ProteinChip technique. In some of these studies, SAA has been reported to be a potential marker for particular cancer status. Multiple variants of SAA have been detected by the SELDI ProteinChip technique in renal cancer patients.[20] The SELDI ProteinChip technique revealed that SAA may be a biomarker for identifying prostate cancer patients with bone lesions, with a sensitivity and specificity of 89.5%.[21]

SAA activates human mast cells, which leads to the degradation of SAA and the generation of an amyloidogenic SAA fragment.[22] SAA is a major acute-phase reactant that increases by as much as 1000-fold during inflammation. SAA is potentially involved in the pathogenesis of several chronic inflammatory diseases: it is the precursor of amyloid A protein deposited in amyloid A amyloidosis, and has also been implicated in the pathogenesis of atherosclerosis and rheumatoid arthritis.[23,24] SAA may be closely related to poor patient outcomes, including left ventricular systolic dysfunction, cardiac rupture and mortality in acute myocardial infarction.[25,26]

Some studies have suggested that the elevation of SAA may be associated with acute allograft rejection of the kidney,[27–30] liver[31] and heart.[32] By contrast, it has also been reported that SAA is inadequate for predicting acute rejection in cardiac allograft.[33] With regard to renal allograft rejection, SAA was shown to be a sensitive marker that rose above 100 mg/l in all cases of rejection, whereas C-reactive protein (CRP) showed little or no response to rejection.[29] We could not confirm whether or not the elevation of SAA was a phenomenon that occurs with all CBT around day 9, as the value of SAA in samples from non-febrile patients at this time point was not analyzed. However, it is unlikely that the elevation of SAA occurs with all allogeneic transplantation, as the phenomenon was not prominent in patients with acute GVHD. The possibility that the elevation of SAA was only a consequence of acute phase change that occurs with high-grade fever could not be completely ruled out, as the elevation of SAA was not confined to CBT; however, the elevation level was higher in PIR than in other conditions. Furthermore, patients who developed graft rejection had markedly higher levels of SAA. Although the reason for these observations is unclear, a previous report on SAA as an indication of allograft rejection has suggested that inflammation and cytokine production induced by an alloreaction may be related to the elevation of SAA in PIR.

In the case of CBT, SAA that increases in relation to PIR, as a factor associated with a poor prognosis of CBT, may be related to allograft rejection. Our retrospective study showed that pre-engraftment CRP values may predict acute GVHD and nonrelapse mortality.[34] Although CRP elevation was also observed during PIR, SAA elevation was more rapid and prominent. The SAA level was above the normal limit in all 13 samples at the day of fever onset, whereas 2 samples were within the normal limit for CRP. The mean SAA level was 121 times the upper normal limit at day 2 of fever onset, whereas the mean CRP level was 10 times the upper normal limit at the same time. SAA or anaphylatoxin C4a alone may lack specificity as a marker for PIR. However, the further analysis of samples obtained from CBT recipients by our method may provide fingerprints of markers useful for the diagnosis of PIR. Identification of peak markers suggests that the SELDI-TOF MS system in combination with other proteomic methods could serve as a potential diagnostic tool in discovering biomarkers for PIR after CBT.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported in part by grants from the Ministry of Health, Labor and Welfare, Japan.

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Int J Hematol (2013) 97:109–116
DOI 10.1007/s12185-012-1229-0

ORIGINAL ARTICLE

Differing impacts of pretransplant serum ferritin and C-reactive protein levels on the incidence of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

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Received: 4 August 2012 / Revised: 21 November 2012 / Accepted: 21 November 2012 / Published online: 7 December 2012

Abstract Studies have suggested an association between pretransplant serum levels of ferritin and C-reactive protein (CRP) and complications of allogeneic hematopoietic stem cell transplantation (HSCT). To evaluate the prognostic impact of these biomarkers on the development of acute and chronic graft-versus-host disease (GVHD), we retrospectively studied 211 patients who underwent allogeneic HSCT for hematologic diseases at our institution. The cumulative incidence rate of chronic GVHD at 3 years was 40.7 %. In the multivariate analysis, elevated CRP levels (≥2 mg/L) were significantly associated with a high incidence of chronic GVHD, whereas high ferritin levels (≥880 ng/mL) showed a tendency, though not statistically significant, to association with a low incidence of chronic GVHD. No significant association was observed between the pretransplant serum ferritin or CRP levels and the incidence of acute GVHD. Multivariate analysis indicated that high pretransplant serum ferritin levels were significantly associated with increases in treatment-related mortality and relapse rates. Overall, an elevated pretransplant serum ferritin level, but not an elevated serum CRP level, is a strong risk factor for overall mortality (hazard ratio, 2.16; $P = 0.002$). Our results also indicate that pretransplant serum CRP levels may be a useful biomarker for predicting the risk of chronic GVHD.

Keywords Ferritin · C-reactive protein · Chronic GVHD · Allogeneic hematopoietic stem cell transplantation

Electronic supplementary material The online version of this article (doi:10.1007/s12185-012-1229-0) contains supplementary material, which is available to authorized users.

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Introduction

Iron overload is frequently observed in patients with hematologic diseases before and after allogeneic hematopoietic stem cell transplantation (HSCT). Many studies have shown that elevated pretransplant serum ferritin levels are associated with lower overall and disease-free survival rates and a higher incidence of treatment-related complications [1–5]. Conversely, several studies have suggested that elevated pretransplant ferritin levels are associated with a lower incidence of chronic graft-versus-host disease (GVHD) [6, 7], and the immunosuppressive effect of iron overload was hypothesized to be an underlying mechanism. However, this association remains controversial because other studies have failed to observe such a relationship [5, 8]. Results are also inconsistent among studies regarding the association between pretransplant serum ferritin levels and the incidence of acute GVHD [4–6, 8].

Serum ferritin is widely used as a surrogate marker of body iron stores. However, ferritin levels are increased by inflammation and iron loading; therefore, it is important to

adjust for the effect of inflammation on ferritin levels. C-reactive protein (CRP), an acute-phase reactant produced by hepatocytes, has been shown to be a reliable biomarker of systemic inflammation [9, 10]. Several studies have suggested that elevated pretransplant or pre-engraftment serum CRP levels are associated with an increased risk of treatment-related mortality (TRM) after allogeneic HSCT [11–14]. Although studies have reported that elevated CRP levels are associated with a higher incidence rate of acute GVHD [11, 12], this finding is not yet fully confirmed. Moreover, the impact of CRP levels on the incidence of chronic GVHD remains unknown.

We previously demonstrated through a multivariate analysis that elevated pretransplant serum ferritin and CRP levels were significantly associated with the development of bacterial infection after allogeneic HSCT [14]. In the present study, we investigated the association between pretransplant serum ferritin and CRP levels and the incidence of acute and chronic GVHD as well as other clinical outcomes of allogeneic HSCT, to determine whether these events could be predicted using these parameters.

Patients and methods

Study population

We retrospectively reviewed the medical records of adult patients who underwent their first allogeneic HSCT for hematologic diseases at the Kyoto University Hospital from January 2000 to December 2010. A total of 211 patients whose pretransplant serum ferritin and CRP profiles were available were included in the analysis. This study was performed in accordance with the Helsinki Declaration and approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine. Written informed consent to undergo the transplantation protocol was obtained from all of the patients.

Serum analysis

The serum ferritin and CRP levels before the start of the conditioning regimen were measured using the standard laboratory technique (reference ranges: ferritin level $\leq$150 ng/mL; CRP level $\leq$2 mg/L) [14].

Statistical analysis

The primary end point was the impact of the pretransplant serum ferritin and CRP levels on the incidence of grade 2–4 acute GVHD and chronic GVHD. In the analysis of chronic GVHD, patients who survived for at least 100 days after allogeneic HSCT were included. The secondary end point was the impact of these 2 markers on the incidence of TRM and relapse, and the overall survival (OS) rate.

The patients were divided into 2 groups depending on their pretransplant serum ferritin or CRP levels. The cutoff point for the CRP levels was 2 mg/dL, the median value. For the ferritin levels, the median value was 470 ng/mL, which was much lower than the cutoff points used in most other studies; therefore, we used 880 ng/mL, which was the higher tertile value, as the cutoff point. The patient and transplant characteristics between the 2 groups were compared using the Mann–Whitney U test or Chi-square analysis, as appropriate. Standard-risk disease was defined as complete remission in cases of acute myeloid leukemia, acute lymphoblastic leukemia, malignant lymphoma, and plasma cell myeloma; as untreated or complete remission in cases of myelodysplastic syndrome and myeloproliferative disorder; as chronic phase in cases of chronic myeloid leukemia; and as nonmalignant disease. High-risk disease was defined as any other hematologic disease status. The conditioning regimen was categorized as either myeloablative or reduced intensity according to the National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research operational definitions [15].

