and 300 ng/ml. After September 1999, CsA was administered as a CIF. The dose of CsA was adjusted to maintain the blood CsA concentration between 250 and 400 ng/ml. CsA concentration was measured at least once a week by fluorescence polarization immunoassay with a specific monoclonal antibody, using whole blood samples.[2] Metabolites of CsA were not measured by this method. The route of CsA administration was converted to oral at a ratio of 1:2 or 1:3 when patients were able to tolerate oral intake at least 3 weeks after transplantation. Acute GVHD was graded as previously described.[3] Prophylaxis against bacterial, fungal, and pneumocystis carinii infection consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim. As prophylaxis against herpes simplex virus infection, acyclovir was given from day −7 to 35. Pre-emptive therapy for cytomegalovirus infection was with ganciclovir, while monitoring cytomegalovirus antigenemia.[4]

Statistical considerations

Standard-risk disease was defined as acute leukemia in complete remission, chronic myelocytic leukemia in the first chronic phase, chemosensitive lymphoma, and myelodysplastic syndrome comprising refractory anemia or refractory anemia with ringed sideroblasts, while others were considered high-risk diseases. Renal dysfunction was defined as an elevation in serum creatinine level to above × 1.5 or × 2.0 the baseline value, except for that clearly caused by the administration of amphotericin B. Patients who received both BM and PBSC grafts were included in the PBSC group.

Probabilities and continuous variables in the two groups were compared using Fisher's exact test and the Mann–Whitney *U*-test, respectively. Cumulative incidences of acute GVHD and relapse were calculated using Gray's method, considering death without acute GVHD or relapse, as a competing risk.[5] Disease-free survival was estimated using the Kaplan–Meier method. Potential confounding factors considered in the analysis were age, sex, donor type (related or unrelated), stem cell source (BM or PBSC), disease risk, conditioning regimen, HLA mismatch, total dose of MTX, and mode of CsA administration.

Results

Patient characteristics

Of the 129 patients analyzed, 58 and 71 patients were in the TD and CIF groups, respectively. The CIF group included a significantly higher proportion of patients with high-risk disease ($P=0.021$), those transplanted from an unrelated donor ($P=0.004$), those who received an HLA-mismatched graft ($P=0.023$), and those who received a PBSC graft ($P=0.0061$) (Table 1). The total dose of MTX was significantly lower in the CIF group (median dose 35 mg/m^2 *vs* 33 mg/m^2, $P=0.0002$). Other characteristics were equivalent between the two groups.

Risk factors for grade II–IV acute GVHD

First, we performed a univariate analysis to evaluate the impact of potential confounding factors on the incidence of grade II–IV acute GVHD. As shown in Table 2, transplant from an unrelated donor, the presence of HLA mismatch, the use of a TBI-containing regimen, a lower total dose of MTX, and CIF of CsA were identified as significant risk factors for the development of grade II–IV acute GVHD. The incidence of grade II–IV acute GVHD in the CIF group (56%) was significantly higher than that in the TD group (27%, $P=0.00022$, Figure 1). Next, we performed a multivariate analysis using the backward stepwise selection method to identify independent risk factors for the development of grade II–IV acute GVHD. Only two factors, CIF of CsA (relative risk 2.59; 95% CI 1.46–4.60, $P=0.0011$) and the presence of HLA mismatch (2.01; 95% CI 1.15–3.53, $P=0.014$), were identified as independent significant risk factors (Table 3A). The impact of these two factors was significant even when adjusted for the total dose of MTX and donor type (Table 3B).

Table 1 Characteristics of the patients

	TD group (n=58)	*CIF group (n=71)*	*P-value*
Sex			
Male	42	53	0.84
Female	16	18	
Age			
<40 years	36	36	0.22
⩾40 years	22	35	
Risk			
Standard	39	33	0.021
High	19	38	
Donor			
Related	42	33	0.004
Unrelated	16	38	
HLA			
Match	50	9	0.023
Mismatch	8	22	
Stem-cell BM	57	59	0.0061
PBSC	1	12	
Regimen non-TBI	19	17	0.33
TBI	39	54	0.33

[a]BM = bone marrow, PBSC = peripheral blood stem cell, TBI = total body irradiation.

Renal toxicity

Renal dysfunction was significantly less frequent in the CIF group than the TD group: 27% *vs* 66% ($P<0.0001$) and 13% *vs* 41% ($P=0.0002$), when we defined renal dysfunction as an elevation of the serum creatinine level to above × 1.5 and × 2.0 the baseline value, respectively (Table 4).

Actual daily dose of CsA

We adjusted the dose of CsA to maintain the target blood level as described above. We compared the actual daily dose of CsA, excluding patients who were converted to oral administration. The actual daily dose of CsA in the CIF

Table 2 Impact of pretransplant factors on the incidence of acute GVHD by univariate analyses

	Incidence of acute GVHD	*P-value*
Sex		
Male	45%	0.55
Female	38%	
Age		
<40 years	44%	0.82
⩾40 years	42%	
Risk		
Standard	37%	0.15
High	51%	
Donor		
Related	36%	0.014
Unrelated	54%	
HLA		
Match	37%	0.0026
Mismatch	63%	
Stem cell		
BM	42%	0.33
PBSC	55%	
Regimen		
Non-TBI	30%	0.041
TBI	48%	
MTX		
<35 mg/m^2	60%	0.0025
⩾35 mg/m^2	36%	
CsA		
TD	27%	0.00022
CIF	56%	

BM = bone marrow, PBSC = peripheral blood stem cell, TBI = total body irradiation, MTX = methotrexate, CsA = cyclosporine A, TD = twice-daily infusion, CIF = continuous infusion.

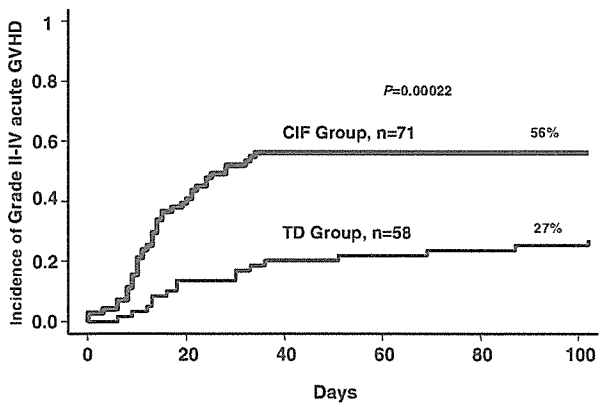

Figure 1 Cumulative incidence of grade II–IV acute GVHD grouped by the mode of CsA administration (TD = twice-daily infusion, CIF = contin-continuous infusion).

group was consistently significantly lower than that in the TD group during the first 4 weeks after transplantation (Figure 2).

Table 3 Impact of pretransplant factors on the incidence of acute GVHD by multivariate analysis: (a) independent significant risk factors identified by multivariate analysis using backward stepwise selection; (b) impact of HLA mismatch and the mode of cyclosporine A administration adjusted for the total methotrexate dose and the donor type (CsA = cyclosporine A, MTX = methotrexate, TD = twice-daily infusion, CIF = continuous infusion)

		Relative risk (95% CI)	*P-value*
	(A)		
HLA	Mismatch *vs* match	2.01 (1.15–3.53)	0.014
CsA	CIF *vs* TD	2.59 (1.46–4.60)	0.0011
	(B)		
HLA	Mismatch *vs* match	1.89 (1.04–3.45)	0.038
CsA	CIF *vs* TD	1.98 (0.98–4.00)	0.056
MTX	⩾35 mg/m^2 *vs* <35 mg/m^2	1.51 (0.80–2.87)	0.20
Donor	Unrelated *vs* related	1.36 (0.78–2.38)	0.28

Table 4 Difference in the incidence of renal dysfunction by the mode of cyclosporine A administration (TD = twice-daily infusion, CIF = continuous infusion)

		(−)	*(+)*	*P-value*
Incidence of serum creatinine >1.5 × baseline value				
CsA	TD	20	38 (66%)	<0.001
	CIF	52	19 (27%)	
Incidence of serum creatinine >2.0 × baseline value				
CsA	TD	34	24 (40%)	0.0002
	CIF	62	9 (13%)	

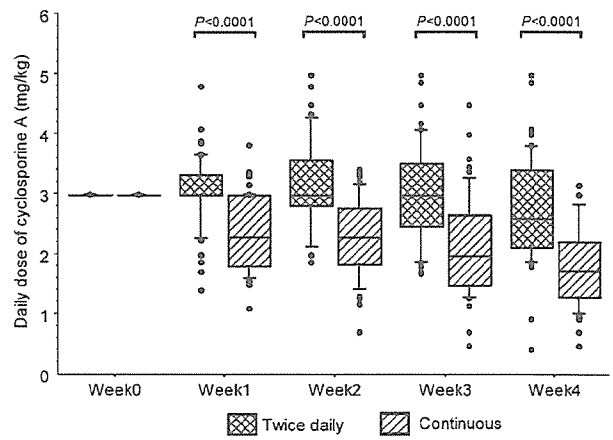

Figure 2 Actual daily dose of CsA grouped by the mode of administration. The box-and-whisker plot shows 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by dots.

Transplant outcome

The CIF of CsA was shown to significantly decrease the incidence of relapse, after adjusting for disease status before transplantation (relative risk 0.41, 95% CI 0.18–0.95, $P = 0.038$). This resulted in significantly better disease-free

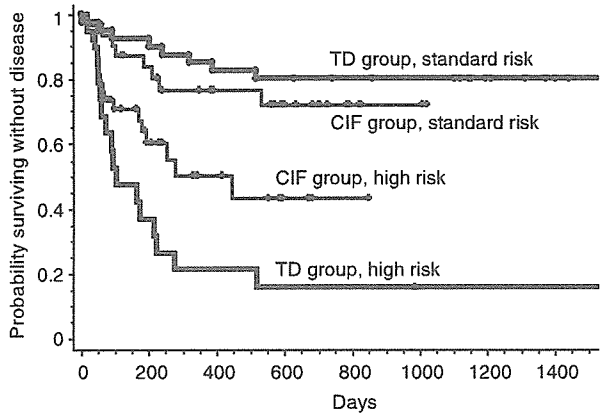

Figure 3 Disease-free survival grouped according to the mode of CsA administration, stratified by the disease status (TD = twice-daily infusion, CIF = continuous infusion).

survival in the CIF group than in the TD group among high-risk patients (43 *vs* 16% at 2 years, $P=0.039$, Figure 3), whereas there was no significant difference in disease-free survival between the two groups among standard-risk patients (72 *vs* 80% at 2 years, $P=0.45$).

