Table 2 Clinical characteristics and outcome of the patients

Patient no.	Sex/ age	Immuno-phenotype	WBC ($\times 10^9$/L)	Ph chromosome	MRD markers	Transplantation	MRD day 100	Outcome	
1	M/28	B	16.6	No	IgH	No	Positive	REL d195	D d218
2	F/59	B	13	Yes	IgH, $p190^{BCR/ABL}$	No	Positive	REL d245	D d617
3	M/53	B	1.7	Yes	IgH, $p210^{BCR/ABL}$	No	*	REL d159	D d280
4	M/50	B	12.2	No	TCRδ	Yes	Negative	REL d494	D d518
5	M/77	B	16.8	Yes	IgH, $p190^{BCR/ABL}$	No	Positive	REL d807	D d1003
6	F/25	T	38	No	TCRγ	Yes	Negative	CR d2040	A d2040
7	F/47	B	3.7	Yes	IgH, $p190^{BCR/ABL}$	Yes	Negative	CR d1989	A d1989
8	M57	B	23	No	IgH	No	Positive	REL d141	D d502
9	F/37	B	3	Yes	TCRδ, $p190^{BCR/ABL}$	Yes	Negative	CR d769	A d769
10	F/60	B	3	No	TCRδ	No	Positive	REL d995	A d1892
11	F/63	B	3272.5	Yes	$p210^{BCR/ABL}$	No	Positive	REL d49	D d309
12	F/65	B	141.1	Yes	TCRδ, γ, $p190^{BCR/ABL}$	No	Positive	REL d47	D d320
13	M/52	B	10.4	Yes	$p210^{BCR/ABL}$	No	Negative	CR d483	A d483
14	M/23	T	8.4	No	TCRδ, γ	Yes	Negative	CR d1604	A d1604
15	M/21	T	10	No	TCRγ	Yes	Positive	REL d280	D d385
16	F/60	B	2	No	IgH	No	Negative	CR d1463	A d1463
17	F/31	B	6	No	IgH	Yes	Negative	REL d382	D d501
18	M/15	B	6.3	No	IgH	Yes	Negative	CR d1339	A d1339
19	M/62	B	2.2	No	IgH	No	Positive	REL d889	D d1138
20	F/19	T	3.3	No	TCRδ, γ	Yes	Positive	CR d1169	A d1169
21	F/79	B	*	No	IgH	No	Positive	REL d346	D d360
22	F/19	B	155.2	No	IgH	Yes	Negative	CR d469	D d469
23	M/72	B	3.8	Yes	$p190^{BCR/ABL}$	No	Negative	CR d948	A d948
24	M/46	B	*	Yes	$p190^{BCR/ABL}$	Yes	*	REL d399	A d964
25	F/50	B	4	Yes	$p190^{BCR/ABL}$	Yes	Negative	CR d840	A d840
26	M/33	B	230	Yes	$p190^{BCR/ABL}$	No	Negative	CR d720	A d720
27	M/72	B	*	Yes	$p190^{BCR/ABL}$	No	–	CR d49	D d49
28	F/26	B	24.9	Yes	$p190^{BCR/ABL}$	Yes	*	CR d738	A d738
29	F/68	B	*	Yes	$p190^{BCR/ABL}$, $p210^{BCR/ABL}$	No	*	REL*	D d352
30	M/25	B	11.3	No	IgH	Yes	Negative	CR d640	A d640
31	F/26	B	8.6	No	IgH	Yes	*	REL d158	D d354
32	F/31	B	24.1	Yes	$p190^{BCR/ABL}$, $p210^{BCR/ABL}$,	Yes	Negative	REL d262	A d325
33	M/28	B	16.8	Yes	$p190^{BCR/ABL}$	No	Negative	CR d120	A d120
34	M/77	B	36.7	Yes	$p190^{BCR/ABL}$	No	Positive	CR d125	A d125

Ph Philadelphia, *REL* relapse, *D* dead, *A* alive

* The data were unknown

2.7 Statistical analysis

Survival curves were plotted according to the method of Kaplan and Meier, and comparison of the curves was made using log-rank tests. Overall survival (OS) was measured from the date of induction therapy until death. Relapse-free survival (RFS) was measured from the date of induction therapy until the date of relapse or death, whichever occurred first. Univariate and multivariate analyses were performed to evaluate the independent prognostic factors for OS and RFS by a Cox regression model.

3 Results

3.1 Identification of MRD-PCR targets

Eighteen patients were Ph chromosome positive and their MRD was detected by BCR/ABL fusion transcripts. Thirty IGH/TCR gene rearrangements were found at diagnosis in the 22 ALL patients: 19 IGH (two different kinds in five patients), 6 TCRδ, and 5 TCRγ gene rearrangements. Eight patients had two targets and 14 patients had one target (Table 3). MRD was analyzed by both

Table 3 MRD studies

Total	34
BCR/ABL transcripts	12
$p190^{BCR/ABL}$	8
$p210^{BCR/ABL}$	2
$p190^{BCR/ABL}$ and $p210^{BCR/ABL}$	2
IGH, TCRδ, TCRγ gene rearrangement patterns	16
Monoclonal	10
IGH	6
TCRδ	2
TCRγ	2
Diclonal	6
2 clones of IGH	4
TCRδ and TCRγ	2
BCR/ABL and IGH/TCR rearrangements	6
$p190^{BCR/ABL}$ and IGH	2
$p190^{BCR/ABL}$ and 2 clones of IGH	1
$p210^{BCR/ABL}$ and IGH	1
$p190^{BCR/ABL}$ and TCRδ	1
$p190^{BCR/ABL}$ and TCRδ, TCRγ	1
Number of samples	231
Median (range)	6 (2–22)
Bone marrow	220
Peripheral blood	11

BCR/ABL fusion transcripts and IGH/TCR gene rearrangements in six patients. The results obtained by the two methods were concordant. In five patients, MRD was also detected by flow cytometry (BCR/ABL, 4; IGH gene rearrangement, 1).

Patients achieved first CR after a median period of 35 days (range 15–105 days). Sixteen patients (47.1%) received allogeneic stem cell transplantation following induction and consolidation chemotherapy. Seventeen patients relapsed and 17 patients remained in CR.

We analyzed 27 of the 34 patients who could be examined for MRD on day 100. The OS rate (45.0%) and RFS rate (40.0%) at 2 years in CR patients with MRD level $\geq 10^{-3}$ ($n = 12$) were significantly lower than those in CR patients with MRD level $<10^{-3}$ ($n = 15$) (OS rate 79.0%, RFS rate 79.4%) (log-rank test, $P = 0.017$ and 0.0007). A lower MRD value on day 100 after induction therapy was associated significantly with longer survival (Fig. 1a, b). Two patients were excluded from the study because of relapse before day 100 after induction therapy.

Monitoring of MRD enabled prediction of relapse in 10 of the 14 relapsed patients whose results of MRD analyses were available before relapse. The median time from molecular to clinical relapse was 65 days (range 12–305 days).

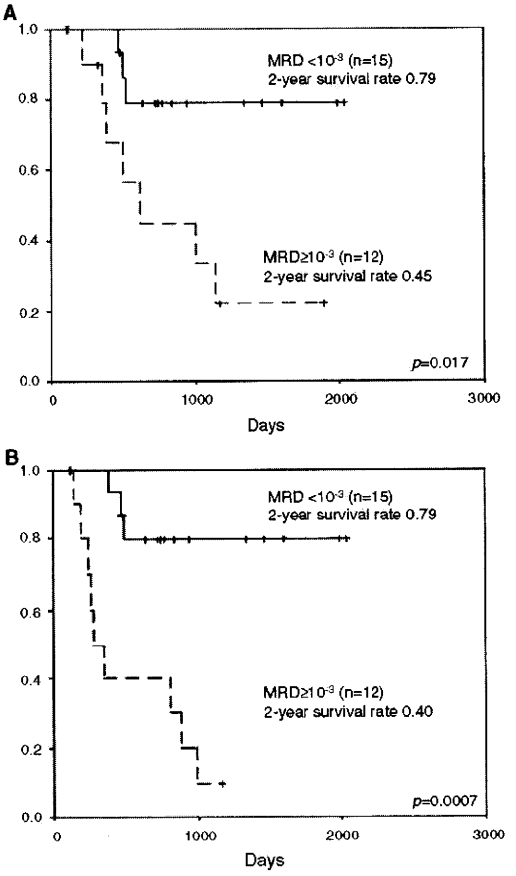

Fig. 1 Comparison of overall survival (OS, **a**) and relapse-free survival (RFS, **b**) by MRD detection on day 100 after induction therapy

3.2 Comparison of the results obtained by flow cytometry and PCR analysis

We compared MRD levels determined by both flow cytometry and PCR analysis in 27 follow-up BM samples from 5 patients (Table 4). Four of them had Ph-positive ALL and MRD was analyzed by BCR/ABL fusion transcripts. The other patient was analyzed by IGH gene rearrangement.

Concordance between the flow cytometric and PCR results was obtained in 17 (63.0%) of the 27 samples ($P = 0.057$). No significant changes regarding immunophenotype were observed when MRD phenotypes at relapse were compared to the original phenotypes at diagnosis.

