risk	54	7	116	8	45	6	0.59
Intermediate risk	615	81	1215	80	622	81	
Poor risk	54	7	105	7	58	8	
Not available	32	4	83	5	44	6	
Conditioning							
TBI regimen	634	84	1245	82	621	81	0.25
Non-TBI regimen	121	16	274	18	148	19	
GVHD prophylaxis							
CsA-based	337	45	833	55	418	54	<0.001
Tacrolimus-based	418	55	686	45	351	46	
No of HLA mismatch by DNA typing							
0	584	77	1183	78	608	79	0.90
1 locus	158	21	306	20	146	19	
2 or more loci	13	2	30	2	15	2	
Year of transplantation							
1993–1996	70	9	227	15	113	15	<0.001
1997–2000	158	21	500	33	293	38	
2001–2003	329	44	509	34	230	30	
2004–2005	198	26	283	19	133	17	

Abbreviation: MDS = myelodysplastic syndrome.

Acute and chronic GVHD

The cumulative incidences of grades II–IV acute GVHD in children and adults were 50 and 43%, respectively. Multivariate analysis showed no statistically significant association of cell dose with incidences of grades II–IV acute GVHD in children and adults.

Table 3 Variables associated with relapse in (a) children and (b) adults

Variable	*Early-stage disease*						*Advance- stage disease*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
(a)												
n = 358									*n = 158*			
Cell dose (× 10⁸/kg)												
3.0 4.6	1.00		1.00	1.00		1.00						
<3.0	1.06	(0.60 1.87)	0.84	0.99	(0.56 1.75)	0.98	1.18	(0.66 2.14)	0.57	1.03	(0.54 1.95)	0.93
⩾4.6	1.22	(0.70 2.14)	0.48	1.20	(0.69 2.09)	0.52	0.98	(0.54 1.81)	0.96	0.95	(0.53 1.72)	0.87
Recipient age												
Linear	0.95	(0.90 1.01)	0.14	0.99	(0.92 1.07)	0.83						
Donor age												
Linear	1.01	(0.99 1.04)	0.37	0.96	(0.92 0.99)	0.02	0.96	(0.92 0.99)				0.021
Cytogenetics												
Intermediate risk	1.00		1.00		1.00							
Good risk	Unevaluable[a]	<0.001	Unevaluable[a]	<0.001	1.71	(0.8 3.67)	0.16					
Poor risk	1.43	(0.76 2.69)	0.27	1.42	(0.76 2.65)	0.27	0.78	(0.27 2.24)	0.64			
ABO mismatch												
Match	1.00						1.00			1.00		
Major mismatch	1.11	(0.64 1.91)	0.72				0.48	(0.24 0.94)	0.031	0.48	(0.23 0.98)	0.043
Minor mismatch	0.80	(0.40 1.61)	0.54				0.66	(0.33 1.31)	0.23	0.25		
HLA mismatch												
Match	1.00						1.00					
Mismatch	0.95	(0.61 1.48)	0.81				0.63	(0.38 1.04)	0.072			
Recipient sex												
Male	1.00						1.00					
Female	0.97	(0.61 1.55)	0.90				0.92	(0.56 1.52)	0.76			
Donor sex												
Male	1.00						1.00					
Female	1.11	(0.70 1.76)	0.67				0.99	(0.61 1.63)	0.98			
Female donor to male recipient												
No	1.00						1.00					
Yes	1.20	(0.72 2.02)	0.48				1.17	(0.69 2)	0.56			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	0.62	(0.36 1.06)	0.08				0.67	(0.38 1.21)	0.18			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	0.91	(0.57 1.45)	0.68				1.02	(0.62 1.67)	0.93			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	0.86	(0.44 1.70)	0.67				1.29	(0.64 2.6)	0.47			
2001 2003	1.02	(0.53 1.96)	0.95				1.20	(0.61 2.39)	0.60			
2004 2005	0.72	(0.32 1.61)	0.42				0.99	(0.4 2.44)	0.98			
(b)												
n = 1883									*n = 1160*			
Cell dose (× 10⁸/kg)												
2.3 3.4	1.00			1.00			1.00			1.00		
<2.3	1.13	(0.85 1.49)	0.41	1.09	(0.82 1.44)	0.56	1.20	(0.94 1.55)	0.14	1.21	(0.94 1.56)	0.13
⩾3.4	0.61	(0.43 0.85)	0.0042	0.60	(0.43 0.85)	0.004	0.91	(0.70 1.18)	0.48	0.90	(0.70 1.17)	0.44
Recipient age												
Linear	0.99	(0.98 1.00)	0.28				0.99	(0.98 1.00)	0.015	0.99	(0.98 1.00)	0.0088
Donor age												
Linear	0.99	(0.97 1.00)	0.088				0.99	(0.98 1.00)	0.20			
Cytogenetics												
Intermediate risk	1.00						1.00					
Good risk	0.97	(0.60 1.58)	0.91				1.33	(0.89 1.99)	0.16			
Poor risk	1.43	(0.91 2.24)	0.12				1.00	(0.66 1.51)	0.98			

Table 3 Continued

Variable	*Early-stage disease*						*Advance- stage disease*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
ABO mismatch												
Match	1.00						1.00			1.00		
Major mismatch	1.10	(0.83 1.46)	0.52				0.70	(0.55 0.90)	0.0045	0.71	(0.56 0.92)	0.0081
Minor mismatch	0.97	(0.70 1.36)	0.88				0.77	(0.59 1.02)	0.07	0.76	(0.58 1.01)	0.055
HLA mismatch												
Match	1.00						1.00			1.00		
Mismatch	0.92	(0.70 1.22)	0.57				0.73	(0.57 0.92)	0.0093	0.73	(0.57 0.93)	0.01
Recipient sex												
Male	1.00						1.00					
Female	1.11	(0.87 1.43)	0.40				1.08	(0.87 1.33)	0.47			
Donor sex												
Male	1.00						1.00					
Female	1.05	(0.81 1.35)	0.72				0.90	(0.73 1.13)	0.37			
Female donor to male recipient												
No	1.00						1.00					
Yes	0.87	(0.62 1.22)	0.41				0.81	(0.61 1.09)	0.17			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	1.36	(0.95 1.95)	0.10				1.08	(0.82 1.42)	0.58			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.50	(1.17 1.92)	0.0014	1.49	(1.16 1.91)	0.0017	1.07	(0.87 1.31)	0.53			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	1.20	(0.77 1.86)	0.42				1.06	(0.74 1.52)	0.74			
2001 2003	1.59	(1.05 2.43)	0.03				1.24	(0.87 1.76)	0.23			
2004 2005	2.02	(1.27 3.19)	0.0028				1.19	(0.81 1.76)	0.37			

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]Hazard ratio was unevaluable because of no events.

The cumulative incidences of limited or extensive chronic GVHD in children and adults were 34 and 45%, respectively. Multivariate analysis in children showed a statistically significant association of $<3.0 \times 10^8$/kg with higher incidences of chronic GVHD in advanced-stage diseases (hazard ratio, 2.46; 95% CI, 1.17–5.17; $P = 0.017$). Multivariate analysis in adults showed no statistically significant association of cell dose with incidences of chronic GVHD.

NRM

The cumulative incidences of NRM at 5 years in children and adults were 21 and 39%, respectively. Multivariate analysis showed no statistically significant association of cell dose with incidences of NRM in children (Supplementary Table S1a) and adults (Supplementary Table S1b). Causes of NRM according to cell dose were not statistically different in children. As a cause of NRM in adults, the proportions of idiopathic pneumonia syndrome were statistically different according to cell dose (13, 14 and 23% for <2.3, 2.3–3.4 and $>3.4 \times 10^8$/kg, respectively; $P = 0.002$).