Discussion

To summarize the findings of this study, the CIF of CsA with a target level of 250–400 ng/ml significantly increased the incidence of grade II–IV acute GVHD, but significantly decreased the incidences of renal dysfunction and relapse, which resulted in better disease-free survival in high-risk patients. However, disease-free survival was not improved in standard-risk patients, probably because the incidence of relapse was originally low in these patients. Therefore, this mode of CsA administration may not be appropriate for standard-risk patients.

There are at least two possible explanations for why the incidence of acute GVHD was higher in the CIF group. First, the total dose (or the area under the curve) of CsA may be important. Second, it may be important to achieve a peak CsA concentration. It was impossible to draw a definite conclusion from this study. However, considering that the actual daily dose of CsA was gradually decreased in the CIF group after transplantation, a target level of 250–400 ng/ml might be too low to prevent GVHD adequately, although this target level has been used in recent large randomized controlled trials.[6,7] Miller *et al*[8] adjusted the dose of CsA as a CIF to maintain the blood CsA level between 450 and 520 ng/ml. In this setting, the mean actual dose of CsA was maintained between 2.87 and 3.15 mg/kg during the first 4 weeks after transplantation. Therefore, this higher target level may be more appropriate when comparing the mode of CsA administration, although it needs to be confirmed by measuring the area under the curve.

The major shortcoming of this study was that this was not a randomized controlled trial and there were some uncontrolled variables that might have caused bias. However, the impact of the mode of CsA administration remained significant after adjusting for these uncontrolled variables, as shown in Table 3B. We are planning a randomized controlled trial to confirm these results.

Acknowledgements

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Post-transplant complications

Predictors for severe cardiac complications after hematopoietic stem cell transplantation

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Summary:

The value of pre-transplant factors for predicting the development of cardiac complications after transplantation has been inconsistent among studies. We analyzed the impact of pre-transplant factors on the incidence of severe cardiac complications in 164 hematopoietic stem cell transplant recipients. We identified eight patients (4.8%) who experienced grade III or IV cardiac complications according to the Bearman criteria. Seven died of cardiac causes a median of 3 days after the onset of cardiac complications. On univariate analysis, both the cumulative dose of anthracyclines and the use of anthracyclines within 60 days before transplantation affected the incidence of severe cardiac complications ($P = 0.0091$ and 0.011). The dissociation of heart rate and body temperature, which reflects 'relative tachycardia', was also associated with a higher incidence of cardiac complications ($P = 0.024$). None of the variables obtained by electrocardiography or echocardiography were useful for predicting cardiac complications after transplantation, although the statistical power might not be sufficient to detect the usefulness of ejection fraction. On a multivariate analysis, the cumulative dose of anthracyclines was the only independent significant risk factor for severe cardiac complications. We conclude that the cumulative dose of anthracyclines is the most potent predictor of cardiac complications and the administration of anthracyclines should be avoided within two months before transplantation.

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Cardiac complications due to conditioning regimens are well recognized, and these include congestive heart failure, fatal arrhythmia, and cardiac tamponade. The incidence of such complications has varied among studies, from less than 1% to more than 26%.[1,9] The use of high-dose cyclophosphamide in the conditioning regimen has been considered to be the main cause of cardiac toxicity.[2,3,6,9] On the other hand, the usefulness of pre-transplant cardiologic evaluation for predicting cardiac complications is still controversial.[1,2,4,8,9] Braverman *et al*[3] and Fujimaki *et al*[5] showed that the incidence of severe cardiac complications was higher among patients with a low ejection fraction (EF), while Hertenstein *et al* found that there was no correlation between pre-transplant cardiac function and the development of life-threatening cardiac events.[8,9] Recently, Nakamae *et al*[8] and Akahori *et al*[1] reported that QTc dispersion and QTc interval, respectively, were good predictors for cardiac complications, which suggested that electrocardiography (ECG) before transplantation may be useful. In this study, we analyzed the impact of variables obtained by ECG and echocardiography (ultrasound cardiography; UCG) on the incidence of life-threatening cardiac complications after hematopoietic stem cell transplantation.

Patients and methods

Patients

Of the 207 adult patients who underwent hematopoietic stem cell transplantation for the first time between June 1995 and March 2003 at the University of Tokyo Hospital, Japan, we retrospectively reviewed the records of 164 patients for whom a standard 12-lead ECG and UCG within 3 months before transplantation was available. Patient characteristics are shown in Table 1. Acute leukemia in first or second remission, chronic myeloid leukemia in chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, lymphoma or solid cancers in remission, and severe aplastic anemia were defined as standard-risk diseases, while others were considered high-risk diseases. In all, 132 patients underwent allogeneic transplantation, while 31 and one underwent autologous and syngeneic transplantation, respectively. Cyclophosphamide at more than 100 mg/kg was used in 129 patients (79.9%) and ifosfamide at 12 g/m^2 was used in one patient (0.6%). Total body irradiation was applied in 89 patients (54.2%).

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Table 1 Patient characteristics

	Severe cardiac complications		*P*-value
	Positive ($n=8$)	*Negative* ($n=156$)	
Sex (M/F)	3/5	102/54	0.14
Age > 40 years	3/8 (37.5%)	79/156 (50.6%)	0.72
Disease status (standard/high)	4/4	94/62	0.72
History of cardiac disease	0/8 (0%)	13/153 (8.4%)	>0.99
Ferritin level	738 (96.5–1379.9)	632.4 (204.9–1469.7)	0.40
Cumulative dose of anthracyclines			
Low (0–200 mg/m^2)	1	75	0.0031*
Intermediate (201–400 mg/m^2)	2	53	
High (>400 mg/m^2)	5	24	
Anthracycline within 60 days	5/8 (62.5%)	27/142 (19.0%)	0.011*
Radiation involving heart	1/8 (12.5%)	8/152 (4.6%)	0.39
ECG			
ECG abnormality	25.0%	14.7%	0.35
QT interval (ms)	370.3 (302.6–438.0)	379.1 (347.5–410.7)	0.47
QTc interval (ms)	416.4 (369.4–463.4)	424.4 (396.5–452.3)	0.45
QT dispersion (ms)	45.1 (28.2–62.0)	51.3 (30.4–72.2)	0.44
QTc dispersion (ms)	50.9 (33.3–68.5)	57.4 (34.0–80.8)	0.45
UCG			
EF (< 55%)	2/8 (25.0%)	16/142 (11.2%)	0.20
LAD (mm)	31.3 (23.5–39.1)	32.8 (26.9–38.7)	0.48
LVDd (mm)	48.0 (44.0–52.0)	48.0 (43.3–52.7)	0.99
LVDs (mm)	33.6 (28.7–38.5)	31.4 (26.9–35.9)	0.18
IVSth (mm)	8.5 (7.1–9.9)	8.8 (7.3–10.3)	0.55
PWth (mm)	8.4 (7.1–9.7)	8.6 (7.1–10.1)	0.64
E/A ratio	1.51 (0.71–2.31)	1.41 (0.83–1.99)	0.75
Vital			
Heart rate (beats/min)	80 (57–103)	76 (63–90)	0.50
Systolic blood pressure (mmHg)	108 (82–134)	112 (99–124)	0.46
HR–BT index (>25)	3/8 (37.5%)	8/138 (7.2%)	0.024*
Regimen			
Includes high-dose Cy or IFM	62.5%	80.8%	0.20
Includes TBI	4/8 (50.0%)	85/156 (54.5%)	>0.99
Stem cell			
Auto/allo	3/5	28/128	0.17
Bone marrow/peripheral blood	5/3	94/62	>0.99

*Statistically significant.

Evaluation of pre-transplant factors

QT intervals were measured manually from the beginning of the QRS complex to the end of the T wave. The average of two consecutive QT intervals was calculated as the QT interval for each lead and the QT interval for each patient was calculated as the mean QT interval of all available leads. QT dispersion was defined as the difference between the longest and shortest QT interval. Each value was corrected with Bazett's formula. QT dispersion could not be determined in three patients either because there were fewer than six readable leads ($n=2$) or due to frequent premature ventricular contractions ($n=1$).

Left ventricular EF and the E/A mitral Doppler ratio were evaluated by UCG as indices of systolic and diastolic functions, respectively. The cutoff of EF was determined as 55%, because the best *P*-value was obtained at this cutoff by univariate analyses. The following variables were also evaluated: left atrial dimension (LAD), left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), end-diastolic intraventricular septal thickness (IVth), and left ventricular posterior wall thickness (PWth).

Blood pressure, heart rate (HR), and body temperature (BT) were calculated as the means of respective values measured on 2 consecutive days prior to the conditioning regimen. The dissociation of HR and BT, called the HR–BT index, was calculated to evaluate 'relative tachycardia' as follows, assuming that the normal HR was 80 beats/min at 37°C and increased by 20 beats/min with an increase in BT of 1°C:

$$\text{HR–BT (beats/min)} = \text{HR (beats/min)} - [80 + (\text{BT (°C)} - 37) \times 20]$$

The mean HR–BT calculated from values measured on 2 consecutive days was used for the analysis.

The cumulative dose of anthracyclines was calculated as the equivalent dose of native doxorubicin, assuming that the cardiac toxicity at an equal dose is 0.5, 0.8, 3.4, 0.6, 1.6, and 0.1 for daunorubicin, pirarubicin, mitoxantrone,

epirubicin, idarubicin, and aclarubicin, respectively.[10 13] The dose of anthracyclines was then categorized into low (0–200 mg/m^2), intermediate (201–400 mg/m^2), and high (>401 mg/m^2) groups. Other potential confounding factors considered in the analysis included the history of irradiation involving the heart, presence or absence of anthracycline administration within 60 days before transplantation, and pre-transplant serum ferritin levels.

Evaluation of regimen-related cardiac toxicity

Regimen-related cardiac toxicity was graded according to Bearman grade.[2] Only cardiac complications that developed within 28 days after transplantation were considered regimen-related cardiac toxicity. Grade III–IV cardiac complications were defined to be severe.

Statistical analysis

For univariate analyses, continuous variables in the two groups were compared using the unpaired *t*-test or the Mann–Whitney *U* test, whereas categorical variables were compared using the χ^2 test or Fisher's exact test. Factors associated with at least borderline significance ($P<0.10$) on univariate analysis were subjected to a multivariate analysis using backward stepwise logistic regression. *P* values of less than 0.05 were considered statistically significant.