Table 4 Informative CD combinations used for flow cytometry (FC)

Case	Type	Marker combinations used at follow-up	MRD results		
			PCR+ FC+	PCR− FC−	PCR− FC+
9	ALL(B)	CD58/CD45/CD34/CD19 CD13/CD10/CD34/CD19 TdT/CD10/CD34/CD19	1		
23	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD58/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	5	3	7
24	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD33/CD34/CD19	3		2
25	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	1	1	1
26	Ph-ALL(B)	CD58/CD45/CD34/CD19 CD10/KOR-SA/CD34/CD19 TdT/CD10/CD34/CD19	3		

Of the ten samples that were MRD positive by flow cytometry but MRD negative by PCR, very low levels (below 10^{-4}) could be detected by flow cytometry in five samples from two patients. The other five samples from three patients were MRD positive by flow cytometry (0.18, 0.16, 0.39, 0.42, and 2.53%) but MRD negative by PCR.

3.3 Other prognostic factors

In addition, we examined factors correlated with OS and RFS by the log-rank test. In the log-lank test, a lower MRD value on day 100 was associated significantly with longer survival (Table 5). However, we could not determine other factors, such as age, WBC count at diagnosis, Ph chromosome, immunophenotype, sex, days to achieve CR, and type of treatment (chemotherapy or transplantation). Therefore, our data provide evidence that molecular MRD status on day 100 is a strong predictor of outcome in adult ALL.

3.4 Relative risk of relapse

Looking for informative predictors of the achievement of molecular CR on day 100, we used univariate and multivariate analyses to investigate the role of conventional clinical findings such as age younger than 55 years or not, WBC count at diagnosis, Ph chromosome, immunophenotype, sex, days to achieve CR, and MRD levels on day 30. Univariate analysis showed that MRD positivity on day 100 was associated with age older than 55 years and MRD positivity on day 30, but there were no significant associations in multivariate analysis.

4 Discussion

There is increasing evidence that MRD has strong prognostic significance in adult patients with ALL. Mortuza et al. [7] found that MRD positivity detected especially at 3–6 months after induction therapy in adults with B-ALL was associated with an increased risk of relapse. Holowiecki et al. [2] reported that MRD equal or greater than 0.1% of BM cells after induction was a strong and independent predictor for relapse in both standard and high-risk groups.

We also showed that OS and RFS at 2 years in CR patients positive for MRD by PCR-based detection of BCR/ABL transcripts or IGH/TCR gene rearrangements were significantly lower than those in CR patients negative for MRD.

Three highly specific and sensitive methodologies for MRD detection are available: multiparameter flow cytometric immunophenotyping, RQ-PCR-based detection of fusion gene transcripts or breakpoints, and PCR-based detection of clonal immunoglobulin and T cell receptor gene rearrangements [8].

PCR-based detection of rearranged IGH/TCR genes is currently the most broadly applied MRD technique owing to its high level of standardization, its well-defined quantitative range and good sensitivity, as well as applicability in the majority of ALL patients [3]. To reach a higher level of sensitivity, DNA sequencing of the junctional regions is required to design tumor-specific primers and/or probes.

A major drawback of using rearranged immune genes as MRD-PCR targets is the possible occurrence of continuing rearrangements during the course of therapy and during follow-up, which can lead to false-negative PCR results [9].

Table 5 Univariate and multivariate analyses of prognostic factors associated with overall survival and relapse-free survival (log-rank test)

Variables	Univariate								Multivariate					
	Overall survival				Relapse-free survival				Overall survival			Relapse-free survival		
	N	Hazard ratio	95% CI	*P* value	*N*	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (years)														
<35	14	1.59	(0.55–4.59)	0.39	14	1.83	(0.63–5.29)	0.26						
≥35	20				19									
<55	21	2.34	(0.87–6.30)	0.09	21	2.28	(0.85–6.14)	0.10	0.42	(0.01–14.67)	0.63	0.64	(0.04–10.26)	0.75
≥55	13				12									
WBC ($\times 10^9$/L)														
<30 (B), <100 (T)	25	3.60	(0.93–13.91)	0.06	25	3.05	(0.82–11.38)	0.10	4.03	(0.12–134.40)	0.44	4.00	(0.24–66.21)	0.33
≥30 (B), ≥100 (T)	5				5									
Gender														
Female	17	0.88	(0.33–2.36)	0.80	16	1.10	(0.41–2.92)	0.85						
Male	17				17									
Ph chromosome														
Negative	16	1.03	(0.37–2.83)	0.95	16	1.11	(0.40–3.04)	0.84						
Positive	18				17									
Time to CR (days)														
<30	10	1.18	(0.38–3.73)	0.77	10	0.62	(0.22–1.76)	0.37						
≥30	23				22									
Immunophenotype														
B-lineage	30	0.30	(0.04–2.34)	0.25	29	0.27	(0.04–2.10)	0.21						
T-lineage	4				4									
Transplantation														
No	18	0.34	(0.12–0.97)	0.04	17	0.40	(0.14–1.10)	0.08	0.74	(0.03–21.18)	0.86	0.75	(0.06–10.16)	0.83
Yes	16				16									
MRD on day 30														
Negative	7	1.13	(0.35–3.70)	0.84	7	1.64	(0.51–5.25)	0.41						
Positive	18				17									
MRD on day 100														
Negative	15	4.54	(1.17–17.67)	0.03	15	7.14	(1.91–26.66)	0.003	7.46	(1.21–45.91)	0.03	9.60	(1.83–50.49)	0.008
Positive	12				12									

Ph Philadelphia

Relapse could not be predicted in 4 of the 14 relapsed patients in this study. In two of them, TCRδ or IGH gene rearrangement was not detected even at the time of relapse. Clonal TCRδ rearrangement in particular can be lost during the follow-up period preceding relapse [10], and continuing rearrangements during the disease course occur in 10–40% of cases depending on the target used [11].

One specific advantage of flow cytometry over PCR-based assays is that it allows direct quantification of MRD, rather than extrapolating it from amounts of PCR product [9]. However, the immunophenotype of leukemic cells may change during progression of the disease, and if these changes affect markers used for monitoring MRD, a false-negative finding may result [12, 13].

In this study, concordance between the flow cytometric and PCR results was obtained in 17 (63.0%) of the 27 samples. Comparative analyses showed that more concordant results could be obtained for both methods at the level of 10^{-3} and 10^{-4} [14, 15]. In three cases, some samples showed discordant results. In these cases, MRD might be overestimated by flow cytometry because normal cells cannot be easily distinguished from leukemic cells in studies of MRD [6]. The combination use of the two methods may offset the possibility of false-negative and false-positive MRD results due to these events [16].

We did not find a significant prognostic value of MRD in patients who received allogeneic transplantation. It has been shown that MRD detected before transplantation was a significant predictor of failure after transplantation [17, 18]. However, Patel et al. [19] suggested that molecularly determined MRD pre-transplant was not a risk factor for relapse in patients receiving allogeneic stem cell transplantation in the first CR. It has also been shown that MRD status after allogeneic transplantation was an important predictor of outcome in adults with ALL [7, 18]. The MRD status before and after transplantation was not correlated with survival in this study, which may be explained by the small number of patients who received transplantation.

Results of log-rank tests showed that age, WBC count at diagnosis, sex, Ph-positivity, and immunophenotype were not associated with prognosis or relapse. Generally, advanced age of the patient and high WBC count at diagnosis with acute leukemia are related to poor prognosis. In this study, we did not intervene in clinical decisions concerning treatment of the patients, but MRD level on day 100 after induction chemotherapy was only a good prognostic factor to monitor relapse and classify the groups as those with good or poor prognosis.

Although limitation of MRD assessment for prediction of extramedullary relapse is recognized [19], MRD methods can be used to predict outcome on the basis of early response to therapy and to recognize leukemia relapse [20].

Thorn et al. [21] suggested that MRD levels calculated by the quantification of BCR/ABL transcripts were higher than levels obtained by flow cytometry and by quantitative PCR of rearranged IGH/TCR genes. However, whether the results of these methods coincide with those of PCR amplification of fusion transcripts is not yet established [22]. Further progress in assessing MRD and improving the prognosis in adult patients with ALL in association with MRD is expected.

In this study, we investigated the prognostic value of MRD in adult patients with ALL. We found that ALL patients with MRD level $<10^{-3}$ on day 100 had significantly better OS and RFS than those of ALL patients with MRD level $\geq 10^{-3}$. Therefore, MRD analysis is useful for monitoring the prognosis of ALL patients. PCR analysis and flow cytometry were both useful for the detection of MRD. However, both methods can yield false-negative and false-positive results, and improvements are needed for further optimization and standardization to assess MRD. It is important to investigate an appropriate way to choose among these methods or the use of them in tandem.

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Reduced intensity conditioning regimen with fludarabine, busulfan, and low-dose TBI (Flu-BU2-TBI): Clinical efficacy in high-risk patients

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Reduced intensity conditioning (RIC) regimens are widely used in allogeneic stem cell transplantation (SCT). In this study, we retrospectively investigated the clinical outcomes of RIC with fludarabine (Flu; 180 mg/m^2), intravenous busulfan (BU; 6.4 mg/kg) or oral BU (8 mg/kg), and low-dose total body irradiation (TBI; 4 Gy) (Flu-BU2-TBI) in 66 patients (median age: 54.5 years) with various hematological malignancies. Thirty-eight patients (58%) were high-risk patients (median age: 56 years). The overall survival rate at 2 years of the high-risk patients was 64.5%, which was comparable to the survival rate of 70.9% in standard-risk patients (P = 0.68). The relapse rates at 2 years in the standard-risk and high-risk patients were 16 and 28%, respectively, and day 100 treatment-related mortality rates were 0 and 6%, respectively. The Flu-BU2-TBI regimen for high-risk patients showed therapeutic effects equivalent to those for standard-risk patients and favorable outcomes compared with those of other previous RIC regimens. Am. J. Hematol. 84:243–248, 2010. © 2010 Wiley-Liss, Inc.

