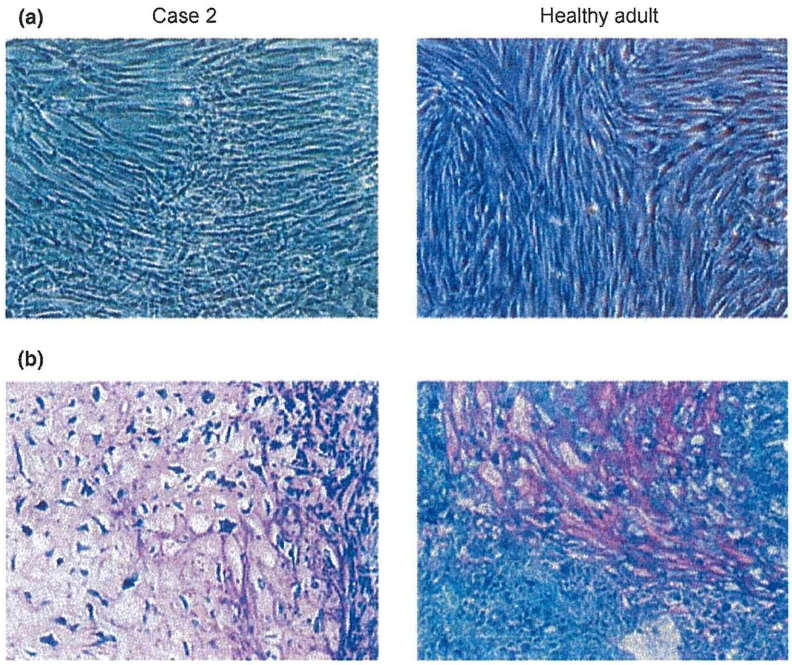

Fig. 1. Mesenchymal stem cells of BM from a haemophilia patient (case 2) and a healthy adult. (a) Morphology of the cultured cells from a haemophilia patient (case 2) and a healthy adult. They were spindle-shape like MSCs (×100). (b) Chondrocyte differentiation of BMMSCs from a haemophilia patient (case 2) and a healthy adult. Cells were stained with toluidine blue, and the stained cells were chondrocytes.

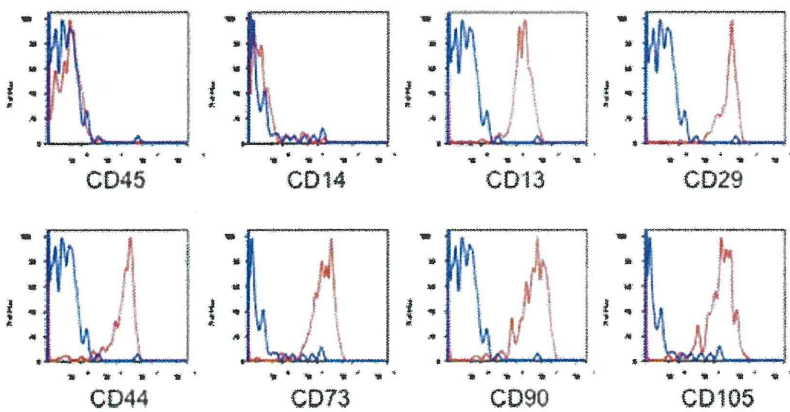

Fig. 2. Flow cytometric profiles of BMMSCs derived from a haemophilia patient (case 3). Cultured cells were stained with phycoerythrin (PE)-conjugated CD45, CD13, CD29, CD44 and CD90, and fluorescein isothiocyanate (FITC)-conjugated CD14, CD73 and CD105. Red and blue lines indicate data from the sample of case 3 and negative control respectively (×200).

All the cell numbers of MSCs derived from 2 mL of BM blood in three haemophilia patients were more than one million as well as a healthy adult (Table 1). Our previous experience demonstrated that 10 million of BMMSCs were enough to repair substantial range of articular cartilage defect in OA patients [3]. Consequently, we need to obtain more than 20 mL of BM blood from each patient to repair articular cartilage defects in haemophilic arthropathy, and it is possible under local anaesthesia.