Acute and chronic GVHD were defined and graded according to conventional criteria [16, 17]. Depending on whether it developed before or after day +100, GVHD was classified as acute or chronic, respectively. To eliminate the effect of a competing risk, the cumulative incidence was assessed using methods described elsewhere [18]. The competing event in the cumulative incidence analyses was defined as death without an event of interest. OS was estimated using the Kaplan–Meier methods. The Cox proportional hazards model was applied.

The following items were added as confounders: the age of the recipient (<50 or $\geq$50 years), the sex of the recipient (male or female), diagnosis (myeloid or lymphoid malignancies, or nonmalignant diseases), risk of disease (standard or high risk), source of stem cells (HLA-matched- or HLA-mismatched-related donor graft, unrelated bone marrow, or unrelated cord blood), conditioning regimen (myeloablative or reduced intensity), and prophylaxis against GVHD (tacrolimus or cyclosporine based). Stepwise backward selection procedures were used with a variable retention criterion of $P < 0.05$ to identify important confounders; these confounders, as well as the serum ferritin and CRP levels, were then included in the final model.

$P < 0.05$ was considered to be statistically significant. All the analyses were conducted using the Stata (version 11; StataCorp LP, College Station, TX, USA) and R version 2.13.0 software (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of patients and transplants

The characteristics of the patients and transplants are shown in Table 1 and Supplementary Table S1. The median age of the patients was 48 years (range 17–69 years). The primary diseases were myeloid malignancies, lymphoid malignancies, and nonmalignant diseases in 115, 88, and 8 patients, respectively. A total of 90 patients (43 %) had a high-risk disease. No patient received T cell-depleted grafts. A myeloablative regimen was used in 116 patients (55 %). There was no significant difference in the patient and transplant characteristics between the low- and high-ferritin groups, except in the serum CRP levels ($P = 0.011$). The patients in the high-CRP group were more likely to be male ($P < 0.001$) and have a high-risk disease ($P < 0.001$).

Acute and chronic GVHD

Among all patients, the median follow-up period for the survivors after allogeneic HSCT was 41.2 months (range 1.2–132.6 months). A total of 188 patients survived for 100 days or longer after transplantation.

The cumulative incidence rates of grade 2–4 acute GVHD at 100 days after transplantation were 35.8 % [95 % confidence interval (CI) 27.9–43.7 %] and 32.5 % (95 % CI 22.0–43.5 %) in the low- and high-ferritin groups, respectively, and 34.9 % (95 % CI 26.2–43.7 %) and 34.4 % (95 % CI 25.2–43.8 %) in the low- and high-CRP groups, respectively (Fig. 1, panels a, b). With regard to grade 3 or 4 acute GVHD, the cumulative incidence rates were 7.9 % (95 % CI 4.2–13.1 %) and 14.1 % (95 % CI 7.2–23.3 %) in the low- and high-ferritin groups, respectively, and 10.7 % (95 % CI 5.9–17.3 %) and 9.1 % (95 % CI 4.5–15.8 %) in the low- and high-CRP groups,

Table 1 Characteristics of patients and transplants according to pretransplant serum ferritin and CRP levels

Variables	Low ferritin (<880 ng/mL) $n = 140$	High ferritin (≥880 ng/mL) $n = 71$	P value	Low CRP (<2 mg/L) $n = 112$	High CRP (≥2 mg/L) $n = 99$	P value
Age at transplant						
Median (range), years	47.5 (17–69)	50 (20–66)	0.46	46.5 (17–69)	49 (17–66)	0.26
Sex, *n* (%)			0.15			<0.001
Male	70 (50)	43 (61)		47 (42)	66 (67)	
Female	70 (50)	28 (39)		65 (58)	33 (33)	
Disease, *n* (%)			0.082			0.54
Myeloid malignancies	73 (52)	42 (59)		64 (57)	51 (52)	
Lymphoid malignancies	64 (46)	24 (34)		43 (38)	45 (45)	
Nonmalignant diseases	3 (2)	5 (7)		5 (4)	3 (3)	
Risk of disease, *n* (%)			0.83			<0.001
Standard	81 (58)	40 (56)		80 (71)	41 (41)	
High	59 (42)	31 (44)		32 (29)	58 (59)	
Source of stem cells, *n* (%)			0.72			0.73
HLA[a]-matched related	44 (31)	18 (25)		31 (28)	31 (31)	
HLA[a]-mismatched	14 (10)	10 (14)		11 (10)	13 (13)	
Unrelated bone marrow	62 (44)	33 (46)		54 (48)	41 (41)	
Unrelated cord blood	20 (14)	10 (14)		16 (14)	14 (14)	
Conditioning regimen, *n* (%)			0.76			0.13
Myeloablative intensity	78 (56)	38 (54)		67 (60)	49 (49)	
Reduced intensity	62 (44)	33 (46)		45 (40)	50 (51)	
GVHD prophylaxis, *n* (%)			0.84			0.53
Tacrolimus-based	114 (81)	57 (80)		89 (79)	82 (83)	
Cyclosporine based	26 (19)	14 (20)		23 (21)	17 (17)	
Serum CRP level, *n* (%)			0.011			
<2 mg/L	83 (59)	29 (41)				
≥2 mg/L	57 (41)	42 (59)				

[a] HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, -B, and -DR antigens

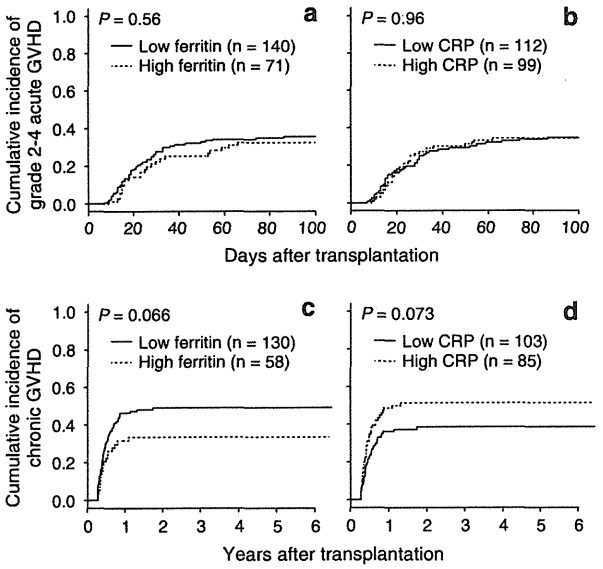

Fig. 1 Cumulative incidence of grade 2–4 acute GVHD according to pretransplant **a** ferritin and **b** CRP levels, and chronic GVHD according to pretransplant **c** ferritin and **d** CRP levels. **a**, **c** *Solid line* low-ferritin group (<880 ng/mL), *dotted line* high-ferritin group (≥880 ng/mL). **b**, **d** *Solid line* low-CRP group (<2 mg/L), *dotted line* high-CRP group (≥2 mg/L). The statistical significance between the 2 groups was calculated using the Gray test

respectively. There was no significant difference in the incidence rates of grade 2–4 and 3 or 4 acute GVHD between the low- and high-ferritin groups or between the low- and high-CRP groups.