Introduction

Over the past decade, a reduced intensity conditioning (RIC) regimen has been used extensively in allogeneic hemetopoietic stem cell transplantation (SCT) in an attempt to improve clinical outcomes in elderly or complicated patients. The introduction of SCT with RIC has allowed the application of SCT to a much wider patient population by reducing the toxicity and exploiting the graft-versus-leukemia effect (GVL) as the primary curative approach [1]. Several protocols have been investigated to determine the optimal RIC regimen [2–5], but the issue is still in controversy.

The antineoplastic effects of chemotherapy or radiation as a conditioning regimen for SCT are required, especially for SCT for patients with refractory disease or without remission. However, there has been little investigation of appropriate choices for conditioning regimen based on risk and it remains unclear that conditioning regimens have a beneficial effect on each patient. The reports for RIC regimens including relatively large numbers of high-risk patients are much more likely to be associated with an increased incidence of relapse and a decreased probability of survival [6–8].

A conditioning regimen with busulfan (BU) in combination with the nucleoside analog fludarabine (Flu) is clinically well tolerated and is gradually replacing myeloablative-conditioning regimens, such as BU combined with cyclophosphamide (CY) or CY combined with total body irradiation (TBI) [6,9–14], but there are many modified regimens using various doses of Flu or BU and with or without antithymocyte globulin (ATG) and TBI [15–18]. Although several studies have shown clinical efficacy of a conditioning regimen with myeloablative doses of BU combined with Flu, those studies included relatively young patients and the results, therefore, cannot be directly applied to elderly high-risk patients [12–16]. Furthermore, the transition from oral BU to intravenous BU (i.v. BU) may have some effects on clinical outcomes of these protocols.

In this study, we investigated the efficacy of an RIC regimen with Flu, nonmyeloablative dose of BU, and low-dose TBI (Flu-BU2-TBI) in high-risk patients and we compared the clinical outcomes of Flu-BU2-TBI with those of other RIC regimens.

Patients and Methods

Patients and donors. This study is a retrospective analysis of SCT outcomes in 66 patients who received allogeneic hemetopoietic SCT at our institution between August 2003 and February 2009 and were treated with an RIC regimen consisting of Flu-BU2-TBI. High-risk disease status was defined as acute leukemia beyond second remission or without hematological remission, chronic myeloid leukemia with accelerated phase and blastic crisis, myelodysplastic syndrome (MDS), according to the World Health Organization criteria, beyond refractory anemia (RA) or RA with ringed sideroblasts, and multiple relapsing or chemo-resistant malignancies. Human leukocyte antigen (HLA) serotyping for HLA-A, HLA-B, and HLA-DR was performed in all patients. In unrelated transplant recipients, HLA genotyping was performed by intermediate-resolution polymerase chain reaction analysis. Unrelated bone marrow donors were selected through the agency of the Japan Marrow Donor Program donor center. Donors who donated peripheral blood stem cells (PBSC) received 400 μg/m^2/day of recombinant human G-CSF (filgrastim) subcutaneously for 5 days before peripheral blood stem cell collection. Peripheral blood stem cell donors are currently restricted to relatives in Japan.

Conditioning regimen and graft-versus-host disease prophylaxis. Patients received Flu at 30 mg/m^2 intravenously for 6 days (total of 180 mg/m^2, days −7 to −2), i.v. BU at 3.2 mg/kg intravenously or oral BU at 4 mg/kg for 2 days (days −3 to −2) and 4 Gy of TBI (day −1). Phenytoin was administered from day −4 to day 0 as an anticonvulsant

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Conflict of interest: Nothing to report

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agent. Oral BU was used from August 2003 to October 2006, and i.v. Bu was used from November 2006 to February 2009. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine A (CyA) or FK506 from day −1 and short-term methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6). Only one patient was given FK506 alone because of a history of methotorexate-induced liver toxicity. CyA and FK506 were started at doses of 2–3 mg/kg/day and 0.02–0.03 mg/kg, respectively, by continuous intravenous drip. The doses of CyA and FK506 were adjusted on the basis of target plasma concentration (CyA [related/unrelated]: 300–400/350–450 ng/ml, FK506 [related/unrelated]: 5–15/10–15 ng/ml). G-CSF (filgrastim) was started at a dose of 300 μg/m^2/day intravenously from day 1 or 5 until engraftment was achieved. Antibiotic prophylaxis was used to prevent bacterial, fungal, viral, and *Pneumocystis carinii* infections by levofloxacin (300 mg/day orally from day −14), micafungin or voriconazole (50 or 100 mg/day intravenously or 400 mg/day orally from day −14), acyclovir (1,000 mg/day orally from day −7), and sulf-methoxazole/trimethoprim (after achieving engraftment).

Evaluation of clinical response (engraftment, toxicity, GVHD, and relapse). Neutrophil engraftment was defined as the first of 2 days with an absolute neutrophil count greater than 0.5 × 10^9/l. Failure to achieve engraftment by day 30 was considered as primary graft failure. Toxicity after SCT was graded according to the National Cancer Institute Common Toxicity Criteria. GVHD was graded and staged according to standard criteria [19]. Acute GVHD was evaluated for all patients other than those who failed to achieve engraftment or died before day 30. Donor chimerism was assessed by fluorescent in situ hybridization with X and Y chromosome probes in sex-mismatched transplants and by DNA microsatellite analysis as described previously [20].

Statistical analysis. The χ^2-test or Student's *t*-test was used to evaluate the difference in baseline characteristics, disease, disease status at conditioning, and source of progenitor cells of the patients, and transplant outcomes between the standard- and high-risk groups. Overall survival (OS) was defined as the time from SCT to death from any cause. Treatment-related mortality (TRM) was defined as death due to any cause other than relapse or refractory disease. OS was calculated using the Kaplan-Meier method. Relapse and TRM were calculated by the cumulative incidence method. The log-rank test was used for comparison of these curves. A Cox proportional hazard model was used to determine the significance of multiple variables in determining the outcomes. Only variables with $P < 0.2$ by univariate analysis were retained in the model.

Results

Patients and donor characteristics

This study included a total of 66 consecutive patients that received SCT with RIC regimens, either standard-risk ($n = 28$) or high-risk patients ($n = 38$), in a single institution over a 6-year period. The patients and donor characteristics are summarized in Table I. Median follow-up period was 356 (33–1403) days in all patients and 475 (33–1403) days in surviving patients. Thirty-three patients (50%) had AML ($n = 16$) or MDS ($n = 17$), and 17 patients (26%) had non-Hodgkin lymphoma (NHL). In the high-risk group, 28 patients (74%) had active disease (standard-risk: $n = 3$ [11%], $P < 0.01$), and 31 patients (82%) received SCT from unrelated donors (standard-risk: $n = 20$ [71%], $P = 0.33$). Nine patients (standard-risk: $n = 1$, high-risk: $n = 8$) received SCT from HLA-mismatched donors, and the remaining 57 patients (standard-risk: $n = 27$, high-risk: $n = 30$) received SCT from HLA-matched donors ($P = 0.04$). More patients were treated with oral BU in the standard-risk group ($n = 17$ [61%]) than in the high-risk group ($n = 14$ [17%], $P = 0.05$). Seventeen patients have received previous autologous transplantations (standard-risk: $n = 2$, high-risk: $n = 15$, $P < 0.01$) and four patients have received previous allogeneic transplantations (standard-risk: $n = 1$, high-risk: $n = 3$, $P = 0.47$).

Engraftment, acute GVHD, and VOD

Clinical outcomes of transplantation are shown in Table II. Neutrophil engraftment was achieved in 63 (95%) of the patients (standard-risk: $n = 28$ [100%], high-risk: $n = 35$ [92%], $P = 0.13$) at median day 16 (standard-risk: day 16 [10–21], high-risk: day 16 [12–25], $P = 0.28$). Platelet engraftment above 20,000/μl occurred in 58 (88%) of the patients (standard-risk: $n = 25$ [89%], high-risk: $n = 33$ [87%], $P = 0.76$) at median day 27 (standard-risk: day 28 [15–157], high-risk: day 26 [13–68], $P = 0.42$), and platelet engraftment over 50,000/μl occurred in 58 (88%) of the patients (standard-risk: $n = 25$ [89%], high-risk: $n = 33$ [87%], $P = 0.76$) at median day 29 (standard-risk: day 31 [15–175], high-risk: day 27 [16–85], $P = 0.12$). Complete chimerism was achieved in 58 (88%) of the patients (standard-risk: $n = 25$ [89%], high-risk: $n = 33$ [87%], $P = 0.76$) at median day 27 (standard-risk: day 24.5 [14–92], high-risk: day 27 [15–64], $P = 0.91$). Graft failure without autologous recovery occurred in 1 patient (2%), who successfully achieved engraftment with the second transplantation using another conditioning regimen. The patient received bone marrow transplantation from a related donor in the first SCT and peripheral blood SCT in the second SCT from the same donor. Of the 63 evaluable patients (standard-risk: $n = 28$, high-risk: $n = 35$), 47 (75%) developed acute GVHD (standard-risk: $n = 23$ [82%: 95% CI 68–96%], high-risk: $n = 24$ [69%: 95% CI 54–85%], $P = 0.29$). Acute GVHD Grade III or IV occurred in 11 patients (standard-risk: $n = 4$ [28%: 95% CI 2–53%], high-risk: $n = 7$ [31%: 95% CI 10–52%], $P = 0.85$). There was only one patient with VOD, in the standard-risk group, and the patient recovered from VOD and is alive in remission. National Cancer Institute Grade II–IV/Grade III–IV elevation of bilirubin (15%/5%), aspartate aminotransferase (15%/3%), and alanine aminotransferase (23%/17%) were common findings in the patients. No adverse toxicities such as hepatic toxicity, renal damage, mucositis, or nausea requiring treatment interruption occurred.