In addition, chromosomal analysis revealed that the cultured cells from BM cells of three haemophilia patients had normal karyotype (Table 1), suggesting little possibility of the transformation of BMMSCs during the present culture. Thus, MSCs capable of proliferating *in vitro* and differentiating into chondrocytes were safely generated from BM cells of haemophilia patients similarly with those from healthy adult, indicating the feasibility of the regenerative medicine using BMMSCs to repair articular cartilage defects in the patients with haemophilic arthropathy.

Some of adult patients with haemophilia have chronic viral infection. To apply the regenerative medicine using BMMSCs to such patients, it is important to validate that our culture system of BMMSCs does not provoke the reactivation of the viruses. This possibility is now under investigation.

Table 1. Characteristics of patients and a healthy adult, and their BMMSCs.

Case	1	2	3	Healthy adult
Age	23	20	19	55
Type of haemophilia	A	A	A	–
Cell number/2 mL BM blood*	1.5×10^6	1×10^6	1×10^6	1×10^6
Karyotype of MSC†	46, XY (10/10)	46, XY (10/10)	46, XY (3/3)	46, XY (5/5)
Chondrocyte formation	+	+	+	+

*The number indicates the average of two cultures of 2 mL of BM blood.
†The number in each parenthesis indicates the number of cells analyzed.

Besides the transplantation of autologous BMMSCs, autologous chondrocyte implantation (ACI) may be considerable for the repair of articular cartilage defects in the patients with haemophilic

arthropathy. However, ACI needs the excision of healthy cartilage tissue even though it is in nonweight bearing areas. The excision procedure is too invasive to operate upon haemophilia patients because they have a significant bleeding tendency. In this regard, as autologous BMMSC transplantation is less invasive, it is more feasible for the treatment of haemophilic arthropathy than AIC.

As mentioned above, BMMSCs from patients with haemophilia have the potentials to proliferate and differentiate into chondrocytes *in vitro*, similarly with healthy adult. Therefore, it must be promising to treat the articular defects in patients with haemophilic arthropathy by the transplantation of autologous BMMSCs, as this procedure is safe and needs less invasive intervention.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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A case of pigmented villonodular synovitis in conjunction with a platelet release defect in a paediatric patient

V. BALAKRISHNAN* and A. L. DUNN†
**Emory College of Arts and Sciences, Emory University, Atlanta, GA, USA; and †Division of Pediatric Hematology/Oncology, Department of Pediatrics, Emory University/Children's Healthcare of Atlanta, Aflac Cancer Center and Blood Disorders Service, Atlanta, GA, USA*

Introduction

Pigmented villonodular synovitis (PVNS), which has also been known as diffuse type giant cell tumour, is a rare, benign, proliferative lesion of the synovium of unclear aetiology. It has an incidence of 4–9 cases per million. The process can be diffuse – mainly affecting large joints such as hips or knees, or localized/nodular most commonly involving smaller joints such as the fingers. The affected synovium may invade and destroy surrounding bone and soft tissues [1]. Patients often present with complaints of joint pain and swelling. In PVNS of the hip, instances of extreme pain are usually caused by haemorrhage into the joint space [2]. The aetiology of PVNS remains unknown and diagnosis remains difficult, although MRI helps to characterize it to an extent [3]. Agonist-specific deficiencies in the platelet aggregation response are a cause of excessive bleeding, and inherited variants of the agonist receptors cause issues with signalling pathways [4]. Inherited platelet disorders typically cause mucocutaneous bleeding such as epistaxis, gum bleeding, menorrhagia and bruising, however, more severe bleeding can be manifested [5].