Results

Severe cardiac complications after transplantation

Eight patients (4.9%) developed grade III–IV cardiac complications within 28 days after transplantation (Table 1). Characteristics of the eight patients are shown in Table 2. Manifestation of cardiac complications was mainly pulmonary congestion in five (patients 1, 2, 3, 7, and 8), while two had severe hypotension (patients 4 and 5). All had primary cardiac dysfunction, not secondary to other causes. Five developed cardiac toxicity during the preparative regimen and three of them died prior to hematopoietic stem cell transplantation. The remaining three patients developed cardiac toxicity 5, 6, and 11 days after transplantation, respectively. Seven died of cardiac causes a median of 3 days (range 0–45 days) after the onset of cardiac complications.

Risk factors for severe cardiac complications

The relationships between possible confounding factors and the development of severe cardiac complications are shown in Table 1. Patient age, sex, and disease status were not associated with cardiac complications. None of the patients who developed cardiac complications had a history of cardiac disease before transplantation, while 12 of 153 patients who did not develop such complications had a prior history of cardiac disease (0% *vs* 8.4%, $P>0.99$), including angina pectoris in two, arrhythmia in six, congestive heart failure in one, leukemic infiltration of the heart in two, and surgery for tetralogy of Fallot in one. Ten patients had diabetes mellitus, five had hypertension, and one had hyperlipidemia before transplantation, but none of them developed cardiac complications after transplantation. As for prior treatments, both the cumulative dose of anthracyclines and the use of anthracyclines within 60 days before transplantation affected the incidence of severe cardiac complications ($P=0.0091$ and 0.011, respectively; Figure 1).

There was no difference in the ECG findings, including QTc interval and QTc dispersion, between those who developed severe cardiac complications and those who did

Table 2 Cardiac complications during the first 30 days

	Age/sex	*Disease status*	*Anthracycline dose (mg/m^2)*	*EF (%)*	*Conditioning regimens*	*Onset*	*Outcome (Bearman grade)*
1	30/F	ALL CR2	1054	52	ETP 40 mg/kg CY 40 mg/kg fTBI 12 Gy/6	day 5	Severe CHF (IV), died on day 31
2	42/F	AML CR2	>800	45	BU 16 mg/kg FLU 120 mg/m^2	day −3	Severe CHF (IV), died on day 36
3	27/M	NHL NR	465	55	L-PAM 140 mg/m^2 BU 8 mg/kg fTBI 12 G/6 fr	day 6	Severe CHF (IV), died on day 51
4	24/M	GCT PR	0	79	IFM 12 g/m^2 CBDCA 1600 mg/m^2 ETP 1600 mg/m^2	day −4	Cardiogenic shock, (IV), died on day -2
5	31/F	NHL CR2	384	65	CBDCA 1600 mg/m^2 ETP 1600 mg/m^2 CY 100 mg/kg AraC 4 g/m^2	day −1	Cardiogenic shock, (IV), died on day-1
6	55/M	ALL CR1	307	59	CY 120 mg/kg fTBI 12 Gy/6 fr	day −1	Cardiac tamponade, (IV), died on day 2
7	46/F	NHL NR	520	69	CBDCA 1600 mg/m^2 ETP 1600 mg/m^2 CY 100 mg/kg AraC 4 g/m^2	day −2	Severe CHF, (IV), died on day −1
8	38/F	AML CR2	408	63	CY 120 mg/kg fTBI 12 Gy/6 fr	day 11	Severe CHF, (III), alive on day 686

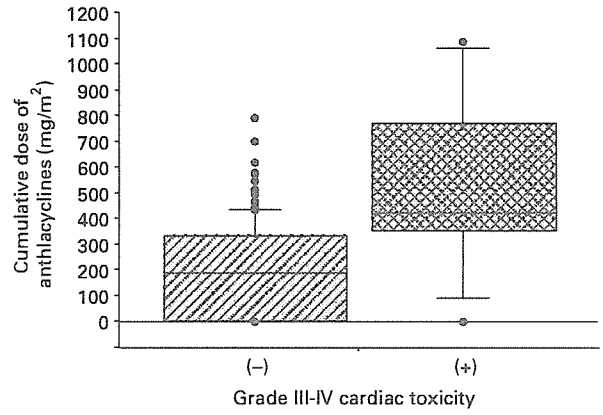

Figure 1 The cumulative dose of anthracyclines was compared in patients who developed grade III–IV cardiac complications and others. The box-and-whisker plot shows 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by dots.

not. Furthermore, no difference was observed in the UCG findings, including EF and the E/A ratio, between the two groups. Impaired EF was more frequently observed in patients who developed severe cardiac complications, but the difference was not statistically significant (25% *vs* 11.2%, $P=0.25$). Heart rate and systolic blood pressure before transplantation were not correlated with the incidence of severe cardiac complications. However, a high HR–BT index, which reflected 'relative tachycardia', was associated with a higher incidence of cardiac complications (37.5% *vs* 7.2%, $P=0.024$).

The use of high-dose cyclophosphamide or ifosfamide was less frequent in patients who developed severe cardiac complications. This may have been because high-dose cyclophosphamide tended to be avoided in patients who were considered to be at higher risk for severe cardiac complications. The use of TBI did not affect the incidence of cardiac complications ($P>0.99$).

By multivariate analysis, the cumulative dose of anthracyclines was identified as the only independent significant risk factor for severe cardiac complications, with an odds ratio of 4.33 (95% CI 1.48–12.7, $P=0.0075$) for changes between categories.

Discussion

Cardiac toxicity due to the conditioning regimen is a well-recognized complication after hematopoietic stem cell transplantation.[2,3,9] The incidence of severe cardiac complications was 4.9% in this series, which is consistent with the values in previous reports (0.9%–26%).[1,9] We found that the cumulative dose of anthracyclines correlated independently with the development of grade III–IV cardiac complications. Five of the 29 patients (17.2%) who had received more than 400 mg/m^2 of anthracyclines developed severe cardiac complications. Furthermore, among this population, four of the 12 patients (33%) who had received anthracyclines within 60 days before transplantation developed severe cardiac complications, whereas these were seen in only one of 14 patients (7.1%) who had not received anthracyclines within 60 days, although this difference was not statistically significant ($P=0.15$).

The predictive value of pre-transplant cardiac evaluation has been inconsistent among studies. Braverman *et al* and Fujimaki *et al* showed that a reduced pre-transplant EF could be a predictive factor,[3,5] while Hertenstein *et al* showed that the incidence of life-threatening cardiac toxicity was not significantly increased in patients with reduced EF.[9] In this study, the incidence of severe cardiac toxicity was higher in the reduced EF (<55%) group, but this difference was not statistically significant (11.1% *vs* 4.5%, $P=0.25$). It is possible that the number of patients was too small to detect the difference; the statistical power of this study to detect the difference was only 15%. In addition, we tended to use less toxic regimens for patients with a reduced pre-transplant EF. This might also explain why a high-dose cyclophosphamide regimen was used less frequently in patients who developed severe cardiac complications.

In this study, we closely analyzed the correlation between severe cardiac complications and variables obtained by ECG or UCG, but none were useful for predicting cardiac complications after transplantation, although the usefulness of EF should not be excluded. On the other hand, relative tachycardia as shown by a high HR–BT index may reflect reduced cardiac reserve. In fact, a high HR–BT was associated with a higher incidence of severe cardiac complications on univariate analysis. Although this was not confirmed by multivariate analysis, it may be worthwhile to further evaluate the impact of this variable, since the HR–BT index can be determined easily without any cost. As another marker for cardiac reserve, Zangari *et al* showed that the increment of EF during excise was useful to predict overall peritransplant mortality, suggesting that pre-transplant cardiac reserve may be important in predicting transplant outcome.[14]

In conclusion, patients who had received a high cumulative dose of anthracyclines, particularly more than 400 mg/m^2, were at the highest risk for severe cardiac complications. Clinical interventions to prevent cardiac toxicity, such as the use of reduced-intensity conditioning or angiotensin-converting enzyme inhibitor as a cardioprotectant, should be evaluated in such patients.[15] Also, the administration of anthracyclines should be avoided within 2 months before transplantation.

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Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program

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There is little information available regarding the outcome of unrelated bone marrow transplantation (BMT) for non-Hodgkin lymphoma (NHL). Therefore, we retrospectively analyzed the data of 124 patients who underwent unrelated BMT through the Japan Marrow Donor Program (JMDP) between July 1992 and August 2001. The overall survival (OS), progression-free survival (PFS), cumulative incidences of disease progression, and nonprogression mortality at 3 years after BMT were 49.7%, 42.6%, 24.5%, and 32.9%, respectively, with a median follow-up duration of 565 days among survivors. The incidence of grades II-IV acute graft-versus-host disease (GVHD) was 40.9%. Recipient age, previous history of autologous transplantation, and chemosensitivity at transplantation were independent prognostic factors for OS and PFS. The development of grades II-IV acute GVHD was associated with lower incidence of disease progression after transplantation, which suggested the existence of a graft versus lymphoma effect. Unrelated BMT should be considered as a treatment option for patients with high-risk NHL without an HLA-matched related donor. (Blood. 2004;103:1955-1960)

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Introduction

Hematopoietic stem cell transplantation for non-Hodgkin lymphoma (NHL) has been mainly performed using an autologous graft, because the incidence of treatment-related mortality after allogeneic transplantation is as high as 57%.[1] However, relapse is a frequent cause of treatment failure after autologous transplantation.[2,3] The lower relapse rate after allogeneic transplantation and the recent development of supportive treatments to decrease the risk of treatment-related mortality have facilitated the use of allogeneic transplantation for NHL.[1] However, an HLA-matched sibling is available for less than half of the patients. Transplantation from an unrelated donor is a possible alternative for patients who do not have a suitable related donor. To date, however, little information is available regarding the outcome of allogeneic transplantation from an unrelated donor for NHL. Therefore, we retrospectively analyzed the outcome of unrelated bone marrow transplantation for NHL using the database of the Japan Marrow Donor Program (JMDP). The purpose of this study was to elucidate the feasibility of unrelated bone marrow transplantation for NHL and to evaluate the impact of a potential graft-versus-lymphoma effect.

Patients and methods

Patients and transplantation procedure

From July 1992 to August 2001, 124 patients with non-Hodgkin lymphoma (NHL) underwent bone marrow transplantation from a serologically HLA-A, -B, and -DR matched unrelated donor identified through the Japan Marrow Donor Program (JMDP). The application of unrelated transplantation was decided at each center. Fourteen of 19 patients who underwent unrelated transplantation in the first complete remission (CR1) had high-grade lymphoma.

