Table 1. *Patient Characteristics*

	CST	RIST	P-Value
No. of patients	52	80	
Sex, male/female	25/27	50/30	
Median age, years (range)	40 (3-55)	53 (20-68)	<.01
Disease status at conditioning, N (%)			
AML	20 (38)	15 (19)	
Relapse 1	12	6	
Relapse ≥2	5	5	
Primary refractory	3	4	
MDS (including overt AML)	15 (29)	24 (30)	
Relapse 1	1	2	
Untreated	6	11	
Primary refractory	8	11	
ALL	5 (10)	2 (3)	
Relapse 1	4	1	
Relapse 2	1	1	
CML	5 (10)	3 (4)	
Accelerated phase	3	0	
Blastic crisis	2	3	
NHL	7 (13)	33 (40)	
Relapse 1	2	6	
Relapse ≥2	2	16	
Primary refractory	3	11	
Others*	0	3 (4)	
Chemotherapy within 2 months before HCT, N (%)	33 (63)	52 (65)	
Leukemia/MDS	30	23	
Lymphoma	3	26	
Others*	0	3	
HCT-CI score, N (%)			.03
0	33 (63)	28 (35)	
1-2	11 (21)	31 (39)	
≥3	8 (16)	21 (26)	
Conditioning regimen, N (%)			
TBI/CY	34 (65)	0	
BU/CY	13 (25)	0	
Fludarabine-based (± TBI)†	0	68 (85)	
Cladribine-based (±TBI)‡	0	12 (15)	
Others	5 (10)	0	
Donor type, N (%)			.1
HLA-matched related donor	17 (33)	41 (51)	
HLA-mismatched related donor	5 (9)	7 (9)	
Unrelated donor	30 (58)	32 (40)	
Stem cell source, N (%)			<.01
G-CSF mobilized PBSC	21 (40)	49 (61)	
BM	27 (52)	20 (25)	
CB	4 (8)	11 (14)	
GVHD prophylaxis, N (%)			<.01
Cyclosporine§	1 (2)	52 (65)	
Cyclosporine/MTX¶	49 (94)	28 (35)	
Tacrolimus	1 (2)	0	
Tacrolimus/MTX	1 (2)	0	
Prior HCT, N (%)	4 (8)	8 (10)	.65

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; TBI, total-body irradiation; CY, cyclophosphamide; BU, busulfan; HLA, human leukocyte antigen; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; GVHD, graft-versus-host disease; MTX, methoterexate.

*Others included 1 chronic lymphocytic leukemia and 2 multiple myeloma patients.

†Twenty-three patients received 4 Gy TBI.

‡Four patients received 4 Gy TBI.

§Including 7 with antithymocyte globulin.

¶Including 10 with antithymocyte globulin.

of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5 × 10^9/L of peripheral blood. The diagnosis and clinical grading of acute and chronic GVHD (aGVHD, cGVHD) were performed according to established criteria [22-24]. CR was defined as lower than 5% blasts in the bone marrow, with a neutrophil count >1.5 × 10^9/L and a platelet count >100 × 10^9/L in leukemia/MDS patients, and according to the International Workshop Criteria [25] in lymphoma patients.

Statistical Analysis

The endpoints of the study were progressive disease/relapse (PD), NRM, overall survival (OS), and progression-free survival (PFS). OS, NRM, and PD were defined as the time between stem cell infusion to the event. PFS was defined as the time between stem cell infusion to PD or death from any cause, whichever occurred earlier. OS and PFS were estimated by the Kaplan-Meier method [26]. NRM and PD were estimated by the cumulative incidence. The chi-square test or Fisher's exact test was used to evaluate the differences in the clinical characteristics of the CST and RIST groups. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of survival, NRM, and PD after HCT over time across patient subgroups.

Multiple Cox regression models were used for multivariate risk factor analysis for PD, NRM, OS, and PFS after HCT. Clinical factors evaluated in the PD, NRM, OS, and PFS analyses were patient age at the time of HCT (continuous), HCT-CI (0, 1-2, 3 or more), conditioning (CST, RIST), donor (HLA-matched related, HLA-mismatched related or unrelated), disease type (leukemia/MDS, lymphoma), and chemotherapy within 2 months before HCT (yes, no). Logistic regression analysis was performed to identify prognostic factors that were associated with the achievement of CR. In addition to the variables examined in the Cox analysis, blast percentage (≥20%, <20%) in the bone marrow or peripheral blood and the serum lactate dehydrogenase (LDH) level (normal, elevation) before HCT were included for the analysis of CR in patients with leukemia/MDS and those with lymphoma, respectively. We considered 2-sided P-values of <.05 to be statistically significant. Statistical analyses were performed with the SAS version 8.2 (SAS Inc, Cary, NC).

RESULTS

Patient Characteristics

The characteristics of all patients who underwent CST (n = 52) or RIST (n = 80) are summarized in Table 1. The median age of the RIST group was significantly higher than that of the CST group (53 years versus 40 years, $P < .01$). A large number of patients in both groups had acute myeloid leukemia (AML) or MDS (CST 67%, RIST 49%), and the RIST group included a higher population of patients with malignant lymphoma (CST 13%, RIST 40%). All malignant lymphomas (n = 40) were non-Hodgkin's lymphoma, including aggressive (n = 16), highly aggressive (n = 15), and indolent (n = 9) lymphomas. The distribution of lymphoma subtypes was similar between the 2 groups. Disease status at transplantation included primary refractory (n = 42), refractory relapse (n = 65), blastic crisis, or accelerated phase of CML (n = 8) and untreated disease (n = 17). The distribution of disease status and the proportion of patients who received chemotherapy within 2 months before HCT were similar between the 2 groups. The RIST group contained higher proportions of patients with an HCT-CI score of 1 or more (CST 37%, RIST 65%) and those who received G-CSF-mobilized PBSC (CST 40%, RIST 61%) than the CST group.

In the leukemia/MDS patients (n = 89), the median percentage of blasts (82 patients in bone marrow and 7 patients in peripheral blood) in both groups were similar (CST 29%, RIST 30%). In patients with malignant lymphoma, serum LDH was elevated above the upper normal limit in 3 of 7 (43%) in the CST group compared to 23 of 33 (70%) in the RIST group.

Engraftment and GVHD

The clinical course and response are detailed in Table 2. The median duration of follow-up in surviving patients is 1123 days (range: 367-2044 days) in the CST group and 899 days (range: 334-1961 days) in the RIST group. Neutrophil engraftment was observed in 48 patients (92%) and 75 patients (94%), at a median of 17 days and 12 days, respectively. Engraftment was not confirmed in the remaining 9 patients because of death or PD within 28 days after HCT. The incidences of grade II-IV and grade III-IV aGVHD were similar in the CST and RIST groups (50% versus 50% and 23% versus 28%, respectively). The incidences of cGVHD and chronic extensive GVHD were also similar (46% versus 49% and 34% versus 38%, respectively).

Disease Response

The probabilities of achieving CR as the best response were similar after CST and RIST (77% and 64%, respectively) (Table 2). To examine the possible risk factors for achieving CR, we separately analyzed patients with leukemia/MDS and those with lymphoma using a logistic regression analysis (Table 3). Conditioning regimen (RIST) did not influence the CR rate in patients with leukemia/MDS (odds ratio [OR] 1.11, 95% confidence interval [CI] 0.40-3.07,

Table 2. *Clinical Course and Response*

	CST (N = 52)	RIST (N = 80)
Median follow-up of surviving patients, days	1123 (367–2044)	899 (334–1961)
Engraftment of neutrophils, N (%)	48 (92)	75 (94)
Median day (range)	17 (10–35)	12 (5–43)
Acute GVHD, N (%)		
Grade II–IV	26 (50)	40 (50)
Grade III–IV	12 (23)	22 (28)
CR*, N (%)	40 (77)	51 (64)
Leukemia/MDS (n = 89), CR/total	35/45	35/44
Lymphoma (n = 40), CR/total	5/7	14/33
Causes of NRM, N (%)	15 (29)	26 (33)
GVHD	6	11
Infection		
fungus	0	4
CMV	0	1
bacterial	4	7
Interstitial pneumonitis	2	1
Others†	3	2

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; CR, complete remission; NRM, nonrelapse mortality; CMV, cytomegalovirus.

*CR as the best response after transplantation.

†Others included acute myocardial infarction, subarachnoid hemorrhage, and pulmonary alveolar haemorrhage in the CST group, and cerebral hemorrhage and unknown in the RIST group.

P = .84) or in those with lymphoma (OR 0.29, 95% CI 0.05-1.75, P = .18). In the leukemia/MDS patients, those who received chemotherapy within 2 months before HCT (OR 0.32, 95% CI 0.09-1.05, P = .06) and transplant from donors other than an HLA-matched relative (OR 0.28, 95% CI 0.08-1.06, P = .06) tended to have a lower CR rate, whereas the blast percentage (≥20%) of bone marrow or peripheral blood was not associated with the CR rate. In lymphoma patients, chemotherapy within 2 months before HCT was the only factor that was significantly associated with a low CR rate (OR 0.04, 95% CI 0.005-0.40, P < .01), whereas serum LDH elevation did not influence the CR rate.

