Impact of hepatitis C virus infection on clinical outcome in recipients after allogeneic hematopoietic cell transplantation

Hideki Nakasone,[1,2,3*] Saiko Kurosawa,[3,4] Kimikazu Yakushijin,[3,5] Shuichi Taniguchi,[3,6] Makoto Murata,[7] Kazuhiro Ikegame,[8] Takeshi Kobayashi,[9] Tetsuya Eto,[10] Koichi Miyamura,[11] Hisashi Sakamaki,[9] Yasuo Morishima,[12] Tokiko Nagamura,[13] Ritsuro Suzuki,[14] and Takahiro Fukuda[3,4]

The impact of hepatitis C virus (HCV) infection on outcomes following allogeneic hematopoietic cell transplantation (HCT) remains a matter of debate. We have retrospectively examined the significance of HCV infection among recipients who received allogeneic HCT, using a Japan transplant outcome registry database between 2006 and 2009. Among 7,831 recipients, 136 were HCV-positive. The rate of hematopoietic recovery was lower in the HCV-positive group (neutrophil recovery of 500 × 10^9/L or higher: 79% vs. 87% at Day 30, $P = 0.087$; platelet recovery of 50 × 10^9/L or higher: 57% vs. 65% at Day 60, $P = 0.012$). The HCV-positive group had a significantly higher incidence of nonrelapse mortality 38% vs. 25% at 2 years, $P < 0.01$) and inferior overall survival (41% vs. 51% at 2 years, $P < 0.01$). A multivariate analysis revealed that HCV seropositivity was associated with an independent risk for higher nonrelapse mortality (hazard ratio: 1.65, $P < 0.01$) and inferior overall survival (hazard ratio: 1.39, $P < 0.01$). The incidences of death due to hepatic problems (8% vs. 2%, $P < 0.01$), bacterial infection (10% vs. 4%, $P < 0.01$), or graft failure (5% vs. 2%, $P = 0.084$) tended to be higher in the HCV-positive group. HCV infection had an adverse impact on the clinical outcome following HCT, especially in the setting of unrelated transplantation. Careful evaluation before embarking on HCT and intensive assessment against complications are warranted in HCV-infected recipients. Am. J. Hematol. 88:477–484, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Since allogeneic hematopoietic cell transplantation (HCT) was introduced about 50 years ago, the procedure has spread widely because of its potential to cure hematological diseases [1]. Recent progress in HCT has been associated with the development of stem cell sources such as peripheral blood or cord blood, alternative donors, novel strategies of immunosuppression, and reduced-intensity conditioning (RIC) regimens. However, many recipients often experience various complications, including organ failure, infection, and acute and chronic graft-versus-host disease (aGVHD and cGVHD, respectively). The identification of risk factors for these complications may help to further improve transplant outcomes.

The hepatitis C virus (HCV) was identified in 1989 [2]. It is estimated that over 2 million and 130–170 million people suffer from HCV infection in Japan and worldwide, respectively [3–5]. Because of HCV infection, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma can develop during long-term follow-up [6]. Previously, HCV was transmitted mainly by blood exposure, such as by transfusion, but recent systematic screening has reduced the proportion of transfusion-transmitted infection [7,8]. However, HCV has remained an important clinical concern because HCV-positive recipients represent 6% of long-term survivors even in the postscreening era [7,8].

The impact of HCV on HCT outcomes has remained a matter of debate. Early retrospective studies in 1990s showed that HCV infection was not associated with an increased risk for either long-term mortality or liver complications among bone marrow transplant (BMT) survivors for at least 2 years [9,10], and these results were verified by a prospective 10-year observation that included both allogeneic and autologous HCT [11]. Therefore, HCV infection was not considered a major problem in HCT for a long time [12]. However, a long-term observation revealed that HCT recipients with HCV progressed to cirrhosis more rapidly than non-HCT patients with HCV [7,13]. Furthermore, a recent case–control study by a Brazilian group reported that HCV infection was an independent risk factor for inferior survival [14]. This discrepancy may be due to the small numbers of HCV recipients and the differences in patient backgrounds. Most of these studies included less than 50 HCV-positive recipients before HCT. In addition, most of the earlier studies in the 1990s included younger recipients (a median of less than 30 years) and few, if any, cases of HCT other than related BMT [9–11,13,15]. On the other hand, the patients in the recent study by the Brazilian group included relatively older recipients (a median of 49

Additional Supporting Information may be found in the online version of this article.

[1]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [2]Division of Blood and Marrow Transplantation, Stanford University School of Medicine, Stanford, California; [3]Working Group for HSCT Complications of the Japan Society for Hematopoietic Cell Transplantation, Japan; [4]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [5]Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [6]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [7]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [8]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [9]Hematology Division, Tokyo Metropolitan Cancer & Infectious Disease Center, Komagome Hospital, Tokyo, Japan; [10]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [11]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [12]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [13]Department of Cell Processing and Transfusion, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [14]Department of HSCT Data Management and Biostatistics, Nagoya University, Nagoya, Japan

Conflicts of interest: Nothing to report.

*Correspondence to: Hideki Nakasone, MD, PhD, Division of Blood and Marrow Transplantation, Stanford University School of Medicine, 269 West Campus Drive, CCSR #2210 (Miklos' Lab), Stanford, CA 94305. E-mail: nakasone-tky@umin.ac.jp

Received for publication 26 February 2013; Accepted 5 March 2013

Am. J. Hematol. 88:477–484, 2013.

Published online 6 March 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23436

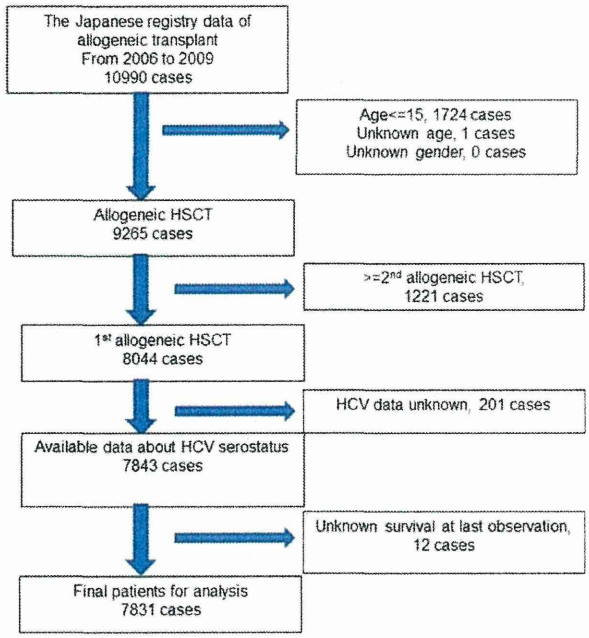


Figure 1. Scheme of patient selection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

years) and more patients who received transplant from unrelated donors (10 of 31 HCV-positive recipients) [14].

As recent progress in the modalities of HCT has spread the indications for allogeneic HCT, the impact of HCV on the clinical outcome needs to be reassessed among recent HCT patients according to the patient background.

Patients and Methods

Patient selection

The patient data were obtained from the Japan transplant outcome registry database by the Transplant Registry Unified Management Program confirmed in 2010 [16]. Eligible patients included all adult recipients (16 years or older) who received their first allogeneic HCT between January 2006 and December 2009 and for whom information on age, gender, HCV serostatus at transplantation, and survival status at last observation were available (Fig. 1). The median duration of follow-up for survivors was 691 days after HCT. This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and approved by the institutional review board at Saitama Medical Centre, Jichi Medical University.

Definitions of categories

HCV infection was reported according to the presence of anti-HCV antibody. European group for blood and marrow transplant (EBMT) risk score was recalculated as far as we could according to the previous report [17]. Since peripheral blood stem cell transplantation (PBSCT) from an unrelated donor was not available in Japan in the era of this study, HCT was categorized into three groups: related BMT/PBSCT, unrelated BMT, and unrelated cord blood transplantation (CBT). HLA mismatch was defined as incompatibility between the recipient and donor when at least a one-antigen mismatch was detected at serological levels of HLA-A, B, or DR. The intensity of conditioning was classified as myeloablative conditioning (MAC) or RIC determined based on the report by Giralt et al. [18]. Briefly, regimens that included TBI >8 Gy, melphalan ≥ 140 mg/m^2, or oral busulfan ≥ 9 mg/kg (iv busulfan ≥ 7.2 mg/kg) were classified as MAC. Other regimens were classified as RIC [18]. Neutrophil recovery was defined as the continuous achievement of neutrophil counts of 500×10^6/L or higher. Platelet recovery was also assessed from the perspective of the achievement of platelet counts of 50×10^9/L or higher. The diagnosis and severity of GVHD were based on the clinical grading score [19,20]. Sinusoidal obstruction syndrome (SOS) was reported based on the clinical symptoms [21,22]. Causes of death were determined based on "the primary cause of death" reported by the attending physicians. When the primary cause of death was GVHD or multiorgan failure (MOF), the causes of death were divided into liver GVHD and GVHD without liver involvement and into MOF with hepatic failure and MOF without hepatic failure based on the secondary causes of death, respectively. Fatal hepatic problems were defined as SOS, liver GVHD, hepatic failure due to uncertain causes, and MOF with hepatic failure. Furthermore, the primary cause of death was replaced by "the secondary cause of death" when the secondary cause of death was "rejection," "relapse," or "secondary malignancy."