The cumulative incidence rates of chronic GVHD at 3 years after transplantation were 49.3 % (95 % CI 40.1–57.8 %) and 33.5 % (95 % CI 21.6–45.8 %) in the low- and high-ferritin groups, respectively, and 38.6 % (95 % CI 29.0–48.2 %) and 51.4 % (95 % CI 40.0–61.7 %) in the low- and high-CRP groups, respectively (Fig. 1, panels c, d). The patients in the high-ferritin group tended to have a low incidence of chronic GVHD, although this association was not significant in the multivariate analysis [≥880 vs. <880 ng/mL; hazard ratio (95 % CI), 0.64 (0.38–1.09); $P = 0.099$; Table 2]. A subgroup analysis showed that the negative effect of the ferritin levels on the incidence of chronic GVHD was significant only in patients with myeloid malignancies ($n = 103$; hazard ratio, 0.46; $P = 0.040$), but not in patients with lymphoid malignancies ($n = 78$; hazard ratio, 1.11; $P = 0.79$). The multivariate analysis showed that the elevated serum CRP levels (≥2 vs. <2 mg/L) were significantly associated with the increased incidence of chronic GVHD [hazard ratio (95 % CI), 1.71 (1.07–2.74); $P = 0.024$; Table 2]. The effect of the CRP levels on the incidence of chronic GVHD was similar regardless of the primary disease (myeloid or lymphoid malignancies). There was no significant difference

Table 2 Multivariate analysis of acute and chronic GVHD

	Hazard ratio (95 % CI)	*P* value
Grade 2–4 acute GVHD		
Conditioning regimen		
Myeloablative intensity	1.00	Reference
Reduced intensity	0.60 (0.37–0.97)	0.037
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	0.86 (0.52–1.43)	0.57
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.13 (0.70–1.81)	0.62
Grade 3–4 acute GVHD		
Risk of disease		
Standard	1.00	Reference
High	3.93 (1.52–10.14)	0.005
Conditioning regimen		
Myeloablative intensity	1.00	Reference
Reduced intensity	0.39 (0.15–1.02)	0.055
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.10 (0.87–5.09)	0.10
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	0.57 (0.22–1.43)	0.23
Chronic GVHD[a]		
Risk of disease		
Standard	1.00	Reference
High	1.48 (0.93–2.37)	0.098
GVHD prophylaxis		
Tacrolimus-based	1.00	Reference
Cyclosporine based	0.52 (0.27–1.01)	0.052
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	0.64 (0.38–1.09)	0.099
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.71 (1.07–2.74)	0.024

[a] Patients who survived for at least 100 days after transplantation were included in the analysis

in the incidence of extensive chronic GVHD between the low- and high-ferritin groups ($P = 0.43$) or between the low- and high-CRP groups ($P = 0.52$).

A history of acute GVHD is known to be an important risk factor for developing chronic GVHD. Therefore, we also conducted an analysis of the association between the incidence of chronic GVHD and the incidence of prior acute GVHD (none or grade 1 vs. grade 2–4) stratified by the pretransplant ferritin and CRP levels. The cumulative incidence rates of chronic GVHD at 3 years were as

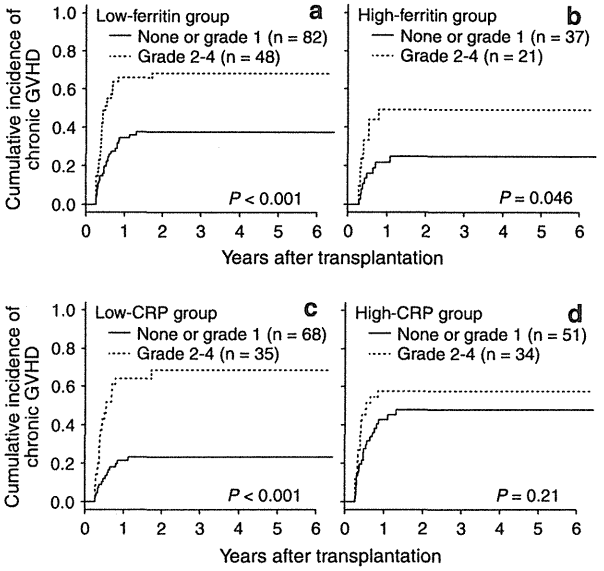

Fig. 2 Cumulative incidence of chronic GVHD according to prior acute GVHD stratified by pretransplant ferritin and CRP levels. **a** Low-ferritin group (<880 ng/mL, $n = 130$), **b** high-ferritin group (≥880 ng/mL, $n = 58$), **c** low-CRP group (<2 mg/L, $n = 103$), and **d** high-CRP group (≥2 mg/L, $n = 85$). *Solid line* no or grade 1 acute GVHD, *dotted line* grade 2–4 acute GVHD. The statistical significance between the 2 groups was calculated using the Gray test

follows: low-ferritin group, 37.8 versus 68.4 %, $P < 0.001$ (Fig. 2, panel a); high-ferritin group, 24.9 versus 49.2 %, $P = 0.046$ (Fig. 2, panel b); low-CRP group, 23.2 versus 68.5 %, $P < 0.001$ (Fig. 2, panel c); and high-CRP group, 47.8 versus 57.5 %, $P = 0.21$ (Fig. 2, panel d). Notably, the patients in the high-CRP group demonstrated a high incidence of chronic GVHD, both in the absence and presence of prior acute GVHD.

TRM, relapse, and OS

At 3 years, the patients in the high-ferritin group had a significantly higher TRM than those in the low-ferritin group (21.5 vs. 9.7 %, $P = 0.026$; Fig. 3, panel a), with the main cause of TRM being infection in both groups [5 of 14 TRM cases in the high-ferritin group ($n = 71$) and 5 of 13 TRM cases in the low-ferritin group ($n = 140$)]. The patients in the high-CRP group had a significantly higher TRM than those in the low-CRP group (20.0 vs. 7.5 %, $P = 0.035$; Fig. 3, panel b). The impact of ferritin on TRM was significant ($P = 0.046$) according to the multivariate analysis, whereas the impact of CRP was not (Table 3).

The cumulative incidence of relapse was similar between the low- and high-ferritin groups and between the low- and high-CRP groups (Fig. 3, panels c, d). However, in the multivariate analysis, the high ferritin levels were significantly associated with the high relapse rate ($P = 0.018$; Table 3).

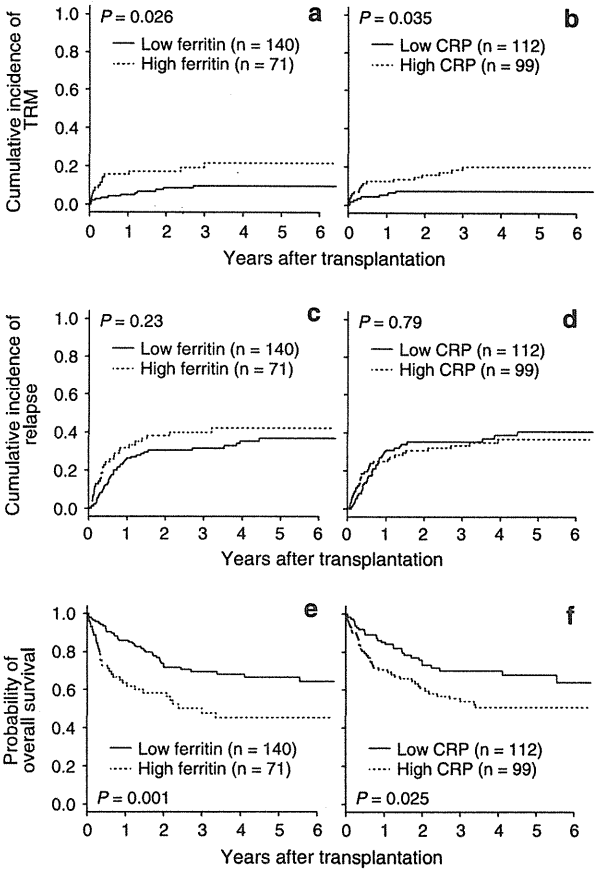

Fig. 3 Cumulative incidence of TRM according to pretransplant **a** ferritin and **b** CRP levels, and relapse according to pretransplant **c** ferritin and **d** CRP levels. The Kaplan–Meier estimate of overall survival according to pretransplant **e** ferritin and **f** CRP levels is also shown. **a**, **c**, **e** *Solid line* low-ferritin group (<880 ng/mL), *dotted line*, high-ferritin group (≥880 ng/mL). **b**, **d**, **f** *Solid line* low-CRP group (<2 mg/L), *dotted line* high-CRP group (≥2 mg/L). The statistical significance between the 2 groups was calculated using the Gray test or the log-rank test. **e**, **f** Note that pretransplant high ferritin levels were significantly associated with inferior overall survival in both univariate ($P = 0.001$) and multivariate ($P = 0.002$) analyses, while high CRP levels were significantly associated with inferior overall survival in only univariate analysis ($P = 0.025$) but not in multivariate analysis ($P = 0.79$; see Table 3)

In the 211 cases, early death within 100 days after SCT occurred in 17 cases, with the cause being TRM in 11 cases and disease relapse in 6 cases. Fifty-seven patients died after day 100, with the cause of death being TRM in 16 cases and disease relapse in 41 cases. At 3 years, the patients in the high-ferritin group had a significantly inferior OS than those in the low-ferritin group (47.7 % vs. 69.5 %, $P = 0.001$; Fig. 3, panel e); the patients in the high-CRP group had a significantly inferior OS than those in the low-CRP group (54.0 % vs. 70.1 %, $P = 0.025$; Fig. 3f). In the multivariate analysis, high ferritin levels ($P = 0.002$), older age ($P = 0.014$), male sex ($P = 0.031$),