Survival, relapse, and treatment-related mortality

Twenty-one patients (32%) died. The causes of death were relapse ($n = 9$), progressive primary disease ($n = 3$), infection ($n = 5$), GVHD ($n = 3$), and late-onset noninfectious pulmonary complication ($n = 1$). The estimated OS rate at 2 years of the high-risk patients was 64.5% (95% CI 46–83%), which was comparable to the OS rate of 70.9% (95% CI 53–89%) in standard-risk patients using the Kaplan–Meier method ($P = 0.68$) (Fig. 1A). The relapse rates at 2 years in the standard-risk and high-risk patients were 16 and 28%, respectively, and day 100 treatment-related mortality rates were 0 and 6%, respectively. There was no statistically significant difference between the two groups in the incidence of relapse by the cumulative incidence method ($P = 0.29$) (Fig. 1B). The causes of nine cases of TRM were GVHD ($n = 3$) and infection ($n = 1$) in the standard-risk patients and infection ($n = 4$) and late-onset noninfectious pulmonary complication ($n = 1$) in the high-risk patients. There was no statistically significant difference between the two groups in the incidence of TRM ($P = 0.79$) (Fig. 1C), but acute GVHD was more commonly observed as the cause of death in the standard-risk group ($P = 0.02$) (Table II).

In univariate and multivariate (Table III) analyses of factors influencing outcomes, age over 55 years at SCT was the most significant predictor of OS. Patients under 55 years of age had an OS rate of 77% (95% CI 61–94%), whereas patients over 55 years of age had an OS rate of 53% (95% CI 34–71%) ($P < 0.01$) in univariate analysis. Multivariate analysis also showed the adverse effect of age (RR 3.89, $P = 0.04$). Disease status at SCT was also a significant predictor of OS in univariate analysis ($P = 0.04$) and had the adverse effect on OS in multivariate analysis ($P = 0.01$).

TABLE I. Patient characteristics

	Total	%	Standard risk	%	High risk	%	*P*-value
Number	66		28	42	38	58	
Male/Female	34/32		14/14		20/18		0.83
Median age (range) (years)	54.5 (27–68)		53 (27–68)		56 (40–66)		0.05
Median follow-up [days][a]	358 [33–1403]		398.5 [33–1403]		309 [45–1124]		0.27
Diagnosis							
AML (CR1)	5		5		0		<0.01
AML (CR2)	6		6		0		
AML (>CR2/nonCR)	5		0		5		
MDS (RA/RARS)	8		8		0		
MDS (>RAEB1)	9		0		9		
ALL (CR1)	1		1		0		
ALL (CR2)	1		1		0		
ALL (>CR2/nonCR)	1		1		0		
CML (CP)	1		1		0		
CML (AP/BC)	1		0		1		
NHL (FL/DLBL/other[b])	17 (12/1/4)		4 (2/0/0)		13 (11/1/2)		
MM	5		0		5		
ATL	5		2		3		
HL	1		0		1		
Disease status at SCT[c]							
Remission	35	53	25	89	10	26	<0.01
Active	31	47	3	11	28	74	
Donor							
Related	15	23	8	29	7	18	0.33
Unrelated	51	77	20	71	31	82	
HLA[d]							
matched (related/unrelated)	57 (14/43)	86 (21/65)	27 (7/20)	96 (25/71)	30 (7/23)	79 (18/61)	0.04
mismatched unrelated (BM)	8	12	0	0	8	21	
mismatched related (PBSC)	1	2	1	4	0	0	
Stem cell source							
BM	60	91	24	86	36	95	0.21
PBSC	6	9	4	14	2	5	
Busulfan							
iv BU	35	53	11	39	24	63	0.05
oral BU	31	47	17	61	14	37	
Previous transplant							
autologous	17	26	2	7	15	39	<0.01
allogeneic	4	6	1	7	3	8	0.47

Abbreviation: CR, complete remission; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non Hodgkin lymphoma; FL, follicular lymphoma; DLBL, duffuse large B cell lympgoma; ATL, adult T-cell lymphoma/leukemia; HL, Hodgkin lymphoma; BM, bone marrow; PBSC, peripheral blood stem cell.

[a] Early death before day 30 (n = 2) were excluded from the analysis.

[b] T cell lymphoma (n = 3). Mandle cell lymphoma (n = 1). NK/T cell lymphoma (n = 1).

[c] Remission: CR in leukemia. CR or partial remission in lymphoma/myeloma and RA in MDS. Active: others.

[d] HLA matched: Donors serologically full-matched with recipients.

TABLE II. Transplantation Outcomes

	Total	%	Standard risk	%	High risk	%	*P*-value
Number	66		28		38		
Engraftment[a]	63	95	28	100	35	92	0.13
Engraftment (day)	16 (10–25)		16 (10–21)		16 (12–25)		0.28
P >20000[a]	58	88	25	89	33	87	0.76
P >50000[a]	58	88	25	89	33	87	0.76
P >20000 (day)	27 (13–157)		28 (15–157)		26 (13–68)		0.42
P >50000 (day)	29 (15–175)		31 (15–175)		27 (16–85)		0.12
Complete chimerism (day)	27 (14–92)		24.5 (14–92)		27 (15–64)		0.91
Graft failure[a]	1	2	0	0	1	3	0.38
Primary	0		0		0		
Secondary	1		0		1		
acute GVHD[a]	47/63	75	23/28	82 (68–96)	24/35	69 (54–85)	0.29
Grades 3–4	11/63	17	4/28	28 (2–53)	7/35	31 (10–52)	0.85
VOD	1	2	1	4	0	0	0.22
Early death (<day30)	2	3	0	0	2	5	0.22
Cause of death							0.09
relapse	9		4		5		0.60
Progressive disease	3		0		3		0.14
aGVHD	3		3		0		0.02
infection	5		1		4		0.34
other	1		0		1		0.42

Abbreviations: GVHD, graft-versus-host disease, VOD, veno-occulusive disease.

[a] Among evaluable patients (Standard: n = 28, High: n = 35) excluding the patients with graft failure and early death before day 30 (Standard: n=0, High: n= 3).

In univariate analysis for relapse, disease status at SCT was a significant predicting factor ($P < 0.01$), and age over 55 years ($P = 0.09$) and related donor ($P = 0.10$) showed a trend for higher incidence of relapse without statistical significance. Sexual mismatch between recipients and donors ($P = 0.02$) and Grades 3–4 acute GVHD ($P = 0.03$) had a significant impact on TRM in univariate analysis.

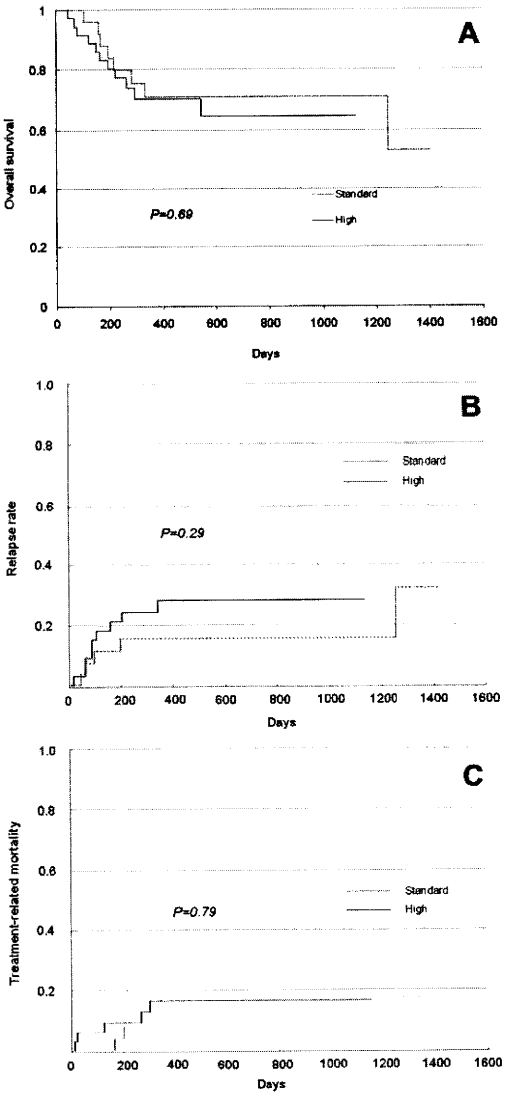

Figure 1. Overall survival of patients receiving RIC with Flu-BU2-TBI comparing the standard-risk with high-risk features (P = 0.69) (A) Incidence of relapse (P = 0.29) (B) and treatment-related mortality (P = 0.79) (C).