Statistical analysis

Categorical and continuous variables were compared using Fisher's exact test and the Mann-Whitney test, respectively. Relapse and nonrelapse mortality (NRM) were considered as competing risk events for each other. The probabilities of relapse and NRM were estimated by cumulative incidence functions, and differences between groups were qualified by Gray's method. The cumulative neutrophil and platelet recoveries and incidences of Grades 2 to 4 aGVHD and cGVHD were also estimated and compared by Gray's method considering death without these events as a competing risk. Overall survival (OS) was estimated by the Kaplan-Meir method and compared by a log-rank test. These probabilities were estimated with a 95% confidence interval (CI). In a multivariate analysis, the Cox proportional hazard model and Gray-Fine's methods were used for OS and the cumulative incidences of events other than OS, respectively, using the following variables: HCV serostatus, gender, age, disease, disease risk, performance status, the presence of prior autologous HCT, EBMT risk score, ABO match, sex match, HLA match, conditioning regimen, GVHD prophylaxis, and donor sources. The hazard ratio (HR) of HCV seropositivity was adjusted for variables with a P-value of less than 0.1 in a univariate analysis with stepwise deletions. While all of the eligible recipients were included in the analysis of neutrophil and platelet recovery, aGVHD, cGVHD, and OS, recipients who had received HCT in nonremission and had never achieved remission after HCT until the last observation were excluded from the analysis of NRM. The impact of HCV was also compared in three subgroups that were stratified according to the donor source: related donors, unrelated BMT, and unrelated CBT. Statistical significance was defined as a two-tailed P-value of less than 0.05. All data management and statistical calculations were performed using Stata version 12.0 and R version 2.13.0.

Results

Patient characteristics

Among the 7,831 recipients who received their first allogeneic HCT between 2006 and 2009, 136 HCV-positive patients were identified. Their characteristics are shown in Table I. The median age of HCV-positive and -negative patients was 49 (range: 18–73) years and 47 (range: 16–82) years, respectively. The HCV-positive group had higher proportions of male patients (67% vs. 59%, $P=0.053$), female to male HCT (30% vs. 22%, $P=0.080$), and tacrolimus-based GVHD prophylaxis (63% vs. 55%, $P=0.076$), although the differences were not significant. There was no difference in other factors, including disease, disease risk, performance status, EBMT risk score, and conditioning regimens, between the two groups (Table I). Regarding infused cell doses, no differences were observed between the two groups when we analyzed according to donor sources: 2.7×10^8/kg vs. 2.6×10^8/kg total nuclear cells (TNC) in related BMT ($P=0.29$), 3.5×10^6/kg vs. 3.7×10^6/kg CD34-positive cells in related PBSCT ($P=0.66$), 2.6×10^8/kg TNC vs. 2.4×10^8/kg TNC in unrelated BMT ($P=0.27$), and 0.24×10^8/kg vs. 0.25×10^8/kg TNC in each in the related CBT ($P=0.83$).

Hematopoietic recovery

The cumulative probability of neutrophil recovery at 30 days after HCT in the HCV-positive group (79% [95% CI: 72–85]) tended to be lower than that in the HCV-negative group (87% [95% CI: 86–88]), but this difference was not significant ($P=0.087$) (Fig. 2A). In subgroup analyses, the rates of neutrophil recovery at 30 days after HCT tended to be lower in the HCV-positive group in the unrelated BMT group (84% vs. 92%, $P=0.094$) and significantly lower in the unrelated CBT group (46% vs. 68%, $P=0.020$). On the

TABLE I. Patient Characteristics

	HCV-positive	%	HCV-negative	%	*P*
Total number	136		7,695		
Age, years. median (range)	49 (18–73)		47 (16–82)		0.11
<50	69	51	4,269	55	
≥50	67	49	3,426	45	0.3
Gender					
Male	91	67	4,511	59	
Female	45	33	3,184	41	0.053
Disease AML	55	40	3,109	40	
ALL	12	9	1,284	17	
MDS	21	15	792	10	
MPN_CML	6	4	345	4	
Other leukemias	1	1	59	1	
Lymphomas	34	25	1,571	20	
MM_PCN	1	1	134	2	
AA_PNH_PRCA	6	4	285	4	
Other diseases	0	0	116	2	0.12
Disease risk					
Standard	81	60	4,667	61	
High	55	40	2,982	39	0.72
Missing	0	0	45	0.6	
Prior SCT					
No	126	93	7,174	93	
Autologous or syngeneic	10	7	521	7	0.73
Performance status					
0 to 1	111	82	6,450	84	
2 to 4	19	14	857	11	0.34
Missing	6	4	388	5	
EBMT risk score					
0–1	7	5	469	6	
2–4	75	55	4,272	56	
5–7	52	38	2,809	37	0.9
Missing	2	1	145	2	
Sex match					
Match	62	46	3,760	49	
Female to male	41	30	1,711	22	
Male to female	24	18	1,711	22	0.08
Missing	9	7	513	7	
ABO					
Match	71	52	3,934	51	
Bidirectional mismatch	12	9	739	10	
Major mismatch	20	15	1,420	18	
Minor mismatch	32	24	1,579	21	0.64
Missing	1	0.7	23	0.3	
Donor source					
Related BMT	15	11	1,189	15	
Related PBSCT	31	23	1,488	19	
Unrelated BMT	64	47	3,260	42	
Unrelated CBT	26	19	1,734	23	0.28
Missing	0	0	24	0.3	
Serological HLA					
Match	89	65	4,923	64	
Mismatch	46	34	2,727	35	0.79
Missing	1	0.7	45	0.6	
Conditioning					
CYTBI ± α	42	31	2,937	38	
BUCY ± α	11	8	668	9	
Other MAC	21	15	955	12	
Flu-based RIC	55	40	2,817	37	
Other RIC	7	5	314	4	0.38
Missing	0	0	4	0.1	
GVHD prophylaxis					
CsA based	47	35	3,355	44	
Tac based	86	63	4,209	55	
Other	3	2	125	2	0.076
Missing	0	0	6	0.1	

"Other diseases" include EB virus-associated disease in 43, solid tumor in 21, hemophagocytic syndrome in 12, primary immunodeficiency in 21, congenital metabolic disorders in 2, and other in 17. High-risk diseases were defined as acute leukemia in the third or more complete remission or in nonremission; CML in the third or more chronic phase, in the accelerated phase, or in blastic crisis; lymphoma and MM in stable or progressive disease status; all plasma cell leukemia; adult T-cell leukemia/lymphoma in nonremission; and all solid tumors. All other diseases were classified as standard risk.

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; PCN, plasma cell neoplasms; AA, aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; PRCA, pure red cell aplasia; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; MAC, myeloablative conditioning; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; Tac, tacrolimus.

other hand, there was no difference in the related donor groups (91% vs. 93% in the HCV-positive and -negative groups, respectively ($P = 0.35$). In a multivariate analysis, the HCV serostatus did not remain significant.

The cumulative probabilities of platelet recovery of 50 × 10^9/L or higher at 60 days after HCT in the HCV-positive group (57% [95% CI: 48–65]) was significantly lower than that in the HCV-negative group (65% [95% CI: 64–66], $P = 0.012$, Fig. 2B). In subgroup analyses, the probability of platelet recovery at 60 days after HCT in the HCV-group was significantly lower among the unrelated BMT group (58% vs. 69%, $P = 0.029$), while there was no difference in the related donor group (71% vs. 75%, $P = 0.21$) and the unrelated CBT group (28% vs. 40%, $P = 0.13$). A multivariate analysis of the whole cohort revealed that HCV seropositivity was significantly associated with a lower rate of platelet recovery of 50 × 10^9/L or higher at 60 days after HCT (HR: 0.73 [95% CI: 0.59–0.92], $P = 0.0067$, Table II and Supporting Information Table I). In multivariate analyses of subgroups according to the donor source, the HCV seropositivity showed an increased risk for lower rate of platelet recovery in the related donors group (HR: 0.73, $P = 0.042$) and unrelated BMT group (HR: 0.72, $P = 0.047$). In unrelated CBT, the difference was not statistically significant (Table II).

Incidences of aGVHD, cGVHD, and SOS

Among the total 7,831 recipients, 2,821 recipients experienced Grades 2–4 aGVHD. The cumulative incidence of Grades 2–4 aGVHD was not different between the two groups (32% [95% CI: 24–40] in the HCV-positive group vs. 36% [95% CI: 35–37] in the HCV-negative group, $P = 0.19$). Among the 4,317 recipients who experienced aGVHD of any grade, the target organs were assessed in 4,305 for whom data were available. The HCV-positive group was significantly more likely to have liver aGVHD (24% vs. 14%, $P = 0.031$).

The 2,208 recipients who experienced cGVHD during the follow-up period were also analyzed. The cumulative incidences of cGVHD were not different between the two groups (31% [95% CI: 23–39] vs. 29% [95% CI: 28–30] at 2 years, $P = 0.66$). The target organs were assessable in 2,183 of the 2,208 recipients with cGVHD. With regard to liver cGVHD, there was no difference between the two groups (45% vs. 37%, $P = 0.33$).

The proportion of patients with SOS in the HCV-positive group (9 of 135 recipients, 7%) tended to be higher than that in the HCV-negative group (274 of 7,655 recipients, 4%, $P = 0.063$). Especially, when we focused on the recipients with MAC, SOS occurred significantly more frequently in the HCV-positive group (7 of 72 recipients, 10%) than in the HCV-negative group (179 of 4,495 recipients, 4%, $P = 0.026$). On the other hand, there was no difference in the incidence of SOS between the two groups among recipients with RIC (3% in each, $P = 0.71$). In a multivariate logistic analysis of the whole cohort, HCV did not remain a significant risk factor for the development of SOS.

Nonrelapse mortality

The cumulative incidence of NRM in the HCV-positive group (38% [95% CI: 28–48] at 2 years) was significantly higher than that in the HCV-negative group (25% [95% CI: 24–27] at 2 years, $P = 0.0063$, Fig. 3A). Notably, for patients aged 50 years or older, NRM in the HCV-positive group was significantly higher than that in the HCV-negative group (54% [95% CI: 36–68] vs. 32% [95% CI: 30–34] at 2 years, $P = 0.0039$). In contrast, differences in NRM according to HCV serostatus were not observed among younger recipients (28% vs. 20% at 2 years, $P = 0.18$).