Table 3 Multivariate analysis of TRM, relapse, and overall mortality

	Hazard ratio (95 % CI)	*P* value
TRM		
Sex		
Male	1.00	Reference
Female	0.37 (0.15–0.92)	0.034
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.19 (1.01–4.75)	0.046
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.67 (0.73–3.82)	0.23
Relapse		
Risk of disease		
Standard	1.00	Reference
High	2.83 (1.74–4.59)	< 0.001
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	1.81 (1.11–2.95)	0.018
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	0.73 (0.45–1.19)	0.21
Overall mortality		
Age at transplant		
<50 years	1.00	Reference
≥50 years	1.80 (1.13–2.87)	0.014
Sex		
Male	1.00	Reference
Female	0.58 (0.36–0.95)	0.031
Risk of disease		
Standard	1.00	Reference
High	2.51 (1.55–4.07)	<0.001
Serum ferritin level		
<880 ng/mL	1.00	Reference
≥880 ng/mL	2.16 (1.34–3.48)	0.002
Serum CRP level		
<2 mg/L	1.00	Reference
≥2 mg/L	1.07 (0.66–1.73)	0.79

and high-risk disease ($P < 0.001$) were significantly associated with a higher mortality rate (Table 3). The high ferritin levels remained to be associated with a high mortality rate among the patients who survived for 100 days or longer after transplantation ($P = 0.032$).

Discussion

In our cohort of 211 patients with hematologic diseases who underwent allogeneic HSCT, we found that an elevated CRP level before transplantation was a significant risk factor for the development of chronic GVHD. We also observed a tendency toward a lower incidence of chronic GVHD in the high-ferritin group, although a significant association was found only in patients with myeloid malignancies. In contrast, the ferritin and CRP levels were not associated with the development of acute GVHD. Consistent with previous reports, we also confirmed that an elevated ferritin level was an adverse prognostic factor for survival [2–8, 14].

Chronic GVHD is the primary cause of late morbidity and nonrelapse mortality after allogeneic HSCT. Although a history of acute GVHD is one of the strongest predictors for the development of chronic GVHD, successful strategies for reducing acute GVHD with combinations of immunosuppressive agents have not resulted in reduced incidence of chronic GVHD [19–21]. In the present study, we demonstrated that an elevated pretransplant CRP level was significantly associated with a high incidence rate of chronic GVHD, without affecting the incidence of acute GVHD (Fig. 1, panels b, d). In addition to the current study, we have analyzed the association between post-transplant (between day +50 and +99) serum CRP levels and the incidence of chronic GVHD and found no significant association between them. When we divided the 186 cases into low- and high-CRP groups based on the post-transplant serum CRP levels (<2 vs. ≥2 mg/L), the incidence of chronic GVHD was 49.4 % in the low-CRP group ($n = 96$) and 40.0 % in the high-CRP group ($n = 90$, $P = 0.43$). Chronic GVHD is considered an immune-mediated syndrome, and its clinical manifestation often resembles autoimmune and other immunological disorders. However, the pathophysiological mechanism underlying chronic GVHD remains poorly understood and there is no reliable marker for predicting and monitoring chronic GVHD. On the other hand, the pathophysiological mechanism underlying acute GVHD is thought to involve the release of proinflammatory cytokines and chemokines from damaged host tissues that activate host antigen-presenting cells and the infused donor T lymphocytes that proliferate and differentiate in response to the host antigen-presenting cells, resulting in target tissue destruction [22]. The difference between the pathophysiological mechanisms of acute and chronic GVHDs may explain our results, which showed that ferritin or CRP levels had no significant influence on the development of acute GVHD. CRP, a surrogate marker of systemic inflammation, has been shown to be a risk factor for the progression of atherosclerosis and future cardiovascular events [23, 24]. In addition, several studies have suggested that CRP itself has a proatherogenic effect [25, 26]. An elevated pretransplant CRP level may be associated with vascular endothelial damage or immune activation in transplant recipients as a

result of minute inflammation. However, whether such pretransplant status can cause an increased incidence of chronic GVHD remains unclear and requires clarification in future studies.

In contrast to the positive association between elevated CRP levels and the development of chronic GVHD, we observed an inverse association trend between high ferritin levels and the development of chronic GVHD, as previously reported by others, though it was not statistically significant ($P = 0.099$), [6, 7]. In a subgroup analysis, we observed a significant association between the high ferritin levels and the development of chronic GVHD in patients with myeloid malignancies, but not in those with lymphoid malignancies. The reason why this association was observed only in those with myeloid malignancies is uncertain. Further well-designed studies are needed to confirm this result.

Ferritin, a heteropolymer comprising 24 H- and L-type subunits, has been suggested to play a role as an immune regulator [27]. Several lines of evidence have demonstrated that ferritin can suppress the proliferation of T cells in response to mitogens, impair the maturation of B cells, and inhibit the proliferation of myeloid cells [28–30]. H-ferritin has been suggested to induce the production of interleukin 10 from regulatory T cells and suppress immune responses [31, 32]. In the light of these findings, among the patients with myeloid malignancies, the decreased incidence of chronic GVHD and significantly higher relapse rate, as determined by the multivariate analysis, in the high-ferritin group (Table 3) may be related to the suppressive effect of ferritin on adaptive immune responses. Given that ferritin levels are increased not only by iron overload but also by other factors such as inflammation, the objective measurement of liver iron content using magnetic resonance imaging [33] will be helpful in providing a more accurate analysis of the association between pretransplant iron overload and the incidence of chronic GVHD.

Several limitations of this study should be mentioned. First, the retrospective study design and heterogeneous background of the diseases and transplantation procedures might have biased the results. Second, posttransplant serum ferritin levels were not evaluated in this cohort owing to the lack of adequate information. We could obtain post-transplant (between day +50 and +99) serum ferritin data from only 28 cases among our cohort. Third, although we included CRP in the multivariate analysis, it was difficult to adjust for the effect of factors other than iron overload on ferritin levels. In a future study, measuring serum ferritin levels and liver iron content using magnetic resonance imaging before and after transplantation, and reanalyzing the effect of iron overload on the outcome may be worthwhile.

In conclusion, our results suggest that the pretransplant serum ferritin levels influenced the incidences of TRM and relapse rate after allogeneic HSCT and that, overall, an elevated pretransplant serum ferritin level is a strong adverse prognostic factor for survival. Furthermore, our results also suggest that a pretransplant serum CRP level may be a useful biomarker for predicting the risk of chronic GVHD.

Acknowledgments The authors would like to thank Rie Goi and Mika Kobayashi for their expert data management and secretarial assistance, and all the staff of our transplant team for their dedicated care of the patients and donors. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Conflict of interest The authors declare that they have no conflict of interest.

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for adult T-cell leukemia (ATL), raising the question about the role of graft-versus-leukemia effect against ATL. In this study, we retrospectively analyzed the effects of acute and chronic graft-versus-host disease (GVHD) on overall survival, disease-associated mortality, and treatment-related mortality among 294 ATL patients who received allogeneic HCT and survived at least 30 days posttransplant with sustained engraftment. Multivariate analyses treating the occurrence of GVHD as a time-varying covariate demonstrated that the development of grade 1-2 acute GVHD was significantly associated with higher overall survival (hazard ratio [HR] for death, 0.65; $P = .018$) compared with the absence of acute GVHD. Occurrence of either grade 1-2 or grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD, whereas grade 3-4 acute GVHD was associated with a higher risk for treatment-related mortality (HR, 3.50; $P < .001$). The development of extensive chronic GVHD was associated with higher treatment-related mortality (HR, 2.75; $P = .006$) compared with the absence of chronic GVHD. Collectively, these results indicate that the development of mild-to-moderate acute GVHD confers a lower risk of disease progression and a beneficial influence on survival of allografted patients with ATL. (*Blood.* 2012;119(9):2141-2148)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm that is causally associated with a retrovirus designated human T-cell leukemia virus type I (HTLV-I).[1-4] HTLV-I is endemic in southwestern Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[3,4] In Japan, more than 1 million people were estimated to be infected with HTLV-I. Although the majority of HTLV-I–infected individuals remain asymptomatic throughout their lives, ~ 5% develop ATL at a median age of 40 to 60 years.[4,5]

ATL is categorized into 4 clinical variants according to its clinical features: smoldering, chronic, acute, and lymphoma types.[6] The acute and lymphoma variants of ATL have an extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections; the median survival time is ~ 13 months with conventional chemotherapy,[7,8] although encouraging results have been recently reported with the use of novel agents such as mogamulizumab.[9-11]

Over the past decade, allogeneic hematopoietic cell transplantation (HCT) has been increasingly performed with the aim of improving dismal prognosis of patients who developed ATL.[12-18] Notably, some patients with ATL who relapsed after allogeneic HCT were shown to achieve remission only with the cessation of immunosuppressive agents, raising the question of whether the graft-versus-leukemia effect against ATL can be induced as part of graft-versus-host reaction.[19,20] In 1 study, among 10 patients who experienced relapse of ATL after transplantation and were withdrawn from immunosuppressive therapy, 8 developed graft-versus-host disease (GVHD), and 6 of them subsequently achieved

Submitted July 17, 2011; accepted January 3, 2012. Prepublished online as *Blood* First Edition paper, January 10, 2012; DOI 10.1182/blood-2011-07-368233.