Discussion

Allogeneic SCT with an RIC regimen is a feasible and effective therapy for patients not eligible for SCT with myeloablative conditioning due to advanced age or comorbidities [1,21]. Flu and CY are equally immunosuppressive, and because of the significantly fewer adverse effects, such as hemorrhagic cystitis or cardiac toxicity, Flu is an excellent candidate to replace CY in a conditioning regimen for SCT [22]. Flu-based regimens including Flu combined with BU or melphalan are therefore extensively used in RIC regimens.

TABLE III. Multivariate Analysis of Factors Predicting OS

		RR	95%*C.I.*	*p*-value
Age	>55 <55	3.89	1.56–11.3	0.004
Diseases status	Active Remission	3.81	1.38–10.6	0.01
Sex	matched mismatched	1.75	0.85–4.75	0.26
Grades 3–4 aGVHD	(+) (−)	2.48	0.74–7.25	0.13

In this study, we retrospectively investigated the SCT outcomes following an RIC regimen, Flu-BU2-TBI, to determine the appropriate cases for the regimen. The 2-year OS rate in the high-risk group (64.5%) in this study was significantly higher than that in other retrospective analyses of patients who received Flu (150–160 mg/m^2) and iv BU (3.2–6.4 mg/kg) (Flu-BU) with 2-year OS rates of 28 [8], 34 [7], and 48% [6] taking into consideration the fact that these analyses included the patients in remission. A direct comparison with results of these studies might be inappropriate since the patient populations in those studies included mainly patients with acute myeloid leukemia (AML) and MDS but our data showed equivalent outcomes in patients with AML/MDS and other diseases (P = 0.92). Moreover, even considering that 13 patients (34%) in the high-risk group had NHL, including 11 patients (29%) with multirelapsing or chemo-refractory follicular lymphoma, the previously reported clinical outcomes of SCT with RIC for NHL are not superior to that for AML/MDS [23]. Two studies on the clinical efficacy of RIC regimens with Flu (180–200 mg/m^2), oral BU (8 mg/kg), and low-dose TBI (2–4 Gy), approximately the same intensity as that in our protocol, showed 1-year OS rates of 41 [18] and 50% [17]. Although the fact that approximately half of our patients received i.v. BU might have had a positive effect on OS, there was no significant difference between i.v. BU and oral BU in univariate analysis for OS (P = 0.57).

The question that we must consider next is the incidence of relapse and TRM. Restricted to high-risk patients, our data showed a relapse rate of 28%, which is lower than that in two previous retrospective analyses of patients who received Flu-BU only, with cumulative incidences of relapse of 61 and 49% in those patients including patients in remission [6,8]. It is possible that concomitant use of BU and TBI had a synergistic antitumor effect on high-risk patients in our cohort. Although TRM of 16% in high-risk patients was not lower but was equivalent to TRM or nonrelapse mortality in those analyses, it follows that our results are fairly good considering a lower incidence of relapse in our patients and the fact that there was a trade-off relationship between decreased risk of relapse and higher TRM in other studies.

In standard-risk patients, on the other hand, TRM was rather high (17%) compared with that in other studies on an RIC regimen with Flu-BU [6,7]. Onishi et al. [18] reported high incidences of a 1-year nonrelapse mortality (46%) and grades 2–4 acute GVHD (48%) in patients who received Flu (180 mg/m^2), oral BU (8 mg/kg), and low-dose TBI (4 Gy). They also reported a high incidence of extensive chronic GVHD (90%), which compromised elderly patients' quality of life and resulted in subsequent pulmonary complications in the later phase. Concomitant use of 4 Gy of TBI and a mucosal barrier injury or unstable blood concentration due to drug absorption variations of oral BU are potential causes of organ toxicity, GVHD, and infection, but these possible associations have not been sufficiently addressed in previous studies [24–27]. An RIC regimen

with Flu and BU without TBI might be potent enough for standard-risk patients, especially those over 55 years of age, considering the fact pointed by Shimoni et al. that an RIC regimen with Flu-BU is associated with improved survival and lower nonrelapse mortality in patients undergoing transplantion in remission and considering our results of univariate or multivariate analysis. Furthermore, Ho et al. and Cho et al. reported that administration of alemtuzumab or ATG in the RIC regimen with Flu-BU reduced the incidence of severe acute GVHD and chronic GVHD [7,28,29]. The addition of alemtuzumab or ATG or the enhancement of prophylaxis for GVHD, especially for unrelated or HLA and sex-mismatched donor-patients, obviously contributes to repression of acute GVHD and should be considered in standard-risk cases with a low relapse risk and without active infection because the most common cause of TRM in our standard-risk group was GVHD [12,16,28–31]. Moreover, two reports on the RIC regimens with Flu (150 mg/m^2) and i.v. BU (6.4 mg/kg) showed older age was not a significant predicting factor for outcomes but Markova et al. reported that age over 45 years was associated with increased nonrelapse mortality and lower survival rate in the RIC regimen with Flu (200 mg/m^2), oral BU (8 mg/kg), and low-dose TBI (2 Gy) [7,17,29]. We may, therefore, reasonably conclude that Flu-BU2-TBI regimen has a beneficial effect for patients with high-risk disease status considering its favorable antitumor effect, but administration of an even more reduced intensity or immunosuppressive regimen in standard-risk patients over 55 years of age and with an unrelated or HLA/sex-mismatched donor, such as Flu-BU only or Flu-BU combined with ATG/alemtuzumab, should also be considered. Our RIC regimen is a feasible and effective conditioning for high-risk patients and standard-risk patients under 55 years not eligible for SCT with myeloablative conditioning due to advanced age or comorbidities.

In addition, our data indicated no significant difference between outcomes in patients with AML/MDS and those with non-AML/MDS, although melphalan-containing regimens are widely used as conditioning regimens to treat lymphoid malignancy, especially acute lymphoid leukemia, and many reports of a Flu-BU-based regimen are actually directed toward AML and MDS [6,8,12,14,29]. Rodrigues et al. [32] reported that the addition of low-dose TBI to an RIC regimen improved survival in patients with lymphoid malignancies. The Flu-BU-TBI regimen is a potential major candidate for RIC in patients with lymphoid malignancy considering the high regimen-related toxicity and NRM of a melphalan-containing regimen, but further larger-scale studies are needed to confirm this issue [7].

The frequency of hepatic VOD was low in our cases, as previous retrospective studies showed that the use of Flu instead of CY results in a lower frequency of hepatic VOD and related deaths [6,12,13,15]. The reported incidence of VOD after a BU-CY regimen ranges from 10 to 50%, whereas that after a Flu-BU regimen is reported to range from 0 to 2%. This difference is likely due to the fact that high-dose CY is closely associated with severe hepatic toxicity due to the depletion of glutathione in hepatocytes, which might be exacerbated by the combination with BU [33]. Moreover, we did not detect a significant difference between the incidence of VOD in the iv BU group and that in the oral BU group, in contrast to a clearly observed difference in the case of a BU-CY regimen [34,35].

On the contrary, a regimen with Flu and myeloablative dose of iv BU, which is a double dose of BU compared to our applied dose, has been reported to be clinically well tolerated and is gradually replacing myeloablative conditioning regimens, such as BU-CY or CY combined with TBI [12–14]. Those studies showed a low nonrelapse mortality rate and a favorable survival rate compared with those in the case of a conventional myeloablative conditioning regimen. However, since the patients in those studies were younger than the patients in our population, a randomized controlled study with a larger number of patients needs to be performed to establish a risk-adjusted treatment strategy.

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ORIGINAL ARTICLE

Heart rate variability during and after peripheral blood stem cell leukapheresis in autologous transplant patients and allogeneic transplant donors

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Abstract Side effects of varying severity are frequent in peripheral blood stem cell harvest (PBSCH). Life-threatening complications associated with PBSCH have also been reported. Heart rate variability (HRV), which reflects sympathovagal balance and autonomic cardiovascular control, has been a subject of intense interest in various diseases precipitating sudden death. Here, we prospectively assessed the impact of leukapheresis on HRV among autologous hematopoietic cell transplant patients and healthy donors. We found that HRV indicators, the standard deviation of normal-to-normal intervals (SDNN) value, the square root of the mean of the sum of squared differences between the adjacent normal-to-normal interval (r-MSSD) value, total frequency (TF), high frequency (HF) and low frequency (LF) powers decreased significantly to morbid levels during leukapheresis (all $P < 0.01$). Morbid changes in SDNN value, TF and LF powers were significantly sustained for 6–9 h after leukapheresis (all $P < 0.05$). Furthermore, TF and LF powers prior to leukapheresis were significantly lower in subjects with symptomatic hypotension than in the other subjects [3282 (3121–4427) vs. 6018 (4983–9816) ms^2, $P = 0.03$; 93 (42–144) vs. 237 (142–360) ms^2, $P = 0.03$, respectively]. Our results suggest that HRV analysis might be of use in evaluating and predicting the adverse effects of cardiovascular complications in PBSCH.