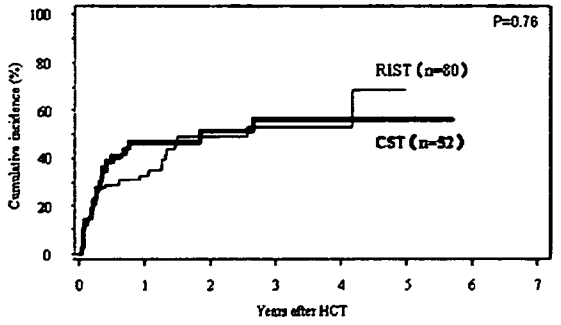

Figure 1. Cumulative incidence of PD. The 2-year probabilities of PD in the CST (51%) and RIST (49%) groups were not significantly different (P = .76).

As shown in Figure 1, the cumulative incidence of PD was not significantly different between the CST and RIST groups. The 2-year probabilities of PD were 51% in the CST group and 49% in the RIST group, which were not significantly different (P = .76). Cox regression analysis was performed to identify factors that were associated with PD. Multivariate analyses in all patients showed that those who received chemotherapy within 2 months before HCT were associated with an increased risk of PD (hazard ratio [HR] 3.93, 95% CI 1.97-7.83, P < .01) (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of PD in any of the patients. To further evaluate the association between risk factors and outcome, we performed a subset analysis in patients who underwent CST or RIST. As a result, chemotherapy within 2

Table 3. *Logistic Analysis of CR Rate in Leukemia/MDS and Lymphoma Patients*

		Leukemia/MDS (N = 89)		Lymphoma (N = 40)	
		Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
HCT-CI	0	1.00		1.00	
	1–2	1.44 (0.43–4.87)	.56	3.33 (0.66–16.7)	.14
	3 or more	0.96 (0.28–3.35)	.95	2.22 (0.40–12.3)	.36
Age		1.00 (0.97–1.04)	.70	1.02 (0.97–1.07)	.49
Conditioning	RIST	1.11 (0.40–3.07)	.84	0.29 (0.05–1.75)	.18
Donor	Alternative*	0.28 (0.08–1.06)	.06	0.95 (0.26–3.42)	.93
Chemotherapy within 2 months before HCT	Yes	0.32 (0.09–1.05)	.06	0.04 (0.005–0.40)	<.01
Blasts†	≥20%	0.62 (0.21–1.80)	.38		
Serum LDH level	Elevation			0.35 (0.09–1.34)	.12

MDS indicates myelodysplastic syndrome; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; RIST, reduced-intensity stem cell transplantation; LDH, lactate dehydrogenase; CI, confidence interval.

*Non-HLA-matched related donor.

†Blast counts in bone marrow (N = 82) or peripheral blood (N = 7).

Table 4. *Multivariate Analysis of PD, NRM, OS, and PFS in All Patients*

Covariates*	N	PD HR (95% CI)	PD P	NRM HR (95% CI)	NRM P	OS HR (95% CI)	OS P	PFS HR (95% CI)	PFS P
Conditioning									
CST	52	1.00		1.00		1.00		1.00	
RIST	80	0.91 (0.53-1.55)	.72	0.99 (0.51-1.96)	.99	0.95 (0.60-1.51)	.83	0.95 (0.63-1.43)	.79
HCT-CI score									
0	65			1.00		1.00		1.00	
1-2	38			3.25 (1.43-7.40)	<.01	1.76 (1.08-2.89)	.02		
3 or more	29			6.61 (2.88-15.2)	<.01	2.62 (1.51-4.56)	<.01	1.63 (1.02-2.62)	.04
Donor									
MRD	58			1.00		1.00			
Alternative†	74			2.77 (1.39-5.54)	<.01	1.80 (1.15-2.82)	.01		
Chemotherapy within 2 months before HCT									
No	47	1.00				1.00		1.00	
Yes	85	3.93 (1.97-7.83)	<.01			1.73 (1.10-2.72)	.02	2.23 (1.44-3.45)	<.01

PD indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; MRD, HLA-matched related donor; HCT, hematopoietic cell transplantation.

*Factors analyzed included age at the time of HCT (continuous), HCT-CI (0, 1-2, 3, or more), conditioning (CST, RIST), donor (MRD, Alternative), disease type (leukemia/MDS, lymphoma) and chemotherapy within 2 months before HCT (yes, no).

†Non-HLA-matched related donor.

months before HCT was associated with an increased risk of PD only in the RIST group, and not in the CST group (Table 5).

NRM

Major causes of NRM for patients in both groups were GVHD and infection (Table 2). More patients died of fungal infection in the RIST group compared to the CST group, but the 2-year probabilities of NRM were not significantly different (36% and 38%, $P = .50$, Figure 2). A Cox regression analysis was performed to identify factors associated with NRM. Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an HLA-mismatched related or unrelated donor (alternative donor) were associated with an increased risk of NRM (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of NRM in any of the patients. A subset analysis revealed that a higher HCT-CI score (1 or more) was associated with increased NRM in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group.

Survival

The 2-year probabilities of OS and PFS were not significantly different between the CST and RIST groups (31% and 38%, $P = .98$, for OS; 28% and

Table 5. *Multivariate Analysis of Outcomes after HCT in the CST and RIST Groups*

	Covariates	CST (N = 52) HR (95% CI)	CST (N = 52) P	RIST (N = 80) HR (95% CI)	RIST (N = 80) P
PD	Chemotherapy within 2 months before HCT		NS	6.16 (2.15–17.7)	<.01
NRM	HCT-CI (1–2)	4.48 (1.26–16.0)	.02		NS
	HCT-CI (3 or more)	10.2 (2.91–35.7)	<.01	2.41 (1.14–5.10)	.02
	Alternative donor*		NS	4.63 (1.96–10.9)	<.01
OS	HCT-CI (1–2)	2.69 (1.23–5.90)	.01		NS
	HCT-CI (3 or more)	4.84 (1.97–11.9)	<.01		NS
	Alternative donor*		NS	3.04 (1.73–5.35)	<.01
PFS	HCT-CI (3 or more)	2.26 (1.01–5.04)	.04		NS
	Chemotherapy within 2 months before HCT	2.10 (1.05–4.19)	.03	2.10 (1.19–3.70)	.01
	Alternative donor*		NS	1.79 (1.06–3.00)	.03

PD, indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; NS; not significant.

*Non-HLA-matched related donor.

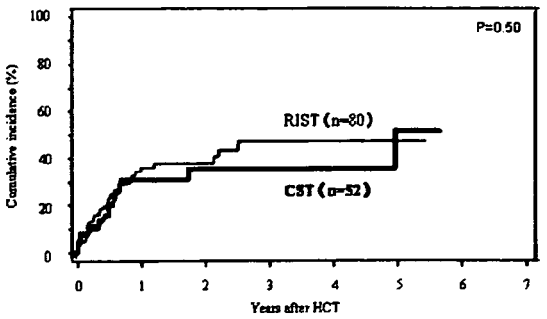

Figure 2. Cumulative incidence of NRM. The 2-year probabilities of NRM in the CST (36%) and RIST (38%) groups were not significantly different (P = .50).

29%, P = .89, for PFS), as shown in Figure 3 and Figure 4. The 2-year probabilities of PD, OS, and PFS were not significantly different between patients who developed grade III-IV aGVHD and those who did not (37% and 44%, P = .39, for PD; 33% and 50%, P = .07, for OS; 27% and 41%, P = .24, for PFS). On the other hand, the 2-year probability of NRM in patients who developed grade III-IV aGVHD was significantly higher than that in those who did not (56% and 21%, P = .004). We also evaluated outcomes in patients who had AML or MDS (CST, n = 35; RIST, n = 39). There was no significant difference in the 2-year probabilities of PD (50% and 51%), OS (37% and 33%), and PFS (34% and 22%) between the CST and RIST groups. On the other hand, the 2-year probability of NRM in the RIST group was significantly higher than that in the CST group (52% and 23%, P = .03).

Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an alternative donor were associated with poor OS, and patients who received chemotherapy within 2 months before HCT were associated with poor OS and PFS (Table 4). After adjusting for these variables, the risks of OS and PFS were not significantly different between the CST and RIST groups. Disease type (leukemia/MDS or lymphoma) was not a significant factor for OS or PFS. Furthermore, subset analyses revealed that a higher HCT-CI score (1 or more) was associated with poor OS and PFS in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group. Patients who received chemotherapy within 2 months before HCT had a poor PFS in both groups.