Relapse

The cumulative incidences of relapse at 5 years in children and adults were 27 and 25%, respectively. Multivariate analysis in children showed no statistically significant association of cell dose with incidences of relapse (Table 3a). Multivariate analysis in adults showed a statistically significant association of $>3.4 \times 10^8$/kg with lower incidences of relapse in early-stage diseases (hazard ratio, 0.60; 95% CI, 0.43–0.85; $P = 0.004$) (Table 3b). Results were similar when CML in chronic phase was excluded from analysis in adults (data not shown).

OS

The median follow-up periods among survivors were 57 months (range, 9–140 months) in children and 55 months (range, 3–147 months) in adults. The OS rates at 5 years among children with early-stage diseases were 67, 75 and 68% for <3.0, 3.0–4.6 and $>4.6 \times 10^8$/kg, respectively ($P = 0.74$; Figure 1a). The OS rates at 5 years among children with advanced-stage diseases were 31, 36 and 40% for <3.0, 3.0–4.6 and $>4.6 \times 10^8$/kg, respectively

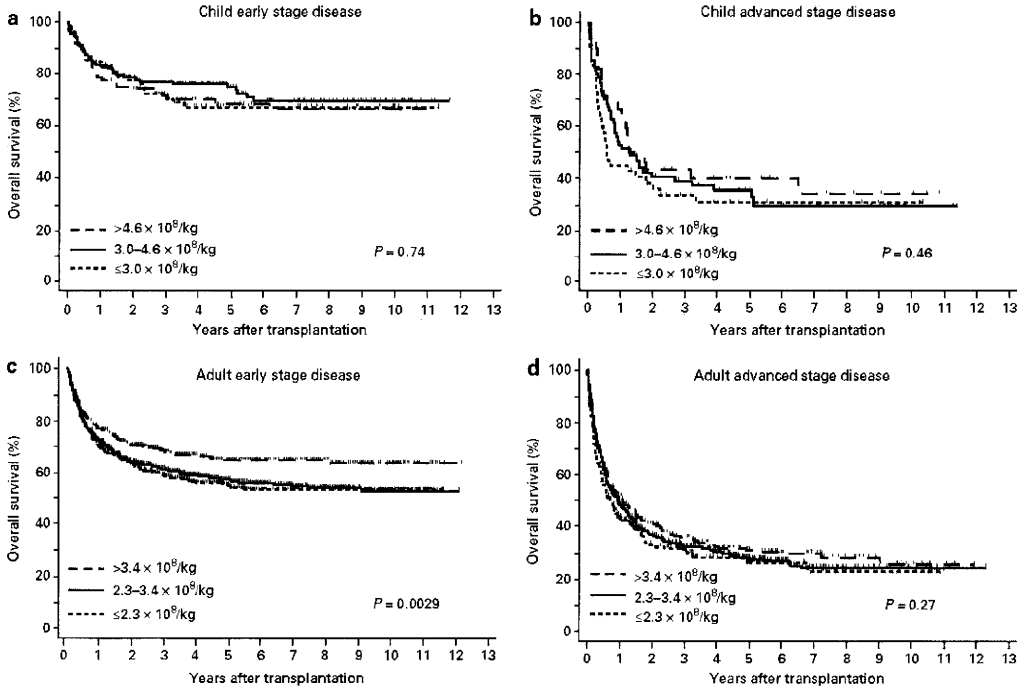

Figure 1 Kaplan–Meier estimates of OS according to cell dose: (**a**) among children with early-stage diseases; (**b**) among children with advanced-stage diseases; (**c**) among adults with early-stage diseases; and (**d**) among adults with advanced-stage diseases.

($P = 0.46$; Figure 1b). The OS rates at 5 years among adults with early-stage diseases were 54, 57 and 65% for < 2.3, 2.3–3.4 and $> 3.4 \times 10^8$/kg, respectively ($P = 0.0029$; Figure 1c). The OS rates at 5 years among adults with advanced-stage diseases were 26, 28 and 31% for < 2.3, 2.3–3.4 and $> 3.4 \times 10^8$/kg, respectively ($P = 0.27$; Figure 1d).

Multivariate analysis in children showed no statistically significant association of cell dose with survival rates (Table 4a). Multivariate analysis in adults showed a statistically significant association of $> 3.4 \times 10^8$/kg with better survival rates only in early-stage diseases (hazard ratio, 0.74; 95% CI, 0.62–0.90; $P = 0.002$) (Table 4b).

Discussion

This study showed that effects of cell dose on transplant outcomes were different among disease stages. Among children, we could not show any statistically significant effects of cell dose except the lower engraftment rates and higher incidences of chronic GVHD associated with $< 3.0 \times 10^8$/kg in advanced-stage diseases. Among adults, cell dose $> 3.4 \times 10^8$/kg was associated with decreased relapse rates and better survival rates in early-stage diseases, whereas cell dose was not associated with outcomes except the lower engraftment rates with $< 2.3 \times 10^8$/kg in advanced-stage diseases.

Although many studies reported that higher cell dose improved OS rates,[8,11,12,18,19] effects of cell dose on relapse and NRM rates were not consistent among studies probably because of the differences in diseases, stages and transplant procedures. Furthermore, it is not practical to analyze child and adult patients together because biology of disease, treatment protocols and harvested total nucleated cells per body wt are likely to differ between them. Therefore, we investigated cell dose effects separately according to disease stages and children or adults, and extended analysis to various outcomes.

Although several studies showed that engraftment rates were improved with higher cell dose,[6,11] our results did not show any statistically significant merits with high cell dose both in children and adults. Low cell dose was associated with worse engraftment rates in advanced-stage diseases in both children and adults. Effects of low cell dose would be particularly great in advanced-stage diseases considering that graft failure occurs more frequently in advanced-stage diseases.[7]

Effects of cell dose on relapse rates were controversial. Although several studies did not show any effects of cell dose on relapse rates,[7,8,11] the results of our study supported those by Rocha *et al.*[13] among patients with AML in the first CR, and those by Barrett *et al.*[20] after