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Keywords Peripheral blood stem cell (PBSC) harvest · Leukapheresis · Heart rate variability · Autologous hematopoietic cell transplant patients and PBSC donors

1 Introduction

Peripheral blood stem cell harvesting (PBSCH) has been widely used for rescue following high-dose chemotherapy, or as an alternative to bone marrow as a stem cell source for allogeneic hematopoietic cell transplantation. The most common side effects are associated with recombinant human granulocyte colony-stimulating factor (rhG-CSF) administration, securing peripheral venous access, or anticoagulation with acid-citrate-dextrose (ACD) solution. These adverse effects are usually transient, not severe and easily controlled with adequate treatment. Severe adverse events in PBSC donors, the majority of which are acute and transient, occur at an incidence of 0.6% [1]. However, although extremely rare, life-threatening complications relating to PBSC donation, including sudden death or transient cardiac arrest, have been reported [2–5]. In several sudden death cases following PBSCH, the underlying mechanisms that led to the occurrence of sudden death have not been clearly described or clarified.

In normal sinus rhythm, the heart rate varies from beat to beat. The impulse generated by the sinus node is affected by the automatic nervous system and various humoral factors. The cardiovascular signal variability of the R–R period (heart rate variability, HRV) is an established tool that can be used to assess autonomic control. HRV assessment enables the evaluation of dynamic changes in the automatic nervous system and humoral factors without an invasive procedure. Recent evidence shows that a decrease in HRV is strongly associated with sudden death

and/or a cardiac event after a myocardial infarction. The usefulness of HRV as a clinical tool has been explored in numerous conditions, such as ischemic sudden death, sustained ventricular tachycardia, myocardial infarction, congestive heart failure, vasovagal syncope, hypertrophic cardiomyopathy, obstructive sleep apnea, diabetic neuropathy and various neurological alterations [6–14]. Two types of analysis, time domain and frequency domain, are included in HRV analysis. In time domain analysis, acknowledged simple markers are the standard deviation of normal-to-normal intervals (SDNN) and the square root of the mean of the sum of squared differences between adjacent normal-to-normal intervals (r-MSSD). In frequency domain analysis, markers include TF, total frequency (0.0001–0.5 Hz); LF, low-frequency power (0.04–0.15 Hz); HF, high-frequency power (0.15–0.4 Hz); and LF/HF ratio. These HRV power spectrum analyses are used to investigate sympathovagal balance, autonomic cardiovascular control and/or target function impairment. The LF component, which is called perfusion rhythmicity, reflects the rennin–angiotensin system or angiokinetic activity. The HF component, called respiratory rhythmicity, reflects breathing variability. Thus, the LF/HF ratio and HF have been used as markers of sympathetic and parasympathetic activity, respectively [15]. The aim of this study was to assess HRV during or after leukapheresis in autologous transplant patients and healthy PBSC donors.

2 Subjects and methods

2.1 Baseline characteristics

In this study, we enrolled 29 subjects (22 allogeneic transplant donors and 7 autologous transplant patients; 10 males, 19 females; median age: 38 years; interquartile range (IQR): 27–53). Median age of the autologous transplant patients and healthy allogeneic donors was 56 (IQR: 33–62) and 35 (IQR: 27–50) years, respectively. Diagnoses of the 7 autologous transplant patients were non-Hodgkin's lymphoma (6 patients) and plasmacytoma (1 patient). All the autologous transplant patients had a history of previous chemotherapy including anthracycline. The median cumulative dose of anthracycline in the 7 autologous transplant patients was 245 mg/m^2 (IQR: 160–297).

The study was conducted in accordance with a protocol approved by the IRB at our institution. Written informed consent was obtained from each patient or healthy donor.

2.2 Peripheral blood collection procedure

Autologous PBSCH was performed during the recovery phase after chemotherapy and was supported by subcutaneous administration of 10 μg/kg/day of rhG-CSF. In allogeneic PBSCH from healthy donors, on the other hand, leukapheresis was initiated following the administration of 10 μg/kg/day of rhG-CSF for 4 days. Of the 29 leukapheresis, 18 were performed using a CS3000 Plus (Baxter, Tokyo, Japan), 4 using an Amicus^{TM} Separator (Baxter, Tokyo, Japan) and 7 using a COBE Spectra (BCT Japan, Tokyo, Japan). In all 7 autologous harvest patients and 7 allogeneic harvest donors, central venous access via the subclavian, internal jugular or femoral vein was secured.

2.3 HRV analysis

In all patients and donors, ambulatory ECG recording was performed for 24 h during the first leukapheresis day but also for 24 h prior to leukapheresis to obtain control data. As control data, we employed the values of HRV indicators obtained during the same time period as leukapheresis on another day before leukapheresis. The data obtained from the 24-h ambulatory ECG recording were stored in a computer. Beat-by-beat cardiac cycle data were obtained by off-line computer analysis methods. The maximum entropy spectral analysis method was used to calculate HRV (MemCalc/CHIRAM version 1, Suwatrust, Tokyo, Japan). This program can perform time domain and frequency domain analyses simultaneously, and is superior to the fast Fourier transform and autoregressive methods in terms of the reproducibility of the original time series. The analysis was automatically performed in short segments and then averaged. In the program, all extrasystolic beats and artifacts were eliminated. We used markers, including heart rate (HR), normal-to-normal intervals (NN), SDNN and r-MSSD in time domain analysis, and TF, LF, HF and LF/HF ratio in frequency domain analysis. The program represents the average values of all markers every 5 min. In the program, TF was defined as the frequency range from 0.0001 to 0.5 Hz and included HF, LF, very low frequency and ultra low frequency. Therefore, at least 3 h of data are needed for TF power measurement; however, since leukapheresis took less than 3 h in 3 of the 29 subjects, TF power during leukapheresis was used in only 26 subjects. We applied the average values of all markers during the leukapheresis periods to assess HRV during leukapheresis, and applied the average values of all markers every 3 h following leukapheresis to assess HRV after leukapheresis.

We compared HRV control data measured for 24 h before leukapheresis between autologous transplant patients and allogeneic transplant donors in all 29 subjects. Control data were available in 26 of the patients, obtained on the day before leukapheresis during the same time period as leukapheresis. We therefore compared HRV data obtained during leukapheresis with control data acquired

during the same time period prior to leukapheresis in 26 evaluable subjects. Furthermore, to evaluate HRV changes after leukapheresis, we compared HRV data obtained during the nine-hour period after leukapheresis with control data obtained during the same time period prior to leukapheresis. This last comparison was possible in 24 subjects.

2.4 Statistical analysis

To evaluate the association between Hb levels just before leukapheresis and HRV indicators, we used Pearson's correlation coefficient. The Mann–Whitney U test was employed to analyze differences in HRV value between autologous transplant patients and healthy donors. The Wilcoxon's rank test was used to compare differences between HRV values during leukapheresis, or transitional changes in HRV values following leukapheresis, with control data measured during the same time period as the measurements taken during or following leukapheresis. Repeated measurements of analysis of variance were used to evaluate the effect of factors [age (>60 or ≤60), sex, weight (>50 or ≤50 kg) and autologous transplant patients] on rate of change in HR and HRV values from before to during leukapheresis. All P values less than 0.05 were considered significant.

3 Results

At HRV measurement, the median processed whole blood volume was 173 ml/kg (IQR: 140–196 ml/kg), the median leukapheresis time was 215 min (IQR: 188–248 min) and the median leukapheresis rate was 43 ml/min (IQR: 37–52 ml/min).

In all subjects, the r-MSSD value, TF, LF and HF powers at baseline showed a significant correlation with Hb levels before leukapheresis [Correlation coefficients: 0.61, 0.45, 0.58 and 0.45 (all $P < 0.05$), respectively]. In the autologous transplant patients, Hb levels before leukapheresis were significantly lower than in the healthy donors [(Median (IQR): 10.2 (9.4–11.5) vs. 13.5 (12.2–14.1) g/dl, respectively, $P = 0.0007$]. The NN and r-MSSD values and LF power were significantly lower in the autologous transplant patients than in the healthy donors in the data for the 24 h prior to leukapheresis [Median (IQR); 755 (709–788) vs. 833 (787–890) ms, $P = 0.03$, 21.8 (15.3–28.0) vs. 30.3 (26.1–39.4) ms, $P = 0.01$, 393 (205–416) vs. 603 (436–761) ms^2, $P = 0.03$, respectively] (Fig. 1).

In all the 26 evaluable subjects, SDNN, r-MSSD, TF, LF and HF values significantly and markedly decreased to morbid levels during leukapheresis (all $P < 0.001$) (Fig. 2). The HR and NN values and LF/HF ratio during leukapheresis did not change significantly compared with the control data. Similarly, among the allogeneic transplant donors, SDNN, r-MSSD, TF, LF and HF values decreased significantly (all $P < 0.05$) and the HR and NN values and LF/HF ratio did not change significantly during leukapheresis. When limited to the autologous transplant patients, HR became significantly elevated during leukapheresis [Median (IQR): 84.3 (77.4–88.4) vs. 93.4 (82.6–97.6) beats/min, respectively, $P = 0.03$]. NN, SDNN, r-MSSD, LF and HF values decreased significantly (all $P < 0.05$) and TF tended to decrease ($P = 0.07$) during leukapheresis. The LF/HF ratio did not change significantly during leukapheresis.