DISCUSSION

Our results suggest that the antileukemia/lymphoma effect of RIST might be comparable to that of CST for hematologic malignancies that are not in remission. We found that a higher HCT-CI score and transplant from an alternative donor were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT because of the acceleration of disease progression were associated with increased risks of PD, poor OS, and PFS. The estimated rates of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group even though the patients who received RIST were significantly older and had significantly higher HCT-CI scores than those who received CST. Several reports have described a similar OS rate in older patients who underwent RIST and CST because the lower NRM rate was offset by a higher PD [5,27,28]. In contrast, Scott et al. [7] found no significant differences in OS, PFS, PD, or NRM between CST and RIST in patients with MDS/AML.

In this study, disease response to the transplantation procedure was similar between the CST and RIST groups when the CR rate is considered the best response, as were the rate and timing of PD. Whereas some reports have shown that PD after HCT was increased in patients who underwent RIST compared to CST [3,5,11], others have found no significant difference [6-8,29]. This discrepancy might result from the differences in disease status at the time of

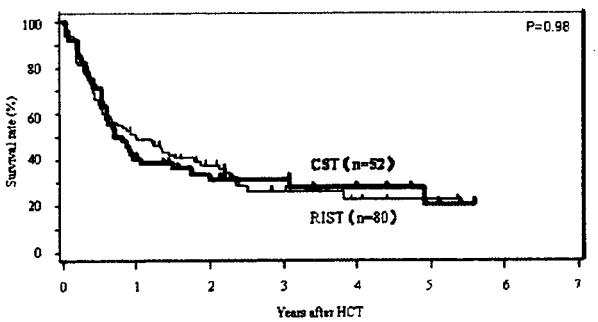

Figure 3. Estimated OS according to the conditioning regimen. The 2-year probabilities of OS in the CST (31%) and RIST (38%) groups were not significantly different (P = .98).

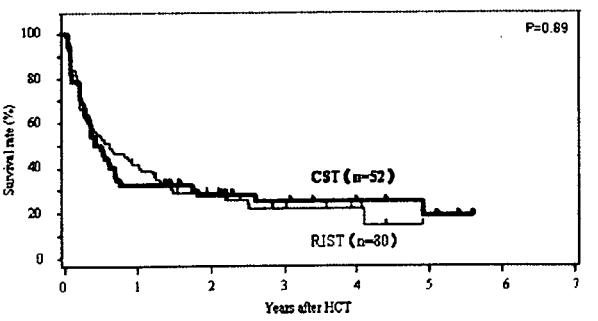

Figure 4. Estimated PFS according to the conditioning regimen. The 2-year probabilities of PFS in the CST (28%) and RIST (29%) groups were not significantly different (P = .89).

transplantation and the intensity of the conditioning regimens. In our study, the median percentage of blasts in leukemia/MDS patients and the distribution of serum LDH levels in lymphoma patients were comparable between the CST and RIST groups. The proportion of patients who required chemotherapy within 2 months before HCT was similar in the 2 groups. Overall, the risk of disease progression was comparable. The lack of a significant difference in PD between the CST and RIST groups in our study may be because the reduced-intensity regimens used in our study were more intense than those in previous reports. Nevertheless, our results suggest that RIST has a comparable antileukemia/lymphoma activity through a GVL effect compared to CST.

Our study found that chemotherapy within 2 months before HCT was the only factor that significantly predicted a lower CR rate in lymphoma patients and tended to be associated with a lower CR rate in leukemia/MDS patients. Furthermore, chemotherapy within 2 months before HCT was also associated with a worse prognosis not only with regard to PD but also for OS and PFS. A subset analysis showed that this negative impact of recent chemotherapy was only seen in RIST patients, and not in CST patients, which suggests that the tempo of the progression of the disease before HCT is especially important in RIST patients. Wong et al. [30] reported that high peripheral blast counts (≥30%) in patients with AML/MDS were associated with poor event-free survival and OS after HCT regardless of the conditioning regimen. In our study, however, ≥20% of blasts in the bone marrow or peripheral blood and serum LDH level elevation did not have a significant impact on the CR rate in leukemia/MDS and lymphoma patients, respectively.

In our study, there was no significant difference in NRM between the CST and RIST groups, which was in contrast to previous reports showing that reduced-intensity regimens were associated with less organ damage, and thus contributed to less NRM [1,4,5,9,27,31-34]. There are several possible explanations for this discrepancy. First, the patients who received RIST were older and had a higher HCT-CI score than those in the CST group. Second, the reduced-intensity conditioning (RIC) we used was more toxic than "truly nonmyeloablative" conditioning. Finally, we tapered immunosuppressive medications rapidly, especially in the RIST group, in an attempt to induce a more potent GVL effect, which resulted in more severe GVHD and subsequent infectious complications. However, our data showed that grade III-IV aGVHD did not contribute to a reduction in the rate of PD or to an overall improvement in survival, which was consistent with a previous report [14], although a high rate of NRM in patients with severe aGVHD may have masked its competing event (ie, PD).

We confirmed that HCT-CI was a significant risk factor for NRM and OS in patients not in remission. HCT-CI has recently been introduced to evaluate pretransplant comorbidities in HCT recipients, which predict well NRM and OS after allogeneic HCT [20]. In this study, the proportion of patients who were not in remission and were associated with comorbidities was 53%, which was higher than the value (42%) in our previous report [21], probably because these patients tended to be heavily pretreated and were forced to pursue HCT in the hope of a rare cure. Interestingly, this negative impact of HCT-CI was only seen in patients who underwent CST, and not in those who underwent RIST. Our data imply that RIC may be preferable in patients with hematologic malignancies not in remission and with a high HCT-CI score by reducing early NRM after transplantation.

Transplant from an alternative donor was another prognostic factor for NRM and OS in this study, which is consistent with previous reports [12,35-38]. Furthermore, an increased risk of NRM and OS associated with alternative donors was observed only in patients who underwent RIST. There are several possible explanations. First, the Japan Marrow Donor Program allows the donation of bone marrow, but not PBSC, from volunteer donors, which has been reported to be associated with poor engraftment and worse outcomes after nonmyeloablative stem cell transplantation [13]. Second, our conditioning regimen including low-dose TBI for RIST from an alternative donor was more toxic than that for RIST from an HLA-matched related donor. Further studies are required to establish optimized conditioning regimens and GVHD prophylaxis for RIST in unrelated pair settings.

In 27 patients who had all of these risk factors (ie, chemotherapy within 2 months beore HCT, HCT-CI score of 1 or more, and transplant from an alternative donor), the 2-year probabilities of NRM, PD, and OS were 56%, 44%, and 21%, respectively, with no significant differences between the CST and RIST groups (data not shown). Therefore, the indications for transplantation in patients with multiple risk factors should be carefully determined.

This study has several inherent limitations. First, the eligibility requirements for CST and RIST were different. Most patients who received RIST were considered ineligible for CST because of age or comorbid conditions. Second, factors other than the conditioning regimen were not entirely comparable between the 2 groups, that is, patient age, underlying diagnosis (leukemia/MDS and lymphoma), donor selection, stem cell source, and GVHD prophylaxis. Third, some of the conventional cytoreductive conditioning regimens we used (ie, use of oral BU and lack of its pharmacologic monitoring) may no longer be considered optimal. Fourth, because the reduced-intensity

regimens used in our study were more intense than those in previous reports, our data may not be generalized to the concept of "reduced-intensity regimen" and there may be circumstances where PD would be more marked. Finally, the follow-up of patients in this study was too short to draw any definite conclusions. Nevertheless, the observed data may still be useful in evaluating the impact of RIST on disease control in patients suffering from a higher risk of disease progression after transplantation.

In conclusion, our results suggest that the antileukemia/lymphoma effect associated with RIST might be comparable to that of CST for hematologic malignancies not in remission, particularly when patients do not require chemotherapy within 2 months before HCT or they had a higher HCT-CI score. To determine the ultimate utility of specific conditioning regimens, controlled prospective trials are needed, with enrolled patients being stratified according to disease activity, hematopoietic stem cell source, and associated comorbidities.

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Case Report

T-Cell Large Granular Lymphocyte Leukemia of Donor Origin After Cord Blood Transplantation

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Abstract

We report the first case of T-cell large granular lymphocyte leukemia of donor origin after a second cord blood transplantation for acute myeloid leukemia, and review the literature regarding rare cases of T-cell–origin posttransplantation lymphoproliferative disorders.