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The online version of the article contains a data supplement.

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complete remission of ATL.[19] Similar observations have been rarely reported in other aggressive mature lymphoid neoplasms,[21] suggesting the unique susceptibility of ATL to graft-versus-host reactions. Recently, a combined analysis of 2 prospective studies including 29 ATL patients in total undergoing allogeneic HCT suggested that development of mild acute GVHD favorably affected overall survival and progression-free survival.[22] However, the impact of GVHD on the outcome of allogeneic HCT in ATL needs to be verified in a much larger cohort. We previously conducted a nationwide retrospective study to evaluate the current results of allogeneic HCT for ATL, and we confirmed that a substantial proportion of patients with ATL can enjoy long-term, disease-free survival after transplantation: the overall survival rate at 3 years among patients who received transplants in complete remission and not in complete remission was 51% and 26%, respectively.[23] Using the same cohort, we further evaluated the effects of acute and chronic GVHD on long-term outcomes of allografted patients with ATL.

Methods

Collection of data

Data on 417 patients with acute or lymphoma type ATL who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN), the 3 largest HCT registries in our country; their roles were detailed previously.[23] The patients were included from 102 transplant centers; the data were updated as of December 2008. The study was approved by the data management committees of JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University Graduate School of Medicine, where this study was organized.

Inclusion and exclusion criteria

Patients were included in the analysis if the following data were available: age at transplantation, sex of the recipient, donor type, stem cell source, agents used in the conditioning regimen and GVHD prophylaxis, the maximum grade and day of occurrence of acute GVHD, and the day of neutrophil recovery. Acute GVHD was reported according to the traditional criteria,[24] except that 1 patient was considered to have late-onset acute GVHD at day 133; neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Patients who missed any of these data (n = 37), who had a history of prior autologous or allogeneic HCT (n = 8), who had received an ex vivo T cell–depleted graft (n = 1), who experienced primary or secondary graft failure (n = 24) were excluded from the analysis. Because the association between the occurrence of acute GVHD and disease-associated mortality was difficult to evaluate in the event of early toxic death, patients who died within 30 days of transplantation (n = 53) also were excluded from the study. Among these 53 patients, 22 were evaluable for acute GVHD: grade 0 in 17 patients, grade 1-2 in 3 patients, and grade 3-4 in 2 patients. Two physicians (J.K. and T.I.) independently reviewed the quality of collected data, and 294 patients in total (158 males and 136 females), with a median age of 51 years (range, 18-79 years), were found to meet these criteria and included in the study: 163 patients from JSHCT, 82 patients from JMDP, and 49 patients from JCBBN. No overlapping cases were identified. Of these 294 patients, the effects of chronic GVHD, reported and graded according to using traditional criteria,[25] were considered evaluable for the 183 patients who survived at least 100 days after transplantation with complete information on the type and the day of occurrence of chronic GVHD.

End points

The primary end point of the study was the effect of acute GVHD on overall survival, defined as the period from the date of transplantation until the date of death from any cause or the last follow-up. The secondary end points of the study included the impact of acute GVHD on disease-associated and treatment-related mortality, and the impact of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL, whereas treatment-related deaths were defined as any death other than disease-associated deaths.

Statistical analysis

The probability of overall survival was estimated by the Kaplan-Meier method. Treatment-related and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[26]: disease-associated death for treatment-related mortality and treatment-related deaths for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Semi-landmark plots were used to illustrate the effects of GVHD on overall survival and cumulative incidence of disease-associated and treatment-related deaths. For patients with acute or chronic GVHD, the probability of overall survival and the cumulative incidences of disease-associated and treatment-related deaths were plotted as a function of time from the onset of acute or chronic GVHD. Day 24.5, the median day of onset for acute GVHD, was termed as the landmark day in patients without acute GVHD. In the case of patients without chronic GVHD, day 116, the median day of onset for chronic GVHD, was termed as the landmark day.

Univariate and multivariate Cox proportional hazards regression models were used to evaluate variables potentially affecting overall survival, whereas the Fine and Gray proportional subdistribution hazards models were used to evaluate variables potentially affecting disease-associated and treatment-related mortality.[27] In these regression models, the occurrence of acute and chronic GVHD was treated as a time-varying covariate.[28] In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and then transferred to the "grade 1-2 acute GVHD group" or to the "grade 3-4 acute GVHD group" at the onset of the maximum grade of acute GVHD. In the analysis of chronic GVHD, patients were assigned to the "no chronic GVHD group" at the time of transplantation and then transferred to the "limited chronic GVHD group" or to the "extensive chronic GVHD group" at the onset of the maximum grade of chronic GVHD. The variables considered were the age group of the recipient ($\leq$ 50 years or $>$ 50 years at transplantation), sex of the recipient (female or male), disease status before transplantation (complete remission, disease status other than complete remission, or unknown), intensity of conditioning regimen (myeloablative, reduced intensity, or unclassifiable), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), type of donor (HLA-matched related donor, HLA-mismatched related donor, unrelated donor for bone marrow, or unrelated cord blood), time from diagnosis to transplantation (within 6 months, $>$ 6 months, or unknown), and year of transplantation (1995-2002 or 2003-2005). We classified the intensity of conditioning regimen as myeloablative or reduced intensity based on the working definition by Center for International Blood and Marrow Transplant Research if data on dosage of agents and total-body irradiation (TBI) used in the conditioning regimen were available.[29] For 110 patients for whom such information was not fully available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by treating clinicians. The cutoff points for year of transplantation were chosen such that we could make optimal use of the data with a proviso that the smaller group contained at least 30% of patients. In the analysis of the effect of chronic GVHD, the prior history of grade 2-4 acute GVHD also was added to the multivariate models. We also assessed the interaction between acute GVHD and the intensity of conditioning regimen in the multivariate models. Only factors with a P value of less than .10 in univariate analysis were included in the multivariate models. In addition, the heterogeneities of the effects of grade 1-2 or grade 3-4 acute GVHD on overall survival according to background transplant characteristics were evaluated by the forest plots stratified by variables included in the regression analyses. Furthermore, landmark analysis treating the development of acute GVHD as a time-fixed covariate was performed to confirm

Table 1. Characteristics of patients and transplants

Variable	No. of patients, n = 294 (%)
Age group at transplant, y	
≤ 30	7 (2)
> 30-40	30 (10)
> 40-50	109 (37)
> 50-60	123 (42)
> 60	25 (9)
Sex	
Male	158 (54)
Female	136 (46)
Disease status	
Complete remission	99 (34)
Not in complete remission	178 (61)
Unknown	17 (6)
Conditioning regimen	
Myeloablative	102 (34)
Reduced intensity	128 (44)
Unclassifiable	64 (22)
GVHD prophylaxis*	
Cyclosporine-based	195 (66)
Tacrolimus-based	94 (32)
Other	5 (2)
Source of stem cells	
Bone marrow	132 (45)
Peripheral blood	111 (38)
Bone marrow + peripheral blood	2 (1)
Cord blood	49 (17)
Type of donor†	
HLA-matched related	132 (45)
HLA-mismatched related	31 (11)
Unrelated, bone marrow	82 (28)
Unrelated, cord blood	49 (17)
Time from diagnosis to transplant	
≤ 6 mo	141 (48)
> 6 mo	141 (48)
Uncertain/missing	12 (4)
Year of transplant	
1995-1999	22 (7)
2000-2002	91 (31)
2003-2005	181 (62)
Follow-up of survivors	
Median time, mo (range)	42.8 (1.5-102.3)

Data are numbers (%) unless specified otherwise.

*Cyclosporine-based indicates cyclosporine with or without other agents; tacrolimus-based indicates tacrolimus with or without other agents.

†HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, B, and DR antigens.

the results of analyses treating the occurrence of acute GVHD as a time-varying covariate; the landmark day was set at day 68 after transplantation, the date until when more than 95% of patients developed acute GVHD.