Transplantation was performed according to the protocol of each center, and therefore the conditioning regimen and graft-versus-host disease (GVHD) prophylaxis varied among patients (Table 1). However, 90% of the patients received a total body irradiation (TBI)–containing conditioning regimen. Prophylaxis against GVHD was performed with cyclosporine A or tacrolimus combined with methotrexate with or without corticosteroid in all but one patient. At transplantation, 60 patients were in complete remission (CR) and 60 were not (non-CR). Among the 43 patients whose CR status was reported in detail, 19, 18, 5, and 1 were in CR1, CR2, CR3, and CR4, respectively. Seventy-six patients had chemosensitive disease at transplantation, whereas 33 patients had chemoresistant disease. In this study we defined patients who achieved CR or partial remission (PR) before transplantation as chemosensitive, and patients with responses less than PR were defined as chemoresistant in the same way as previous studies.[4,5] Before unrelated donor transplantation, 18 of 101 patients had undergone high-dose therapy and autologous stem cell transplantation (HDT/ASCT). Information regarding previous treatments except for HDT/ASCT or the results of genomic typing were not available in the dataset.

Histology

The JMDP requested the histologic subtype of NHL according to a unique classification system that was a slight modification of the Working Formulation.[6] However, the respective centers used different classification systems, such as the Working Formulation, Kiel,[7] Lymphoma Study Group

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A complete list of the centers in Japan that participated in the bone marrow transplantations for non-Hodgkin lymphoma facilitated by the Japan Marrow Donor Program (JMDP) appears in the "Appendix."

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Table 1. Patient characteristics

Characteristic	Value
Sex, n, M/F	78/46
Median age at transplantation, y (range)	29 (1-59)
Median interval from diagnosis to transplantation, d (range)	470 (183-2329)
Histology, n	
Low-grade	10
Follicular lymphoma	9
Small lymphocytic lymphoma	1
Intermediate-grade	42
Peripheral T-cell lymphoma, unspecified	14
NK-cell lymphoma	12
Anaplastic large cell lymphoma	6
Diffuse large B-cell lymphoma	5
Angioimmunoblastic lymphoma	1
Mantle cell lymphoma	1
High-grade	60
Lymphoblastic lymphoma	39
Adult T-cell leukemia/lymphoma	15
Burkitt lymphoma	5
Unclassified	12
Previous history of HDT/ASCT, n	
Yes	18
No	83
ND	23
Disease status at transplantation, n	
CR	60 (CR1 19, CR2 18, CR3 5, CR4 1)
Non-CR	60
ND	4
Chemosensitivity at transplantation, n	
Sensitive	76
Resistant	33
ND	15
Conditioning regimen, n	
TBI-containing regimen	111
Non-TBI regimen	13
GVHD prophylaxis, n	
CsA ± MTX ± steroid	76
TCR ± MTX ± steroid	44
CsA + TCR ± MTX ± steroid	3
MTX alone	1

HDT/ASCT indicates high-dose therapy and autologous stem cell transplantation; CR, complete remission (CR1, CR2, CR3, CR4; the first, second, third, and fourth CR, respectively); ND, not described; TBI, total body irradiation; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; TCR, tacrolimus.

(LSG),[8] Revised European-American Classification of lymphoid neoplasms (REAL),[9] and World Health Organization (WHO) systems.[10] In this study, we grouped the histology into low grade, intermediate grade, and high grade as usually accepted in daily practice. There were 10, 42, and 60 patients with low-, intermediate-, and high-grade lymphoma, respectively. Histologic subtypes in detail are described in Table 1. The histologic subtype or grade was unclassified in 12 cases. Transplantation for lymphoblastic lymphoma (LBL) and adult T-cell leukemia/lymphoma (ATLL) was included as in other studies focusing on allogeneic transplantation for NHL.[11,12]

Data management and statistical considerations

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and annually after transplantation. Overall survival (OS) was defined as days from transplantation to death from any cause. Progression-free survival (PFS) was defined as days from transplantation to disease progression or death from any cause. Nonprogression mortality was defined as death without disease progression. Patients who were alive at the last follow-up date were censored. Survival was calculated using the Kaplan-Meier method. To evaluate the influence of confounding factors for survival, the log-rank test was used for univariate analyses and proportional hazard modeling was used for multivariate analyses. Cumulative incidences of acute GVHD and disease progression were calculated using the Gray method,[13] considering death without acute GVHD and death without disease progression as respective competing risks. The effects of acute and chronic GVHD on survival and disease progression were analyzed among patients who survived without disease progression at 60 and 150 days after transplantation, respectively.[14,15] This landmark method was used to exclude bias that may arise from including patients who died too early to develop GVHD in the group without GVHD.

Results

Survival and disease progression

Of the 124 patients, 69 were alive with a median follow-up duration of 565 days (range, 82 to 2217 days) after transplantation (Table 2). The overall 3-year OS and PFS were 49.7% and 42.6%, respectively (Figure 1A). Cumulative incidences of disease progression and nonprogression mortality at 3 years were 24.5% and 32.9%, respectively (Figure 1B). Disease progression was observed in 26 patients, and the median time from transplantation to disease progression was 109 days (range, 0 to 1079 days). Notably, only 1 patient developed disease progression more than 500 days after

Table 2. Transplantation outcome

	Value
Alive/dead, n	69/55
Median follow-up for survivors, d (range)	565 (82-2217)
Cause of death	
Progression, n	17
Median days after transplantation (range)	165 (2-1106)
Death without progression, n	36
Median days after transplantation (range)	72 (8-718)
GVHD, n	10
Infection, n	9
IP, n	6
VOD, n	3
Renal failure, n	2
ARDS, n	2
Others: pericarditis, hemorrhage, cerebral infarction, RRT, n	4
Not described, n	2
Disease progression, n	26
Median days after transplantation (range)	109 (0-1079)
Engraftment, n	
Engraftment	115
Rejection	2
Death within 20 days	7
Acute GVHD, n*	
Grade 0	31
Grade I	37
Grade II	30
Grade III	7
Grade IV	10
Chronic GVHD, n†	
None	47
Limited	17
Extensive	24
Not described	5

GVHD indicates graft-versus-host disease; IP, interstitial pneumonitis; VOD, venoocclusive disease; ARDS, acute respiratory distress syndrome; RRT, regimen-related toxicity.

*Acute GVHD was evaluated among patients who achieved engraftment and survived more than 20 days after transplantation.

†Chronic GVHD was evaluated among patients who survived more than 100 days after transplantation.

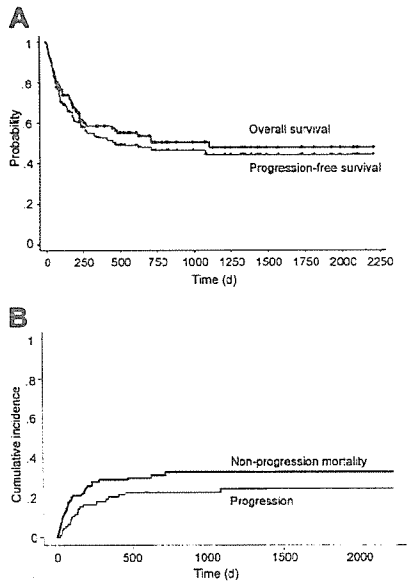

Figure 1. Survival, progression, and nonprogression mortality after transplantation. Overall survival, progression-free survival (A), and cumulative incidences of disease progression and nonprogression mortality (B) after unrelated bone marrow transplantation for non-Hodgkin lymphoma.

transplantation. The cause of death was related to disease progression in 17, whereas 36 died without disease progression (Table 2). The major cause of transplantation-related death within 100 days after transplantation was GVHD in 8, infection in 5, venoocclusive disease in 3, acute respiratory distress syndrome in 2, interstitial pneumonitis in 2, renal failure in 2, and other causes in 2.

Engraftment and GVHD

Seven patients died within 20 days after transplantation, and therefore engraftment could not be evaluated. In the others, 2 rejected the graft and 115 achieved engraftment. Among the latter 115 patients, 47 developed grades II-IV acute GVHD (Table 2) with a cumulative incidence of 47.5% (Figure 2). Seven and 10 patients experienced grade III and IV acute GVHD, respectively. Among the 93 patients who were alive at 100 days after transplantation, 17 and 24 developed limited and extensive chronic GVHD, respectively.

Influence of pretransplantation factors

We evaluated the effects of pretransplantation factors on OS after transplantation and identified 3 independent significant risk factors: chemosensitivity before transplantation (chemosensitive versus chemoresistant, relative risk 0.28, 95% confidence interval [CI] 0.15-0.52, $P < .0001$); previous history of HDT/ASCT (yes versus no, relative risk 0.40, 95% CI 0.20-0.79, $P = .0087$); and patient age (less than 40 years versus 40 years or more, relative risk 0.42, 95% CI 0.22-0.81, $P = .0092$) at transplantation (Tables 3 and 4; Figure 3A-C). These 3 factors were also identified as independent risk factors for PFS (data not shown), probably because only a few patients survived after disease progression and the OS and PFS curves were almost superimposed. We further analyzed the impact of disease status among patients who had chemosensitive disease at bone marrow transplantation; 19 were in first CR, 24 were in a later CR, and 16 were in PR. However, there was no significant difference in OS or PFS among them (data not shown). Among 13 deaths in patients with previous history of HDT/ASCT, 11 were from transplantation-related causes before day 100 (median, 56; range, 13 to 97 days after transplantation). Two patients died from

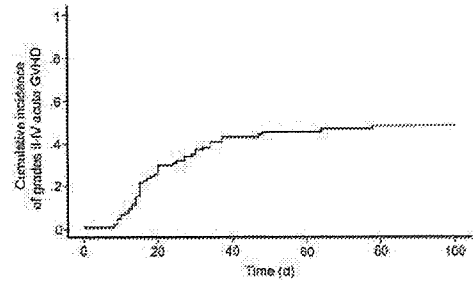

Figure 2. Cumulative incidence of grades II-IV acute graft-versus-host disease.

disease progression on day 48 and 458, respectively. However, 5 of 6 patients who survived more than 100 days after transplantation were progression free at a median follow-up of 1339 days (range, 493 to 2217 days) after transplantation. Furthermore, we evaluated the impact of histologic grade on outcome. However, there was no significant difference in OS, PFS, or cumulative incidence of disease progression among these histologic grades (Figure 3D and data not shown).