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Key words: Bone marrow, Epstein-Barr virus, Polymerase chain reaction, Posttransplantation lymphoproliferative disorders, T-cell receptor

Introduction

T-cell large granular lymphocyte leukemia (LGL; LGLL) is characterized by the monoclonal proliferation of $CD3^+$, and $CD8^+$ LGLs, with abundant cytoplasm and fine or coarse azurophilic granules.[1,2] Reactive expansion of LGL in the peripheral blood has been occasionally reported during viral infection and in recovery phase of allogeneic hematopoietic stem cell transplantation (HSCT).[3,4]

Posttransplantation lymphoproliferative disorder (PTLD) is a characteristic lymphoid proliferation or the development of lymphoma in a setting of decreased T-cell immune surveillance, typically in recipients of solid organ transplantation or allogeneic HSCT. Most reported cases of PTLD are of B-cell origin, in association with Epstein-Barr virus (EBV) infection, which leads to monoclonal or, less frequently, polyclonal proliferation of B cells. Most of the rare cases of T-cell PTLD were reported after solid organ transplantation, with very rare cases after allogeneic HSCT.

In this report, we describe the unique clinical and laboratory findings of a patient with γδ T-cell LGLL of cord donor origin after a second cord blood transplantation for acute myeloid leukemia.

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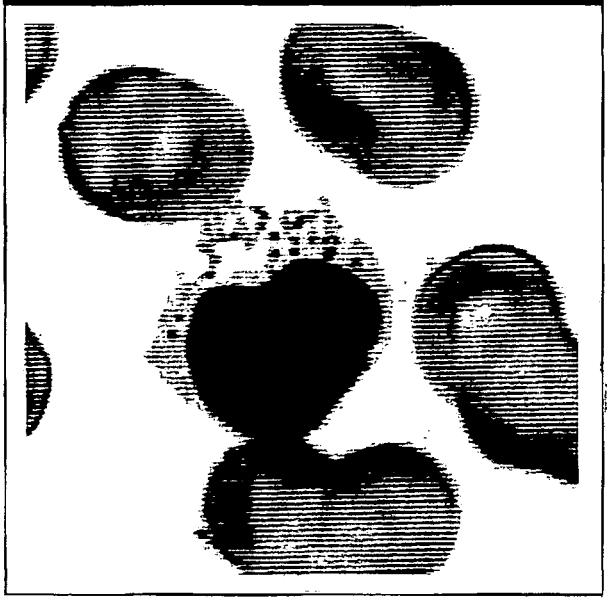
Case Report

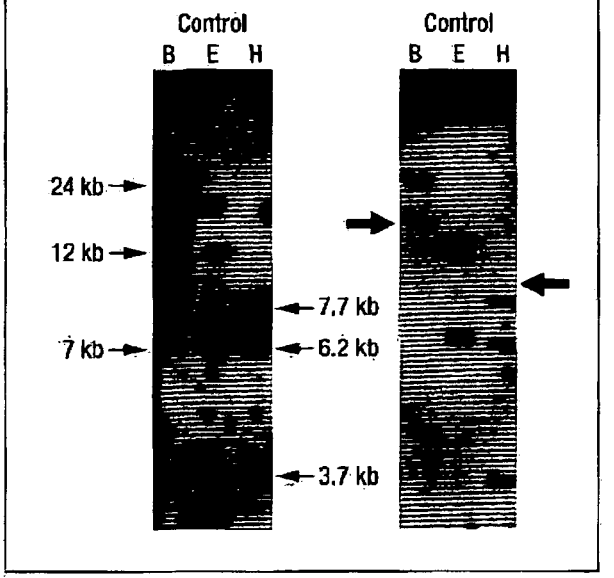
A 58-year-old Japanese man with acute myeloid leukemia (French-American-British classification; M2) in second complete remission received allogeneic HSCT from an unrelated female cord blood donor. The conditioning regimen consisted of total body irradiation of 12 Gy in 6 fractions from day −6 to −4, and cyclophosphamide 60 mg/kg once daily intravenously on days −3 to −2 (total dose, 120 mg/kg). He received human leukocyte antigen–loci mismatched (2 by serology and 2 by DNA typing) unrelated cord blood, which contained 3.03×10^7 nucleated cells/kg in January 2003. Cyclosporine and short-term methotrexate were used as graft-versus-host disease prophylaxis. However, hematologic recovery was not observed up to day 40, and we concluded that this was a case of primary graft failure without leukemia relapse because the results of interphase fluorescence in situ hybridization analysis on days 23, 30, and 37 on bone marrow (BM) samples were negative. Because his condition remained good, we planned a second cord blood transplantation with a reduced-intensity regimen, which consisted of fludarabine 30 mg/kg once daily intravenously from days −8 to −3 (total dose 180 mg/kg), busulfan 4 mg/kg orally on days −6 and −5 (total dose 8 mg/kg), and total body irradiation of 4 Gy in 1 fraction on day −1. Cyclosporine and mycophenolate mofetil 15 mg/kg twice daily were administered. On day 51 of the initial transplantation in March 2003, human leukocyte antigen–loci mismatched (2 by serology and 3 by DNA typing) male cord blood, containing 2.6×10^7/kg nucleared cells, was infused. Neutrophil engraftment was observed by day 33 after second transplantation. Acute and chronic graft-versus-host disease did not develop, and cyclosporine was tapered off in November 2003.

Figure 1 T-Cell Large Granular Lymphocyte Leukemia Stained with May-Giemsa on the Peripheral Blood Smear



The predominant cells were typical of LGLs with abundant cytoplasm and fine or coarse azurophilic granules.
Hematoxylin and eosin stain; original magnification ×1000.

Figure 2 Southern Blots of T-Cell Receptor β-Chain Gene Rearrangements



DNA from BM of this patient was hybridized with a TCR Cβ1 probe.
Arrows indicate rearranged bands.
Abbreviations: B = Bam H1; E = Eco R; H = Hind III

In February 2004, 10 months after the second cord blood transplantation, he developed anorexia, abdominal distention with fluid accumulation, and edema in the lower extremities. A computed tomography scan showed gross ascites and mild pleural effusion but no sign of enlarged lymph nodes or hepatosplenomegaly. The peripheral white blood cell count was 10,300/μL (10.3 × 10^9/L), and 30% of the cells had a morphology of medium to large lymphocytes with abundant azurophilic granules in the cytoplasm, as shown in Figure 1. The hemoglobin level was 8.8 g/dL (88 g/L), and the platelet count was 192 × 10^3/μL (1.92 × 10^9/L).

A retrospective review of the peripheral blood smears disclosed that the appearance of LGL coincided with the tapering off of immunosuppression 3 months before the admission.

Flow cytometry examination of the peripheral blood mononuclear cells showed a homogeneous population of T-cell LGLs positive for CD2, CD3, CD8, CD56, and T-cell receptor (TCR)–γδ, but negative for CD4 and TCR-αβ. The BM biopsy specimen histologically showed 10% of hypocellular gelatinous marrow with diffuse infiltration of medium to large lymphoid cells. Immunoperoxidase studies on sections of BM showed strong expression of T-cell–restricted intracellular antigen-1, partially positive staining of CD8 and granzyme B, but no expression of CD3 or CD20. Southern blot analysis of the BM cells revealed a clonal rearrangement of the TCR-β chain, as shown in Figure 2 and TCR-δ chain (data not shown).

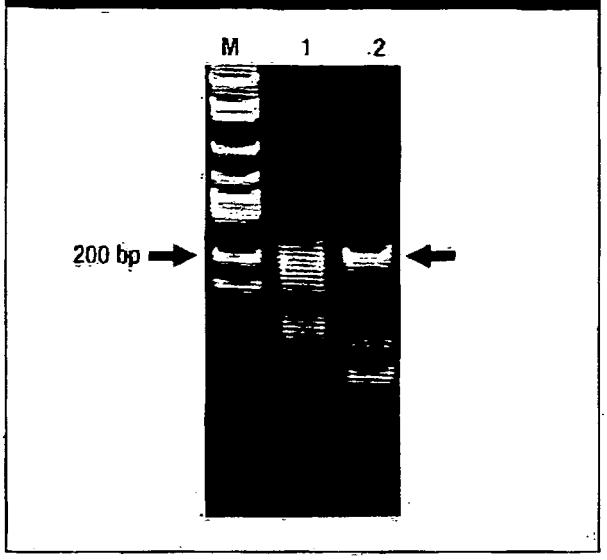
Abdominal paracentesis was performed with milky chylous fluid, and a flow cytometry examination showed results similar to those in the peripheral blood. Multiprimer-based polymerase chain reaction (PCR) analysis of ascitic cells also showed clonal rearrangement of the TCR-δ chain, as shown in Figure 3. The primer sets were used in the following locations: Vδ1, 5'-AAA GTG GTC GCT ATT CTG TC-3'; Vδ2A, 5'-GCA CCA TCA GAG AGA GAT GA-3'; Jδ, 5'- TGG TTC CAC AGT CAC ACG GG-3'; Dδ3B, 5'-TTG TAC CAC CGT GCG TAT CC-3'. The amplified 200 base-pair PCR products of the TCR-δ chain were then cloned into the pCR-TOPO vector. The DNA sequences of 3 clones amplified by vectors were identical and had high homology to TCR-δ chain including a 197 base-pair sequence (data not shown). This sequence also involved the forward and reverse primers Vδ1 and Jδ, respectively, described previously.