Table 4 Variables associated with OS in (a) children and (b) adults

Variable	*Early stage disease (n = 358)*						*Advanced stage disease (n = 158)*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
(a)												
n = 358							*n = 158*					
Cell dose (× 10⁶/kg)												
3.0 4.6	1.00			1.00			1.00			1.00		
<3.0	1.15	(0.72 1.85)	0.56	1.09	(0.68 1.75)	0.73	1.59	(0.85 2.95)	0.14	1.39	(0.87 2.20)	0.17
⩾4.6	1.18	(0.74 1.89)	0.49	1.18	(0.74 1.89)	0.48	0.99	(0.63 1.56)	0.96	0.87	(0.53 1.43)	0.59
Recipient age												
Linear	1.01	(0.95 1.07)	0.86				1.04	(0.98 1.10)	0.20			
Donor age												
Linear	1.02	(1.00 1.05)	0.11				1.01	(0.98 1.04)	0.41			
Cytogenetics												
Intermediate risk	1.00						1.00					
Good risk	0.75	(0.27 2.06)	0.58				1.18	(0.55 2.56)	0.67			
Poor risk	1.09	(0.60 1.96)	0.79				1.20	(0.60 2.39)	0.61			
ABO mismatch												
Match	1.00						1.00					
Major mismatch	1.40	(0.88 2.22)	0.15				0.87	(0.54 1.39)	0.55			
Minor mismatch	1.49	(0.89 2.51)	0.13				0.71	(0.41 1.25)	0.24			
HLA mismatch												
Match	1.00			1.00			1.00					
Mismatch	1.72	(1.30 2.27)	<0.001	1.72	(1.30 2.27)	<0.001	1.11	(0.77 1.60)	0.58			
Recipient sex												
Male	1.00						1.00					
Female	1.04	(0.70 1.54)	0.86				1.25	(0.85 1.85)	0.25			
Donor sex												
Male	1.00						1.00					
Female	1.26	(0.85 1.87)	0.25				0.72	(0.49 1.07)	0.10			
Female donor to male recipient												
No	1.00						1.00			1.00		
Yes	1.10	(0.71 1.70)	0.68				0.63	(0.40 0.99)	0.05	0.57	(0.35 0.91)	0.02
Conditioning												
Non-TBI regimen	1.00						1.00					
BI regimen	1.01	(0.59 1.72)	0.98				1.26	(0.74 2.15)	0.40			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.07	(0.71 1.60)	0.75				0.83	(0.56 1.22)	0.34			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	0.74	(0.44 1.25)	0.27				1.10	(0.65 1.87)	0.73			
2001 2003	0.59	(0.34 1.03)	0.06				0.87	(0.51 1.49)	0.61			
2004 2005	0.69	(0.35 1.36)	0.29				0.90	(0.46 1.76)	0.76			
(b)												
n = 1883									*n = 1160*			
Cell dose (× 10⁶/kg)												
2.3 3.4	1.00			1.00			1.00			1.00		
<2.3	1.05	(0.88 1.25)	0.59	1.06	(0.89 1.26)	0.54	1.10	(0.93 1.31)	0.25	1.15	(0.97 1.37)	0.11
⩾3.4	0.75	(0.62 0.90)	0.002	0.74	(0.62 0.90)	0.002	0.94	(0.79 1.11)	0.47	0.94	(0.80 1.12)	0.52
Recipient age												
Linear	1.01	(1.01 1.02)	<0.001	1.01	(1.01 1.02)	<0.001	1.00	(1.00 1.01)	0.61			
Donor age												
Linear	1.01	(1.00 1.02)	0.01	1.01	(1.00 1.02)	0.02	1.00	(0.99 1.01)	0.42			
Cytogenetics												
Intermediate risk	1.00						1.00			1.00		
Good risk	0.79	(0.59 1.06)	0.12				1.05	(0.78 1.41)	0.75	1.04	(0.77 1.40)	0.80
Poor risk	1.09	(0.82 1.45)	0.56				1.59	(1.24 2.04)	<0.001	1.61	(1.26 2.07)	<0.001

Table 4 Continued

Variable	*Early stage disease (n = 358)*						*Advanced stage disease (n = 158)*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
ABO mismatch												
Match	1.00			1.00			1.00					
Major mismatch	1.16	(0.98 1.37)	0.08	1.18	(1.00 1.40)	0.05	1.10	(0.94 1.30)	0.23			
Minor mismatch	1.08	(0.89 1.31)	0.42	1.12	(0.92 1.36)	0.26	1.11	(0.93 1.33)	0.26			
HLA mismatch												
Match	1.00			1.00			1.00			1.00		
Mismatch	1.41	(1.22 1.63)	<0.001	1.38	(1.19 1.60)	<0.001	1.34	(1.18 1.53)	<0.001	1.31	(1.15 1.50)	<0.001
Recipient sex												
Male	1.00						1.00					
Female	0.88	(0.75 1.02)	0.08				0.96	(0.83 1.10)	0.55			
Donor sex												
Male	1.00						1.00					
Female	1.00	(0.86 1.16)	0.97				0.96	(0.83 1.11)	0.56			
Female donor to male recipient												
No	1.00						1.00					
Yes	1.11	(0.93 1.34)	0.25				1.06	(0.89 1.27)	0.50			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	0.90	(0.74 1.08)	0.26				1.00	(0.83 1.19)	0.97			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.04	(0.90 1.20)	0.60				0.85	(0.74 0.97)	0.02			
Year of transplantation												
1993 1996	1.00			1.00			1.00			1.00		
1997 2000	0.75	(0.60 0.93)	0.009	0.79	(0.63 0.99)	0.04	0.77	(0.62 0.95)	0.014	0.79	(0.63 0.98)	0.032
2001 2003	0.82	(0.66 1.02)	0.072	0.80	(0.64 1.00)	0.053	0.70	(0.56 0.87)	0.001	0.72	(0.58 0.90)	0.005
2004 2005	0.92	(0.72 1.19)	0.54	0.85	(0.65 1.11)	0.23	0.66	(0.51 0.85)	0.001	0.68	(0.53 0.88)	0.003

Abbreviations: CI = confidence interval; HR = hazard ratio.

identical twin BMT. Interestingly, our results showed lower relapse rates not associated with higher incidences of acute GVHD, which was also observed in the studies by Rocha *et al.*[13] and by Barrett *et al.*[20] GVL effect is influenced by disease types and stages possibly because of the differences in expression of tumor Ags, co-stimulatory molecules, resistance to killing and growth patterns.[21,22] It has been demonstrated that the GVL effect works more efficiently for minimal residual disease than for active disease.[23,24] Therefore, it is reasonable that decreased relapse rates with $\geqslant 3.4 \times 10^8$/kg was limited to early-stage diseases. Although it may be argued that patients with CML in chronic phase greatly influence the outcomes,[25] the results were similar even if these patients were excluded from analysis.

What are effector cells of cell dose effect? Calculated with the published data,[26] 1×10^8/kg nucleated BM cells include 8×10^6/kg T cells, 3×10^6/kg B cells and 2×10^6/kg nature killer cells. Considering the cell dose used in adaptive immunotherapies with these cells,[27–29] this number of T cells can alter the outcome but that of nature killer cells will not. Therefore, we speculated that T cells would be the most likely population affecting relapse rates. As the registry did not have data as to graft composition during the study period, we could not confirm this hypothesis in our data. Using total nucleated cells as the surrogate for cell dose may have limitations because some studies showed that more specific fractions, such as $CD34^+$ cell dose also predicted transplant outcomes.[30,31] Future studies analyzing the effect of subpopulations in grafts are warranted.

Many previous studies reported that higher cell dose decreased NRM, particularly related to infection.[7,8,12,32] However, no significant effects of cell dose on NRM rates were observed in our study. To address this discrepancy, we performed a further analysis on causes of NRM according to cell dose, which showed no significant differences in the proportions of deaths from infection both in children and adults. This would partly account for the discrepancy.

In light of the study which reported that 7×10^7/kg nucleated cells are enough to induce GVHD after donor leukocyte infusion,[33] higher cell dose may result in increased incidences of GVHD. However, most of the previous studies showed that cell dose had no effect on acute GVHD or that higher cell dose decreased acute GVHD.[7,8,18] They speculated a possible effect of accessory cells, such as MSCs, and a possibility that higher cell dose decreased early post transplant infections that might

amplify GVHD. Our results were compatible with these reports. We could not explain why $<3.0 \times 10^8$/kg resulted in increased incidences of chronic GVHD among children with advanced-stage diseases.