Correspondence: Amy L. Dunn, Emory Children's Center, 2015 Uppergate Drive Room 426-1, Atlanta, GA 30322, USA.
Tel.: +404-727-1608; fax: +404-727-3681;
e-mail: amy.dunn@choa.org

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Case report

A 7-year-old previously healthy male presented to the emergency department with complaints of left hip pain, limp and fever. Multiplanar MRI of the pelvis was performed and demonstrated a moderate left hip effusion along with abnormal signal intensity in the adductor magnus muscle and distal epiphysis of the left fibula. No intra-articular mass was noted. An arthrocentesis was performed and it revealed hemosiderotic fluid. All cultures were negative. A PT, aPTT, factor VIII and IX were performed and were all normal. The patient's pain improved after surgery and he was discharged home.

Because possible joint haemorrhage was noted at the time of surgery the patient was referred to the outpatient haematology clinic. His history was positive for trauma-related bruising. He did not have

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ORIGINAL ARTICLE

Reduced-intensity allogeneic stem cell transplantation for patients aged 50 years or older with B-cell ALL in remission: a retrospective study by the Adult ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation

This article has been corrected since Advance Online Publication and an erratum is also printed in this issue.

H Kanamori[1], S Mizuta[2], S Kako[3], H Kato[4], S Nishiwaki[5], K Imai[6], A Shigematsu[7], H Nakamae[8], M Tanaka[1], K Ikegame[9], T Yujiri[10], T Fukuda[11], K Minagawa[12], T Eto[13], T Nagamura-Inoue[14], Y Morishima[15], R Suzuki[16], H Sakamaki[17] and J Tanaka[18]

We retrospectively assessed the outcome and pretransplantation predictors of the outcome in 118 patients aged ⩾50 years who received fludarabine-containing reduced-intensity allo-SCT (RIST) for B-cell ALL in the first or second CR. Eighty patients received transplants from unrelated donors. Seventy-eight patients were positive for the Ph chromosome. The median follow-up period was 18 months and the 2-year OS rate was 56%. The 2-year cumulative incidence of relapse and non-relapse mortality was 28% and 26%, respectively. The incidence of grades II–IV and III–IV acute GVHD was 46% and 24%, respectively. After 2 years, the incidence of chronic GVHD was 37%. Multivariate analysis of pretransplant factors showed that a higher white blood cell count (⩾30×10^9/L) at diagnosis (hazard ratio (HR) = 2.19, $P = 0.007$) and second CR (HR = 2.02, $P = 0.036$) were significantly associated with worse OS, whereas second CR (HR = 3.83, $P < 0.001$) and related donor (HR = 2.34, $P = 0.039$) were associated with a higher incidence of relapse. Fludarabine-containing RIST may be a promising strategy for older patients with B-cell ALL in their first remission.

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Keywords: ALL; elderly; reduced-intensity SCT

INTRODUCTION

The overall CR rate is very high (80–90%) for adult ALL due to the efficacy of induction therapy with relatively low toxicity, which allows many patients to receive postremission therapy. However, adult ALL has a poor long-term outcome, with the 5-year OS rate being only 39–50% despite aggressive chemotherapy[1,2] and declining to 15% for patients over 50 years old.[3] At present, allogeneic hematopoietic SCT (allo-HSCT) is thought to be the most potent therapy for prevention of relapse in adult ALL patients. A recent large-scale prospective study showed that allo-HSCT from matched sibling donors achieved a better outcome compared with chemotherapy or autologous transplantation.[4] However, Goldstone *et al.*[4] reported that TRM is unacceptably high for high-risk older patients and this counteracts the reduced risk of relapse.[4] Therefore, reduced-intensity conditioning allo-HSCT (RIST) is performed in older patients and those who are unsuitable for myeloablative conditioning with the aim of reducing TRM, although its antileukemic efficacy is uncertain.[5–7] In general, the relationship between age and the prognosis of ALL patients aged between 20 and 65 years shows a continuum.[3] Because most older patients are excluded from clinical studies, very few prospective trials have investigated the efficacy of chemotherapy and/or allo-HSCT tailored for older patients. Therefore, more clinical data are needed to establish the optimum transplant strategy for elderly patients with ALL. Accordingly, the objectives of this study were to analyze the outcome and identify pretransplant outcome predictors in older patients with B-cell ALL undergoing RIST.