The results of all of the previously mentioned studies indicated the clonal expansion of T cells compatible with a diagnosis of T-cell LGLL with γδ T-cell phenotype involving peripheral blood, BM, and ascites.

Donor-recipient DNA chimerism was analyzed by comparing the short tandem repeat findings for the donor blood sample and pretransplantation recipient samples. Eleven short tandem repeat loci were analyzed by PCR using an AmpFlSTR SGM Plus® kit. The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539), as shown in Figure 4. These results further confirmed that the expanded γδ T-LGL cells were exclusively of second cord blood transplantation donor origin.

Serologic examination showed no evidence of viral infection. Real-time PCR analysis revealed a high load of EBV (7.9 × 10^3 copies/10^6 cells). However, in situ hybridization studies of BM cells did not reveal EBV-encoded small RNA, and Southern blot analysis of BM cells also showed no band for

Figure 3 Polymerase Chain Reaction for T-Cell Receptor δ Gene Rearrangement



(1) Negative control; and (2) patient's sample of frozen neoplastic lymphoid cells in ascites. A clonal band was identified at approximately 200 base pairs.
Abbreviations: bp = base pairs; M = molecular weight marker

clonal EBV genomes. Chromosome analysis demonstrated a normal 46, XY karyotype in all 20 cells examined.

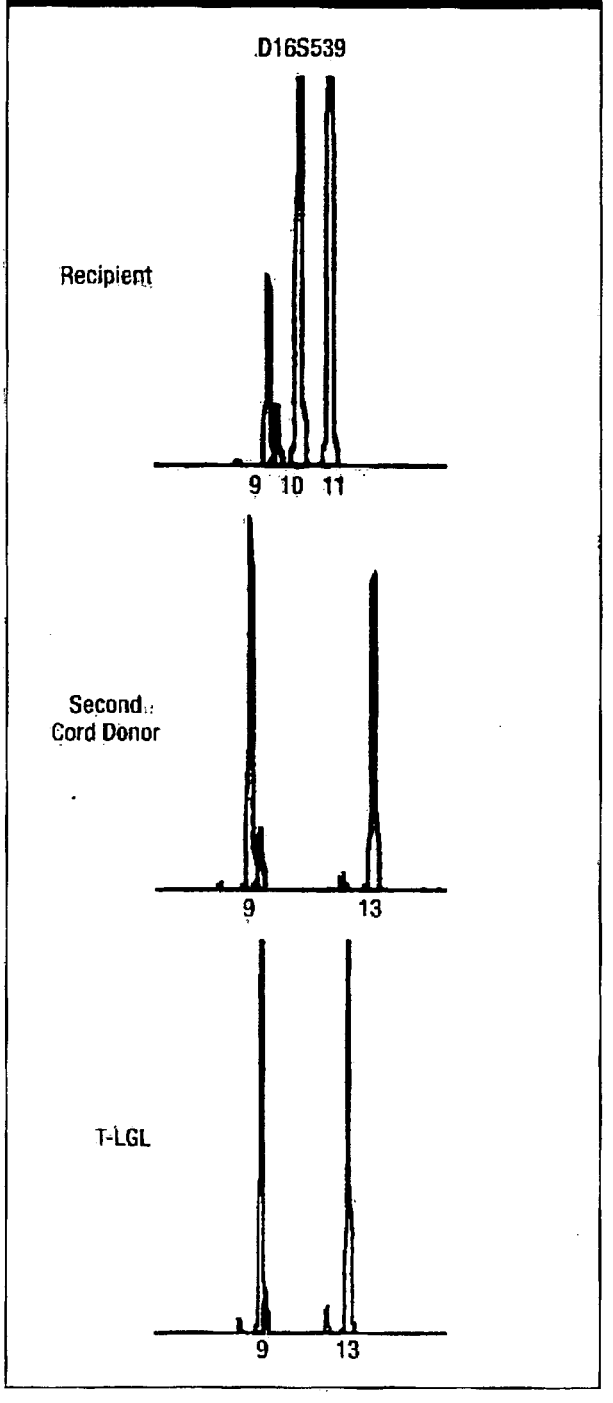
After admission, his abdominal distention and dyspnea with hypoxemia progressed rapidly with spiking fever. A computed tomography scan demonstrated acute respiratory distress syndrome. Because we found no evidence of bacterial or fungal infection or drug-induced pneumonia, cyclosporine and methylprednisolone were started immediately but with no effect, and he died of acute respiratory failure 1 week later. A postmortem lung biopsy showed extensive diffuse alveolar damage without the T-LGL cell's involvement; on the other hand, the leukemic cell involvement in Glisson's sheath was shown by a liver biopsy.

Discussion

In this case, the increase in LGLs developed 7 months after the second cord blood transplantation, and the kinetics of LGLs correlated with the tapering off of immunosuppression, which suggested the possibility that lymphocytosis might have been associated with reactive expansion because of viral infection or an alloimmune reaction. However, our case showed *TCR-β* and *TCR-δ* gene rearrangement by Southern blot analysis and *TCR-δ* gene rearrangement by PCR and cytotoxic T-cell immunophenotype, which were comparible with T-cell LGLL.

Most cases of PTLD, usually of B-cell origin, are associated with EBV infection and represent the EBV-induced monoclonal expansion of B cells in conditions with decreased T-cell immune surveillance.[5,6] Although there have been some reports of EBV-associated PTLD after cord blood transplantation,[7-10] the incidence of PTLD of T-cell origin has been reported to be only 4%-14% with a less frequent association with EBV.[6,11]

Figure 4 Donor-Recipient DNA Chimerism Analysis by Comparing the Short Tandem Repeat



The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539).

In our case, because a high viral load of EBV was detected by real-time PCR analysis, we initially speculated that γδ T-LGLL was EBV-associated PTLD, but this was later denied based on the results of EBV-encoded small RNA in situ

Table 1A Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation[7,13-16]

Study	Case Number	Age/Sex	Donor	Diagnosis	Origin	Involved Organ
Zutter et al[13]	1	14/Male	Sibling*	Lymphoblastic lymphoma	Recipient	Lymph node, BM
Zutter et al[13]	2	9/Male	Sibling*	Lymphoblastic lymphoma	ND	Pericardium, pleura
Zutter et al[13]	3	2/Female	Father	NHL (polymorphic)	Donor	Lung, liver, spleen
Wang et al[14]	4	13/Male	Sibling*	NHL (diffuse large)	Recipient	Lymph node
Sirvent et al[7]	5	ND/ND	ND	LGL ($\alpha\beta$)	ND	PB, BM
Collins et al[15]	6	11/Male	ND	NHL (polymorphic)	ND	Lymph node, brain
Au et al[16]	7	39/Male	Unrelated	LGL	Donor	PB, BM
Our Case	8	58/Male	UCB	LGL ($\gamma\delta$)	Donor	PB, BM, ascites, liver

*Human leukocyte antigen–matched sibling.
Abbreviations: ND = not determined; NHL = non-Hodgkin lymphoma; PB = peripheral blood; UCB = unrelated cord blood

Table 1B Literature Review of T-Cell Posttransplantation Lymphoproliferative Disorder After Hematopoietic Stem Cell Transplantation[7,13-16]

Study	Case Number	Time to PTLD* (Days)	EBER-ISH	Rearrangement	Survival† (Days)
Zutter et al[13]	1	1290	Not determined	TCR-γ (SB)	851
Zutter et al[13]	2	630	Not determined	Not determined	180
Zutter et al[13]	3	39	Not determined	Polyclonal	11
Wang et al[14]	4	601	Negative	TCR-γ (PCR)	> 1170
Sirvent et al[7]	5	300	Negative	TCR-β (SB)	≥ 690
Collins et al[15]	6	90	Negative	Not determined	29
Au et al[16]	7	180	Negative	TCR-γ (PCR)	134
Our Case	8	330	Negative	TCR-β (SB), TCR-δ (SB, PCR)	30

*Time from transplantation to PTLD.
†Survival time from diagnosis of PTLD.
Abbreviations: EBER-ISH = EBV-encoded small RNA in-situ hybridization; SB = Southern blotting

hybridization stains and Southern blot EBV terminal repeat analysis. Therefore, the clinical significance of EBV infection in this case remains undetermined.

Most previously reported cases of T-cell PTLD developed after solid organ transplantation,[12] and there have been only 7 previously documented cases of T-cell PTLD after allogeneic HSCT, as summarized in Table 1.[7,13-16] Posttransplantation lymphoproliferative disorder was of donor origin in 3 of 8 total cases, including our case, of recipient origin in 2, and of undetermined origin in the remaining 3. No correlation has been demonstrated between EBV and T-cell PTLD after HSCT.