In the analysis of subgroups stratified according to donor source, HCV seropositivity had no impact on NRM in the related HCT group (21% at 2 years, in each subgroup,

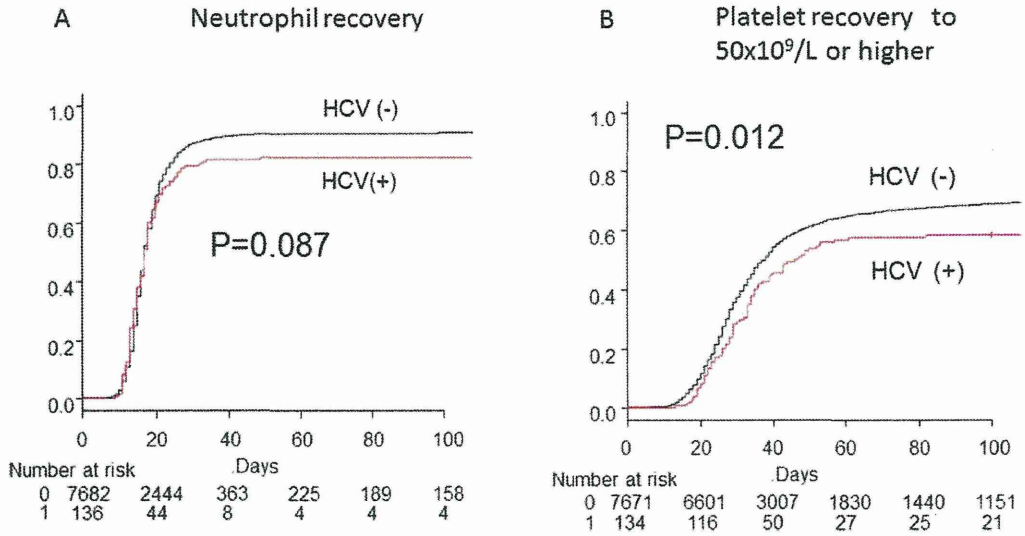


Figure 2. Comparison of the probabilities of hematopoietic recovery between the HCV-positive and -negative groups: (A) neutrophil engraftment and (B) platelet recovery to 50 × 10^9/L or higher. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$P = 0.88$, Fig. 3B). On the other hand, the adverse impact of HCV seropositivity on NRM was prominent in HCT from unrelated donors. In the unrelated BMT group, NRM in the HCV-positive group (46% [95% CI: 31–60] at 2 years) was significantly higher than that in the HCV-negative group (27% [95% CI: 25–29] at 2 years, $P = 0.0062$, Fig. 3C). Similarly, in the unrelated CBT group, NRM in the HCV-positive group (51% [95% CI: 23–73] at 2 years) tended to be higher than that in the HCV-negative group with borderline significance (31% [95% CI: 28–33] at 2 years, $P = 0.055$, Fig. 3D).

A multivariate analysis of the whole cohort revealed that HCV seropositivity was independently associated with a significantly increased risk of NRM after adjusting for age, gender, disease risk, performance status, EBMT score, the presence of prior autologous HCT, sex match, ABO match, HLA match, donor sources, and GVHD prophylaxis (HR 1.65 [95% CI: 1.19–2.30], $P = 0.0029$, Table II and Supporting Information Table I). In multivariate analyses of subgroups stratified according to donor source, the adverse impact of HCV seropositivity on NRM was observed only in HCT from unrelated donors (HR: 1.85 [95% CI: 1.23–2.80, $P = 0.0034$] in the unrelated BMT group and HR: 2.51 [95% CI: 1.19–5.31, $P = 0.016$] in the unrelated CBT group, Table II).

Overall survival

During the observation period, 3,648 of the 7,831 recipients died. The OS in the HCV-positive group (41% [95% CI: 32–50] at 2 years) was significantly lower than that in the HCV-negative group (51% [95% CI: 50–53] at 2 years, $P = 0.0070$, Fig. 4A). For patients aged 50 years or older, the OS in the HCV-positive group was significantly inferior to that in the HCV-negative group (22% [95% CI: 12–33] vs. 43% [95% CI: 41–45] at 2 years, $P < 0.0001$). No difference in OS was found among the younger recipients (60% vs. 58% at 2 years, $P = 0.55$).

In the analysis of subgroups stratified according to the donor source, the impact of HCV on OS was not seen in the related donor group (52% [95% CI: 36–66] at 2 years in the HCV-positive group vs. 54% [95% CI: 52–56] at 2 years in the HCV-negative group, $P = 0.76$, Fig. 4B). On the other hand, the adverse impacts of HCV on OS were significantly prominent in HCT from unrelated donors. In the unrelated BMT group, the OS in the HCV-positive group (39% [95% CI: 26–51] at 2 years) was significantly inferior to that in the HCV-negative group (55% [95% CI: 53–57] at 2 years, $P = 0.0054$, Fig. 4C). Similarly, in the unrelated CBT group, the OS in the HCV-positive group (28% [95% CI: 12–46] at 2 years) was also significantly inferior to that in the HCV-negative group (41% [95% CI: 39–44] at 2 years, $P = 0.039$, Fig. 4D).

A multivariate analysis of the whole cohort revealed that HCV seropositivity was independently associated with an significantly increased risk of inferior survival after adjusting for age, gender, age, disease, disease risk, performance status, EBMT score, sex match, HLA match, donor sources, conditioning regimen, and GVHD prophylaxis (HR: 1.39 [95% CI: 1.08–1.77], $P = 0.0096$, Table II and Supporting Information Table I). In multivariate analyses of

TABLE II. Impact of HCV-seropositivity on platelet recovery to 50 × 10^9/L or higher, NRM, and OS in a multivariate analysis

	Overall		Related donors		Unrelated BMT		Unrelated CBT	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Platelet recovery to 50 × 10^9/L or higher	0.73 (0.59–0.92)	0.0067	0.73 (0.53–0.99)	0.042	0.72 (0.52–1.00)	0.047	1.03 (0.53–1.99)	0.93
Nonrelapse mortality	1.65 (1.19–2.30)	0.0029	1.11 (0.52–2.39)	0.79	1.85 (1.23–2.80)	0.0034	2.51 (1.19–5.31)	0.016
Overall survival	1.39 (1.08–1.77)	0.0096	1.27 (0.81–1.96)	0.29	1.56 (1.13–2.17)	0.0076	1.78 (1.06–2.98)	0.029

HR of HCV serostatus was shown after adjusting for the factors of P less than 0.1 in univariate analysis with stepwise deletions among gender, age, disease, disease risk, performance status, EBMT score, the presence of prior autologous HCT, ABO match, sex match, HLA match, conditioning regimen, GVHD prophylaxis, and donor sources.

BMT, bone marrow transplantation; CBT, cord blood transplantation; HR, hazard ratio; CI, confidence interval.

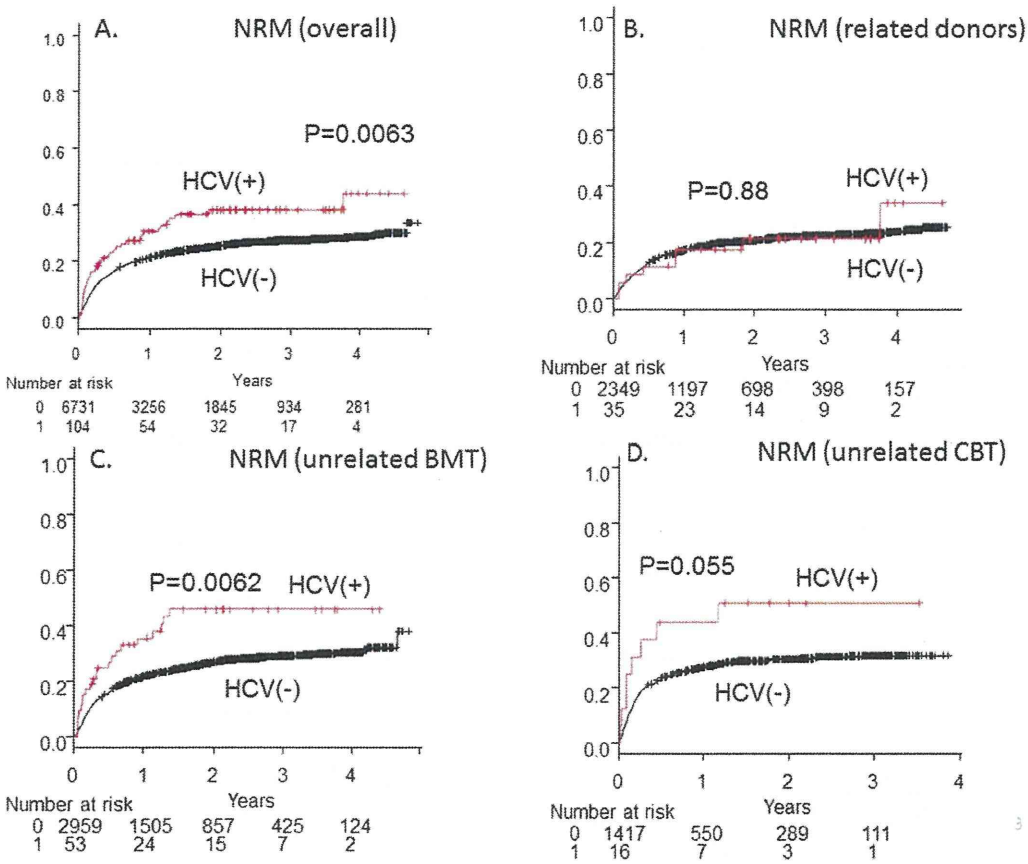


Figure 3. Comparison of the probabilities of NRM between the HCV-positive and -negative groups: (A) overall patients, (B) in the subgroup of related donors, (C) in the subgroup of unrelated BMT, and (D) in the subgroup of unrelated CBT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

subgroups stratified according to donor source, the adverse impact of HCV seropositivity remained significant, especially in HCT from unrelated donors (HR: 1.56 [95% CI: 1.13–2.17, $P = 0.0076$] in the unrelated BMT group and HR: 1.78 [95% CI: 1.06–2.98, $P = 0.029$] in the CBT group, Table II).