PATIENTS AND METHODS

Patient selection and data sources

This study enrolled patients aged 50 years or older who received RIST for B-cell ALL in the first or second remission between 2000 and 2009 in Japan.

[1]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [2]Department of Hematology, Fujita Health University Hospital, Toyoake, Japan; [3]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [4]Division of Molecular Medicine, Aichi Cancer Center Research Institute, Nagoya, Japan; [5]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [6]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [7]Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [8]Department of Hematology, Osaka City University Graduate School of Medicine, Osaka, Japan; [9]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [10]Third Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan; [11]Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan; [12]Division of Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [13]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [14]Department of Cell Processing and Transfusion, Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [15]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [16]Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [17]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan and [18]Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan. Correspondence: Dr H Kanamori, Department of Hematology, Kanagawa Cancer Center, 1-1-2 Nakao, Asahi-ku, Yokohama 241-8515, Japan.
E-mail: kanamori@kcch.jp
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Data were provided by the Japan Society of Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP) and the Japan Cord Blood Bank Network (JCBBN). Information on transplantation was collected at 100 days after allo-HSCT, whereas the data concerning survival, disease status and long-term complications, including chronic GVHD and second malignancies, were renewed annually from follow-up forms. This study was approved by the data management committees of the JSHCT, JMDP and JCBBN. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki Principles.

Graft sources

Peripheral blood stem cell (PBSC) donation from unrelated donor was not permitted until 2009 in Japan. If recipients have no suitable related donors, physicians choose alternative graft sources according to recipient's condition and institutional strategy. HLA matching of related donor–recipient pairs was mainly performed using serologic typing methods. HLA matching of unrelated BM and umbilical cord blood (CB) was performed using low- or high-resolution molecular typing for HLA-A, -B and -C, and high-resolution molecular typing for HLA-DRB1.

Study end points and definitions

The primary endpoints of the study were non-relapse mortality (NRM), relapse, leukemia-free survival (LFS) and OS. NRM was defined as death while in remission, and relapse was defined as hematological recurrence of leukemia. LFS was defined as survival without evidence of relapse or progression and OS was calculated from the date of allo-HSCT. Death from any cause was treated as an event and surviving patients were censored at the date of last contact. The day of engraftment was defined as the first of 3 consecutive days on which the ANC was $\geqslant 0.5 \times 10^9$/L. Acute and chronic GVHD were diagnosed and graded according to established criteria.[8,9] We defined a reduced-intensity regimen as having the following dosage levels: BU <9 mg/kg, melphalan $\leqslant 140$ mg/m^2 and TBI <500 cGy (single or fractionated) or <800 cGy (fractionated).[10]

Statistical analysis

The final date of analysis was 30 November 2010. We compared demographic factors and disease characteristics according to the donor source by using Fisher's exact test for categorical data and the Mann–Whitney *U*-test for continuous variables. LFS and OS were estimated by the Kaplan–Meier method. The Cox proportional hazards model was used for univariate and multivariate analyses. Gray's test was used to compare the cumulative incidence curves for relapse and NRM.[11] Death without acute GVHD was defined as the competing event for acute GVHD, whereas death without neutrophil engraftment and second transplantation without engraftment were the competing events for neutrophil engraftment, NRM and second transplantation without relapse were the competing events for relapse, and relapse and second transplantation were the competing events for NRM. The proportional hazard regression model of Fine and Gray[12] was used for univariate and multivariate analyses of these competing risks. All covariates with $P<0.10$ according to univariate analysis were entered into the multivariate model. All tests were two-sided and $P<0.05$ was considered to indicate significance. Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R software (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6–3) that includes functions frequently used in biostatistics.[13]