Influence of acute and chronic GVHD

We analyzed the relationship between the development of acute GVHD and the transplantation outcome. In this study, all but 2 patients developed acute GVHD before day 60. Thus, we defined day 60 as a landmark for this analysis. The cumulative incidence of disease progression at 3 years after transplantation was significantly lower in patients who developed grades II-IV acute GVHD (5.9% versus 33.2%, $P = .0053$; Figure 4B). This effect was preserved even when it was adjusted for the chemosensitivity before transplantation using proportional hazard modeling (relative risk 0.15, 95% CI 0.03-0.65, $P = .012$). This inverse correlation between the development of acute GVHD and disease progression

Table 3. Prognostic factors in univariate analyses

	3-year OS, %	P
Sex		.70
Male	47.7	
Female	53.1	
Age at transplantation		.0036
Less than 40 y	57.1	
40 y or more	28.7	
Histology		.80
Low grade	60.0	
Intermediate grade	53.2	
High grade	47.6	
Previous HDT/ASCT		.0006
Yes	27.8	
No	56.3	
Chemosensitivity		< .0001
Chemosensitive	63.2	
Chemoresistant	22.8	
Disease status		.0011
CR	61.4	
Non-CR	32.0	
Preparative regimen		.29
TBI-containing	50.8	
Non-TBI	40.0	
Days from diagnosis to transplantation		.53
Less than 365 d	44.8	
365 d or more	52.1	

OS indicates overall survival; HDT/ASCT, high-dose therapy and autologous stem cell transplantation; CR, complete remission; TBI, total body irradiation.

Table 4. Prognostic factors in multivariate analysis

	Relative risk	95% CI	P
Age less than 40 y	0.42	0.22-0.81	.0092
No previous HDT/ASCT	0.40	0.20-0.79	.0087
Chemosensitive disease	0.28	0.15-0.52	< .0001

CI indicates confidence interval.

suggested the existence of a graft-versus-lymphoma (GVL) effect. However, there was no significant difference in 3-year OS (61.4% versus 58.8%, $P = .63$; Figure 4A) or PFS (58.9% versus 48.9%, $P = .28$) between patients with and without grades II-IV acute GVHD. When we classified patients into those who developed grades III-IV acute GVHD and those who did not, there was a trend for lower incidence of disease progression ($P = .13$) but worse OS ($P = .075$) in patients with acute GVHD. The influence of chronic GVHD was evaluated similarly, with day 150 after transplantation defined as a landmark. However, the cumulative incidence of disease progression at 3 years after transplantation was not different between those with and without chronic GVHD (12.3% versus 14.5%, $P = .80$).

Results in specific histologic subtypes

Kaplan-Meier estimates of OS of patients with peripheral T-cell lymphoma (n = 14), natural killer (NK)–cell lymphoma (n = 12), LBL (n = 39), and ATLL (n = 15), which were the 4 major histologic subtypes in this study, are shown in Figure 5. OS of patients with peripheral T-cell lymphoma, which is considered to be associated with poor prognosis,[16-18] appeared to be favorable after unrelated allogeneic transplantation with several long-term survivors (3-year OS, 75.0%), although this study contained only a small number of patients. In contrast, the result for ATLL was poor, and there were no survivors beyond 500 days after transplantation.

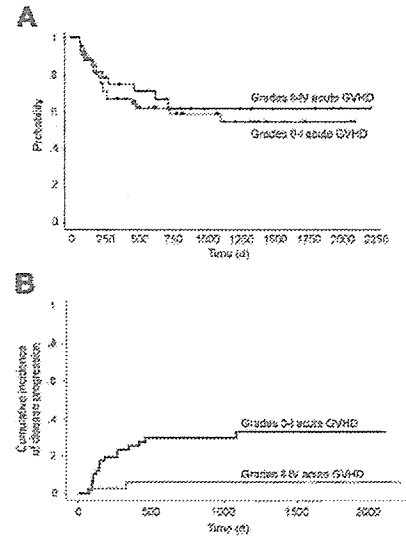

Figure 4. Survival and progression according to development of acute GVHD. OS (A) and cumulative incidence of disease progression (B) grouped by the development of grades II-IV acute graft-versus-host disease among patients who were alive without disease progression at 60 days after transplantation.

Discussion

In this study, we analyzed the outcome of bone marrow transplantation from an unrelated donor for NHL performed through the JMDP. In a similar study from the National Marrow Donor Program (NMDP),[19] both OS and PFS were estimated to be 30% at 2 years. The outcome in the present study appeared to be more favorable than that in the NMDP study, which could be attributed to the lower incidence of grades III-IV acute GVHD in the present study (15% versus 30%). This observation is compatible with previous studies showing lower incidence of acute GVHD among Japanese than among whites, which might reflect less diverse genetic background in Japan.[20,21]

The association between the development of GVHD and reduced disease progression rate has been inconsistent among previous studies.[4,11,22] In this study, the development of grades II-IV acute GVHD was associated with a lower incidence of disease progression after transplantation. This result supports the existence of a potential GVL effect. Because the previous studies nearly exclusively included transplantation from an

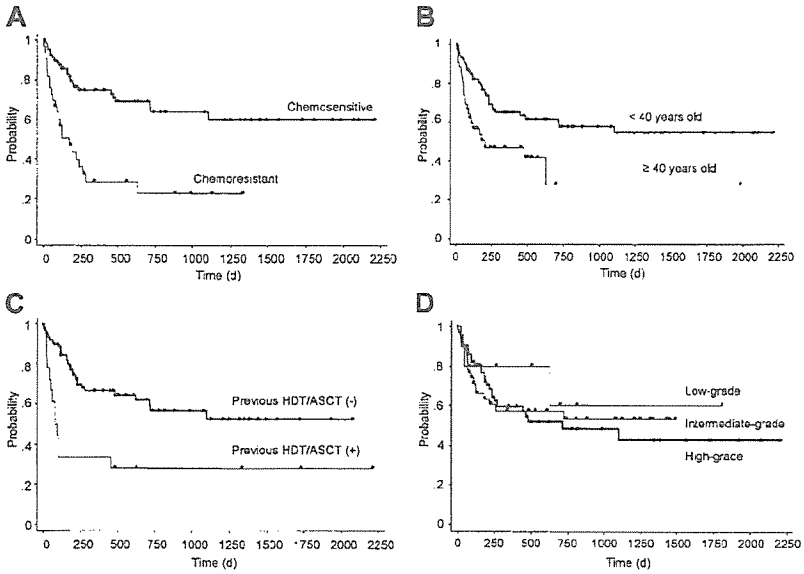

Figure 3. Overall survival according to pretransplantation factors. OS grouped by chemosensitivity at transplantation (A), age (B), previous history of autologous transplantation (HDT/ASCT) (C), and histologic grade (D).

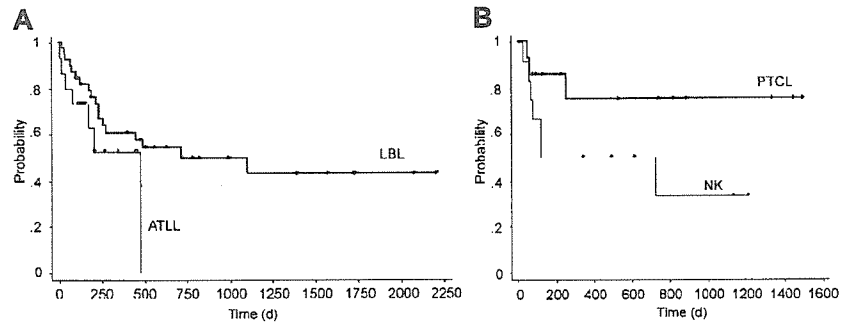

Figure 5. Overall survival of specific histologic subtypes. (A) Adult T-cell leukemia/lymphoma (ATLL) and lymphoblastic lymphoma (LBL); (B) peripheral T-cell lymphoma (PTCL) and NK-cell lymphoma (NK).

HLA-matched sibling, the use of unrelated donor might have facilitated the GVL effect. Nevertheless, there was no difference in OS or PFS between patients with and without grades II-IV acute GVHD, because the decreased incidence of disease progression was counterbalanced by the increased incidence of transplantation-related mortality. An association between the development of chronic GVHD and the incidence of disease progression was not observed. We suppose that the main reason we failed to observe this association is insufficient statistical power due to the paucity of disease progression (only 9 patients) beyond day 150, a landmark for the analysis.

Chemosensitivity at transplantation was identified as a major prognostic factor for OS. Patients with chemosensitive disease at transplantation were associated with lower nonprogression mortality, a lower incidence of disease progression, and better survival. These results may raise the question whether the effect of allogeneic transplantation was from the GVL effect or from high-dose therapy before transplantation. However, this study strongly suggested the existence of the GVL effect, because the incidence of disease progression was significantly lower in patients who developed acute GVHD, even after adjusted for chemosensitivity before transplantation. Previous history of HDT/ASCT was also a strong prognostic factor. Because the median survival for patients with NHL who had a relapse after HDT/ASCT is extremely short (less than 12 months),[23] allogeneic transplantation using conventional or reduced-intensity conditioning is being evaluated in this population. In this study, 11 patients (61%) with a previous history of HDT/ASCT died within 100 days after transplantation from transplantation-related causes. On the other hand, most of the patients who survived beyond day 100 were progression free with long follow-up, suggesting a benefit of allogeneic transplantation to suppress disease progression. Therefore, strategies to decrease transplantation-related mortality are important, especially for patients after HDT/ASCT failure. Allogeneic transplantation with reduced-intensity conditioning is an option that deserves further evaluation.[24]

LBL, ATLL, peripheral T-cell lymphoma, and NK-cell lymphoma were the 4 major histologic subtypes in this population. The composition of the histologic subtypes in this study was different from that of NHL in general and that in previous studies focusing on allogeneic transplantation for NHL.[4,12] Higher ratios of peripheral T-cell lymphoma, NK-cell lymphoma, and ATLL would, at least in part, be a reflection of the histologic population of NHL in Japan.[25] Because the long-term results with conventional therapy and/or HDT/ASCT for ATLL had been always dismal,[26] allogeneic transplantation even for patients in CR is being tested in a clinical trial in several centers in Japan.[27] The long-term results for peripheral T-cell lymphoma and NK-cell lymphoma with conventional therapy, especially in patients with advanced or relapsed disease, were also poor.[16-18,28] The high ratio of LBL might be a reflection of Japanese physicians' preference to perform allogeneic transplantation for LBL in CR1.

