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ORIGINAL ARTICLE

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

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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.

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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
	$<3.0\times10^8$/kg (n = 140)		*3.0–4.6 $\times10^8$/kg (n = 248)*		*$\geqslant4.6\times10^8$/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 $<3.0\times10^8$/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 $<2.3\times10^8$/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 (× 10^8/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 (× 10^8/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 $<3.0\times10^8$/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 $>3.4\times10^8$/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 $>3.4\times10^8$/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 $>4.6\times10^8$/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 $>4.6\times10^8$/kg, respectively

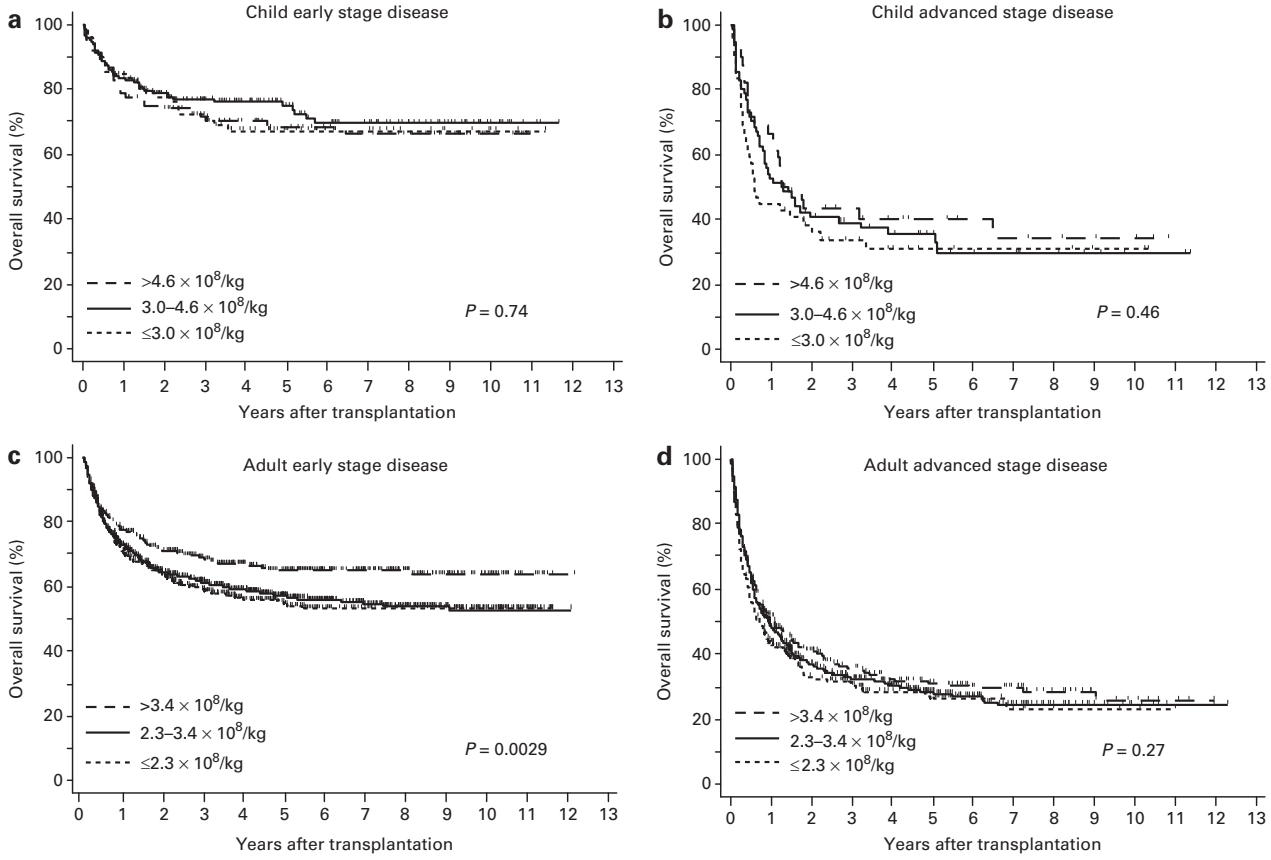

Figure 1 Kaplan–Meier estimates of OS according to cell dose: (**a**) among children with early-stage diseases; (**b**) among children with advanced-stage diseases; (**c**) among adults with early-stage diseases; and (**d**) among adults with advanced-stage diseases.

($P=0.46$; 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 $>3.4\times10^8$/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 $>3.4\times10^8$/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 $>3.4\times10^8$/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 $<3.0\times10^8$/kg in advanced-stage diseases. Among adults, cell dose $>3.4\times10^8$/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 $<2.3\times10^8$/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 (× 10^8/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 (× 10^8/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.[34] 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.

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