Advanced age (>60) significantly affected HR elevation during leukapheresis in comparison to baseline (Mean ± SD: 76.4 ± 12.9 and 87.7 ± 9.4 beats/min, $P = 0.02$). However, the factors including age (>60 or ≤60), sex, weight (>50 or ≤50 kg) and autologous transplant did not significantly affect the degree of decrease in HRV values during leukapheresis from the baseline.

Furthermore, r-MSSD power improved almost to control levels 6–9 h following leukapheresis (Table 1). On the other hand, SDNN, TF and LF values did not normalize to control levels even 6–9 h following leukapheresis (all $P < 0.05$). Furthermore, HF also did not completely normalize to control levels even 6–9 h following leukapheresis; however, this was not statistically significant ($P = 0.22$).

Of the 29 harvest cases, symptomatic hypotension occurred during leukapheresis in 2 subjects and about 3 h after leukapheresis in 1 subject. All 3 subjects were female, their systolic blood pressure decreasing significantly from 108, 96 and 114 mmHg to 82, 72 and 76 mmHg, respectively. In a 47-year-old female, anginal chest pain and dyspnea occurred; in a 52-year-old female, nausea, blurry vision and chest oppression were evident; and another 52-year-old female experienced nausea and dizziness with hypotension. However, symptomatic hypotension immediately improved with saline infusion, tilting the patient head-down, or discontinuance of leukapheresis. Notably among the HRV indicators, in the three subjects with symptomatic hypotension, TF and LF powers were significantly lower prior to leukapheresis than those in the other subjects [3282 (3121–4427) vs. 6018 (4983–9816) ms^2, $P = 0.03$; 93 (42–144) vs. 237 (142–360) ms^2, $P = 0.03$, respectively].

4 Discussion

In the present study we detected that the time domain indicators including SDNN and r-MSSD, and the frequency domain indicators including TF, HF and LF markedly decreased during leukapheresis and that this decrease was sustained over several hours after leukapheresis.

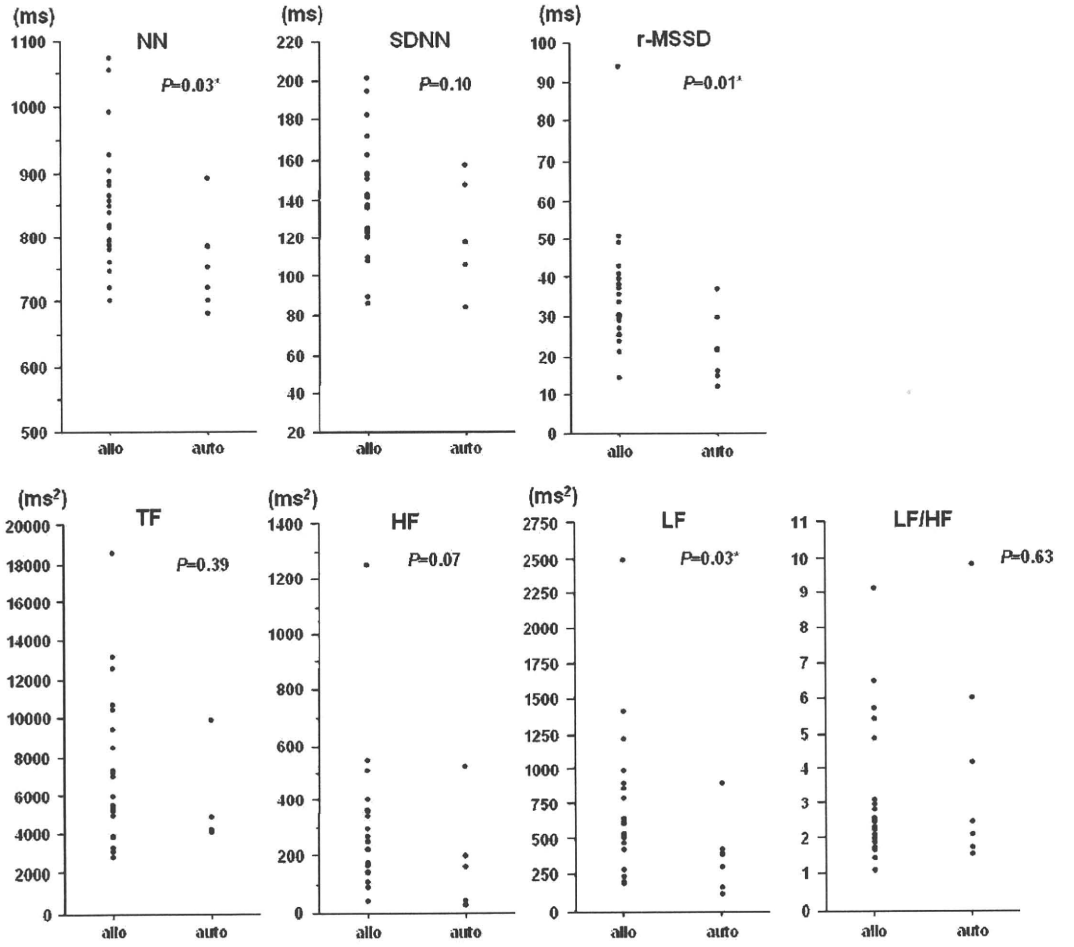

Fig. 1 Comparison of heart rate variability (HRV) indicator values between autologous transplant patients and healthy donors. NN, r-MSSD and LF were significantly lower in the autologous hematopoietic cell transplant patients than in the healthy donors. *auto* autologous hematopoietic cell transplant patients, *allo* allogeneic hematopoietic cell transplant healthy donors, *NN* normal-to-normal intervals, *SDNN* standard deviation of normal-to-normal intervals, *r-MSSD* square root of mean of sum of squared differences between adjacent normal-to-normal intervals, *TF* total frequency, *HF* high frequency, *LF* low frequency

Interestingly, in subjects who had symptomatic hypotension, TF and LF powers at baseline were significantly lower than for subjects without adverse cardiovascular effects.

It is reported that in patients with chronic heart failure, those with an SDNN value of less than 44 ms are at risk of cardiac events and all-cause mortality [16]. Surprisingly, in the 6 (86%) out of the 7 autologous transplant patients and in 7 (32%) out of the 22 allogeneic transplant donors, the SDNN value decreased to less than 44 ms during leukapheresis. Additionally, in 2 out of the 3 subjects with symptomatic hypotension, SDNN values decreased to less than 44 ms during leukapheresis.

Although we cannot clearly explain the underlying mechanism of HRV alteration during leukapheresis, we speculate that such a major change in HRV indicators was induced by heightened sympathetic activity and parasympathetic withdrawal, mediated by a hemodynamical or neural effect of the leukapheresis procedure and/or other pathogenesis, including altered concentrations of electrolytes in serum and metabolic alkalosis [17]. Both r-MSSD and HF are known to reflect parasympathetic activity; thus the significant reduction in the r-MSSD value and HF power suggested parasympathetic activity was reduced during leukapheresis. It has been reported that

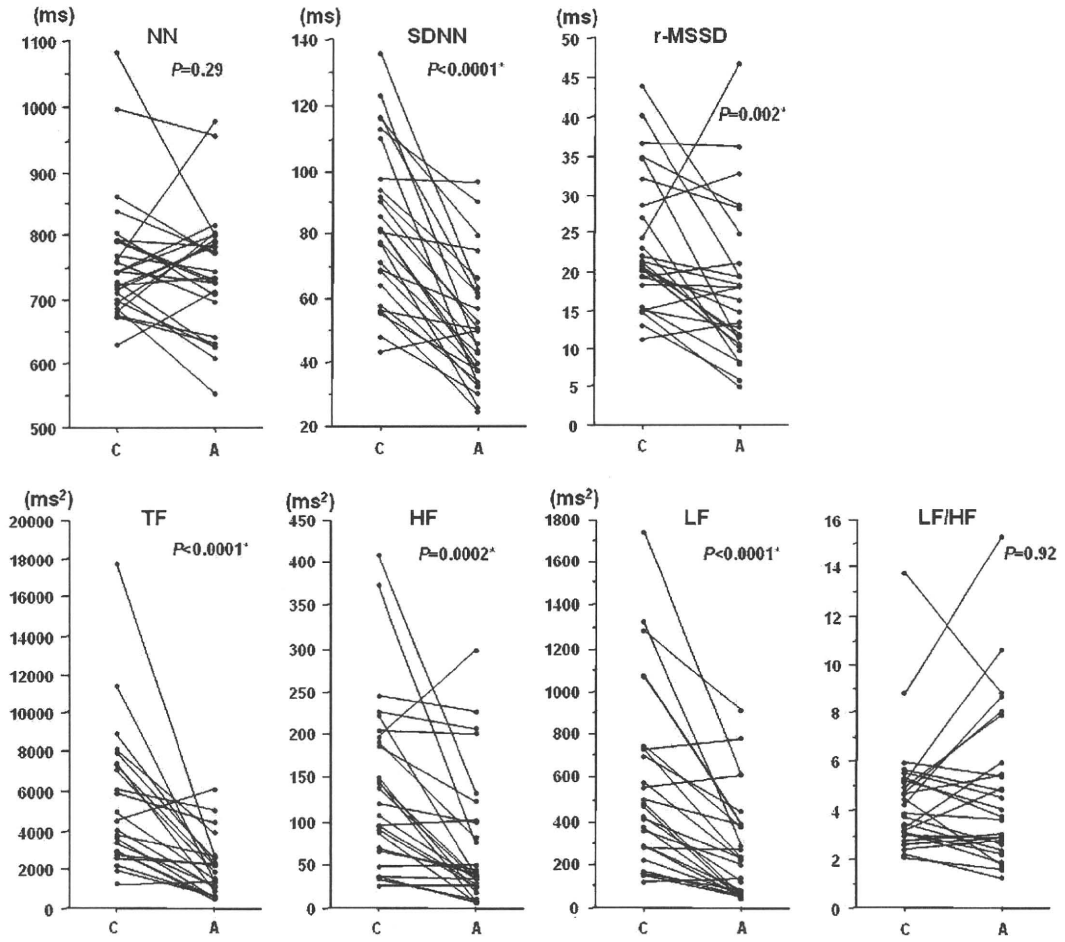

Fig. 2 Comparison of control data and heart rate variability (HRV) indicator values during leukapheresis. Many HRV indicators decreased significantly during leukapheresis. *C* control HRV values, *A* HRV values during leukapheresis, *NN* normal-to-normal intervals, *SDNN* standard deviation of normal-to-normal intervals, *r-MSSD* square root of mean of sum of squared differences between adjacent normal-to-normal intervals, *TF* total frequency, *HF* high frequency, *LF* low frequency