Transplantation outcome in each histologic subtype should be evaluated further to select patients who will benefit from unrelated transplantation. In this study, there was no difference in OS or PFS among the 3 grades. Although no patient with low-grade lymphoma had disease progression at a median follow-up of 513 days, the number of patients was too small and follow-up period was too short to draw a definite conclusion. Based on the results of this study, unrelated donor bone marrow transplantation deserves to be evaluated in patients with peripheral T-cell lymphoma and NK-cell lymphoma, considering the poor results after conventional chemotherapy for these subtypes.[16-18,28] Finally, although the outcome of patients with ATLL was poor in this study, this treatment strategy should not be abandoned because both the number of the patients and the follow-up duration were not enough.

In conclusion, allogeneic bone marrow transplantation from an unrelated donor appeared to be a feasible treatment option for patients with high-risk NHL. Further study is required to determine detailed indications for unrelated transplantation for NHL, including histologic subtype and disease status.

Appendix

The following centers in Japan participated in the bone marrow transplantations for NHL facilitated by the JMDP: Hokkaido University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Iwate Medical University Hospital, Tohoku University Hospital, Yamagata University Hospital, National Cancer Center Central Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Itabashi Hospital, Jikei University Hospital, Keio University Hospital, University of Tokyo Hospital, National Tokyo Medical Center, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, Chiba University Hospital, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Jichi Medical School Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Niigata Cancer Center Hospital, Saku Central Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Aichi Cancer Center, Showa Hospital, Kanazawa University Hospital, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Kansai Medical University Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kyoto University Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Ehime Prefectural Central Hospital, National Okayama Medical Center, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Kumamoto National Hospital, and Oita Medical University Hospital.

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Effect of graft-versus-host disease on the outcome of bone marrow transplantation from an HLA-identical sibling donor using GVHD prophylaxis with cyclosporin A and methotrexate

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The effect of graft-versus-host disease (GVHD) on relapse incidence and survival has been analyzed in several studies, but previous studies included heterogeneous patients. Therefore, we analyzed the data of 2114 patients who received unmanipulated bone marrow graft from an HLA-identical sibling donor with a GVHD prophylaxis using cyclosporin A and methotrexate. Among the 1843 patients who survived without relapse at 60 days after transplantation, 435 (24%) developed grade II–IV acute GVHD. Among the 1566 patients who survived without relapse at 150 days after transplantation, 705 (47%) developed chronic GVHD. The incidence of relapse was significantly lower in patients who developed acute or chronic GVHD, but disease-free survival (DFS) was significantly inferior in patients who developed acute GVHD. A benefit of 'mild' GVHD was only seen in high-risk patients who developed grade I acute GVHD. The strongest association between GVHD and a decreased incidence of relapse was observed in patients with standard-risk acute myelogenous leukemia/myelodysplastic syndrome. In conclusion, the therapeutic window between decreased relapse and increased transplant-related mortality due to the development of GVHD appeared to be very narrow.
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Introduction

Graft-versus-host disease (GVHD) is the main cause of treatment failure after allogeneic hematopoietic stem cell transplantation (HSCT). On the other hand, an antitumor effect induced by GVHD, the so-called graft-versus-leukemia (GVL) effect, was recognized in the 1970s.[1,2] The impact of GVHD on relapse incidence and survival has been analyzed in several large studies.[1–7] While GVHD has an apparent antitumor effect, the positive effect is counterbalanced by increased transplant-related mortality, which generally results in a worse transplant outcome in patients who develop GVHD, although two studies have suggested that 'mild' GVHD confers a survival benefit.[4,7] Previous studies included heterogeneous patients with regard to donor source, *ex vivo* graft manipulation, and the GVHD prophylaxis regimen. Such differences may have affected analyses of the GVL effect. In addition, recent advances in supportive treatments may have improved the outcome of patients who developed GVHD. Therefore, the aim of this study was to re-evaluate the influence of GVHD on bone marrow transplant outcome in non-T-cell-depleted transplantation from an HLA-identical sibling donor, which was performed between 1991 and 2000 with a GVHD prophylaxis using cyclosporin A (CsA) and methotrexate.

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Materials and methods

Study population

The Japan Society for Hematopoietic Cell Transplantation (JSHCT) collects data from each transplant center by means of the standardized report form. The follow-up reports were submitted annually after transplantation. A total of 3356 patients, who underwent allogeneic HSCT for the first time between 1991 and 2000 for chronic myelocytic leukemia (CML), acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL), and myelodysplastic syndrome (MDS), were reported to the JSHCT.[8] Only patients who received bone marrow graft from an HLA-identical sibling donor using a GVHD prophylaxis regimen consisting of CsA and methotrexate were included in this study. Those less than 16 years old, those who received graft from a syngeneic donor, those who received manipulated graft, those who received a reduced-intensity/nonmyeloablative conditioning, and those who received peripheral blood or cord blood graft were excluded. Finally, the data on August 2001 of 2114 patients were analyzed. This study was approved by the Committee for Nationwide Survey Data Management of JSHCT.

Transplantation procedure

The conditioning regimen before HSCT was either a total body irradiation-based regimen (61%, mainly combined with cyclophosphamide) or a chemotherapy-based regimen (39%, mainly a combination of busulfan and cyclophosphamide). Acute and chronic GVHD were scored according to the traditional Seattle criteria.[9,10]

Statistical considerations

Engraftment was defined as a neutrophil count greater than 500/mm^3 for 3 consecutive days. Engraftment failure was diagnosed as when engraftment was not achieved at any time after transplantation. Disease-free survival (DFS) was defined as days from a landmark point (described below) to disease relapse or death from any cause. Nonrelapse mortality was defined as death without relapse. Patients who were alive at the last follow-up date were censored. DFS was calculated using the Kaplan–Meier method. To evaluate the influence of confounding factors for acute GVHD and survival, the log-rank test and proportional-hazards modeling were used for univariate and multivariate analyses, respectively. Cumulative incidences of relapse and nonrelapse mortality were calculated using Gray's method, considering each other event as a competing risk.[11] Effects of acute and chronic GVHD on DFS and relapse were analyzed among patients who achieved engraftment and were surviving without relapse at 60 and 150 days after transplantation, respectively.[2,5] This landmark method was used to exclude bias that may have arisen from including patients who died or had a relapse too early to develop GVHD in the group without GVHD.[12,13] Potential confounding factors considered in the analysis were recipient age, sex, disease status, year of transplantation, and conditioning regimen. Factors associated with at least borderline significance ($P<0.10$) in the univariate analysis were subjected to a multivariate analysis using backward stepwise selection of covariates. All P-values were two-sided and P-values of 0.05 or less were considered statistically significant.

Acute leukemia in first or second remission, CML in first or second chronic phase, and MDS without leukemic transformation were considered standard-risk diseases, while others were considered high-risk diseases. The effect of acute GVHD was evaluated by comparing grade 0–I *vs* grade II–IV acute GVHD, except for the evaluation of the effect of 'mild GVHD'.

Results

Characteristics of the patients

The characteristics of the patients are summarized in Table 1. The underlying disease was AML in 737 (35%), ALL in 498 (24%), CML in 619 (29%), and MDS in 260 (12%). There were 1284 males and 830 females with a median age of 35 years (range 16–60 years). Engraftment was achieved in 2065 patients. Among the 1843 patients who survived without relapse at 60 days after transplantation, data with regard to acute GVHD were available in 1819, of whom 537, 308, 91, and 36 developed grade I, II, III, and IV acute GVHD, respectively, with an incidence of grade II–IV acute GVHD of 24%. Pretransplant factors that significantly affected the incidence of grade II–IV acute GVHD were higher age (⩾40 years old, RR 1.36, 95% CI 1.11–1.66. $P=0.0025$) and male sex (RR 1.29, 95% CI 1.04–1.58, $P=0.018$). Among the 1566 patients who survived without relapse at 150 days after transplantation, data with regard to chronic GVHD were available in 1514, of whom 705 (47%) developed chronic GVHD including 208 and 270 with limited and extensive chronic GVHD, respectively (grade not described in 227 patients).

Effects of acute GVHD on relapse, nonrelapse mortality, and DFS

The cumulative incidence of relapse and nonrelapse mortality was 23 and 32% at 5 years, respectively, in patients who

Table 1 Patients' characteristics

	AML	*ALL*	*CML*	*MDS*	*Total*
Transplanted	$n=737$	$n=498$	$n=619$	$n=260$	$n=2114$
Engrafted	$n=715$	$n=483$	$n=610$	$n=257$	$n=2065$
DFS ⩾60 days	$n=622$	$n=423$	$n=572$	$n=226$	$n=1843$
DFS ⩾150 days	$n=520$	$n=342$	$n=513$	$n=191$	$n=1566$
Sex					
Male	413 (56%)	302 (61%)	417 (67%)	152 (58%)	1284 (61%)
Female	324 (44%)	196 (39%)	202 (33%)	108 (42%)	830 (39%)
Age (years)					
<40	483 (66%)	370 (74%)	348 (56%)	149 (57%)	1350 (64%)
⩾40	254 (34%)	128 (26%)	271 (44%)	111 (43%)	764 (36%)
Disease risk					
Standard	535 (73%)	364 (73%)	504 (81%)	215 (83%)	1618 (77%)
High	202 (27%)	134 (27%)	115 (19%)	45 (17%)	496 (23%)
Use of total body irradiation					
No	282 (38%)	105 (21%)	325 (53%)	109 (42%)	821 (39%)
Yes	455 (62%)	393 (79%)	294 (47%)	151 (58%)	1293 (61%)
Acute GVHD (grade II–IV)[a]					
No	481 (79%)	319 (77%)	429 (76%)	155 (69%)	1384 (76%)
Yes	131 (21%)	95 (23%)	138 (24%)	71 (31%)	435 (24%)
ND	10	9	5	0	24
Chronic GVHD[b]					
No	308 (61%)	189 (58%)	223 (45%)	89 (48%)	809 (53%)
Yes	196 (39%)	137 (42%)	275 (55%)	97 (52%)	705 (47%)
ND	16	16	15	5	52

ND = not described.
[a]Among patients who survived more than 60 days without relapse.
[b]Among patients who survived more than 150 days without relapse.

developed grade II–IV acute GVHD, and 29 and 12% in those who did not (Figure 1a). The development of grade II–IV acute GVHD significantly decreased the incidence of relapse ($P=0.029$), but was significantly associated with a greater risk of nonrelapse mortality ($P<0.0001$). Two factors were identified as independent significant risk factors for relapse by a multivariate analysis using backward stepwise selection of covariates; high-risk disease and the absence of grade II–IV acute GVHD (Table 2a). However, the benefit of decreased relapse associated with acute GVHD was outweighed by the increased nonrelapse mortality, which produced an inferior DFS at 5 years with grade II–IV acute GVHD in both standard-risk (51 *vs* 65%, $P<0.0001$) and high-risk patients (25 *vs* 32%, $P=0.07$) (Figure 2a).