Causes of death

Overall, 77 (57%) of the 136 HCV-positive recipients and 3,571 (46%) of the 7,685 HCV-negative patients died during the follow-up period. The distributions of the causes of death seemed different between the two groups. The incidences of fatal hepatic problems in the HCV-positive group (8% vs. 2%, $P = 0.00034$) and fatal bacterial infection/sepsis (10% vs. 4%, $P = 0.0048$) were significantly higher than those in the HCV-negative group. In addition, the HCV-positive group had a trend of higher incidences of death due to graft-failure (5% vs. 2%, $P = 0.084$, Table III). Notably, among older recipients (50 years or older), the HCV-positive group had significantly higher incidences of fatal hepatic problems (12% vs. 3%, $P < 0.001$), fatal bacterial infection/sepsis (18% vs. 6%, $P < 0.001$), and death due to graft-failure (8% vs. 3%, $P = 0.046$). In contrast, there was no difference among younger recipients.

In an analysis of subgroups stratified according to the donor source, there was no significant difference in the incidences of fatal hepatic problems, death due to graft-failure, and fatal bacterial infection/sepsis in the related donor group (Table III). On the other hand, in the unrelated BMT group, the HCV-positive group showed a significantly higher incidence of fatal hepatic problems (14% vs. 3%, $P < 0.0001$, Table III). Furthermore, in the unrelated CBT group, HCV-positive patients had fatal bacterial infection (23% vs. 7%, $P = 0.0087$) and higher incidences of death due to graft-failure (15% vs. 4%, $P = 0.022$, Table III).

Regarding hepatocellular carcinoma, we did not found any death due to it during the short observational period.

Discussion

In this large cohort from a Japanese registry database, we showed that HCV seropositivity had adverse impacts on platelet recovery, NRM, and OS. Furthermore, this is the first to reveal that the impacts of HCV on NRM and OS differed according to the donor source and recipient age by subgroup analyses. HCV did not have an adverse impact on NRM or OS in HCT from related donors or in younger recipients, which was compatible with early studies [9–11]. On the other hand, HCV had prominent adverse effects on NRM and OS in unrelated BMT, unrelated CBT, and older recipients, which was compatible with a recent report from a Brazilian group that included unrelated donors and older recipients [14]. Therefore, we should pay attention to HCV seropositivity, especially in HCT from unrelated donors or for older recipients.

One of the reasons for the adverse impact of HCV on survival was the increased incidence of hepatic problems such as SOS and liver aGVHD. HCV is known to be a risk factor for severe SOS, although this has been controversial [9,11,23]. In our study, SOS was significantly more frequent in the HCV-positive group among recipients of MAC but not

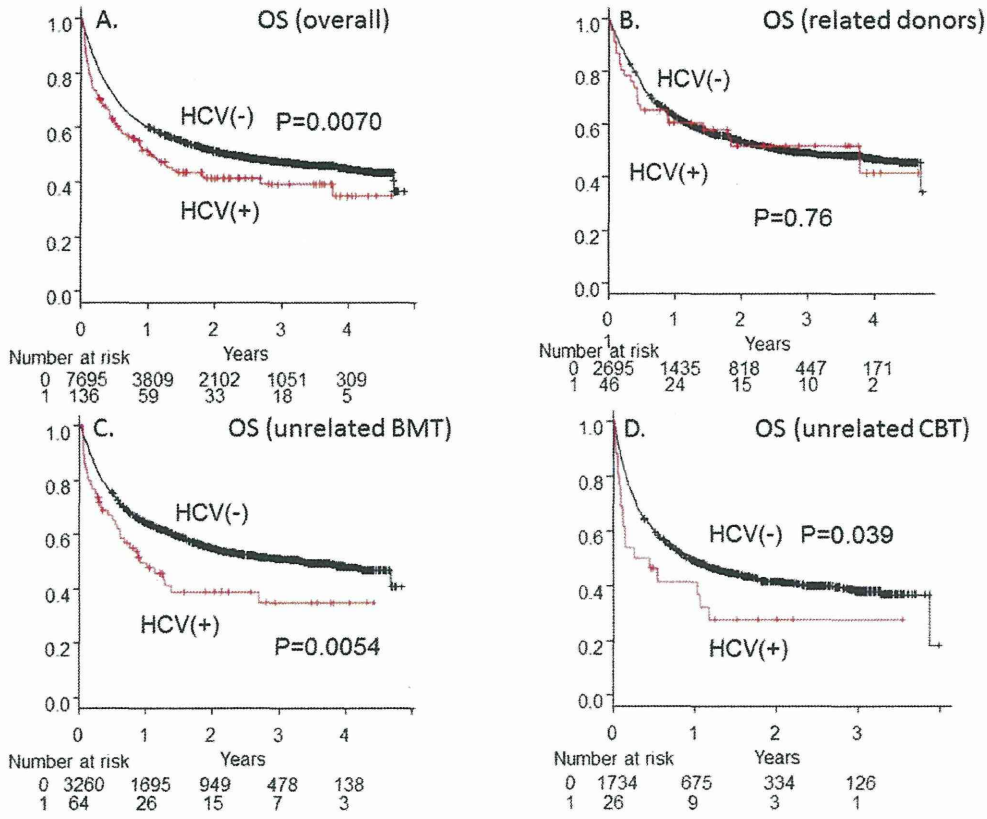


Figure 4. Comparison of the probabilities of overall survival between the HCV-positive and -negative groups: (A) overall patients, (B) in the subgroup of unrelated related donors, (C) in the subgroup of unrelated BMT, and (D) in the subgroup of unrelated CBT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of RIC, which is at least partially because the recent use of RIC and the development of supportive therapies might reduce the risk for SOS [24]. In addition, HCV is also known to alter intrahepatic cytokine profiles and deteriorate hepatic injuries [25,26]. Therefore, hepatic problems, including SOS and liver GVHD, might tend to become severe and fatal in recipients with HCV. Another possible explanation is that prolonged immunosuppression, particularly in unrelated BMT, may enhance the replication of HCV and liver dysfunction, which may result in fatal hepatitis or liver failure [23,27,28]. Otherwise, reduced and dysfunctional T cells after HCT might not be able to suppress HCV reactivation. A long-term observation reported that transplant recipients with HCV developed liver cirrhosis more rapidly than nontransplant patients with HCV [13]. The adverse impact of HCV on hepatic problems including cirrhosis and hepatocellular carcinoma might become further prominent after long-term follow-up even in our cohort.

To the best of our knowledge, this study is the first to show that pretransplant HCV infection was associated with a lower rate of platelet recovery. Thrombocytopenia is frequently observed in HCV-infected patients with chronic hepatitis [29]. The presence of hypersplenism and a low thrombopoietin level are thought to be responsible for the thrombocytopenia in HCV-infected patients [30]. In addition, HCV is known to infect CD34-positive hematopoietic progenitor cells and to suppress their maturation like megakaryocytes [31,32]. These facts might also contribute to the delay of platelet recovery following HCT in the HCV-positive group.

The current analysis further suggested that recipients with HCV would be susceptible to fatal bacterial infections, especially unrelated CBT recipients. An excess of bacterial infections in HCT recipients with HCV has been reported previously [13,14], and a similar susceptibility to bacterial infection has also been described in HCV-positive patients who receive solid organ transplantation or dialysis [33,34]. HCV-infected patients have been reported to have dysfunctional phagocytes, T cells, and B cells [35,36], as well as the impaired maturation of hematopoietic progenitor cells [31,32]. These findings suggest that the defense mechanisms against bacterial infections are impaired in recipients with HCV. The appropriate strategy for preventing infection in recipients with HCV should be explored further.

This analysis had several limitations as a result of its retrospective nature, and all information was based on the reports by attending physicians, not on a central review. The HCV-positive group might include more patients with a worse disease status, such as those who had to receive HCT despite the presence of HCV infection and liver dysfunction, although there were no differences in the patient backgrounds including disease risk, performance status, and EBMT score before HCT. In addition, the registry database had no information on baseline liver functions, viral loads of HCV, pathological grades, and the presence of cirrhosis at clinical events, which are supposed to be important for risk stratification of HCV-positive patients. Therefore, the HCV-positive group might include more recipients with highly damaged liver functions such as cirrhosis. However, when we analyzed the association

TABLE III. Causes of death

	Overall			Related donors			Unrelated BMT			Unrelated CBT		
	HCV (136)	HCV-negative (7,695)	*P*	HCV (46)	HCV-negative (2,695)	*P*	HCV (64)	HCV-negative (3,260)	*P*	HCV (26)	HCV-negative (1,734)	*P*
Total death incidence	57% (77)	46% (3,571)	0.019	48% (22)	45% (1,222)	0.77	58% (37)	42% (1,383)	0.015	69% (18)	55% (962)	0.17
Fatal hepatic problems	8.1% (11)	2.2% (173)	0.00034	2.2% (1)	1.9% (52)	0.6	14% (9)	2.6% (85)	<0.0001	3.8% (1)	2.1% (36)	0.43
SOS	2.2% (3)	1.1% (82)	–	0.0% (0)	0.9% (24)	–	3.1% (2)	1.1% (37)	–	3.8% (1)	1.2% (21)	–
Liver aGVHD	0.7% (1)	0.1% (7)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (5)	–	0% (0)	0% (0)	–
Liver cGVHD	0% (0)	0.0% (2)	–	0.0% (0)	0.1% (2)	–	0% (0)	0% (0)	–	0% (0)	0% (0)	–
Hepatic failure due to uncertain cause	4.4% (6)	0.9% (71)	–	2.2% (1)	0.8% (22)	–	7.8% (5)	1.1% (36)	–	0% (0)	0.7% (13)	–
MOF with hepatic failure	0.7% (1)	0.1% (11)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (7)	–	0% (0)	0.1% (2)	–
Fatal bacterial infection	10% (14)	4.4% (336)	0.0048	6.5% (3)	3.2% (87)	0.19	7.8% (5)	3.9% (128)	0.11	23.1% (6)	7.0% (121)	0.0087
Death due to graft Failure	5.1% (7)	2.4% (187)	0.084	2.2% (1)	1.8% (48)	0.57	3.1% (2)	2.1% (68)	0.39	15% (4)	4.1% (71)	0.022

"–" indicates that statistical assessment was not performed in a subcategory of fatal hepatic problems.
BMT, bone marrow transplantation; CBT, cord blood transplantation; SOS, sinusoidal obstruction syndrome; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; MOF, multiple organ failure.

between HCV seropositivity and the presence of "hepatic-moderate/severe" in HCT-comorbidity index [37], on which we have started to gather information since 2008, we did not find any significant associations (data not shown), although HCV-seropositive patients in this cohort might be equal to patients with possible chronic HCV-hepatitis. On the other hand, strength of this study is that it involved the largest number of recipients with HCV of all studies to date and is the first to reveal the impact of HCV seropositivity on the clinical outcome of HCT in subgroups stratified according to the donor source. These findings in addition to liver functional tests could help a further risk stratification and management of HCV-positive recipients.