Generally, most cases of B-cell posttransplantation lymphoproliferative disorder after HSCT develop within the first 5 months, because the balance between proliferating EBV-infected B cells and cytotoxic T cells cannot be controlled with the unrecovered lymphocyte components.[17] In solid organ transplantation, EBV-positive cases tend to occur earlier than EBV-negative cases, ie, a median interval of 6-10 months compared with 4-5 years.[6,7] Some cases of T-cell PTLD have a longer interval between the day of transplantation and the occurrence of PTLD than in B-cell PTLD. The donor source of transplantation included sibling (3 cases), father (1 case), unrelated (1 case), cord (our case), and not described (2 cases). Therefore, whereas there has been very little experience with cases after cord blood transplantation, all 8 cases of PTLD in the literature are of B-cell origin.[8-11] Our case is the first report of PTLD of T-cell origin after cord blood transplantation and might reflect very intense immunosuppression passing through consecutive cord blood transplantation.

It has been reported that T-cell PTLD has a worse prognosis than B-cell PTLD in a solid organ transplantation setting. In 1 series of 6 cases presenting with T-cell non-Hodgkin lymphoma as PTLD, pulmonary involvement was reported in 5 cases and marrow infiltration in 4 cases. All patients showed aggressive courses.[18] Of importance is that of 8 patients with T-cell PTLD after HSCT: 3 patients who died within 30 days had extranodal involvement in the lung, liver, spleen, brain, and/or ascites.

Conclusion

We have reported an unusual case of EBV-negative, T-cell PTLD as γδ T-cell LGL of donor origin after a second cord blood transplantation. The occurrence of T-cell PTLD after HSCT is extremely rare, and the efficient accumulation of knowledge and further research are needed to establish the oncogenic mechanism and appropriate therapeutic maneuvers in this disease entity.

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Hyperglycemia During the Neutropenic Period Is Associated With a Poor Outcome in Patients Undergoing Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation

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Background. Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) frequently require support with parenteral nutrition and immunosuppressive drugs, which introduce the risk of hyperglycemia. Van den Berghe et al. showed that the strict glucose control improved the outcome of patients treated in the intensive care unit, and this point was evaluated in this study in a HSCT setting.

Methods. A cohort of 112 consecutive adult patients treated by myeloablative allogeneic HSCT between January 2002 and June 2006 was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, preexisting infectious diseases, preexisting neutropenia or previous allogeneic HSCT. The remaining 91 patients were categorized according to mean fasting blood glucose (BG) level in the neutropenic period after conditioning: normoglycemia (BG <110 mg/dL, n=28), mild hyperglycemia (110 to 150 mg/dL, n=49), and moderate/severe (>150 mg/dL, n=14). The primary endpoint was the occurrence of febrile neutropenia (FN) and documented infection during neutropenia, and the secondary endpoints included organ dysfunction according to the definition used by van den Berghe, acute graft-versus-host disease (GVHD), overall survival, and nonrelapse mortality (NRM).

Results. Although the incidence of FN or documented infections was similar between the three groups, hyperglycemia was significantly associated with an increased risk of organ dysfunction, grade II–IV acute GVHD, and NRM.

Conclusions. While the results suggested an association between the degree of hyperglycemia during neutropenia and an increased risk of posttransplant complications and NRM, the possibility that intensive glucose control improves the outcome after HSCT can only be confirmed in a prospective randomized trial.

Keywords: Allogeneic transplantation, Hyperglycemia, Nonrelapse mortality, Acute graft-versus-host disease.

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Van den Berghe et al. showed with patients nursed in the intensive care unit (ICU) that the rigid control of hyperglycemia with intensive insulin therapy to keep the blood glucose level at 80–110 mg/dL reduced morbidity, including infections, and mortality compared to patients who received standard care maneuvers that maintained the level at <200 mg/dL (*1–3*). Although these results have been confirmed in several subsequent studies (*4–7*), the precise mechanism that underlies this association is unclear. In animal models, it has been shown that insulin itself has a direct inhibitory effect on the inflammation process (*8, 9*). However in human studies, it has been suggested that these benefits could be directly attributed to intense glucose control rather than to any pharmacological activity of administered insulin per se (*3, 4*).

Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) suffer from serious complications including infection, graft-versus-host disease (GVHD) and organ dysfunction. They are also at higher risk of hyperglycemia due to the use of steroids for the treatment of graft-versus-host disease (GVHD), prolonged total parenteral nutrition (TPN), immunosuppressive drugs, and infectious complications (*10, 11*). This makes them susceptible to numerous serious complications, including multiple organ failure (*12–14*). In this study, we evaluated whether hyperglycemia during the cytopenic pe-

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riod after conditioning for HSCT could be a significant risk factor for the subsequent clinical course.

PATIENTS AND METHODS

Patient Characteristics

A cohort of 112 consecutive adult patients who received myeloablative allogeneic HSCT between January 2002 and June 2006 at the National Cancer Center Hospital (Tokyo, Japan) was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, pre-existing infectious diseases or neutropenia before HSCT, and previous allogeneic HSCT. The remaining 91 patients were subjected to further analysis, and their characteristics are listed in Table 1. Their median age was 36 years (range, 18–57 years), and their diagnosis included acute myeloid leukemia (AML, n=41), acute lymphoblastic leukemia (ALL, n=21), non-Hodgkin lymphoma (NHL, n=13), myelodysplastic syndrome (MDS, n=10), and chronic myelogenous leukemia (n=6). Standard-risk patients included those with acute leukemia in first complete remission, chronic leukemia in first chronic phase, MDS in refractory anemia, and NHL in complete remission, and the remaining patients were categorized as high-risk. Forty-six and 45 patients received a graft from a related donor and an unrelated donor, respectively. Stem cell sources included bone marrow (n=46), peripheral blood (n=41), and cord blood cells (n=4). In this study, only two patients were diagnosed as type 2 diabetes mellitus before HSCT, which reflects the low prevalence of this condition in Japan, especially in younger patients who can be the target of allogeneic HSCT with a myeloablative conditioning regimen. These two diabetic patients were included in the moderate and severe hyperglycemia group. None of the patients, including these two patients, had major organ dysfunction or diabetic complications before HSCT. For the transplantation procedure, signed informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

All patients received a myeloablative conditioning regimen that included oral busulfan (BU) plus cyclophosphamide (CY, n=45), CY plus 12 Gy total body irradiation (TBI, n=43) or cytarabine (CA) plus CY plus TBI (n=3: Table 1). GVHD prophylaxis included cyclosporine- (n=62) and tacrolimus-based regimens (n=29), with an additional short course of methotrexate (MTX) in 89 patients. Granulocyte

TABLE 1. Patient characteristics

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Blood glucose, median mg/dl (range)	104 (81–109)	120 (110–150)	168 (150–211)
Age, median years (range)	31 (21–52)	36 (18–57)	45 (30–57)
<40	20 (71)	32 (65)	4 (29)
≥40	8 (29)	17 (35)	10 (71)
Sex			
Male	9 (32)	34 (69)	8 (57)
Female	19 (68)	15 (31)	6 (43)
Disease risk			
Standard	16 (57)	18 (37)	6 (43)
High	12 (43)	31 (63)	8 (57)
Conditioning			
TBI-containing	11 (39)	26 (53)	9 (64)
Non-TBI-containing	17 (61)	23 (47)	5 (36)
GVHD prophylaxis			
Cyclosporine-based	24 (86)	33 (67)	5 (36)
Tacrolimus-based	4 (14)	16 (33)	9 (74)
Relation to donor			
Related	19 (68)	24 (49)	3 (21)
Unrelated	9 (32)	25 (51)	11 (79)
Stem cell source			
Bone marrow	11 (39)	24 (49)	11 (79)
PBSC	16 (57)	22 (45)	3 (21)
Cord blood	1 (4)	3 (6)	0 (0)
HLA match			
Match	25 (89)	34 (69)	10 (71)
Mismatch	3 (11)	15 (31)	4 (29)

Data are n (%) unless noted.
TBI, total body irradiation; GVHD, graft-versus-host disease; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

colony-stimulating factor (G-CSF) was administered in all patients from day +6 after transplantation until engraftment. Most patients received ciprofloxacin (200 mg orally three times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and varicella zoster virus until the cessation of immunosuppressive agents. Prophylaxis against *Pneumocystis jiroveci* infection consisted of trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the cessation of immunosuppressive agents. Patients who developed fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin, aminoglycosides and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5×10^9/L.

Grouping of Patients

Patients were categorized according to the mean blood glucose (BG) level in the preengraftment neutropenic period: normoglycemia BG maintained at <110 mg/dL (group 1, n=28), mild hyperglycemia at 110–150 mg/dL (group 2, n=49), and moderate/severe hyperglycemia at >150 mg/dL (group 3, n=14). Blood glucose level was routinely tested in the morning at least three times a week. Daily caloric intake was calculated by dietitian following the chart record.