To test the hypothesis that the development of grade I acute GVHD, a form of 'mild' GVHD, may improve transplant outcome,[4,7] we analyzed the relapse incidence, nonrelapse mortality and DFS, grouped according to the grade of acute GVHD. Nonrelapse mortality at 5 years showed a continuous increase of 9, 17, 26, 39, and 73%, respectively, with increasing acute GVHD grade from 0 to IV (Figure 3b). Relapse incidence at 5 years tended to be lower with higher acute GVHD grade, but the difference among grade 0, I and II acute GVHD was not statistically significant (31, 26, 27, 16, and 7%, respectively, for grades 0, I, II, III, and IV acute GVHD, Figure 3a). The development of grade I acute GVHD was associated with an inferior DFS in standard-risk patients (61 *vs* 68% at 5 years, $P=0.0070$, Figure 4a), but a superior DFS in high-risk patients (43 *vs* 25% at 5 years, $P=0.018$, Figure 4b). These effects were shown to be independently significant by multivariate analyses (Table 3).

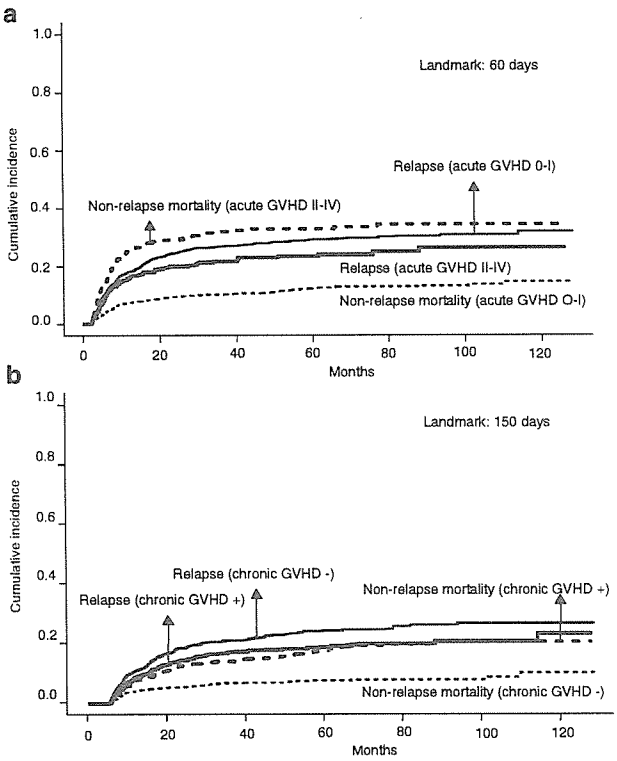

Figure 1 Cumulative incidences of relapse and nonrelapse mortality. (a) Effect of acute GVHD in patients who survived more than 60 days without relapse. (b) Effect of chronic GVHD in patients who survived more than 150 days without relapse.

Table 2 Development of acute and chronic GVHD reduces the risk of relapse: (a) Impact of acute GVHD and (b) chronic GVHD in patients who survived more than 60 days and 150 days without relapse

Covariates	*Relative risk (95% CI)*	*P-value*
(a)		
Acute GVHD		
0–I ($n=1384$)	1.00	0.0075
II–IV ($n=435$)	0.73 (0.59–0.92)	
Disease status		
Standard risk ($n=1468$)	1.00	<0.00001
High risk ($n=351$)	3.01 (2.48–3.64)	
(b)		
Chronic GVHD		
No ($n=809$)	1.00	0.0039
Yes ($n=705$)	0.71 (0.57–0.90)	
Disease status		
Standard risk ($n=1282$)	1.00	<0.00001
High risk ($n=232$)	2.72 (2.11–3.49)	

Effects of chronic GVHD on relapse, nonrelapse mortality, and DFS

The cumulative incidences of relapse and nonrelapse mortality at 5 years were 19 and 18%, respectively, in patients who developed chronic GVHD and 24 and 8% in those who did not

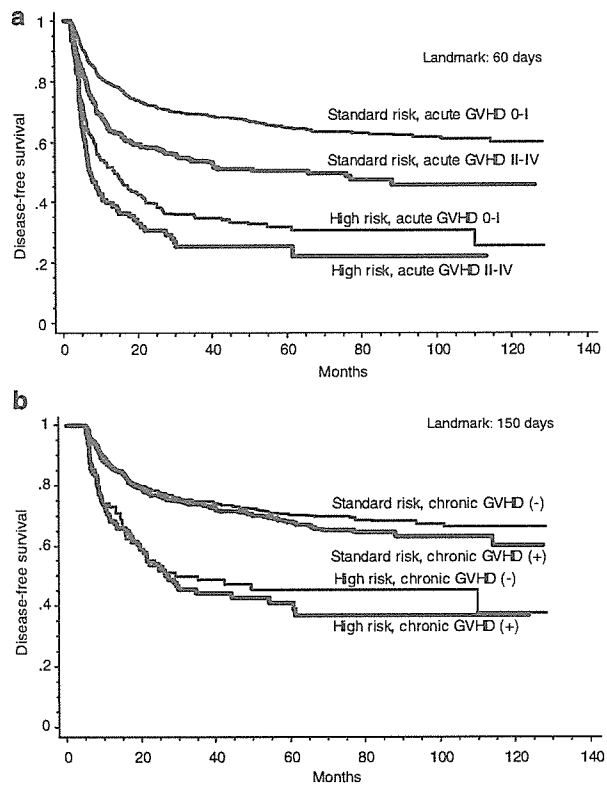

Figure 2 DFS after transplantation. (a) Effect of acute GVHD in patients who survived more than 60 days without relapse. (b) Effect of chronic GVHD in patients who survived more than 150 days without relapse.

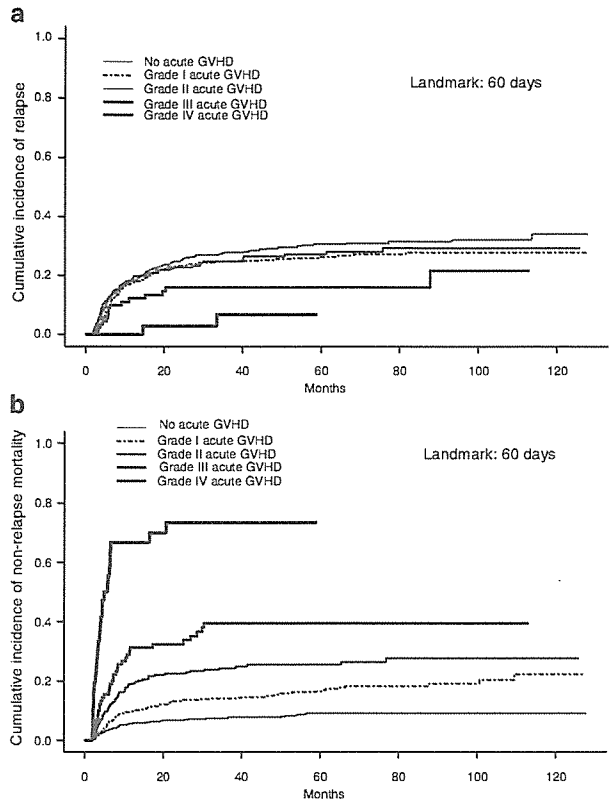

Figure 3 Cumulative incidences of relapse (a) and nonrelapse mortality (b) in patients who survived more than 60 days without relapse, grouped according to the grade of acute GVHD.

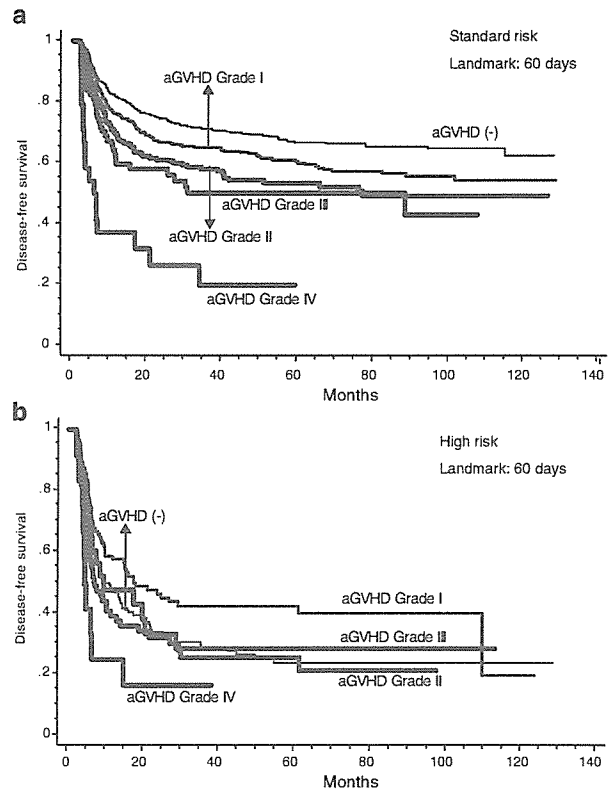

Figure 4 DFS grouped according to the grade of acute GVHD. (a) Standard- and (b) high-risk patients.

(Figure 1b). Patients who developed chronic GVHD had a lower incidence of relapse ($P=0.023$), but had a significantly greater risk of nonrelapse mortality ($P<0.0001$). High-risk disease and the absence of chronic GVHD were identified as independent significant risk factors for relapse (Table 2b). However, DFS at 5 years was similar between those who developed chronic GVHD and those who did not (68 *vs* 71%, $P=0.31$ and 41 *vs* 46%, $P=0.58$ for standard-risk and high-risk disease, respectively), since the decreased incidence of relapse was counterbalanced by the increased incidence of nonrelapse mortality (Figure 2b).

The development of limited chronic GVHD, another form of 'mild' GVHD, did not affect survival in either standard- or high-risk patients (76 *vs* 71% at 5 years, $P=0.39$ and 49 *vs* 46% at 5 years, $P=0.78$, respectively (Figure 5).

Effects of the combination of acute and chronic GVHD on DFS

We classified patients into four groups according to the combination of acute and chronic GVHD (no GVHD, acute GVHD alone, chronic GVHD alone, and both acute and chronic GVHD) to evaluate whether a specific combination of acute and chronic GVHD may improve DFS. However, the development of acute GVHD was shown to be associated with inferior survival in both standard- and high-risk patients, regardless of the development of chronic GVHD (Figure 6).