Results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CI). All tests were 2-sided, and a P value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp).

Results

Characteristics of patients and transplants

Characteristics of the patients and transplants are shown in Table 1. Most of the patients received transplants at the age of 41 to 60 years (median, 51 years). The disease status at transplantation was mainly defined as other than complete remission. The intensity of conditioning regimen was classified as myeloablative in 102 (35%) patients and reduced intensity in 128 (44%) patients; the remaining 64 (22%) patients were reported to receive cyclophosphamide plus TBI in 16 patients; busulfan plus cyclophosphamide in 15 patients; busulfan plus melphalan in 1 patient; purine analog-containing regimen in 6 patients; and other TBI-based regimens in 26 patients, although the intensity of these regimens was considered unclassifiable because of lack of dosage information. Cyclosporine-based prophylaxis against GVHD was used in more than half of patients. Patients underwent transplantation using HLA-matched related donor in 132 patients (45%), HLA-mismatched related donor in 31 patients (11%), unrelated bone marrow donor in 82 patients (28%), and unrelated cord blood unit in 49 patients (17%). Half of the patients received transplants within 6 months of diagnosis. The median time of follow-up among the survivors was 42.8 months (range, 1.5-102.3 months).

Effects of acute GVHD on overall survival

The median onset day of acute GVHD of any grade after transplantation was 24.5 (range, 5-133). Acute GVHD of grades 1-4, 2-4, and 3-4 occurred in 202 patients (69%), 150 patients (51%), and 65 patients (22%), respectively. The effect of acute GVHD on overall survival was evaluated using semi-landmark plots with reference to the following 3 categories: no acute GVHD, grade 1-2 acute GVHD, and grade 3-4 acute GVHD (Figure 1A). The impact of grade 1-2 or grade 3-4 acute GVHD on overall survival also was evaluated by forest plots stratified by background characteristics of patients and transplants (Figure 2). These analyses revealed that development of grade 1-2 acute GVHD was consistently associated with higher overall survival compared with the absence of acute GVHD, whereas occurrence of grade 3-4 acute GVHD was consistently associated with lower overall survival, except that adverse impact of grade 3-4 acute GVHD was not observed in the subgroups of patients who received transplants from an HLA-matched related or HLA-mismatched related donor. Multivariate analysis treating an occurrence of acute GVHD as a time-dependent covariate also confirmed the positive impact of grade 1-2 acute GVHD (HR, 0.65; 95% CI, 0.45-0.93; P = .018) and the adverse impact of grade 3-4 acute GVHD on overall survival (HR, 1.64; 95% CI, 1.10-2.42; P = .014; Table 2). Patients who received reduced intensity conditioning and myeloablative conditioning had similar rates of overall survival by both univariate (HR of reduced intensity vs myeloablative transplant, 1.19; 95% CI, 0.85-1.68; P = .318) and multivariate analysis (HR, 0.95; 95% CI, 0.61-1.47; P = .814). There was no interaction effect between conditioning intensity and grade 1-2 (P = .704) or grade 3-4 acute GVHD (P = .891) on overall survival. The effect of each grade of acute GVHD on overall survival was additionally evaluated. It showed that only grade 2 acute GVHD was associated with superior overall survival, whereas only grade 4 acute GVHD was associated with inferior survival (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). In the landmark analysis treating an occurrence of acute GVHD as a time-fix covariate, consistent results were obtained for patients who survived at least 68 days (landmark day), although the adverse impact of grade 3-4 acute GVHD on overall survival became no longer significant (supplemental Table 2).

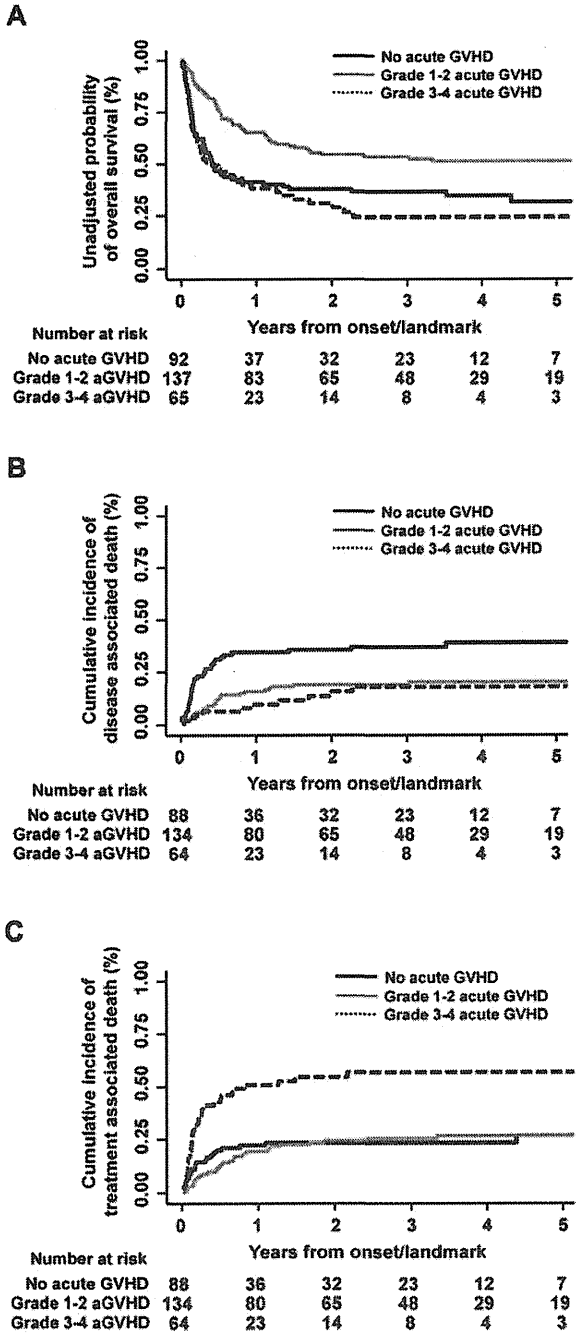

Figure 1. Semi-landmark plots for effects of acute GVHD. Semi-landmark plots illustrating the effects of acute GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

Effects of acute GVHD on disease-associated and treatment-related mortality

We next evaluated the effects of acute GVHD on disease-associated and treatment-related mortality (Figure 1B-C). Disease-associated mortality was defined as cumulative incidence of death directly attributable to relapse or progression of ATL, whereas treatment-related mortality was calculated as cumulative incidence of any death not included in disease-associated deaths. Multivariate analysis revealed that disease-associated mortality was lower in the presence of grade 1-2 and grade 3-4 acute GVHD compared with the absence of acute GVHD (grade 1-2 acute GVHD: HR, 0.54; 95% CI, 0.32-0.92; $P = .023$ and grade 3-4 acute GVHD: HR, 0.44; 95% CI, 0.22-0.90; $P = .024$; Table 2), and each grade of acute GVHD showed consistent inverse association with disease-associated mortality (supplemental Table 1). Although the risk of treatment-related mortality was not higher in the presence of grade 1-2 acute GVHD, development of grade 3-4 acute GVHD was significantly associated with higher treatment-related mortality compared with the absence of acute GVHD (HR, 3.50; 95% CI, 2.01-6.11; $P < .001$; Table 2). Patients undergoing reduced intensity transplantation and those undergoing myeloablative transplantation had similar risks of disease-associated death (HR, 0.99; 95% CI, 0.46-2.13; $P = .975$) and treatment-related death (HR, 0.98; 95% CI, 0.60-1.59; $P = .928$) by multivariate analysis. There was no interaction effect between conditioning intensity and grade 1-2 or grade 3-4 acute GVHD on disease-associated mortality and treatment-related mortality. Of 95 patients who experienced treatment-related deaths, 27 patients succumbed to infectious complications: bacterial in 13 patients, viral in 7 patients (including 3 cases of cytomegalovirus disease), viral and bacterial in 1 patient, fungal in 5 patients, and no specific organism reported in 1 patient. The proportions of patients who died of infectious complication among those without acute GVHD (n = 92), those with grade 1-2 (n = 137), and those with grade 3-4 acute GVHD (n = 65) were 4%, 9%, and 17%, respectively (supplemental Table 3). By multivariate analysis, development of grade 3-4 acute GVHD was significantly associated with higher risk of death related to infection (HR, 4.74; 95% CI, 1.51-14.8; $P = .008$), whereas the adverse influence on the infection-related deaths was less evident in the presence of grade 1-2 acute GVHD (HR, 2.17; 95% CI, 0.72-6.56; $P = .169$).