RESULTS

Background of transplantation

The patient and graft characteristics are summarized in Table 1. A total of 187 patients aged $\geqslant 50$ years received RIST for ALL. Of these, 35 patients in non-remission, 32 patients with non-B cell (uncertain in 20, T cell in 10 and null cell in 2) and 2 patients aged $\geqslant 70$ years were excluded in this analysis. There were 118 patients in the study cohort and their median age was 59 years (range: 50–69 years). There were early pre-B-cell type in 6 patients, pre-B-cell type in 34 and common type in 78 according to immunophenotype classification. The median WBC count at diagnosis was 15.6×10^9/L (range: 0.8–1967 $\times 10^9$/L). BM was the most common source of stem cells (55%), followed by cord blood (CB) (24%) and PB (21%). The median time from diagnosis to RIST was 200 days (range: 75–3372 days). TBI was used for 74 patients and its dosages were as follows: 200 cGy in 13 patients, 300 cGy in 9, 400 cGy in 52 and 600 cGy in 1.

Transplantation was carried out on HLA-matched related donors in 33 patients, HLA-mismatched related donors in 5, HLA-matched unrelated donors in 47 and HLA-mismatched unrelated donors in 33. RIST from unrelated donors was significantly more frequent in patients aged 60–69 years compared with those aged 50–59 years. T-cell depletion was performed in six patients (five patients with antithymocyte globulin and 1 with antilymphocyte globulin). The median time from diagnosis to RIST from related and unrelated donors were 154 days (range: 75–617 days) and 229 days (range: 79–3372 days), respectively ($P=0.029$). Furthermore, use of TBI and GVHD prophylaxis showed significant differences among patients with different donor sources (Table 1).

Engraftment

The median time until neutrophil engraftment after transplantation was 16 days (range: 9–39 days). Three patients died before day 35 without achieving neutrophil recovery. Sustained engraftment was achieved in 113 of the remaining 115 patients, whereas primary graft failure was confirmed in two patients who received CB transplantation. One patient died of primary graft failure on day 60, but the other was salvaged by repeat transplantation. The median time to platelet count recovery ($\geqslant 20 \times 10^9$/L) was 26 days (range: 0–154 days). Seven patients died within 60 days after transplantation and stable engraftment of platelets was seen in 102 of the 111 patients who survived beyond day 60.

Acute and chronic GVHD

The incidence of GVHD according to donor type is shown in Table 2. The cumulative incidence of grade II–IV and grade III–IV acute GVHD was 46% and 24%, respectively. Stem cell and donor sources were not associated with the incidence or grade of acute GVHD. The cumulative incidence of chronic GVHD after 2 years was 37%. Limited chronic GVHD was noted in 16 patients (16%), whereas 24 patients (24%) had extensive chronic GVHD. After RIST from related donors, there was a significantly higher incidence of chronic GVHD compared with after RIST from unrelated donors ($P=0.012$). Also, RIST with PB from related donors was associated with a significantly higher incidence of chronic GVHD than when BM or CB was the source (63% vs 36% and 37%, respectively; $P=0.019$).

Outcome

The median follow-up period for the survivors was 18 months (range: 2–77 months). The 2-year LFS, OS, cumulative relapse rate and NRM were 66%, 56%, 28% and 26%, respectively (Figure 1). Detailed results, including the incidence of GVHD, are shown in Table 2, with stratification by donor source. Fifteen patients (14 with NRM and 1 with relapsed leukemia) died within 100 days after transplantation. They included 12 of the 57 patients receiving fludarabine + melphalan, but the conditioning regimen did not have a significant impact on 2-year OS (68% for fludarabine + i.v. BU, 64% for fludarabine + oral BU, 47% for fludarabine + melphalan and 63% for fludarabine + CY; $P=0.472$). When OS at 2 years was stratified according to stem cell source, it was 56%, 55%, 43% and 47% ($P=0.301$) for related BM, related PB, unrelated BM and unrelated CB, respectively. In addition, the 2-year OS of patients with ($n=78$) and without ($n=40$) the Ph chromosome was 58% and 52%, respectively ($P=0.997$). In this study, the information of pre- and post-transplant treatment with tyrosine kinase inhibitors was obtained in only 45 and 9 patients,