To date, the strategy against HCV infection, such as peginterferon and ribavirin therapy, has shown a sustained viral remission rate of close to 50% [38,39]. Regarding HCT recipients, treatment for HCV infection after HCT has been reported to be efficient in the specific population [40]. Recent progress in novel direct-acting antiviral agents might also be beneficial among HCV-positive recipients [41]. These anti-HCV therapies would play a critical role for the control and prevention of possible HCV-induced complications after HCT, as well as risk stratification by liver functional tests.

In summary, HCV seropositivity had an adverse impact on the clinical outcome following HCT, especially in the setting of unrelated HCT and in older patients. Careful evaluation before embarking on HCT and intensive assessment against complications are warranted in HCV-infected recipients. We may need to pay more attention to hematopoietic recovery and bacterial infections as well as hepatic problems in recipients with HCV. Based on these findings, a further prospective observation is warranted to overcome the adverse impact of HCV.

Acknowledgments

The authors appreciate the contributions of all the physicians and data managers at the centers that provided valuable data on transplantation to the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN). They also thank all of the members of the Transplant Registry Unified Management committees in the JSHCT, JMDP, and JCBBN for their dedicated management of data.

Author Contributions

H.N. designed the study, analyzed data, and wrote the manuscript. S.K. and K.Y. gave advice regarding the methods, analyzed data, and wrote the manuscript. S.T., M.M., K.I., T.K., T.E., and K.M. collected data. H.S., Y.M., and T.N. collected data and were responsible for the management of data from JSHCT, JMDP, and JCBBN, respectively. R.S. analyzed and managed the unified registry database and wrote the manuscript. T.F. analyzed data, wrote the manuscript, and was responsible for the study and the Complication-WG of the JSHCT.

References

1. Powles R. 50 years of allogeneic bone-marrow transplantation. Lancet Oncol 2010;11:305–306.
2. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359–362.
3. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–567.
4. .World Health Organization. Media Centre. Hepatitis C. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/. Accessed July 9, 2012.
5. Ministry of Health Labour and Welfare. Information Examination Medical Treatment Enlightenment. About hepatitis. Available from: http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou09/hepatitis_about.html. Accessed July 9, 2012.
6. Sharara AI, Hunt CM, Hamilton JD. Hepatitis C. Ann Intern Med 1996;125:658–668.

7. Peffault de Latour R, Ribaud P, Robin M, et al. Allogeneic hematopoietic cell transplant in HCV-infected patients. J Hepatol 2008;48:1008–1017.
8. Locasciulli A, Testa M, Valsecchi MG, et al. The role of hepatitis C and B virus infections as risk factors for severe liver complications following allogeneic BMT: A prospective study by the Infectious Disease Working Party of the European Blood and Marrow Transplantation Group. Transplantation 1999;68:1486–1491.
9. Ljungman P, Johansson N, Aschan J, et al. Long-term effects of hepatitis C virus infection in allogeneic bone marrow transplant recipients. Blood 1995;86:1614–1618.
10. Tomás JF, Pinilla I, García-Buey ML, et al. Long-term liver dysfunction after allogeneic bone marrow transplantation: Clinical features and course in 61 patients. Bone Marrow Transplant 2000;26:649–655.
11. Strasser SI, Myerson D, Spurgeon CL, et al. Hepatitis C virus infection and bone marrow transplantation: A cohort study with 10-year follow-up. Hepatology 1999;29:1893–1899.
12. Socié G, de Latour RP, McDonald GB. Hepatitis C virus and allogeneic stem cell transplantation still matters! Haematologica 2009;94:170–172.
13. Peffault de Latour R, Lévy V, Asselah T, et al. Long-term outcome of hepatitis C infection after bone marrow transplantation. Blood 2004;103:1618–1624.
14. Ramos CA, Saliba RM, de Pádua L, et al. Impact of hepatitis C virus seropositivity on survival after allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Haematologica 2009;94:249–257.
15. Ivantes CA, Amarante H, Ioshii SO, et al. Hepatitis C virus in long-term bone marrow transplant survivors. Bone Marrow Transplant 2004;33:1181–1185.
16. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J Hematol 2007;86:269–274.
17. Gratwohl A. The EBMT risk score. Bone Marrow Transplant 2012;47:749–756.
18. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant 2009;15:367–369.
19. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825–828.
20. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204–217.
21. McDonald GB, Hinds MS, Fisher LD, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: A cohort study of 355 patients. Ann Intern Med 1993;118:255–267.
22. Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation 1987;44:778–783.
23. Maruta A, Kanamori H, Fukawa H, et al. Liver function tests of recipients with hepatitis C virus infection after bone marrow transplantation. Bone Marrow Transplant 1994;13:417–422.
24. Hogan WJ, Maris M, Storer B, et al. Hepatic injury after nonmyeloablative conditioning followed by allogeneic hematopoietic cell transplantation: A study of 193 patients. Blood 2004;103:78–84.
25. García-Monzón C, Sánchez-Madrid F, García-Buey L, et al. Vascular adhesion molecule expression in viral chronic hepatitis: evidence of neoangiogenesis in portal tracts. Gastroenterology 1995;108:231–241.
26. Kurkijärvi R, Yegutkin GG, Gunson BK, et al. Circulating soluble vascular adhesion protein 1 accounts for the increased serum monoamine oxidase activity in chronic liver disease. Gastroenterology 2000;119:1096–1103.
27. Henry SD, Metselaar HJ, Van Dijck J, et al. Impact of steroids on hepatitis C virus replication in vivo and in vitro. Ann N Y Acad Sci 2007;1110:439–447.
28. Vento S, Cainelli F, Mirandola F, et al. Fulminant hepatitis on withdrawal of chemotherapy in carriers of hepatitis C virus. Lancet 1996;347:92–93.
29. Streiff MB, Mehta S, Thomas DL. Peripheral blood count abnormalities among patients with hepatitis C in the United States. Hepatology 2002;35:947–952.
30. Giannini E, Borro P, Botta F, et al. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. J Hepatol 2002;37:572–577.
31. Sansonno D, Lotesoriere C, Cornacchiulo V, et al. Hepatitis C virus infection involves CD34(+) hematopoietic progenitor cells in hepatitis C virus chronic carriers. Blood 1998;92:3328–3337.
32. El Barbary MA, Saad AE, Attia FM, et al. Thrombocytopenia in patients with chronic hepatitis C: a possible role of HCV on platelet progenitor cell maturation. Angiology 2010;61:304–313.
33. López-Medrano F, Fernández-Ruiz M, Morales JM, et al. Impact of hepatitis C virus infection on the risk of infectious complications after kidney transplantation: Data from the RESITRA/REIPI cohort. Transplantation 2011;92:543–549.
34. Reddy S, Sullivan R, Zaiden R, et al. Hepatitis C infection and the risk of bacteremia in hemodialysis patients with tunneled vascular access catheters. South Med J 2009;102:374–377.
35. Ono Y, Watanabe T, Matsumoto K, et al. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor-alpha and lipopolysaccharide in patients' blood. J Infect Chemother 2004;10:200–207.
36. Pavio N, Lai MM. The hepatitis C virus persistence: How to evade the immune system? J Biosci 2003;28:287–304.
37. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: A new tool for risk assessment before allogeneic HCT. Blood 2005;106:2912–2919.
38. Rosen HR. Clinical practice. Chronic hepatitis C infection. N Engl J Med 2011;364:2429–2438.
39. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
40. Peffault de Latour R, Asselah T, Lévy V, et al. Treatment of chronic hepatitis C virus in allogeneic bone marrow transplant recipients. Bone Marrow Transplant 2005;36:709–713.
41. Asselah T, Marcellin P. Direct acting antivirals for the treatment of chronic hepatitis C: One pill a day for tomorrow. Liver Int 2012;32(suppl 1):88–102.

Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia

Yoshiko Atsuta,[1] Junya Kanda,[2] Minoko Takanashi,[3] Yasuo Morishima,[4] Shuichi Taniguchi,[5] Satoshi Takahashi,[6] Hiroyasu Ogawa,[7] Kazuteru Ohashi,[8] Yuju Ohno,[9] Yasushi Onishi,[10] Nobuyuki Aotsuka,[11] Tokiko Nagamura-Inoue,[12] Koji Kato,[13] and Yoshinobu Kanda,[2] on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

[1]Department of Hematopoietic Stem Cell Transplantation Data Management / Biostatistics, Nagoya University Graduate School of Medicine, Nagoya; [2]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; [3]The Japanese Red Cross Tokyo Blood Center, Tokyo; [4]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya; [5]Department of Hematology, Toranomon Hospital, Tokyo; [6]Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo; [8]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [9]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Kitakyushu; [10]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [11]Department of Hematology and Oncology, Japanese Red Cross Narita Hospital, Narita; [12]Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; and [13]Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

ABSTRACT

Recent advances in unrelated cord blood transplantation have increased chances and options available in allogeneic stem cell transplantation. The effect of HLA disparity on outcomes after cord blood transplantation was studied recently in mainly pediatric populations. Results showed that HLA matching in combination with total nucleated cell dose positively affects survival. The effect of HLA disparity after single-unit cord blood transplantation may be different in adults because their total nucleated cell dose is much lower compared to pediatric patients. We investigated the effect of HLA disparity on the outcome of single-unit unrelated cord blood transplantation separately in 498 children aged 15 years or under (HLA-A, HLA-B low-resolution, and HLA-DRB1 high-resolution matched [6/6], n=82, and one locus- [5/6], n=222, two loci- [4/6], n=158, three loci- [3/6] mismatched, n=36) and 1,880 adults (6/6, n=71; 5/6, n=309; 4/6, n=1,025; 3/6, n=475) with leukemia. With adjusted analyses, in children, 4/6 showed significantly increased risks of overall mortality (relative risk [RR]=1.61, P=0.042) and transplant-related mortality (RR=3.55, P=0.005) compared to 6/6. The risk of grade 2 to 4 acute GVHD was increased in 5/6 (RR=2.13, P=0.004) and 4/6 (RR=2.65, P<0.001). In adults, the risk of mortality did not increase with the number of mismatched loci (RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6). The risk of relapse was significantly decreased in 4/6 (RR=0.67, P=0.034). The risk of transplant-related mortality (TRM) or acute GVHD was not increased in 5/6 or 4/6. The effect of HLA disparity on transplant outcome differed between children and adults. In children, an increased number of mismatched HLA loci correlated with an increased risk of mortality. In adults, there was no increase in mortality with an increase in the number of mismatched HLA loci.