Outcome Measures

The primary outcome measure was the occurrence of febrile neutropenia (FN) and documented infection including bacteremia, pneumonia and central venous catheter infection in the neutropenic period. Secondary outcome measurements were organ dysfunction in the neutropenic period, acute GVHD, overall survival (OS) and nonrelapse mortality (NRM). Organ dysfunction was defined with reference to van den Berghe (*5–7*) as follows: 1) hypercreatininemia: serum creatinine level ≥2.0 mg/dL or more than twice the baseline; 2) hyperbilirubinemia: serum total bilirubin level ≥2.0 mg/dL; and 3) increased inflammatory markers: serum C-reactive protein (CRP) level ≥15 mg/dL. Acute GVHD was graded by the Consensus Criteria (*15*).

Statistical Analyses

Standard descriptive statistics were used. The Student's *t*-test, chi-square, and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. Multiple logistic regression analysis was conducted to ascertain odds ratios (ORs) and 95% confidence intervals (CIs). OS was estimated using Kaplan-Meier curves. The cumulative incidences of NRM were estimated based on a Cox regression model for the cause-specific hazards by treating progressive disease or relapse as a competing event. Cox proportional hazard models were used for multivariate analysis of variables on NRM and OS after HCT. Clinical factors that were assessed for their association with NRM and OS included patient age, sex, conditioning regimen (TBI-based vs. non-TBI-based), donor [human leukocyte antigen (HLA)-matched vs. HLA-mismatched, related vs. unrelated], GVHD prophylaxis (cyclosporine-based vs. tacrolimus-based) and disease risk (standard vs. high). Factors with $P<0.10$ in the univariate analyses were subjected to a multivariate analysis. A level of $P<0.05$ was defined as statistically significant. All P values are two-sided. All analyses were performed using SPSS 10.0 statistical software (Chicago, IL).

RESULTS

Patients and Transplantation Characteristics

The median ages of the patients in the normoglycemia, mild hyperglycemia, and moderate/severe hyperglycemia groups were, respectively, 31, 36, and 45 years. The percentages of patients who received graft from an unrelated donor were 32%, 51%, and 79%, and the percentages of patients who received GVHD prophylaxis with tacrolimus were 14%, 33%, and 74%. To clarify the risk factor to be included in moderate and severe hyperglycemia group, logistic analysis was performed, which showed older age and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.04$, OR 3.9 (1.1–14.0), and $P=0.01$, OR 5.5 (1.5–20.3), respectively], and there was a trend that patients who received stem cell from unrelated donor were associated with moderate and severe hyperglycemia [$P=0.07$, OR 3.6 (0.9–14.2)]. Multiple logistic analysis showed age more than 40 years old and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.042$, OR 4.1 (1.1–15.7), and $P=0.01$, OR 5.8 (1.5–22.1), respectively].

Although in practice we generally keep the parenteral glucose dose relatively low to avoid severe metabolic complications including hyperglycemia and hyperlipidemia during the acute phase of allogeneic HSCT, the possibility that the dose of parenteral nutrition affects the blood glucose level should be explored. We calculated the total caloric intake by combining both oral and parenteral nutrition. Although the mild hyperglycemia group received significantly more parenteral nutrition than the normoglycemia group (group 1 694+322 kcal/day vs. group 2 969+383 kcal/day), overall there was no essential difference in caloric intake between the three groups (1070+303 kcal/day, 1190+393 kcal/day, 1045+530 kcal/day, respectively). The median duration of the follow-up time in surviving patients was 809 days (range, 132-1530 days) in group 1, 369 days (105–1550 days) in group 2, and 587 days (170–774 days) in group 3. Described as hydrocortisone-equivalent dose, the median dose of corticosteroid used during neutropenia was 0 mg (0–1610 mg) in group 1, 100 mg (0–9700 mg) in group 2, and 375 mg (0–2468 mg) in group 3. Statistically more dose of corticosteroid was used in group 2 and group 3, compared with group 1.

Primary Endpoints

The incidence of FN and documented infections is summarized in Table 2. The incidences of FN and documented infections including bacteremia, pneumonia, and central venous catheter infection in groups 1, 2 and 3 were, respectively, 89% and 32% (25%, 4% and 11%), 88% and 20% (16%, 6% and 6%), and 98% and 43% (36%, 14% and 14%). Overall, no statistically significant difference was observed between the three groups in the incidence of infectious episodes, including FN and documented infections.

TABLE 2. Endpoints

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Febrile neutropenia	23 (89)	43 (88)	13 (98)
Documented infection	9 (32)	10 (20)	6 (43)
Bacteremia	7 (25)	8 (16)	5 (36)
Pneumonia	1 (4)	3 (6)	2 (14)
Central-venous catheter infection	3 (11)	3 (6)	2 (14)
Organ dysfunction			
Hypercreatininemia	1 (4)	4 (8)	4 (29)
Hyperbilirubinemia	3 (11)	11 (22)	6 (43)
Increased inflammatory markers	4 (14)	15 (31)	9 (64)

Data are n (%).
Hypercreatininemia, serum creatinine level ≥2.0 mg/dl or more than twice of baseline; hyperbilirubinemia, serum bilirubin level ≥2.0 mg/dl; increased inflammatory markers, serum C-reactive protein level ≥15 mg/dl.

Secondary Endpoints

The incidence of hypercreatininemia was 4% in group 1, 8% in group 2 and 29% in group 3, as summarized in Table 2, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 1.1–108.6; $P=0.018$) and group 2 (OR 4.5, 95% CI 1.0–21.1; $P=0.043$). The incidence of hyperbilirubinemia was, respectively, 11%, 22% and 43%, in the three groups, and that in group 3 was significantly higher than that in group 1 (OR 6.3, 95% CI 1.3–30.9; $P=0.017$). The incidence of increased inflammatory markers was, respectively, 14%, 31% and 64%, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 2.4–49.5; $P<0.001$) and group 2 (OR 4.1, 95% CI 1.2–14.3; $P=0.022$). Multiple logistic regression analysis showed that the degree of hyperglycemia was associated with hypercreatininemia, hyperbilirubinemia, and increased inflammatory markers (Table 3).

TABLE 3. Multiple logistic regression analysis for organ dysfunction and multiple variate analysis for acute GVHD, nonrelapse mortality, and overall survival

Outcomes and variables	Odds/hazard ratio	95% CI	P value
Multiple logistic regression analysis			
Hypercreatininemia			
Hyperglycemia	5.2	1.1–24.6	0.039
Hyperbilirubinemia			
Hyperglycemia	4.9	1.6–14.9	0.005
Increased inflammatory markers			
Hyperglycemia	6.7	2.2–20.3	0.001
Tacrolimus-based	6.9	1.6–30.5	0.011
Multivariate analysis (Cox-proportional hazard model)			
Acute GVHD			
Hyperglycemia	2.3	1.2–4.3	0.013
Disease risk (high)	2.3	1.0–5.1	0.047
HLA mismatch	2.8	1.3–5.9	0.009
Nonrelapse mortality			
Hyperglycemia	2.9	1.2–6.6	0.013
Disease risk (high)	2.7	0.9–8.7	0.091
Overall survival			
Hyperglycemia	2.0	1.1–3.6	0.019
TBI-containing	2.3	1.1–5.0	0.035
Disease risk (high)	1.9	0.9–4.1	0.10

Odds ratios are presented for multiple logistic regression analysis; hazard ratios are presented for multivariate analysis.
GVHD, graft versus host disease; TBI, total body irradiation.

The cumulative incidence of grade II–IV acute GVHD is shown in Figure 1. The degree of hyperglycemia was associated with a higher incidence of grade II–IV acute GVHD

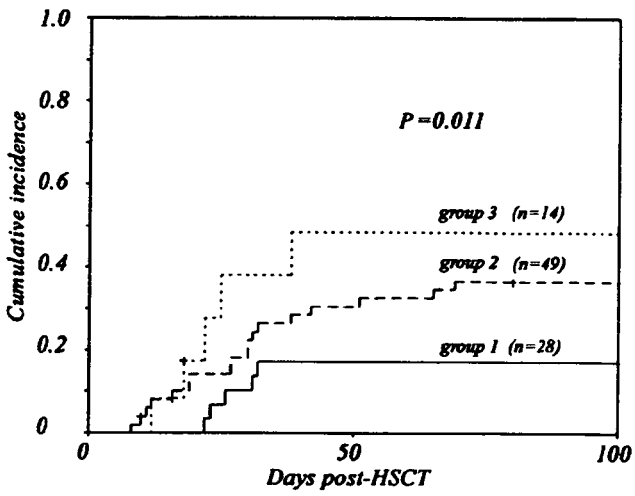

FIGURE 1. Cumulative incidence of acute GVHD grade II–IV stratified according to the mean glucose level during neutropenia. Group 1 included patients with normoglycemia, group 2 included patients with mild hyperglycemia, and group 3 included patients with moderate and severe hyperglycemia.