Effects of chronic GVHD on overall survival and mortality

Chronic GVHD was evaluated in 183 patients who survived at least 100 days after transplantation. The median day of chronic GVHD occurrence after transplantation was 116 (range, 100-146 days). Limited and extensive chronic GVHD occurred in 29 (16%) and 63 patients (34%), respectively. Semi-landmark plots were constructed to illustrate the effects of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality with reference to the following subgroups: no chronic GVHD, limited chronic GVHD, and extensive chronic GVHD (Figure 3). In multivariate analysis treating an occurrence of chronic GVHD as a time-dependent covariate, neither overall survival nor disease-associated mortality was significantly associated with severity of chronic GVHD, whereas treatment-related mortality was higher in the presence of extensive chronic GVHD (HR, 2.75; 95% CI, 1.34-5.63; $P = .006$) compared with the absence of chronic GVHD (Table 3). The proportions of patients who died of infectious complication among those without chronic GVHD (n = 91), those with limited chronic GVHD (n = 29), and those with extensive chronic GVHD (n = 63) were 7%, 10%, and 8%, respectively. In multivariate analysis, no statistically significant association was found between infection-related death and the occurrence of either limited ($P = .289$) or extensive GVHD ($P = .836$).

Discussion

To our knowledge, this is the largest retrospective study to analyze the impact of acute and chronic GVHD on clinical

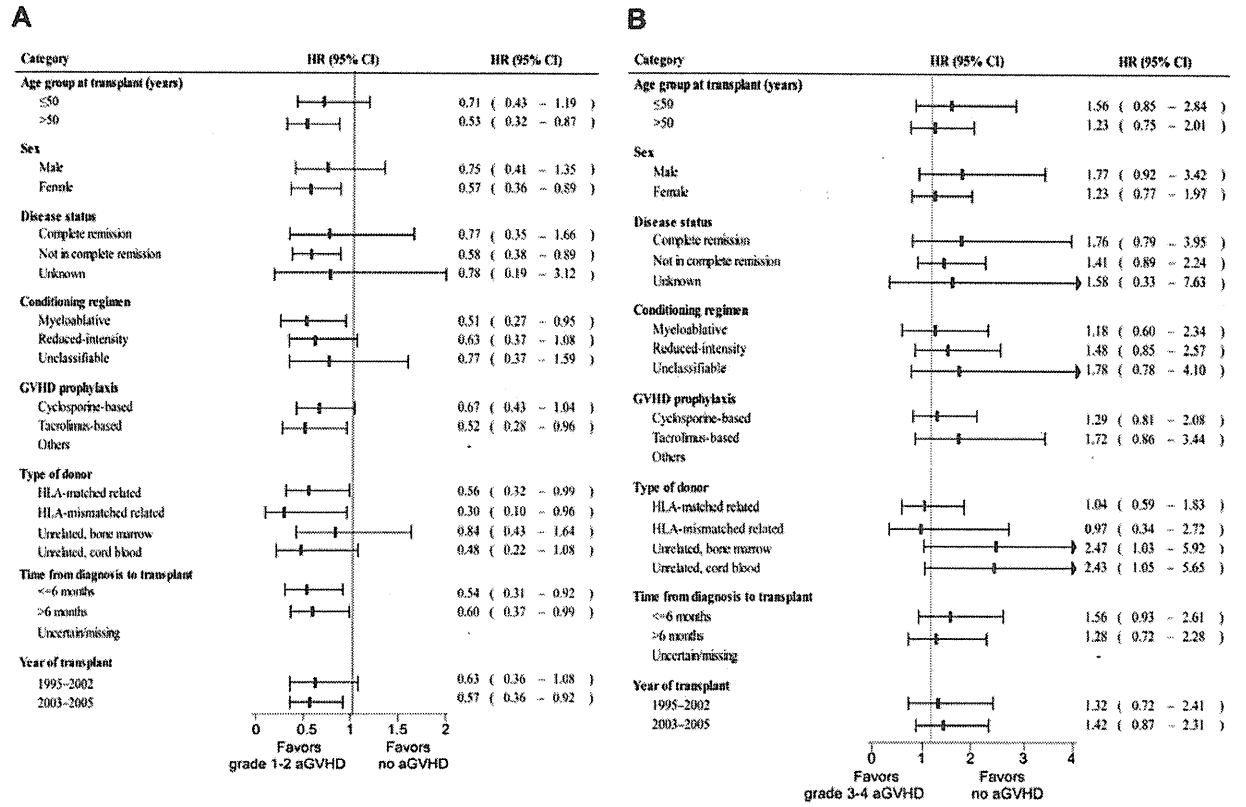

Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1-2 (A) and grade 3-4 acute GVHD (B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group," and horizontal lines represent the corresponding 95% CI. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1-2 and grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the HR comparing limited chronic GVHD versus the absence of chronic GVHD

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Overall survival*				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	.004	0.65 (0.45-0.93)	.018
Grade 3 or 4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	.099	1.64 (1.10-2.42)	.014
Disease-associated mortality†				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	.005	0.54 (0.32-0.92)	.023
Grade 3 or 4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	.010	0.44 (0.22-0.90)	.024
Treatment-related mortality‡				
Grade 1 or 2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	.649	1.22 (0.72-2.07)	.461
Grade 3 or 4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	< .001	3.50 (2.01-6.11)	< .001

*Other significant variables were sex of recipient, female (reference, 1.00) and male (HR, 1.70; 95% CI, 1.24-2.32; $P = .001$); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44-2.92; $P < .001$), and status not known (HR, 2.21; 95% CI, 1.15-4.22; $P = .017$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04-2.84; $P = .036$), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94-2.06; $P = .096$), and unrelated cord blood (HR, 1.86; 95% CI, 1.22-2.83; $P = .004$).

†Other significant variables were achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62-5.47; $P < .001$), and status not known (HR, 0.96; 95% CI, 0.21-4.49; $P = .963$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00-4.55; $P = .049$), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81-2.61; $P = .214$), and unrelated cord blood (HR, 1.25; 95% CI, 0.63-2.49; $P = .517$).

‡Another significant variable was achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74-1.84; $P = .498$) and status not known (HR, 2.31; 95% CI, 1.04-5.15; $P = .040$).

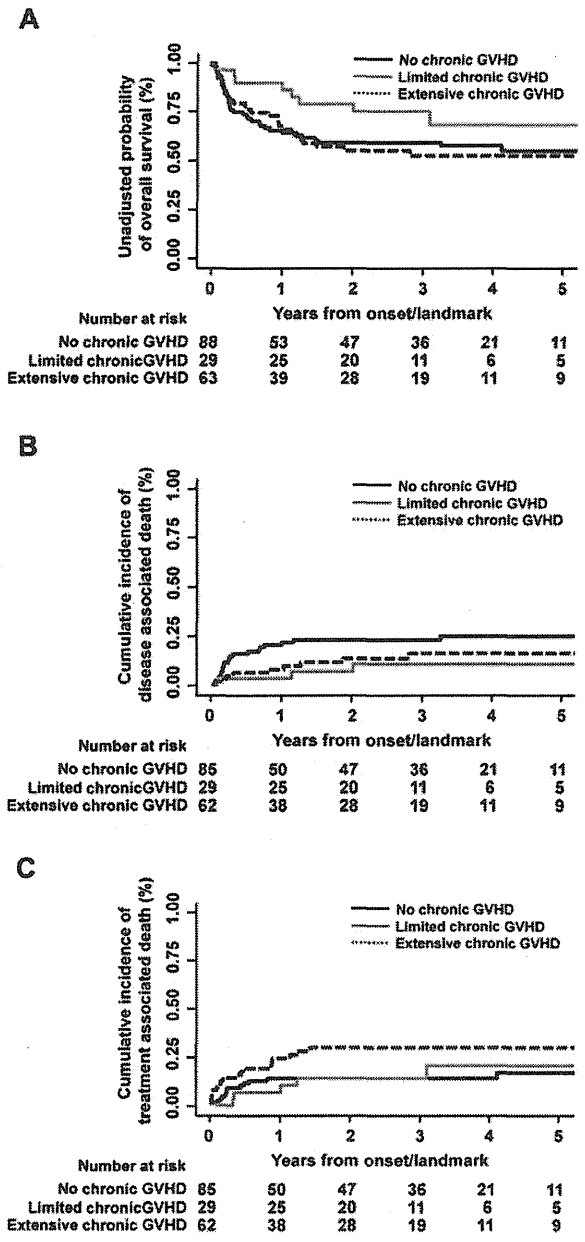

Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-leukemia responses compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD was relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] In contrast, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade 1 acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that posttransplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I–specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I–negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms, including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may

Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

Outcome	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	.353	0.72 (0.35-1.50)	.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	.131	1.40 (0.86-2.30)	.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	.183	0.45 (0.14-1.44)	.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	.563	0.80 (0.39-1.64)	.536
Treatment-related mortality‡				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	.316	1.56 (0.63-3.87)	.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	.004	2.75 (1.34-5.63)	.006

*There was no significant variable.
†There was no significant variable.
‡There was no other significant variable.

lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but also for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Second, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Third, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Last, the effect of multiple testing should be taken into account for the interpretation of the secondary end points.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

Acknowledgments

The authors are indebted to all the physicians and data managers at the centers who contributed valuable data on transplantation for adult T-cell leukemia to the JSHCT, the JMDP, and the JCBBN. They also thank all the members of the data management committees of JSHCT, JMDP, and JCBBN for their dedicated management of data.