Introduction

Recent advances in unrelated cord blood transplantation (UCBT) have provided increased opportunities for patients with hematologic malignancies to receive hematopoietic stem cell transplantation (HSCT). This has led to an increased number of UCBT procedures over the past decade.[1,2] Clinical comparison studies of cord blood and bone marrow from unrelated donors have shown comparable results, which indicates that cord blood is a reasonable alternative donor / stem cell source.[5-12] These studies support the use of HLA-A, HLA-B, low-resolution and HLA-DRB1 zero- to two-loci-mismatched UCB for patients with leukemia in the absence of an HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched unrelated adult donor, and the use of UCB as a first-line option when a transplant is urgently required.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched bone marrow is currently the first alternative for HLA-identical sibling donors.[13-16] An increase in the number of HLA mismatches, antigen-level, or high-resolution, at HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci from 8/8 to 7/8, or 7/8 to 6/8 was associated with higher mortality with an approximately 10% reduction in survival in UBM recipients.[12,13,15] Since HLA mismatches are better tolerated after UCB with a lower incidence of severe graft-*versus*-host disease (GVHD), up to two HLA antigen mismatches of HLA-A, HLA-B, low resolution and HLA-DRB1 high resolution are considered in the current CB selection algorithm. Several reports have recently described the effect of HLA disparity on the transplant outcomes after UCBT.[9,17,18] Eapen *et al.* reported the pos-

 doi:10.3324/haematol.2012.076042
The online version of this article has a Supplementary Appendix.
Manuscript received on August 17, 2012. Manuscript accepted on January 9, 2013.
Correspondence: y-atsuta@med.nagoya-u.ac.jp

sibility of a better outcome in HLA 6/6 matched UCB in 35 recipients, and Barker *et al.* confirmed these results with a larger number of UCB recipients.[9,18] However, these studies, which assessed the effect of HLA disparity on the outcome of single-unit CBT, were mainly conducted in pediatric populations in which the infused cell dose is much greater than that in adult recipients.

The aim of this study was to assess the effect of HLA disparity on the transplant outcomes after single-unit UCBT in pediatric and adult recipients. The accumulation of single-unit CBT in adult recipients has enabled us to assess separately the effect of HLA disparity on CBT outcomes in children and adults.

Design and Methods

Study design and data source

For this retrospective observational study, recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 cord blood banks in Japan are affiliated with the JCBBN. JCBBN collected the recipients' clinical information at 100 days post-transplant through the Transplant Registry Unified Management Program (TRUMP) of the Japan Society of Hematopoietic Cell Transplantation (JSHCT).[19] Information on survival, disease status, and long-term complications including chronic graft-*versus*-host disease and second malignancies is renewed annually. Patient consent is not required for TRUMP registration of the JSHCT for the registry data consists of anonymized clinical information. This study was approved by the data management committees of the JSHCT and the JCBBN, and by the institutional review boards of Saitama Medical Center, Jichi Medical University and Nagoya University Graduate School of Medicine, Japan.

Patients

The subjects were patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), or myelodysplastic syndrome (MDS), who were recipients of their first UCBT between January 2000 and December 2009. Among 2,461 recipients of single-unit UCB with complete HLA-A, HLA-B, low-resolution and HLA-DRB1 high-resolution data, 51 recipients with 4 HLA mismatches were excluded. Thirty recipients who did not receive GVHD prophylaxis and 2 recipients for whom information regarding the conditioning regimen was missing were excluded. A total of 2378 single-unit UCB recipients (498 children aged 15 years or under at transplant, and 1880 adults aged 16 years or over at transplant) were subjects for analysis.

HLA typing

Histocompatibility data for low-resolution typing for the HLA-A, HLA-B, and HLA-DR loci and high-resolution typing for HLA-DRB1 were obtained from the TRUMP database which includes HLA information provided by cord blood banks or transplant centers. The level of HLA typing in the present study was HLA-A, HLA-B, low-resolution, and HLA-DRB1 high-resolution, as in other studies in Europe and North America. However, according to current practice in Japan, mismatches in HLA-DR loci were counted at the low-resolution level at UCB unit selection. Therefore, results regarding the effect of HLA mismatches in HLA-A, HLA-B, and HLA-DR low-resolution are also provided (*Online Supplementary Table S1*). Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients compared to HLA class I mismatched recipients. Thus, in Japan, a single-DRB1-mismatched UBM donor is preferred over a single-A-mismatched UBM or single-B-mismatched UBM donor.[15,20] This background affected HLA typing strategy of HLA-DR low-resolution typing instead of high-resolution typing for selection of cord blood units in Japan. This observation may explain the fact that the frequency of 4/6 grafts is higher in this cohort than in cohorts in Europe and the USA.

Definitions

The primary outcome of the analyses was overall survival, defined as time from transplant to death from any cause. Several secondary end points were also analyzed. Neutrophil recovery was defined as an absolute neutrophil count of at least 0.5×10^9/L cells per cubic millimeter for three consecutive points; platelet recovery was defined as a count of at least 50×10^9 platelets per cubic millimeter without transfusion support. The recipients of reduced-intensity conditioning were also defined with the criteria above, according to the previous report that confirmed complete donor chimeras of all engrafted patients after CBT with reduced-intensity conditioning.[21] Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[22,23] Relapse was defined as the recurrence of underlying hematologic malignant diseases. Transplant-related death was defined as death during a continuous remission.

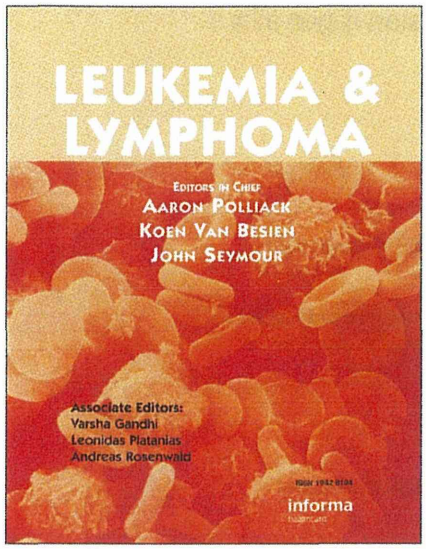
Statistical analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages are shown for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and transplant-related mortality (TRM).[24] Gray's test was used for group comparisons of cumulative incidences.[25] An adjusted comparison of the groups with regard to overall survival (OS) was performed with the use of the Cox's proportional-hazards regression model.[26] For other outcomes with competing risks, Fine and Gray's proportional-hazards model for the subdistribution of a competing risk was used.[27] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for transplant-related mortality (TRM), relapse was the competing event.[28] For acute GVHD, subjects were limited to those who engrafted, and for chronic GVHD, subjects were limited to those who engrafted and survived at least 100 days after transplantation.

The variables considered were the patient's age at transplant (5 years or over *vs.* under 5 years for pediatric recipients, and 50 years or over *vs.* under 50 years for adult recipients; cut-off points were around the median in each group), patient's sex, donor-patient sex mismatch (matched *vs.* male to female *vs.* female to male), donor-patient ABO mismatch (major mismatch *vs.* matched or minor mismatch), diagnosis (AML, ALL, CML or MDS), disease status at conditioning (first or second complete remission (CR) of AML, 1CR of ALL, first chronic phase of CML, and refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases *vs.* advanced for all others), the conditioning regimen (reduced-intensity conditioning *vs.* myeloablative conditioning), and the type of prophylaxis against GVHD (tacrolimus-based *vs.* cyclosporine-based). Conditioning regimens were classified as myeloablative if total-body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used based on the report from the Center for International Blood and Marrow Transplant Research.[29,30] We cat-

informa
healthcare

Just Accepted by *Leukemia & Lymphoma*



Quantitative PCR detection of CEP110-FGFR1 fusion gene in a patient with 8p11 syndrome

Shohei Yamamoto, Yasuhiro Ebihara, Shinji Mochizuki, Toshiro Kawakita, Seiko Kato, Jun Ooi, Satoshi Takahashi, Arinobu Tojo, Nozomi Yusa, Yoichi Furukawa, Naoki Oyaizu, Junichi Watanabe, Ken Sato, Fumihiko Kimura, Kohichiro Tsuji

doi:10.3109/10428194.2013.767455

Letter to the Editor

Quantitative PCR detection of CEP110-FGFR1 fusion gene in a patient with 8p11 syndrome

Shohei Yamamoto[1], Yasuhiro Ebihara[1], Shinji Mochizuki[2], Toshiro Kawakita[3], Seiko Kato[3], Jun Ooi[3], Satoshi Takahashi[3], Arinobu Tojo[3], Nozomi Yusa[4], Yoichi Furukawa[4], Naoki Oyaizu[5], Junichi Watanabe[6], Ken Sato[6], Fumihiko Kimura[6], Kohichiro Tsuji[1,2]

[1]Department of Pediatric Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Division of Stem Cell Processing, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [3]Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [4]Department of Applied Genomics, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [5]Department of Pathology and Laboratory Medicine, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [6]Division of Hematology, Department of Internal Medicine, National Defense Medical College, Saitama, Japan

Corresponding Author: Shohei Yamamoto, Department of Pediatric Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. TEL: +81-3-5449-5694; FAX: +81-3-5449-5428. E-mail: y-shohei@ims.u-tokyo.ac.jp

tions, and the germline sets (A and C) have the advantage of not requiring DNA sequencing or specific custom-made primers.