There are two possible explanations for the discrepancy observed with regard to the effect of cell dose on OS in children and adults. First, a much greater volume of harvested marrow for adults as compared with children (almost twice the volume) might bring about higher contamination of peripheral blood and increase the dose of graft T cells to produce the different effects.[3,4] Second, cell dose effect might be already saturated in children because most children received much more cell dose than adults (7×10^7/kg more at median). Different analytical power between children and adults would not account for the discrepancy as the point estimate of hazard ratio in children with early-stage diseases was more than 1.0 with $>4.6 \times 10^8$/kg (Table 4a).

In summary, our results suggested a strategy to determine an optimal cell dose of BMT according to disease stages to maximize the efficacy of BMT and minimize the risk of donors, although these results should be interpreted with caution because of their retrospective nature. In terms of overall benefits, cell dose of 3.4×10^8/kg or higher is recommended only for adults with early-stage diseases. With the number of patients available for analysis in our study, we could not show any significant benefits associated with 4.6×10^8/kg or higher in children.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

References

1 Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE *et al.* Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975; **292**: 832 843.
2 Kernan NA, Bartsch G, Ash RC, Beatty PG, Champlin R, Filipovich A *et al.* Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med* 1993; **328**: 593 602.
3 Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H *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.
4 Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K *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.
5 Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M *et al.* High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**: 4576 4583.
6 Davies SM, Kollman C, Anasetti C, Antin JH, Gajewski J, Casper JT *et al.* Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. *Blood* 2000; **96**: 4096 4102.
7 Sierra J, Storer B, Hansen JA, Bjerke JW, Martin PJ, Petersdorf EW *et al.* Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching, and marrow cell dose. *Blood* 1997; **89**: 4226 4235.
8 Dominietto A, Lamparelli T, Raiola AM, Van Lint MT, Gualandi F, Berisso G *et al.* Transplant-related mortality and long-term graft function are significantly influenced by cell dose in patients undergoing allogeneic marrow transplantation. *Blood* 2002; **100**: 3930 3934.
9 Storb R, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. *N Engl J Med* 1977; **296**: 61 66.
10 Deeg HJ, Self S, Storb R, Doney K, Appelbaum FR, Witherspoon RP *et al.* Decreased incidence of marrow graft rejection in patients with severe aplastic anemia: changing impact of risk factors. *Blood* 1986; **68**: 1363 1368.
11 Kollman C, Howe CW, Anasetti C, Antin JH, Davies SM, Filipovich AH *et al.* Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood* 2001; **98**: 2043 2051.
12 Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S *et al.* Impact of AB0-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica* 2008; **93**: 1686 1693.
13 Rocha V, Labopin M, Gluckman E, Powles R, Arcese W, Bacigalupo A *et al.* Relevance of bone marrow cell dose on allogeneic transplantation outcomes for patients with acute myeloid leukemia in first complete remission: results of a European survey. *J Clin Oncol* 2002; **20**: 4324 4330.
14 Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H *et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (eds). IARC: Lyon France, 2008.
15 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.
16 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250 259.
17 Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496 497.
18 Paulin T. Importance of bone marrow cell dose in bone marrow transplantation. *Clin Transplant* 1992; **6**: 48 54.
19 Byrne JL, Stainer C, Cull G, Haynes AP, Bessell EM, Hale G *et al.* The effect of the serotherapy regimen used and the marrow cell dose received on rejection, graft-versus-host disease and outcome following unrelated donor bone marrow transplantation for leukaemia. *Bone Marrow Transplant* 2000; **25**: 411 417.
20 Barrett AJ, Ringden O, Zhang MJ, Bashey A, Cahn JY, Cairo MS *et al.* Effect of nucleated marrow cell dose on relapse and survival in identical twin bone marrow transplants for leukemia. *Blood* 2000; **95**: 3323 3327.
21 Han P, Story C, McDonald T, Mrozik K, Snell L. Immune escape mechanisms of childhood ALL and a potential countering role for DC-like leukemia cells. *Cytotherapy* 2002; **4**: 165 175.

22 Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097 2100.
23 Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD *et al.* Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; **300**: 1068 1073.
24 Levine JE, Braun T, Penza SL, Beatty P, Cornetta K, Martino R *et al.* Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. *J Clin Oncol* 2002; **20**: 405 412.
25 Porter DL, Roth MS, McGarigle C, Ferrara JL, Antin JH. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. *N Engl J Med* 1994; **330**: 100 106.
26 Theilgaard-Monch K, Raaschou-Jensen K, Palm H, Schjodt K, Heilmann C, Vindelov L *et al.* Flow cytometric assessment of lymphocyte subsets, lymphoid progenitors, and hematopoietic stem cells in allogeneic stem cell grafts. *Bone Marrow Transplant* 2001; **28**: 1073 1082.
27 Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W *et al.* Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 1995; **86**: 2041 2050.
28 Collins Jr RH, Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 1997; **15**: 433 444.
29 Passweg JR, Stern M, Koehl U, Uharek L, Tichelli A. Use of natural killer cells in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005; **35**: 637 643.
30 Mavroudis D, Read E, Cottler-Fox M, Couriel D, Molldrem J, Carter C *et al.* CD34+ cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood* 1996; **88**: 3223 3229.
31 Bittencourt H, Rocha V, Chevret S, Socie G, Esperou H, Devergie A *et al.* Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002; **99**: 2726 2733.
32 Dominietto A, Raiola AM, van Lint MT, Lamparelli T, Gualandi F, Berisso G *et al.* Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. *Br J Haematol* 2001; **112**: 219 227.
33 Shiobara S, Nakao S, Ueda M, Yamazaki H, Takahashi S, Asano S *et al.* Donor leukocyte infusion for Japanese patients with relapsed leukemia after allogeneic bone marrow transplantation: indications and dose escalation. *Ther Apher* 2001; **5**: 40 45.
34 Batinic D, Marusic M, Pavletic Z, Bogdanic V, Uzarevic B, Nemet D *et al.* Relationship between differing volumes of bone marrow aspirates and their cellular composition. *Bone Marrow Transplant* 1990; **6**: 103 107.

Supplementary Information accompanies the paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)

Bone Marrow Transplantation (2010), 1–6

www.nature.com/bmt

ORIGINAL ARTICLE

Air-leak syndrome following allo-SCT in adult patients: report from the Kanto Study Group for Cell Therapy in Japan

R Sakai[1,2], H Kanamori[1], C Nakaseko[3], F Yoshiba[4], K Fujimaki[5], T Sakura[6], S Fujisawa[2], N Kawai[7], M Onoda[8], T Matsushima[9], A Maruta[1], H Sakamaki[10] and S Okamoto[11] for the Kanto Study Group for Cell Therapy (KSGCT)

[1]*Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan;* [2]*Department of Hematology, Yokohama City University Medical Center, Yokohama, Japan;* [3]*Department of Hematology, Chiba University Graduate School of Medicine, Chiba, Japan;* [4]*Department of Hematology and Oncology, Tokai University School of Medicine, Kanagawa, Japan;* [5]*Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, Yokohama, Japan;* [6]*Leukemia Research Center, Saiseikai Maebashi Hospitals, Maebashi, Japan;* [7]*Department of Hematology, Saitama Medical University, Saitama, Japan;* [8]*Department of Internal Medicine, Chiba Municipal Aoba Hospital, Chiba, Japan;* [9]*Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Gunma, Japan;* [10]*Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan and* [11]*Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan*

We retrospectively investigated air-leak syndrome (ALS), including pneumothorax and mediastinal/s.c. emphysema, following allogeneic hematopoietic SCT. Eighteen patients (1.2%) developed ALS among 1515 undergoing SCT between 1994 and 2005 at the nine hospitals participating in the Kanto Study Group on Cell Therapy. The median onset of ALS was at 575 days (range: 105–1766) after SCT and 14 patients (77.8%) had experienced late onset noninfectious pulmonary complications (LONIPC) before ALS. Chronic GVHD (cGVHD) was the strongest risk factor for ALS (odds ratio 13.5, $P=0.013$ by multivariate analysis). Repeat SCT, male sex and age <38 years at the time of transplantation were also significant risk factors for ALS. Patients with ALS had a significantly worse survival rate than those without ALS (61.5 vs 14.9% at 3 years; $P=0.000$). The main cause of death was respiratory complications in 8 of the 18 patients. In conclusion, ALS is a rare complication of SCT that is more likely to occur in relatively young male patients with cGVHD and/or LONIPC. It is possible that better understanding and treatment of LONIPC may lead to prevention of ALS.