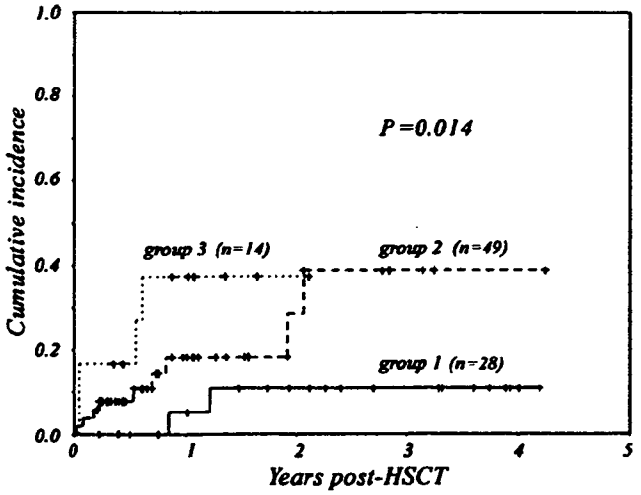

FIGURE 2. Cumulative incidence of treatment-related mortality stratified according to the mean glucose level during neutropenia.

(P=0.002). A Cox proportional hazard model showed that hyperglycemia, high-risk underlying disease, and HLA mismatch were risk factors for grade II-IV acute GVHD (Table 3).

The cumulative incidence of NRM was, respectively, 5%, 17%, and 35% at 1 year, and was significantly related to the degree of hyperglycemia (P=0.014; Fig. 2). The probability of OS was, respectively, 88%, 70%, and 56%, and was significantly associated with hyperglycemia (P=0.008; Fig. 3). A Cox proportional hazard model showed that the degree of hyperglycemia was associated with NRM and OS (Table 3).

DISCUSSION

In this study, we evaluated whether hyperglycemia during the cytopenic period after conditioning for HSCT could be a significant risk factor for the subsequent clinical course. Infectious diseases remain a major cause of morbidity and mortality in patients who receive HSCT, and we speculated that this might be exaggerated in the presence of hyperglycemia. Alternatively, hyperglycemia can be caused by infectious diseases and also aggravates infectious diseases to lead to a vicious cycle, with resultant morbidities that include organ dysfunction and mortality. Theoretically, strict glucose control should prevent this vicious cycle and help to reduce morbidity and mortality in patients after HSCT, as shown previously in ICU settings (*1*, *2*). However, in this study the incidences of FN and documented infections were not different among the three groups. On the other hand, we found that hyperglycemia was associated with organ dysfunction and increased inflammatory markers, which was consistent with previous reports that demonstrated the impact of hyperglycemia on clinical outcomes of patients suffering from nonhematological diseases (*1–3*, *12–14*). Additionally, a multivariate analysis showed that hyperglycemia was a risk factor for acute GVHD.

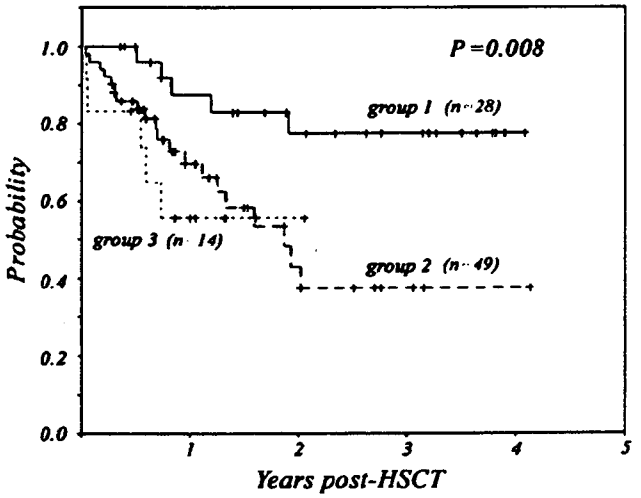

FIGURE 3. Overall survival stratified according to the mean glucose level during neutropenia.

The reason for the association between early hyperglycemia and late complications needs to be clarified. The increase in the levels of circulating cytokines due to hyperglycemia may further aggravate hyperglycemia itself (*16–21*). Therefore, this condition which occurs during the critical period of neutropenia before engraftment may influence the afferent phase of acute GVHD, as suggested by Ferrara et al. Elevated cytokine levels during the afferent phase then lead to subsequent acute GVHD in the effector phase (*22*, *23*). Teshima et al. reported that the effector phase of acute GVHD is not antigen-specific and inflammatory cytokines mediate target destruction (*24*), and other reports have shown that inflammatory cytokines were required in acute GVHD and these molecules can cause tissue damage (*25–27*). With these reports in mind, it is reasonable to speculate that the aggravated production of inflammatory cytokines by hyperglycemia may be a risk factor in the pathogenesis of acute GVHD and organ dysfunction.

This study has several limitations, including heterogeneous patient populations and a retrospective nature. First, hyperglycemia can be caused by infection itself and it has been previously shown that the level of hyperglycemia was correlated with the severity of illness (*4*). In this retrospective study, we could not confirm whether hyperglycemia directly influenced organ dysfunction or increased inflammatory markers. Furthermore, statistically more corticosteroid was used in the group of moderate and severe hyperglycemia, and statistically more parenteral nutrition was used in the group of mild hyperglycemia. However, the observation that hyperglycemia and the severity of illness were independently associated with a worse prognosis has been well confirmed in the ICU setting (*4*), and several prospective studies have shown that intensive glucose control reduced both morbidity and mortality (*1*, *2*). Considering these findings, we suggest that our data still support the possibility that the degree of hyperglycemia was associated with morbidity and mortality in the allogeneic HSCT setting. Second, we must consider that the patients who developed moderate and severe hyperglycemia included older patients, those who received more unrelated grafts, and those who received tacrolimus compared to other groups. In terms of immunosuppressive drugs, tacrolimus has recently become a preferred immunosuppressive drug for GVHD prophylaxis in unrelated or HLA-mismatched HSCT, based on the results of two Japanese studies, which showed that, compared to cyclosporine, tacrolimus was associated with a lower incidence of acute GVHD and better overall survival, which were similar to those in related HSCT, even

after HSCT with alternative donors, including unrelated donors (*28, 29*). Therefore, the effect of unrelated graft and tacrolimus on the incidence of acute GVHD and NRM might not be significant in this study.

The effects of tacrolimus on hyperglycemia, hyperbilirubinemia, and hypercreatininemia need to be clarified. It is well known that hyperglycemia occurs more often in patients receiving tacrolimus than in those receiving cyclosporine (*30–32*). In the present study, patients receiving tacrolimus were more likely to have moderate to severe hyperglycemia. However, the association of hyperbilirubinemia with tacrolimus has not been previously reported and two other studies (*33, 34*) showed that cyclosporine was more likely to cause hyperbilirubinemia than tacrolimus after allogeneic HSCT or kidney transplantation. Although the relative nephrotoxicity attributed to tacrolimus compared to cyclosporine has been controversial (*30, 33, 35*), studies that have reported such nephrotoxicity used a higher target tacrolimus level (>20 ng/ml) (*30, 35*). On the other hand, it has been reported that the use of lower levels of tacrolimus (10–15 ng/ml in our hospital) was associated with reduced complications in allogeneic HSCT (*36, 37*), with no difference in the incidence of hypercreatininemia compared to cyclosporine (*33*). Based on a consideration of all of these results, we think that tacrolimus might not be the direct cause of hypercreatininemia in this study. Finally, due to the nature of this retrospective study, during the period evaluated we did not apply any consistent protocol for glucose control and nutritional support, although we tried to avoid severe hyperglycemia (BG ≥200 mg/dl), which certainly biases the interpretation of the data, although it has been reported that the overall glucose level, rather than the dose of insulin administered, directly influenced the outcome of patients (*3*).

Even with these limitations, we believe that our observation is still of value in considering the clinical impact of the strict control of hyperglycemia during the early phase of HSCT. To confirm our preliminary observation, a prospective pilot study is underway to assess the effect of intensive glucose control after HSCT. If this pilot study shows a beneficial effect of intensive glucose control, a prospective randomized trial would be warranted to confirm the possibility that intensive glucose control improves the outcome after HSCT. Additionally, in this ongoing pilot study, we evaluate the diurnal blood glucose and insulin levels, including postprandial levels, to detect hyperglycemia more precisely before transplantation since the level of HgA1c is affected by both the blood glucose level and the turnover rate of red blood cells, and would not precisely correlate with the true mean blood glucose level in patients who received courses of blood transfusion for anemia.

In conclusion, the association of the degree of hyperglycemia during neutropenia and an increased risk of post-transplant complications and NRM was suggested, but the possibility that intensive glucose control improves the outcome after HSCT would only be confirmed in a prospective randomized trial.

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