Two DNA MRD markers with high sensitivity (at least 10^{-4}) are generally required in MRD intervention clinical trials,[1,9] and in a large cohort of 2854 pediatric precursor B ALL patients, 20% of patients had only one sensitive marker and 8% had none.[9] Four of the 16 cases evaluated in this study had only one sensitive Ig/TCR marker so that availability of *IKZF1*-based MRD testing would have been useful for their risk stratification. Using routine PCR, *IKZF1*Δ3–6 rearrangements were identified in 6% of ALL patients in the ANZCHOG cohort in this study, so inclusion of this marker in standard screening for MRD targets would be an easy way to provide more patients with two sensitive markers.

The concept of using disease-related markers for MRD testing has been already established for fusion transcripts such as BCR-ABL and for gene rearrangements such as for *SIL-TAL1* in T-ALL and for *MLL* rearrangements in infant ALLs.[10] Kuiper *et al.*[4] in an analysis of paired diagnosis and relapse samples from 34 patients found *IKZF1* deletions and nonsense mutations in 14 (41%) patients at diagnosis and showed that all were conserved at relapse, in contrast to other recurrent genetic lesions found at diagnosis such as *PAX5*, *CDKN2A* and *EBF1*. It is therefore likely that this *IKZF1* marker will be at least as stable as Ig/TCR rearrangements, although this will need to be confirmed in more extensive studies.

In summary, we have assessed three ways to measure MRD levels by RQ-PCR for the most common deletion of the *IKZF1* gene found in ALL and demonstrated that all three methods provided robust and sensitive MRD assays for patients with this arrangement. The two primer and probe sets based on germline sequences could be used within a few days of diagnosis to provide quantitative measures of very-early responses to therapy. We expect that *IKZF1* gene deletions (*IKZF1*Δ3–6 and probably others) will provide a useful addition to the repertoire of MRD markers currently available for monitoring MRD in ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge the NH&MRC and the Cancer Council NSW for their financial support of the Australian MRD studies, and clinicians participating in the ANZCHOG Study 8 trial particularly Dr L Dalla Pozza. Standardization and quality control for MRD testing is supported by the EuroMRD (previously ESG-MRD-ALL) group. Children's Cancer Institute Australia for Medical Research is affiliated with both the University of New South Wales and Sydney Children's Hospital.

NC Venn[1], VHJ van der Velden[2], M de Bie[2], E Waanders[3], JE Giles[1], T Law[1], RP Kuiper[3], V de Haas[4], CG Mullighan[5], M Haber[1], GM Marshall[1,6], Norris MD[1], JJM van Dongen[2] and R Sutton[1]

[1]*Children's Cancer Institute Australia for Medical Research, Lowy Cancer Research Centre, Sydney, New South Wales, Australia;*
[2]*Department of Immunology, Erasmus MC, Rotterdam, The Netherlands;*
[3]*Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands;*
[4]*Dutch Childhood Oncology Group, The Hague, The Netherlands;*
[5]*Department of Pathology, St Jude Children's Research Hospital, Memphis, TN, USA and*
[6]*Children's Centre for Cancer and Blood Disorders, Sydney Children's Hospital, Randwick, New South Wales, Australia*
E-mail: rsutton@ccia.unsw.edu.au

REFERENCES

1 Brüggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG *et al.* Standardized MRD quantification in European ALL trials: proceedings of the Second international symposium on MRD assessment in Kiel, Germany, 18–20 September 2008. *Leukemia* 2009; **24**: 521 .
2 Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J *et al.* BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 2008; **453**: 110–114.
3 Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB *et al.* Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med* 2009; **360**: 470–480.
4 Kuiper RP, Waanders E, van der Velden VH, van Reijmersdal S, Venkatachalam R, Scheijen B *et al.* IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia* 2010; **24**: 1258–1264.
5 Waanders E, van der Velden VH, van der Schoot CE, van Leeuwen FN, van Reijmersdal SV, de Haas V *et al.* Integrated use of minimal residual disease classification and IKZF1 alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. *Leukemia* 2011; **25**: 254–258.
6 van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grümayer R *et al.* Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative data. *Leukemia* 2007; **4**: 604–611.
7 van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, Flohr T, Sutton R, Schrauder A *et al.* Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. *Leukemia* 2007; **21**: 706–713.
8 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **8476**: 307–310.
9 Flohr T, Schrauder A, Cazzaniga G, Panzer-Grümayer R, van der Velden V, Fischer S *et al.* Minimal residual disease (MRD)-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia (ALL). *Leukemia* 2008; **22**: 771–782.
10 van der Velden VHJ, Corral L, Valsecchi MG, Jansen MWJC, De Lorenzo P, Cazzaniga G *et al.* Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. *Leukemia* 2009; **23**: 1073–1079.

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Prognostic factors for acute myeloid leukemia patients with t(6;9)(p23;q34) who underwent an allogeneic hematopoietic stem cell transplant

Leukemia (2012) **26,** 1416–1419; doi:10.1038/leu.2011.350; published online 9 December 2011

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often selected as a curative treatment strategy for acute myeloid leukemia (AML). In particular, AML patients with poor cytogenetics at diagnosis are considered for allo-HSCT as the first-line therapy.[1–3] Recently, we have reported that AML with the t(6;9)(p23;q34) abnormality, which predicts a very poor prognosis in patients treated with chemotherapy,[4] is associated with an

outcome in patients receiving allo-HSCT that is comparable to that in patients with a normal karyotype.[5] However, 55% of the AML patients with t(6;9)(p23;q34) eventually had a negative outcome. We herein performed a further analysis for AML patients with t(6;9)(p23;q34) who received allo-HSCT to identify the prognostic factors affecting their overall survival (OS).

A total of 64 *de novo* AML patients with t(6;9)(p23;q34) detected in G-band staining at diagnosis, who received their first allo-HSCT between January 1996 and December 2007, were extracted from the databases of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Cord Blood Bank Network. The cytogenetic data were analyzed according to the Southwestern Oncology Group criteria for each institution, instead of by central review.[2] The clinical data were collected using a standardized report form, which was submitted at 100 days, 1 year and annually after HSCT. This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Written informed consent was obtained in accordance with the Declaration of Helsinki. The OS was defined as the number of days from HSCT until death from any cause. Non-relapse mortality (NRM) was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. The analysis was performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; www.r-project.org).[6] The probability of OS was calculated using the Kaplan–Meier method and compared using the log-rank test. The probabilities of transplant related mortality and disease relapse were compared using the Grey test[7] and were analyzed using the cumulative incidence analysis,[6] while considering relapse and death without disease relapse as respective competing risks. The following variables related to the survival of the adult patients older than 15 years and their clinical data were compared in a univariate analysis: recipient characteristics (age; younger than 35 vs. older than 35 years, gender, performance status at diagnosis; 0 to 2 vs. 3 or 4, FAB classification; M2 or others, positivity for peroxidase in leukemic blasts at diagnosis; less than 50% vs. greater than 50%, cytogenetic abnormality), donor characteristics (age; younger than 35 vs. older than 35 years, gender, sex compatibility, compatibility of cytomegalovirus antibody serostatus, relationship; related vs. unrelated, and ABO compatibility), transplant characteristics (disease status at HSCT; complete remission (CR) vs. non-CR, use of total body irradiation as a preconditioning regimen, source of the graft; bone marrow, peripheral blood stem cell, cord blood (CB)), graft-versus-host disease prophylaxis; cyclosporine versus tacrolimus and the use of methotrexate. Multivariate Cox models were used to evaluate the hazard ratios associated with the prognosis. Covariates found to be significant in the univariate analyses ($P \leqslant 0.10$) were included in the models. For both the univariate and the multivariate analyses, *P*-values were two sided, and outcomes were considered to be significant for $P \leqslant 0.05$.

The characteristics of the 64 AML patients with t(6;9)(p23;q34) were shown in Table 1a. The OS of the seven pediatric patients younger than 14 years old seemed to be better than the OS of the 57 adult patients older than 15 years, although there were no statistically significant differences between the groups (Figure 1a, the probability of 3-year OS in pediatric patients and adult patients was 83% and 48%, respectively ($P = 0.12$)). We performed a further analysis in the 57 adult patients older than 15 years. The univariate analysis showed that the disease status at HSCT was the sole significant prognostic factor affecting the OS (Figure 1b, the probability of 3-year OS in patients with CR and with non-CR at HSCT was 69% and 29%, respectively ($P < 0.003$)), and the number of HLA disparities, M2 in the FAB classification and CB as the source of the graft were calculated to have a *P*-value < 0.1 (Table 1b). No statistically significant tendencies related to gender, gender mismatch between the donor and recipient, recipient cytomegalovirus serostatus or the use of total body irradiation for the preconditioning regimen were observed. The cumulative incidence of relapse and of NRM are shown in Figure 1c; the cumulative incidence of relapse was significantly lower in patients with a CR at HSCT than in patients without CR, although such differences were not seen in the cumulative incidence of NRM between these two groups (the 3-year cumulative incidence of relapse was 25% in CR patients and 58% in non-CR patients ($P = 0.005$), and the 3-year cumulative incidence of NRM was 10% in CR patients and 16% in non-CR patients ($P = 0.85$)). In the multivariate analysis, the disease status at HSCT and FAB-M2 remained the significant variables associated with the OS (Table 1b). The OS of the patients categorized by the combination of the disease status at HSCT and FAB-M2 showed a favorable outcome in FAB-M2 patients with a CR at HSCT (Figure 1d, the probability of 3-year OS in patients with CR/FAB-M2, CR/non-FAB-M2, non-CR/FAB-M2 and non-CR/non-FAB-M2 was 76%, 60%, 43% and not reached, respectively ($P < 0.001$)). In contrast, the patients who

Table 1a. Characteristics of patients with t(6;9)(p23;q34)

	Children (n = 7)	*Adult (n = 57)*
Age, median (range)	9 (6–14)	35 (17–58)
Gender, male/female	1/6	34/23
FAB classification[a]		
M0	0	1
M1	0	7
M2	5	32
M4	1	13
M5	1	2
Status at HSCT, CR/non-CR	5/2	29/28
HLA disparity[b]		
0	2	24
1	2	5
2	0	10
Graft source		
BM	3	32
PBSC	2	12
CB	2	13

Abbreviations: BM, bone marrow; CB, cord blood; CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; PBSC, peripheral blood stem cells. [a]Data not available in 2 adult patients. [b]Data not available in 3 pediatric patients and 18 adult patients.