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (T.U.).

The views expressed in this report are those of authors and do not indicate the views of the JSHCT, JMDP, or JCBBN.

This work is in memory of T.U., who died during the preparation of this manuscript.

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed and analyzed data and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T.N.-I., and S. Kato collected data from JCBBN; and A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, and J.O. interpreted data and reviewed and approved the final manuscript.

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

A list of other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN appears in the online supplemental Appendix.

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Int J Hematol (2011) 93:532–541
DOI 10.1007/s12185-011-0820-0

ORIGINAL ARTICLE

Clinical significance of subcategory and severity of chronic graft-versus-host disease evaluated by National Institutes of Health consensus criteria

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Received: 12 October 2010 / Revised: 21 February 2011 / Accepted: 16 March 2011 / Published online: 5 April 2011

Abstract To evaluate the clinical significance of subcategory and severity of chronic graft-versus-host disease (GVHD) as defined by the National Institutes of Health (NIH) consensus criteria, we retrospectively studied 211 patients with hematologic neoplasms who survived beyond 100 days after allogeneic hematopoietic cell transplantation. Endpoints included chronic GVHD-specific survival (cGSS), duration of immunosuppressive treatment, and non-relapse mortality (NRM). A total of 96 patients fulfilled the NIH diagnostic criteria for cGVHD. In univariable analysis, patients with NIH overlap syndrome tended to exhibit lower cGSS compared to those with NIH classic cGVHD [hazard ratio (HR) = 2.76, $P = 0.060$], while patients with severe cGVHD at onset had a significantly lower cGSS compared to those with mild-to-moderate cGVHD (HR = 3.10, $P = 0.034$). The duration of immunosuppressive treatment was not significantly affected by either subcategory or severity of NIH cGVHD. In multivariable analysis treating cGVHD as a time-dependent covariate, development of overlap syndrome (HR = 3.90, $P = 0.014$) or severe cGVHD at peak worsening (HR = 6.21, $P < 0.001$) was significantly associated with higher risk of NRM compared to the absence of cGVHD. Our results suggest that both the subcategory and severity of NIH cGVHD are partly correlated with cGSS and may play a useful role in distinguishing patients at high risk for NRM, warranting validation of this approach through future prospective studies.

Keywords Hematopoietic cell transplantation · Chronic graft-versus-host disease · NIH consensus criteria

1 Introduction

Chronic graft-versus-host disease (cGVHD) remains a serious complication associated with substantial late morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). In contrast to acute GVHD (aGVHD), which preferentially affects specific organs such as the skin, liver, and gastrointestinal tract, cGVHD presents with protean organ dysfunctions and various degrees of immunodeficiency that is further worsened by immunosuppressive medications used for relieving symptoms associated with GVHD [1]. Previous studies have identified a variety of factors that increase the risk of the development of cGVHD, including a prior history of aGVHD, older patient age, use of alloimmune female donors for male recipients, transplants from unrelated or human leukocyte antigen (HLA)-mismatched donors, and use of peripheral blood grafts [2–10]. In this context, clinical management of cGVHD has increasingly become more important, because recent trends in allo-HCT such as expanding applications of peripheral blood stem cell

A part of this study was presented as an abstract at the 51th Annual Meeting of American Society of Hematology, New Orleans, LA, USA, December 6, 2009.

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transplantation after reduced-intensity conditioning in older patients may increase the incidence of cGVHD [11].

Historically, aGVHD and cGVHD were distinguished based on whether immune-mediated organ dysfunction occurred within 100 days or more than 100 days after transplantation. However, accumulating experience has indicated that clinical manifestations similar to aGVHD can develop even several months after allo-HCT, while GVHD with typical features of the "chronic" form can occur as early as 2 months post-transplantation [12, 13]. Therefore, an arbitrary classification using the timing of GVHD onset is no longer considered appropriate. Another drawback in the management of cGVHD is that the grading criteria for its severity has not been standardized: it is difficult to predict the risk of GVHD-associated mortality by using historic classification that categorizes cGVHD into limited and extensive subtypes [14], because clinical severity as well as organ involvement of patients classified as having extensive cGVHD varies considerably [15–17].

To resolve these issues, the National Institutes of Health (NIH) consensus criteria were recently proposed to standardize the diagnosis and global assessment of cGVHD with a new severity scoring system based on organ-specific manifestations taking functional impact into account [18]. The NIH criteria distinguished two subcategories of cGVHD, "classic cGVHD" without features of aGVHD and "an overlap syndrome" in which characteristic features of both cGVHD and aGVHD are simultaneously present. In particular, features of aGVHD occurring beyond day 100 without manifestations of classic cGVHD are classified as "persistent", "recurrent", or "late-onset" aGVHD. Based on the number of involved organs and the severity within affected organs, each subcategory of cGVHD was graded into mild, moderate, or severe subtype. However, clinical significance of NIH cGVHD subcategory as well as their severity is not fully established, although several studies have shown their impact on overall survival, cGVHD-specific survival (cGSS), and non-relapse mortality (NRM) [19–23].

In the present study, we retrospectively evaluated patients who received allo-HCT for intractable hematologic disorders with special focus on the influences of subcategory and severity of NIH cGVHD on clinical outcomes. Since probabilities of GVHD-specific survival and discontinued immunosuppressive treatment (IST) have been most commonly used as surrogate endpoints representing the clinical resolution of cGVHD [24–26], we analyzed factors associated with these outcomes in patients who developed NIH cGVHD. We also evaluated the impact of the presence or absence of each subtype of NIH cGVHD on NRM.

2 Patients and methods

2.1 Patients

We retrospectively reviewed the medical records of 259 consecutive patients with hematologic disorders who underwent allo-HCT between January 2000 and December 2008 in our department and survived at least 100 days after transplantation. Patients were excluded if they had a history of previous allo-HCT ($n = 24$), rejected graft ($n = 4$), or relapsed before day 100 ($n = 20$); thus, a total of 211 patients were included in the present analysis. No patients received donor lymphocyte infusions before day 100. Patients with malignant hematologic neoplasms were defined as having standard-risk disease if they underwent transplantation in first complete remission or without prior chemotherapy, while those who underwent transplantation in any other status were classified as having high-risk disease. Patients with aplastic anemia were considered to have standard-risk disease. This study was approved by the Ethics Committee of Kyoto University Graduate School of Medicine. Written informed consent for the transplantation protocol was obtained from all patients.

2.2 Transplantation procedure

Patients with malignant hematologic neoplasms received myeloablative or fludarabine-based reduced-intensity conditioning regimens with or without total-body irradiation (TBI) as described elsewhere [27, 28]. Patients with aplastic anemia received conditioning regimens consisting of high-dose cyclophosphamide and horse or rabbit antilymphocyte globulin with or without 2–4 Gy TBI. None of these patients received T-cell-depleted grafts. All patients received GVHD prophylaxis by the use of cyclosporine or tacrolimus combined with or without short-term methotrexate. A proportion of patients given transplants from HLA-mismatched family members or unrelated marrow donors received mycophenolate mofetil in addition to tacrolimus plus methotrexate as GVHD prophylaxis [28]. All patients received supportive care including blood product transfusion and prophylaxis against opportunistic infections according to our institutional protocols [29].

2.3 Evaluation and management of acute and chronic GVHD

All patients were graded for aGVHD using conventional criteria, and the maximum grade until day 100 after transplantation was assigned [30]. Patients who developed grade II–IV aGVHD were initially treated with methylprednisolone or prednisolone usually at a dose of 1–2 mg/kg. Treatment of steroid-refractory aGVHD was variable.