parasympathetic withdrawal is seen in patients with congestive heart failure and parasympathetic withdrawal causes a decrease in HRV [18]. Therefore, we speculated that the suppression of parasympathetic activity might be causally related to critical cardiovascular complications in PBSCH.

In this study, LF power also significantly decreased during leukapheresis. In recent reports, reduced LF spectral power was also identified as a risk of all-cause mortality [19] and sudden cardiac death [20] in chronic heart failure. LF power is more complicated because it is jointly mediated by the sympathetic and parasympathetic nervous systems [21]. Reduced R–R interval variability and parasympathetic activity withdrawal might also be associated with reduced LF power.

In addition, citrate-based anticoagulants, such as the ACD solution used for leukapheresis, decrease the concentration of electrolytes in serum by chelation and cause hypocalcemia, hypomagnesemia [22] and intermittent hypotension [23] in leukapheresis. A previous report showed that electrolyte abnormalities mediated by citrate, such as hypocalcemia, may change HRV [24].

Life-threatening complications associated with PBSC donation reportedly occur after, rather than during, leukapheresis [2, 3]. Notably, our data showed that abnormal HRV indicators persisted 6–9 h after leukapheresis

Table 1 Changes in HRV indicators following leukapheresis

	0–3 h [Median (IQR)]			3–6 h [Median (IQR)]			6–9 h [Median (IQR)]		
	Control	Post-leukapheresis	*P*	Control	Post-leukapheresis	*P*	Control	Post-leukapheresis	*P*
SDNN (ms)	92 (74–110)	75 (65–82)	0.002*	96 (82–110)	69 (60–86)	0.002*	86 (74–103)	75 (60–91)	0.01*
r-MSSD (ms)	26 (15–37)	23 (13–31)	0.002*	30 (24–37)	19 (12–34)	0.001*	33 (22–42)	28 (15–42)	0.34
TF (ms^2)	5996 (3861–10229)	4291 (2925–5188)	0.02*	5723 (3408–8008)	3772 (2760–6092)	0.04*	4909 (3647–7392)	3097 (2556–5145)	0.01*
HF (ms^2)	157 (44–266)	107 (27–196)	0.02*	215 (83–300)	74 (28–235)	0.002*	277 (120–430)	180 (46–417)	0.22
LF (ms^2)	489 (382–751)	471 (136–685)	0.04*	523 (340–846)	287 (160–605)	0.0008*	688 (387–879)	337 (190–579)	0.0004*
LF/HF	3.6 (2.9–5.1)	4.0 (3.3–5.8)	0.27	2.9 (2.3–4.5)	3.1 (2.1–7.3)	0.10	2.6 (1.5–5.5)	2.3 (1.1–3.6)	0.15

IQR interquartile range, *SDNN* standard deviation of normal-to-normal intervals, *r-MSSD* square root of mean of sum of squared differences between adjacent normal-to-normal intervals, *TF* total frequency, *HF* high frequency, *LF* low frequency

* $P < 0.05$

(Table 1). The altered concentration of electrolytes in serum, mediated by ACD solution or hypovolemia, which remained long after leukapheresis, might cause symptomatic hypotension and reduce HRV. Such pathologic conditions might, in extremely rare instances, lead to severe cardiovascular complications in a patient with latent cardiovascular diseases.

In the 3 subjects with symptomatic hypotension, tachycardia was not observed at the onset of hypotension (Subject 1: 82/46 mmHg, 72 beats/min; Subject 2: 72/42 mmHg, 66 beats/min; Subject 3: 76/48 mmHg, 60 beats/min). Vasovagal hypotension, which occasionally occurs during leukapheresis, is a neurally mediated reaction due to blood pressure decreases without compensatory tachycardia. In patients with vasovagal hypotension, bradycardia is therefore often observed. We therefore speculate that vasovagal reflex played a critical role in the development of symptomatic hypotension in these subjects.

Finally, some HRV indicators in the autologous transplant patients were significantly lower than those in healthy donors. In the present study, ages were higher and Hb levels before leukapheresis were lower in autologous transplant patients than in the healthy donors. Furthermore, all patients scheduled to receive an autologous transplant had a history of chemotherapy. Chemotherapy including anthracycline has been reported to reduce the values of HRV indicators [25]. Therefore, decreased values of some HRV indicators might have been caused by advanced age, anemia and/or the cumulative toxicity of chemotherapy, especially that caused by anthracycline drugs.

The major obstacle which precludes translating HRV analysis into clinical practice is that we can analyze HRV during leukapheresis only retrospectively. However, our data suggest that HRV prior to leukapheresis might have potential as a useful non-invasive tool for predicting autonomic or cardiovascular complications. Therefore, in the future, we need to examine the prognostic value of HRV for autonomic or cardiovascular complications in more detail by using a larger cohort.

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Conflict of interest statement All the authors declare no conflict of interest.

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●原 著●

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A Modified Myeloablative Conditioning Regimen for Allogeneic Hematopoietic Stem Cell Transplantation, Consisting of Intravenous Busulfan, Cyclophosphamide and Total Lymphoid Irradiation, in Advanced Leukemia: Hirohisa Nakamae, Yoshiki Terada, Takahiko Nakane, Hideo Koh, Mika Nakamae, Ran Aimoto, Asao Hirose, Yoshiki Hayashi, Mitsutaka Nishimoto, Eri Inoue, Takuro Yoshimura, Atsushi Inoue, Ki-Ryang Koh, Takahisa Yamane and Masayuki Hino (*Dept. of Hematology, Graduate School of Medicine, Osaka City University*)

Summary

In nine patients with advanced acute or chronic leukemia, we performed allogeneic hematopoietic stem cell transplantation (HSCT) following a modified myeloablative conditioning regimen intended to optimize the intensity of conditioning. This regimen consisted of intravenous busulfan 8 mg/kg, cyclophosphamide 120 mg/kg and total lymphoid irradiation 7.5 Gy. The median age of the patients was 30 years (range 18-59). Stem cell sources were related bone marrow in two, related peripheral blood in one, and unrelated bone marrow in six patients. Prophylaxis against acute graft-versus-host disease (GVHD) was cyclosporine and short-term methotrexate. Acute GVHD appeared in six patients (67%), grade II in all. Extensive chronic GVHD occurred in three of seven evaluable patients. The median follow-up period after HSCT was 813 days (248-1,702). Of nine patients, five relapsed or progressed after HSCT. However, no patient relapsed or progressed within 100 days after HSCT. During the full follow-up period, transplant-related mortality (TRM) was not observed. The two-year overall survival and event-free survival were 88.9% and 50.0%, respectively. Our results suggested that we might reduce the incidence of TRM and simultaneously control disease by using an optimized conditioning regimen for HSCT. **Key words**: Modified myeloablative conditioning regimen, Intravenous busulfan, Total lymphoid irradiation (TLI) (*Received Dec. 28, 2009/ Accepted Mar. 3, 2010*)

要旨 同種造血幹細胞移植の移植前処置の強度の最適化を検討する目的で静注 busulfan 8 mg/kg＋cyclophosphamide 120 mg/kg＋total lymphoid irradiation（TLI）7.5 Gy による modified myeloablative conditioning regimen にて進行期の急性，慢性白血病9例に対して同種造血幹細胞移植を行った。年齢の中央値は30（18～59）歳。造血幹細胞源は血縁骨髄2例，血縁末梢血幹細胞1例，非血縁骨髄6例。急性 graft-versus-host disease（GVHD）予防は cyclosporin＋短期 methotrexate 療法で行った。急性 GVHD は9人中6例（67%）に出現し，全例で grade 2 であった。広範型慢性 GVHD は評価可能症例7例中3例に認められた。移植後観察期間中央値は813（248～1,702）日で，9例中5例で移植後に原疾患の再発，再燃を認めたが，移植後100日以内の再発例はなかった。移植全観察期間を通じて移植関連死亡は認めなかった。2年の全生存率は88.9%，無病生存率は50.0%であった。今回の結果から骨髄破壊的前処置を最適化することによって，移植関連死亡を減少させると同時に病勢をコントロールし得る至適な移植前処置が存在する可能性があると考えられた。

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