Bone Marrow Transplantation advance online publication, 31 May 2010; doi:10.1038/bmt.2010.129

Keywords: air-leak syndrome; allo-SCT; late onset noninfectious pulmonary complications; chronic GVHD

Correspondence: Dr R Sakai, Department of Hematology, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan.
E-mail: sakarika@urahp.yokohama-cu.ac.jp
Received 14 October 2009; revised 12 April 2010; accepted 17 April 2010

Introduction

The survival of patients with hematological disorders who receive allo-SCT has shown continuing improvement due to the introduction of various innovative therapeutic approaches. However, organ dysfunction/damage and infection remain problematic for long-term survivors after SCT, especially those with chronic GVHD (cGVHD), and such complications affect both their quality of life and survival. Late onset noninfectious pulmonary complications (LONIPC) are one of the most common manifestations of organ damage during the late phase after allo-SCT,[1–5] and have been reported to be the major cause of death for patients in this phase. Unlike LONIPC, air-leak syndrome (ALS), which includes pneumothorax (PT), mediastinal emphysema (ME) and s.c. emphysema (SE), is a relatively rare complication of SCT.[6–9] Although patients with ALS after SCT had a fatal outcome according to some case reports or small-scale retrospective studies, the clinical features of post transplant ALS remain obscure. The purpose of this study was to clarify the characteristics and risk factors for ALS after SCT, as well as its effect on survival.

Materials and methods

Patients

We retrospectively surveyed 1515 patients aged ⩾15 years who received allo-SCT between January 1994 and March 2005 at nine hospitals participating in the Kanto Study Group on Cell Therapy (KSGCT) in Japan. Detailed clinical data were collected by reviewing the medical records of each institution, whereas baseline pretransplant and post transplant information on the patients was retrieved from the KSGCT database.

Definitions

Air-leak syndrome was diagnosed from chest X-ray films and/or computed tomography scans as follows: PT was diagnosed by detection of extra-alveolar air in the left and/or right hemithorax, ME was defined as the presence of extra-alveolar air in the mediastinal space and SE was defined as extra-alveolar air in the s.c. tissue. Iatrogenic ALS that occurred after procedures such as trans-bronchial lung biopsy or mechanical ventilation was excluded. cGVHD and LONIPC were diagnosed from previously reported criteria.[10,11]

The case report form for ALS patients included the following information: date of diagnosis of ALS, type of ALS (described above), initial symptoms (cough, dyspnea and chest pain), presence of active cGVHD at the onset of ALS (yes or no), type of cGVHD (limited or extensive), presence of LONIPC (bronchiolitis obliterans (BO), bronchiolitis obliterans with organizing pneumonia (BOOP) and interstitial pneumonia (IP)), time from LONIPC to ALS, immunosuppressive therapy at the onset of ALS (prednisolone (PSL), CYA, tacrolimus (FK) and others), treatment of ALS (drain, initiate or increase the dose of immunosuppressive therapy, decrease the dose of immunosuppressive therapy, pleurodesis, observation and others), response to treatment (improved, stable or worsened), outcome and cause of death.

Statistical analysis

To identify risk factors for ALS, we tested the following variables by univariate and multivariate analyses: recipient age and sex, stem cell source, conditioning regimen (conventional vs reduced intensity), use of TBI, number of SCT procedures (first vs second or more), GVHD prophylaxis (CYA based vs FK based), grade of acute GVHD (0–I vs II–IV) and cGVHD (none vs limited or extensive). Comparison of categorical variables was carried out by the χ^2-test or Fisher's exact test, whereas comparison of continuous variables was performed with Student's *t*-test. To evaluate the independence of potential risk factors for ALS, we performed multiple logistic regression analysis. In all analyses, $P<0.05$ was considered to indicate statistical significance. Survival curves after the occurrence of ALS were estimated by the Kaplan–Meier method and the survival of patients with ALS was compared to that of those without ALS by the log-rank test, treating the occurrence of ALS as a time-dependent variable. To determine whether ALS was an independent poor prognostic factor for long-term survival after SCT, we performed Cox proportional hazards analysis including the following variables: recipient age and sex, disease risk (standard risk diseases were acute leukemia in the first or second CR, aplastic anemia, refractory anemia, refractory anemia with ringed sideroblasts, CML in the first chronic phase, multiple myeloma in PR or CR and malignant lymphoma in the first or second CR, whereas all other diseases/states were considered to be high risk), stem cell source, type of conditioning regimen and use of TBI. Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Clinical features of ALS

Air-leak syndrome was diagnosed in 18 patients (1.2% of all patients) after allo-SCT. Table 1 shows a summary of the baseline characteristics of these patients. Sixteen patients were men and the median age was 29.5 years. Grade II–IV acute GVHD occurred in 11 patients (61%) and all but one patient (94%) had cGVHD. The clinical presentation and outcome of ALS are shown in Table 2. The median time of onset was day 575 (range: days 105–1766) after SCT. ALS was classified as PT in seven patients, ME/SE in six patients and PT combined with ME/SE (mixed ALS) in five patients. At the onset of ALS, 16 patients had active cGVHD. Before ALS occurred, 14 patients had also experienced LONIPC, including 4 with BO, 4 with BOOP, 5 with IP and 1 with IP along with BOOP. At the diagnosis of ALS, LONIPC had resolved in two patients (nos. 5 and 18), but persisted in the remaining 12 patients. The median time from the diagnosis of LONIPC to the onset of ALS among the patients who had persistent LONIPC was 74 days (range: 3–1177 days). Seventeen patients (94%) had been treated with steroids and 13 patients (72%) were on steroids at the time of diagnosis of ALS. Treatment of ALS included drainage in 11 patients and pleurodesis in 3. ALS improved in 12 of 18 patients, remained stable in 2 patients and progressed despite treatment in 4 patients. The three patients (nos. 7, 12 and 13) who underwent pleurodesis all had progressive ALS (Table 2).