Table 3 Development of grade I acute GVHD improved survival in high-risk patients but adversely affected survival in standard-risk patients: (a) Standard-risk patients and (b) high-risk patients who survived more than 60 days without relapse

Covariates	*Relative risk (95% CI)*	*P-value*
(*a*)		
Year		
1995–	1.00	0.030
–1994	1.26 (1.02–1.55)	
Age (years)		
⩾40	1.00	0.020
<40	0.78 (0.63–0.96)	
Acute GVHD		
I	1.00	0.0075
0	0.76 (0.62–0.93)	
(*b*)		
Acute GVHD		
I	1.00	0.019
0	1.48 (1.07–2.04)	

Effects of acute and chronic GVHD on relapse and DFS in each disease

The strength of a GVL effect may differ among the underlying diseases. Therefore, we evaluated the effects of acute and

chronic GVHD on relapse in each underlying disease to assess whether the development of GVHD may significantly improve transplant outcome in a specific disease. As shown in Table 4, the antitumor effect was strongest with chronic GVHD in standard-risk AML and MDS, with a marginal significance ($P=0.082$ and 0.068, respectively). Neither acute nor chronic GVHD affected the relapse rate of high-risk diseases. We also evaluated the effect of the combination of acute and chronic

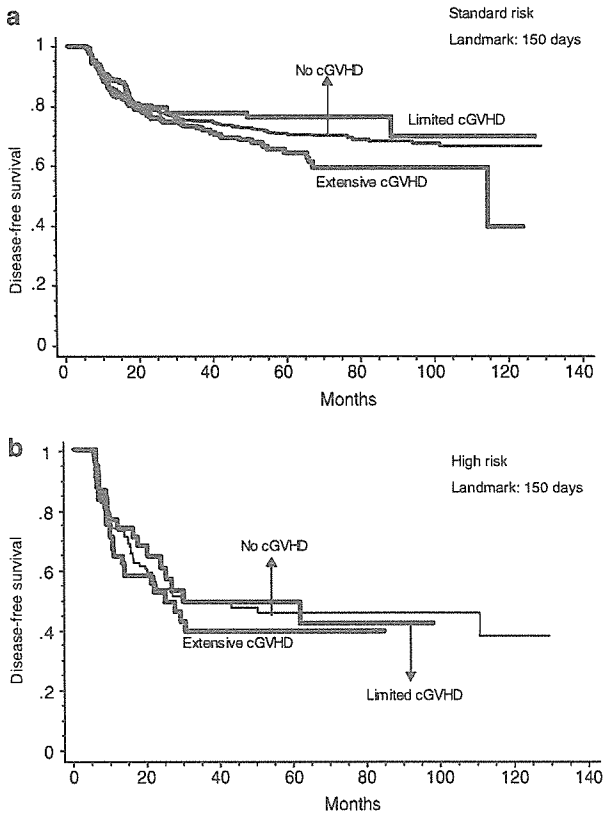

Figure 5 DFS grouped according to the severity of chronic GVHD. (a) Standard- and (b) high-risk patients.

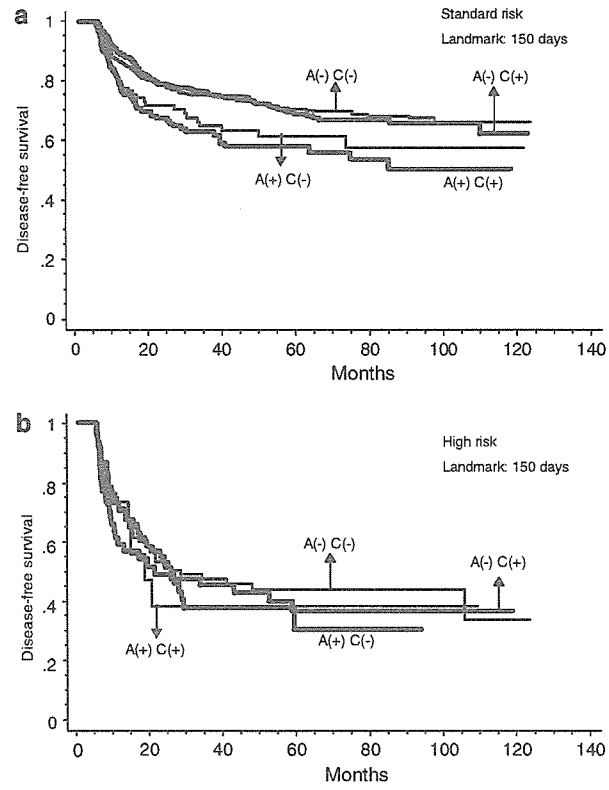

Figure 6 Effects of the combination of acute and chronic GVHD on DFS in patients who survived more than 150 days without relapse. (a) Standard risk and (b) high risk.

Table 4 Effects of acute and chronic GVHD on relapse rate for each hematological malignancy in patients who survived more than 150 days without relapse, stratified by the disease status

	Standard risk		*High risk*	
Covariates	*Relative risk (95% CI)*	*P-value*	*Relative risk (95% CI)*	*P-value*
AML				
RI without GVHD	21%		34%	
Acute GVHD (grade II–IV)	0.87 (0.44–1.70)	0.68	0.97 (0.35–2.69)	0.95
Chronic GVHD	0.63 (0.38–1.06)	0.082	0.76 (0.30–1.95)	0.57
ALL				
RI without GVHD	34%		57%	
Acute GVHD (grade II–IV)	0.72 (0.36–1.43)	0.35	1.12 (0.48–2.62)	0.79
Chronic GVHD	0.67 (0.40–1.10)	0.11	1.12 (0.52–2.43)	0.78
CML				
RI without GVHD	16%		39%	
Acute GVHD (grade II–IV)	0.96 (0.48–1.90)	0.90	0.70 (0.20–2.42)	0.57
Chronic GVHD	0.87 (0.50–1.50)	0.62	0.76 (0.31–1.85)	0.54
MDS				
RI without GVHD	18%		40%	
Acute GVHD (grade II–IV)	1.81 (0.75–4.38)	0.19	1.55 (0.53–4.56)	0.42
Chronic GVHD	0.45 (0.19–1.06)	0.068	1.04 (0.32–3.32)	0.95

RI = relapse incidence at 5 years after transplantation.

GVHD on DFS, but did not find that acute or chronic GVHD had any benefit on DFS in any disease (data not shown).

Discussion

We evaluated the effects of acute and chronic GVHD on transplant outcome in a relatively homogeneous population, with regard to donor source, graft manipulation, and GVHD prophylaxis. The development of acute and/or chronic GVHD was significantly associated with a decreased risk of relapse, while a benefit in DFS was observed only in patients who developed grade I acute GVHD in high-risk disease. The data confirmed the results from a recent publication of the European Group for Blood and Marrow Transplantation,[14] which were limited to CML, and extended the observations to other leukemias.

We compared the incidence of relapse and DFS in patients who had survived without relapse at a specific landmark time. Without this landmark analysis, patients who died or relapsed too early to develop GVHD would have been included in the non-GVHD group, resulting in a bias toward a favorable outcome in the GVHD group. Therefore, some previous studies might have overestimated the positive impact of 'mild' GVHD on DFS.[4,7] However, the landmark analysis is not ideal. First, patients who die or relapse before the landmark time do not contribute to the analysis. Second, selection of the landmark may strongly affect the analysis. Therefore, we selected the landmark before the data analysis. The landmark time for acute GVHD analysis was chosen to be day 60 considering the fact that more than 90% of patients who develop grade II–IV acute GVHD do so within 60 days after transplantation. We selected a landmark of day 150 for chronic GVHD analysis, the same as in previous studies.[2,5] Of course, this analysis of DFS also favors patients who develop GVHD, since more than 80% of GVHD-related deaths occur within 150 days after transplantation.[15] Nonetheless, we did not find that chronic GVHD conferred any benefit on DFS. We re-evaluated the effect of GVHD using landmark point of 20 and 100 days after transplantation for acute and chronic GVHD, respectively, to rule out the possibility that the selection of landmark point might have strongly affected the results of this study. However, major results did not differ by the changes in the landmark points (data not shown).

The association between relapse incidence and chronic GVHD was prominent in standard-risk AML/MDS, but not in CML. This does not mean that the GVL effect is stronger against AML/MDS than CML. Considering the low incidence of relapse among standard-risk CML patients who did not develop GVHD, this result might indicate that the GVL effect can be obtained without apparent GVHD in standard-risk CML. On the other hand, the fact that there was no relationship between the incidence of relapse and the development of both acute and chronic GVHD might suggest that the GVL effect cannot suppress advanced leukemia. However, considering the finding that grade I acute GVHD improved DFS in high-risk patients, it could be more likely explained by a bias caused by the fact that GVHD more often develops in patients with higher-risk disease.[16] Although we analyzed DFS after stratifying patients according to the disease status, each group may still be heterogeneous, and thus patients who developed GVHD may have included a higher proportion of patients with a relatively higher-risk disease than those who did not. It is also possible that cyclosporine tended to be more rapidly tapered in relatively higher-risk patients based on the earlier studies,[1–4,6,7,17] which might have increased the incidence of GVHD in higher-risk patients and made it difficult to find a beneficial effect of GVHD on relapse incidence. In addition, we must notify that the statistical power was not enough to detect a small effect of GVHD in these subgroup analyses.

The major findings in this study do not support a strategy of inducing GVHD to obtain a GVL effect, and such intervention is not recommended as a routine practice. However, recent studies that have included less-intense GVHD prophylaxis[18–20] or prophylactic donor lymphocyte infusion[21,22] to high-risk patients have shown promising results. In addition, a randomized study that compared low-dose CsA at 1 mg/kg/day and high-dose CsA at 5 mg/kg/day showed a better DFS in the low-dose CsA group.[23,24] The increased incidence of acute GVHD in the low-dose CsA group did not translate into higher transplant-related mortality. Taking these results together, the impact of GVHD on DFS may differ depending on the GVHD prophylaxis regimen. GVHD induced by less-intense GVHD prophylaxis may be more manageable than GVHD that occurs during standard GVHD prophylaxis.

In conclusion, there appears to be at most a narrow therapeutic window between a decreased incidence of relapse and increased transplant-related mortality due to the development of GVHD. Although this does not deny the possibilities that strategies for inducing a GVL effect by using less-intense GVHD prophylaxis may have beneficial effects, such intervention should be performed only as part of a well-designed clinical study.

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