Table 1b. Prognostic factors affecting overall survival of adult patients with t(6;9)(p23;q34)

Variables	*Risk factors*	*Univariate*	*Multivariate*		
			HR	*95% CI*	*P-value*
Disease status at HSCT	CR Non-CR	<0.003	1 2.54	1.17-5.51	<0.02
FAB classification	M2 other than M2	0.075	1 3.61	1.59–8.21	<0.003
Number of HLA disparity	0 1 2	0.061		NA	
Source of the graft	BM or PBSC CB	0.076		NA	

Abbreviations: BM, bone marrow; CB, cord blood; CI, confidence interval; CR, complete remission; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; NA, not assessed; PBSC, peripheral blood stem cell.

Bone Marrow Transplantation (2013) **48,** 529–536

www.nature.com/bmt

ORIGINAL ARTICLE

Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan Transplant Outcome Registry

S Kurosawa[1], K Yakushijin[2], T Yamaguchi[3], Y Atsuta[4], T Nagamura-Inoue[5], H Akiyama[6], S Taniguchi[7], K Miyamura[8], S Takahashi[9], T Eto[10], H Ogawa[11], M Kurokawa[12], J Tanaka[13], K Kawa[14], K Kato[15], R Suzuki[4], Y Morishima[16], H Sakamaki[6] and T Fukuda[1]

The outcomes for allogeneic hematopoietic cell transplantation (allo-HCT) are heavily influenced by non-relapse mortality (NRM). We retrospectively assessed the changes in the incidence and causes of NRM after allo-HCT over the past 12 years. NRM, relapse rate and OS were analyzed using the Japan transplant outcome database of 6501 adult patients with acute leukemia or myelodysplastic syndrome who received their first allo-HCT in remission from 1997 through 2008. In multivariate analysis in patients aged 16–49 years, the adjusted hazard ratios (HRs) for NRM for 2001–2004 and 2005–2008 were 0.78 (95% confidence interval, 0.65–0.93) and 0.64 (0.54–0.78), respectively, compared with 1997–2000. The HR for overall mortality in 2005–2008 was 0.81 (0.70–0.93) compared with 1997–2000. In patients aged 50–70 years, the HRs for NRM and overall mortality in 2005–2008 were 0.56 (0.46–0.68) and 0.66 (0.47–0.93), respectively, compared with those in 2001–2004. We found that causes of death that contributed to the changes in NRM varied among subgroups. In conclusion, our study indicated that the incidence of NRM after allo-HCT has significantly decreased over the past 12 years, which has led to an improvement of OS, and also showed reductions in NRM in subgroups consisting of older patients and those who received unrelated cord blood transplantation.

Bone Marrow Transplantation (2013) **48,** 529–536; doi:10.1038/bmt.2012.172; published online 10 September 2012

Keywords: leukemia; allogeneic hematopoietic cell transplantation; non-relapse mortality; GVHD; cord blood transplantation

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) has been recognized as a potent strategy for curing hematological malignancies. However, there have always been concerns about the risk of non-relapse mortality (NRM). As the risk of relapse is known to be significantly reduced after allo-HCT, the outcome of and indications for allo-HCT are heavily influenced by the risk for NRM.

Over the past few decades, many changes have been made to improve the outcome after allo-HCT, including improvements in the conditioning regimen, donor selection, and prophylaxis and treatment for organ complications, GVHD and infectious diseases, which have led to a reduction in NRM.[1–4]

Although an improvement in NRM has been reported in relatively younger patients who have received allo-HCT from a BM or peripheral blood (PB) donor, NRM has not been fully examined in other settings, such as in elderly patients, or in cord blood (CB) transplantation.

To evaluate the effects of these advances, we retrospectively assessed the changes in the incidence and causes of NRM over the past 12 years, using a nationwide registry database of more than 6000 patients who received various types of allo-HCT.

PATIENTS AND METHODS

Data source

The clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP) and the Japan Cord Blood Bank Network (JCBBN). The JSHCT collect clinical data through the Transplant Registry Unified Management Program, as described previously.[5] This study was approved by the data management committees of JSHCT, JMDP and JCBBN, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated the data on patients aged between 16 and 70 years who had AML, acute lymphocytic leukemia (ALL) or myelodysplastic syndrome (MDS), and who received their first allo-HCT between 1997 and 2008. We compared the incidence of NRM after allo-HCT in three consecutive 4-year

[1]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [2]Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [3]Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, Japan; [4]Department of HSCT Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [5]Department of Cell Processing and Transfusion, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [6]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [7]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [8]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [9]Department of Hematology and Oncology, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [10]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [11]Department of Internal Medicine, Division of Hematology, Hyogo College of Medicine, Hyogo, Japan; [12]Department of Haematology & Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [13]Stem Cell Transplantation Center, Hokkaido University Hospital, Sapporo, Japan; [14]Department of Hematology and Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; [15]Division of Haematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan and [16]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan. Correspondence: Dr T Fukuda, Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: tafukuda@ncc.go.jp
Received 15 June 2012; revised and accepted 6 August 2012; published online 10 September 2012

periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without recurrent disease after allo-HCT. Analyses were performed for patients with acute leukemia/MDS in remission or low-risk MDS (refractory anemia with or without ringed sideroblast: RA/RARS). Analyses were performed on the basis of patients' age (16–49 years and 50–70 years) and donor source (HLA-6/6-serum-matched or 1-Ag-mismatched related, unrelated BM and unrelated CB). In the era considered by this study, only BM from unrelated volunteer donors was used in Japan. In 2003, JMDP nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, JMDP required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1, and also recommended high-resolution typing of the C-locus. Conditioning regimens were classified as indicated by Giralt *et al.*[6] The incidences of mortality associated with GVHD, infection and organ failure were analyzed. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized.

Statistical analysis

Data were retrospectively reviewed and analyzed as of June 2011. Among the three time periods, patient characteristics were compared using the χ^2 test. The primary end point of the study was NRM after allo-HCT. Probabilities of NRM and relapse were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM, and with NRM viewed as a competing risk of relapse. The Pepe and Mori test was used to evaluate the differences between groups. For the 151 patients (2%) who were known to have relapsed but whose date of relapse was unavailable, mid-point imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The probability of OS was estimated using the Kaplan-Meier product limit method, and 95% confidence intervals (CIs) were calculated using the Greenwood formula. To compare the OS between groups, the log-rank test was used. Incidences of NRM, relapse and OS were estimated as probabilities at 3 years from allo-HCT. Multivariate analyses for NRM and relapse were performed using competing risk regression by the method of Fine and Gray, and for OS using a Cox proportional hazard regression model. The multivariate analyses were performed separately among patients aged 16–49 years and patients aged 50–70 years, where the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients, 2001–2004 vs 2005–2008 among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL or MDS), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-6/6-Ag-matched sibling vs other family donors, HLA-6/6-Ag-matched unrelated BM, mismatched unrelated BM or unrelated CB) and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. Multivariate analyses were also performed separately for those receiving related allo-HCT, where HLA-6/6-Ag-matched sibling vs other family donors were considered as covariates for the donor source, those receiving unrelated BM transplantation (UBMT), where HLA-6/6-Ag-matched unrelated BM vs mismatched BM were considered as covariates, and those who received UCBT, where the covariates above were examined other than the donor source. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and the SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

RESULTS

Patients

A total of 6501 patients registered from 266 institutions across the country[5] were analyzed, with a median age of 40 years and a median follow-up of 39 months. Characteristics of the patients and

Table 1. Patients' characteristics according to the time period of transplant

Characteristics	*1997–2000,* N *(%)*	*2001–2004,* N *(%)*	*2005–2008,* N *(%)*	P
Total number of patients	1354	2292	2855	
Age at transplant (years)				<0.001
16–34	740 (55)	892 (39)	862 (30)	
35–49	491 (36)	783 (34)	939 (33)	
50–59	116 (9)	489 (21)	743 (26)	
60–70	7 (1)	128 (6)	311 (11)	
Donor source				<0.001
Related BM	511 (38)	367 (16)	504 (18)	
Related peripheral blood	158 (12)	546 (24)	456 (16)	
Unrelated BM	588 (43)	998 (44)	1312 (46)	
Unrelated cord blood	14 (1)	321 (14)	534 (19)	
Others	83 (6)	60 (3)	49 (2)	
Disease type				0.991
AML	699 (52)	1226 (53)	1516 (53)	
ALL	505 (37)	744 (32)	949 (33)	
MDS	150 (11)	322 (14)	390 (14)	
Disease status				0.001
CR1	811 (60)	1288 (56)	1802 (63)	
CR2	311 (23)	552 (24)	654 (63)	
CR3 or beyond	76 (6)	96 (4)	77 (3)	
MDS RA/RARS	83 (6)	202 (9)	267 (9)	
Other remission state/no detailed data	73 (5)	154 (7)	55 (2)	
Conditioning				<0.001
Myeloablative	1131 (84)	1585 (69)	1788 (63)	
Reduced-intensity	21 (2)	394 (17)	689 (24)	
Not categorized	202 (15)	313 (14)	378 (13)	
GVHD prophylaxis				<0.001
CYA-based	1041 (77)	1367 (60)	1354 (47)	
Tacrolimus-based	270 (20)	825 (36)	1373 (48)	
No data available	43 (3)	100 (4)	128 (4)	

Abbreviations: MDS = myelodysplastic syndrome; RA/RARS = refractory anemia with or without ringed sideroblast.