Eleven patients died at a median of 222 days after the occurrence of ALS (range: 6–944 days). Respiratory complications were the direct cause of death in eight patients, including ALS in four, BO in two and IP in two. Patient 10 did not have LONIPC before the occurrence of ALS, but BO appeared after improvement of ALS and became progressively worse. Two nonrespiratory deaths were attributable to relapse of the primary disease and one was due to multiple organ failure that was unrelated to ALS. Among the patients with PT or ME/SE, seven out of nine who responded to treatment are still alive, whereas all four patients whose initial therapy

Table 1 Summary of baseline characteristics of the patients with ALS

No. of patients (%)	18 (1.2%)
Sex (male/female)	16/2
Median age (range)	29.5 (16–53)
Primary disease (AML/ALL/CML/ML)	9/5/2/2
Disease status at HSCT (CR or CP/NR or BC)	14/4
Donor source (RBM/UBM/PB/CB)	6/8/4/0
Conditioning (myeloablative/nonmyeloablative)	17/1
TBI or TLI (yes/no)	15/3
No. of SCT (first/second or more)	5/13
GVHD prophylaxis (CYA based/FK based)	8/8
aGVHD (0–I/II–IV)	6/12
cGVHD (no/limited/extensive)	1/2/15

Abbreviations: aGVHD = acute GVHD; BC = blastic crisis; CB = cord blood; cGVHD = chronic GVHD; CP = chronic phase; FK = tacrolimus; ML = malignant lymphoma; NR = non remission; RBM = related BM; RPB = related PBSCs; TLI = total lymphoid irradiation; UBM = unrelated BM.

Table 2 Clinical presentation and outcome of 18 patients with ALS

Patient no.	*Time from SCT to ALS (days)*	*Type of ALS*	*Initial symptoms*			*Active cGVHD at onset of ALS/ type of cGVHD*	*Active LONIPC at onset of ALS*	*Time from LONIPIC to ALS (days)*	*Immuno-suppression at onset of ALS*	*Treatment of ALS*	*Response to therapy*	*Outcome (cause of death)*
			Cough	*Dyspnea*	*Chest pain*							
1	1332	PT (one side)	+	+	–	Yes/Limit	BOOP	264	—	Drainage	Improved	Alive
2	296	ME/SE	+	+	–	Yes/Ext	BO	36	PSL+FK	Observation	Improved	Alive
3	257	ME/SE	–	–	–	Yes/Ext	IP	20	—	Observation	Improved	Died (IP)
4	636	PT (both sides)	–	+	–	No	—		—	Drainage	Improved	Alive
5	600	PT (one side)	–	–	+	Yes/Ext	(BOOP; resolved)[a]		PSL+FK	Drainage	Improved	Alive
6	1766	ME/SE	–	–	–	Yes/Ext	BOOP	170	PSL+CYA	Increase IS	Improved	Alive
7	806	PT (both sides)	+	+	–	Yes/Ext	BOOP	112	PSL+FK	Drainage, increase IS, pleurodesis	Progressed	Died (ALS)
8	155	PT (one side)	+	+	+	Yes/Ext	BO	3	PSL+CYA	Drainage, increase IS	Improved	Died (primary disease)
9	145	Mixed	–	–	–	Yes/Ext	IP	13	PSL+FK	Observation	Improved	Died (primary disease)
10	185	PT (both sides)	–	–	+	Yes/Ext	—		PSL+FK	Drainage	Unchanged	Died (BO)
11	163	Mixed	–	–	+	Yes/Limit	IP	18	PSL+FK	Drainage	Improved	Died (IP)
12	584	Mixed	+	+	+	Yes/Ext	BO	170	PSL+FK	Drainage, increase IS, pleurodesis	Progressed	Died (ALS)
13	1378	Mixed	+	+	+	Yes/Ext	BOOP/IP	1177	PSL+FK	Drainages, increase IS, pleurodesis	Progressed	Died (ALS)
14	105	ME/SE	–	–	–	Yes/Ext	—		PSL+CYA	Observation	Improved	Alive
15	565	Mixed	–	+	–	Yes/Ext	—		PSL+CYA	Drainage	Improved	Died (MOF)
16	841	ME	+	+	–	Yes/Ext	BO	170	—	Start IS	Unchanged	Died (BO)
17	209	ME/SE	–	+	–	Yes/Ext	IP	23	PSL+CYA	Increase PSL	Progressed	Died (ALS)
18	1438	PT (one side)	–	+	+	No	(IP; resolved)[a]		—	Drainage	Improved	Alive

Abbreviations: ALS = air-leak syndrome; BO = bronchiolitis obliterans; BOOP = bronchiolitis obliterans with organizing pneumonia; Ext = extensive type; FK = tacrolimus; IP = interstitial pneumonia; IS = immunosuppressants; Limit = limited type; ME = mediastinal emphysema; mixed = PT with ME/SE; MOF = multiple organ failure; PSL = prednisolone; PT = pneumothorax; SE = s.c. emphysema.
[a]At the diagnosis of ALS, LONIPC had already resolved.

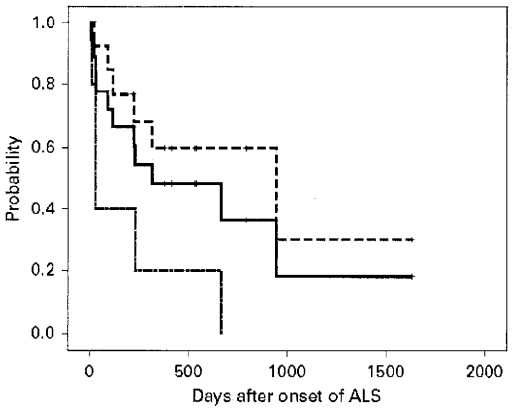

Figure 1 OS after the onset stratified according to the type of ALS. ——— OS of all 18 patients. ---- OS of patients with PT and ME/SE ($n=13$). -·-·- OS of patients with mixed ALS ($n=5$). Patients with PT and ME/SE showed better survival than those with mixed ALS ($P=0.017$).

failed eventually died of LONIPC and/or ALS. In contrast, all five patients with mixed ALS died regardless of their responses to treatment and three of them died of progressive lung disease.

For all 18 patients, OS at 1 year after the onset of ALS was 48.5±12.1% and the 3-year survival rate was 18.2±14.6% (Figure 1). Patients with PT and ME/SE showed better survival than did those with mixed ALS (59.8 vs 20.0% at 1 year and 29.9 vs 0% at 3 years, respectively; $P=0.017$; Figure 1). The patients without active LONIPC at the diagnosis of ALS ($n=6$) had a higher survival rate at 3 years than those with active LONIPC (53.3 vs 0%; $P=0.15$; data not shown).

Risk factors for ALS

Air-leak syndrome was always diagnosed more than 3 months after SCT in this study, so we compared the clinical features of patients with or without ALS who survived for more than 90 days after transplantation (1142 recipients). According to univariate analysis, ALS was significantly more frequent in recipients with cGVHD ($P=0.001$), those who received a second or subsequent SCT ($P=0.043$), younger recipients ($P=0.013$) and male recipients ($P=0.013$; data not shown). We also evaluated the risk factors for ALS by logistic regression analysis in 1047 recipients, after excluding 95 recipients (8.3%) because complete data were not available. The median age of the recipients was 38 years, so we divided them into two groups aged <38 and ≥38 years. Chronic GVHD was identified as the strongest risk factor for ALS (odds ratio (OR), 13.48; $P=0.013$), whereas second or subsequent SCT (OR, 7.91; $P=0.021$), male sex (OR, 4.95; $P=0.038$), age <38 years (OR, 3.55; $P=0.033$) and FK-based GVHD prophylaxis (OR, 3.3; $P=0.025$) were also identified as independent risk factors (Table 3).

Table 3 Multivariate analysis of factors related to ALS

Variable	*HR*	*95% CI*	P-*value*
Recipient age (≥38)	3.55	1.11–11.37	0.033
Recipient sex, male	4.95	1.10–22.36	0.038
Donor source, unrelated	0.62	0.30–1.27	0.19
No. of SCT ≥2	7.91	1.57–39.91	0.021
Reduced-intensity conditioning	0.22	0.02–2.49	0.219
TBI	0.96	0.26–3.54	0.953
GVHD prophylaxis with FK	3.30	1.17–9.36	0.025
Acute GVHD (grade II–IV)	1.51	0.56–4.10	0.193
Chronic GVHD	13.48	1.75–103.89	0.013

Abbreviation: FK, tacrolimus.

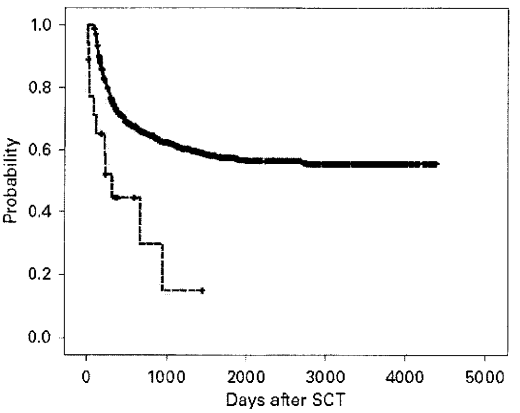

Figure 2 Kaplan–Meier curves for OS after SCT in patients with or without ALS. ——— ALS(−) ($n=1124$). ---- ALS(+) ($n=18$). Recipients with ALS showed significantly worse survival after SCT than those without ALS ($P=0.002$).

Impact of ALS on OS

The patients with ALS had significantly lower survival rates (44.7% at 1 year and 14.9% at 3 years) compared with those without ALS (72.7% at 1 year and 61.5% at 3 years; $P=0.002$; Figure 2). We analyzed factors associated with lower OS among 1079 recipients who lived for more than 90 days after allo-SCT by multivariate Cox regression analysis (63 patients (5.5%) were excluded because of incomplete data). ALS was identified as an independent predictor of worse survival after SCT (OR, 3.468; $P=0.001$), as was a high-risk disease status (OR, 2.851; $P=0.000$) and an age ≥38 years (OR, 1.267; $P=0.016$; Table 4).

Discussion

All forms of thoracic air leak are defined as ALS according to Franquet *et al.*,[12] including spontaneous pneumomediastinum or pneumopericardium, SE, interstitial emphysema and spontaneous PT. There have been only a few reports about ALS associated with SCT. Recently, Toubai *et al.*[8] performed a single-institution retrospective study and found ALS in 5 out of 213 recipients (2.3%)

Table 4 Multivariate analysis of factors associated with worse OS after SCT

Variable	*HR*	*95% CI*	P-*value*
Recipient age (≥38)	1.27	1.05–1.54	0.016
Recipient sex, male	0.89	0.73–1.08	0.231
Donor source, unrelated	1.07	0.88–1.30	0.528
Reduced-intensity conditioning	0.75	0.53–1.06	0.105
TBI	0.97	0.75–1.25	0.795
Disease status, high risk	2.85	2.34–3.48	0.000
ALS	3.47	1.63–7.40	0.001

Abbreviation: ALS = air-leak syndrome.

following allo-SCT, whereas Vogel *et al.*[9] reported ALS in 7 out of 300 recipients (2.3%). The incidence of ALS in the present study was slightly lower than in these previous studies. In this retrospective multicenter study, we tried to identify the characteristics, risk factors and prognosis of ALS after SCT, but the following limitations of our study must be considered. Unrecognized biases might have influenced the results of this retrospective study, especially as patients with asymptomatic ALS could not be detected. All cases of ALS were diagnosed more than 100 days after SCT in our series. Other studies and case reports have also shown that this complication occurs more than 100 days after SCT,[6–9] and all authors have agreed that ALS can be classified as a late complication of allo-SCT.

We confirmed that cGVHD, second or subsequent SCT, male sex, age <38 years and FK-based GVHD prophylaxis were independent risk factors for ALS. Several case reports have indicated that ALS following SCT is associated with severe cGVHD or noninfectious pulmonary complications such as BO.[6,9,13,14] In our series, 17 out of 18 recipients with ALS experienced cGVHD, and this is the first report to confirm statistically that the occurrence of ALS is strongly associated with cGVHD. It has also been reported that cGVHD is a significant risk factor for the development of LONIPC.[1–4] In this series, most ALS patients also had LONIPC based on cGVHD. Although the mechanism leading to chronic pulmonary GVHD is unknown, it is thought that host-reactive donor T cells cause injury to the lungs.[15] Continuous inflammation due to cGVHD may lead to fibrotic change of the peripheral airways that decreases lung compliance. Chronic GVHD appears to cause the progression of LONIPC, resulting in the occurrence of ALS. Our recipients who received a second or subsequent SCT would have suffered pulmonary damage by the conditioning regimen, which could have contributed to the development of ALS, although the mechanism of lung injury differs from that of cGVHD. According to a review of the literature,[6,7,13,14,16,17] ALS occurred after SCT in 17 men and 6 women with a median age of 30 years (range: 8–51 years). Our study confirmed by multivariate analysis that younger (<38 years old) men have a high risk of developing ALS after SCT. FK-based GVHD prophylaxis is generally chosen if stem cells are obtained from high-risk donors for GVHD, such as unrelated or HLA-mismatched related donors. This might explain the association between FK-based GVHD prophylaxis and development of ALS in our study.

The survival rate of patients after the occurrence of ALS was significantly impaired (18.2 ± 14.6% at 3 years), and the prognosis was related to the type of ALS (mixed ALS vs others). Alveolar rupture can occur because of an elevated intra-alveolar pressure, following damage to the alveolar walls, or for both reasons.[9] The subtypes of ALS reflect the extent of pulmonary/thoracic tissue damage, rather than different pathophysiological processes. This may explain the very poor outcome of mixed ALS. It is well known that the prognosis of secondary PT in patients with COPD is worse compared with idiopathic PT because it usually takes longer to reexpand the lung after a chest tube is inserted and failure of treatment is common.[16] It can be suggested that the onset of ALS in recipients with LONIPC leads to more severe lung tissue damage and a poor prognosis.

In conclusion, we were able to identify a subgroup of SCT recipients with a high risk of developing ALS, namely younger (<38 years) men with cGVHD, second or subsequent SCT and/or FK-based GVHD prophylaxis. As ALS is rare and its etiology is multifactorial, trials of new treatments are not feasible. A more promising strategy may be to improve our understanding and treatment of LONIPC, which should then lead to prevention of ALS.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Satoshi Morita and Tetsuji Kaneko from Yokohama City University Medical Center for the expert statistical advice. We also thank all of the dedicated physicians and paramedical staff at the participating transplantation centers who contributed data to this study.

References

1 Duncker C, Dohr D, Harsdorf S, Duyster J, Stefanic M, Martini C *et al.* Non-infectious lung complications are closely associated with chronic graft-versus-host disease: a single center study of incidence, risk factors and outcome. *Bone Marrow Transplant* 2000; **25**: 1263 1268.
2 Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 425 434.
3 Dudek AZ, Mabasetb H, DeFor TE, Weisdorf DF. Bronchiolitis obliterans in chronic graft-versus-host disease: analysis of risk factors and treatment outcomes. *Biol Blood Marrow Transplant* 2003; **9**: 657 666.
4 Patriarca F, Sert C, Sperotto A, Damiani D, Cerno M, Geromin A *et al.* Incidence, outcome, and risk factors of late-onset noninfectious pulmonary complications after unrelated donor stem cell transplantation. *Bone Marrow Transplant* 2004; **33**: 751 758.
5 Jinta M, Ohashi K, Ohra T, Ieki R, Abe K, Kamata N *et al.* Clinical features of allogeneic hematopoietic stem cell transplantation-associated organizing pneumonia. *Bone Marrow Transplant* 2007; **40**: 465 472.

6 Kumar S, Tefferi A. Spontaneous pneumomediastinum and subcutaneous emphysema complicating bronchiolitis obliterans after allogeneic bone marrow transplantation-case report and review of literature. *Ann Hematol* 2001; **80**: 430 435.
7 Shin HJ, Park CY, Park YH, Kim YJ, Mib C-K, Lee S *et al.* Spontaneous pneumothorax developed in patients with bronchiolitis obliterans after unrelated hematopoietic stem cell transplantation: case report and review of the literature. *Int J Hematol* 2004; **79**: 298 302.
8 Toubai T, Tanaka T, Kobayashi N, Honda T, Miura Y, Ogawa T *et al.* Mediastinal emphysema and bilateral pneumothoraces with chronic GVHD in patients after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2004; **33**: 1159 1163.
9 Vogel M, Brodoefel H, Bethge W, Faul C, Hartmann J, Schimmel H *et al.* Spontaneous thoracic air-leakage syndrome in patients following allogeneic hematopoietic stem cell transplantation: causes, CT-follow up and patient outcome. *Eur J Radiol* 2006; **60**: 392 397.
10 Shulmann H, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host disease I man: a clinic pathologic study of 20 long term Seattle patients. *Am J Med* 1980; **69**: 204 217.
11 Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG *et al.* Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. *Br J Haematol* 1998; **100**: 680 687.
12 Franquet T, Gimenez A, Torrubia S, Sabate JM, Rodriguez-Arias JM. Spontaneous pneumothorax and pneumomediastinum in IPF. *Eur Radiol* 2000; **10**: 108 113.
13 Galanis E, Litzow MR, Tefferi A, Scott JP. Spontaneous pneumomediastinum in a patient with bronchiolitis obliterans after bone marrow transplantation. *Bone Marrow Transplant* 1997; **20**: 695 696.
14 Hoshino Y, Hatake K, Mimuro J, Nakamura Y, Ashizawa N, Tsunoda S *et al.* Refractory bilateral pneumothoraces complicated with interstitial pneumonitis after bone marrow transplantation. *Jpn J Clin Hematol* 1993; **34**: 718 722.
15 Cooke KR, Krenger W, Hill G, Martin TR, Kobzik L, Brewer J *et al.* Host reactive donor T cells are associated with lung injury after experimental allogeneic bone marrow transplantation. *Blood* 1998; **92**: 2571 2580.
16 Takahashi N, Maruta A, Hashimoto C, Kato K, Tanabe J, Kodama F *et al.* Idiopathic mediastinal and subcutaneous emphysema in a patient with acute lymphocytic leukemia after allogeneic bone marrow transplantation. *Jpn J Clin Hematol* 2000; **41**: 1158 1163.
17 Doki N, Irisawa H, Saito Y, Sakura T, Miyawaki S. Acute myelogenous leukemia with mediastinal and subcutaneous emphysema following chronic GVHD after allogeneic bone marrow transplantation. *Jpn J Clin Hematol* 2005; **46**: 1038 1043.

Bone Marrow Transplantation (2010) 45, 1077–1083

www.nature.com/bmt

ORIGINAL ARTICLE

Baseline profiles of ocular surface and tear dynamics after allogeneic hematopoietic stem cell transplantation in patients with or without chronic GVHD-related dry eye

Y Wang[1,2], Y Ogawa[1], M Dogru[3,4], Y Tatematsu[1], M Uchino[1], M Kamoi[1], N Okada[1], S Okamoto[5] and K Tsubota[1]

[1]*Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan;* [2]*Department of Ophthalmology, The Eye and ENT Hospital of Fudan University School of Medicine, Shanghai, China;* [3]*Department of Ophthalmology, Tokyo Dental College, Chiba, Japan;* [4]*J&J Ocular Surface and Visual Optics Department, Keio University School of Medicine, Tokyo, Japan and* [5]*Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan*

We evaluated ocular surface alterations in allogeneic hematopoietic stem cell transplantation (HSCT) recipients with or without chronic GVHD-related dry eye in a prospective study. Fifty eyes of 25 post-HSCT patients and 28 eyes of 14 age-matched healthy controls were included. Meibomian gland (MG) obstruction, tear evaporation rate, corneal sensitivity (CS), Schirmer test-I, tear break-up time (BUT) and ocular surface vital staining were examined. Conjunctival impression and brush cytology specimens were collected to evaluate the goblet cell density (GCD) and the inflammatory cell numbers. Obvious MG obstruction, decreased CS and enhanced tear evaporation rate were found in post-HSCT patients compared with normal controls. In addition, decreased conjunctival GCD, increased conjunctival squamous metaplasia and inflammatory cells were noted in cGVHD-related dry eyes compared with normal controls and post-HSCT without dry eye subjects. Furthermore, the conjunctival inflammatory cells were significantly higher in severe dry eyes compared with mild dry eyes ($P=0.03$). We found comprehensive ocular surface alteration in post-HSCT patients, regardless of whether they had cGVHD-related dry eye or not. The results suggest that the extent of inflammatory process seems to have a pivotal role in the outcome of the cGVHD-related dry eye.
Bone Marrow Transplantation (2010) **45,** 1077 1083; doi:10.1038/bmt.2009.312; published online 9 November 2009

Correspondence: Dr Y Ogawa, Department of Ophthalmology, Keio University, School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: yoko@sc.itc.keio.ac.jp or Dr M Dogru, J&J Ocular Surface and Visual Optics Department, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: muratodooru@yahoo.com
Received 29 June 2009; revised 11 August 2009; accepted 12 August 2009; published online 9 November 2009

Keywords: allogeneic hematopoietic stem cell transplantation (HSCT); dry eye; impression cytology; brush cytology; meibomian gland; tear evaporation

Introduction

Chronic GVHD is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT).[1] Ocular surface is one of the target tissues of cGVHD. About 50% of patients develop dry eye or experience a worsening of the pre-existing dry eye after HSCT.[2] Dry eye is a distinctive sign and symptom for the diagnosis of cGVHD.[1] However, the pathogenesis of dry eye associated with cGVHD is still unclear, and effective treatments have not yet been established.[3] Pathogenic studies of dry eye associated with cGVHD depend on the lacrimal gland and conjunctival biopsy.[4–6] It is impossible to follow the alterations of the ocular surface pathologic process after HSCT by repeated biopsy. On the other hand, impression cytology and brush cytology are widely used methods to evaluate the ocular surface pathologic changes.[7] They are noninvasive, repeatable, and useful in following the changes in the ocular surface.[8,9] However, there are few reports on impression cytology changes and brush cytology characteristics in patients with cGVHD-related dry eye.[10] On the other hand, the conditioning regimen including total body irradiation and high incidence of meibomian gland dysfunction (MGD) in post-GVHD patients contributes to the ocular surface and tear function changes.

However, there is no report comparing the tear functions and ocular surface alterations between post-HSCT patients with or without dry eye. In a previous study,[2] we noticed there were two types of dry eye after HSCT. One had severe ocular surface and tear function damage with decreased reflex tearing that occurred soon after the onset of dry eye, whereas the other was mild with normal reflex tearing. There are no data comparing the ocular