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Clinical Factors Predicting the Response of Acute Graft-versus-Host Disease to Corticosteroid Therapy: An Analysis from the GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation

Makoto Murata [1,2,*], Hideki Nakasone [2,3], Junya Kanda [2,3], Takahiko Nakane [3,4], Tatsuo Furukawa [2,5], Takahiro Fukuda [6], Takehiko Mori [7], Shuichi Taniguchi [8], Tetsuya Eto [9], Kazuteru Ohashi [10], Masayuki Hino [4], Masami Inoue [11], Hiroyasu Ogawa [12], Yoshiko Atsuta [13], Tokiko Nagamura-Inoue [14], Hiromasa Yabe [15], Yasuo Morishima [16], Hisashi Sakamaki [10], Ritsuro Suzuki [13]

[1] *Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[2] *GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation*
[3] *Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan*
[4] *Department of Hematology, Graduate School of Medicine, Osaka City University, Osaka, Japan*
[5] *Division of Bone Marrow Transplantation, Niigata University Medical and Dental Hospital, Niigata, Japan*
[6] *Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan*
[7] *Division of Hematology, Keio University School of Medicine, Tokyo, Japan*
[8] *Department of Hematology, Toranomon Hospital, Tokyo, Japan*
[9] *Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan*
[10] *Hematology Division, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan*
[11] *Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan*
[12] *Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan*
[13] *Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[14] *Department of Cell Processing and Transfusion, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[15] *Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan*
[16] *Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan*

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A B S T R A C T

Systemic corticosteroid therapy is recommended as a first-line treatment for acute graft-versus-host disease (GVHD). We performed a retrospective study to identify the factors affecting the response of grade II to IV acute GVHD to systemic corticosteroid therapy using the Japanese national registry data for patients who received first allogeneic hematopoietic cell transplantation with bone marrow (BM) (n = 1955), peripheral blood stem cells (PBSCs) (n = 642), or umbilical cord blood (UCB) (n = 839). Of 3436 patients, 2190 (63.7%) showed improvement of acute GVHD to first-line therapy with corticosteroids. Various factors were identified to predict corticosteroid response. Interestingly, UCB (versus HLA-matched related BM) transplantation was significantly associated with a higher probability of improvement, whereas HLA-matched unrelated BM and HLA-mismatched stem cell sources other than UCB were significantly associated with a lower probability of improvement. HLA-matched related PBSC transplantation was not significantly different from HLA-matched related BM transplantation. Patients without improvement from corticosteroid therapy had a 2.5-times higher nonrelapse mortality and a .6-times lower overall survival rate. The present study demonstrated, for the first time, a higher probability of improvement in grade II to IV acute GVHD with systemic corticosteroid therapy in patients after UCB transplantation than in those after BM and PBSC transplantation. A prospective study is warranted.

INTRODUCTION

Despite prophylactic treatment with immunosuppressive agents, acute graft-versus-host disease (GVHD) remains a major problem after allogeneic hematopoietic cell transplantation (HCT). Several studies have evaluated a variety of agents added to prednisone [1-7], but the use of prednisone or methylprednisolone alone is recommended as a standard first-line treatment for acute GVHD [8]. The response rate is approximately 40% to 60%, and patients unresponsive or resistant to corticosteroid therapy have an increased risk of mortality related to uncontrolled GVHD [2,9-16]. Some clinical factors are reported to be statistically predictive of a response to systemic corticosteroid therapy: HLA-mismatched donor transplantation, unrelated donor transplantation, combination of male recipient and female donor, early onset of GVHD, higher grade of GVHD, and liver or gut involvement of GVHD have lower response rates [2,9,10,14].

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* Correspondence and reprint requests: Makoto Murata, MD, PhD, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa, Nagoya, Aichi 466-8550, Japan.
E-mail address: mmurata@med.nagoya-u.ac.jp (M. Murata).

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These significant factors were identified in retrospective studies in which most or all patients underwent bone marrow (BM) transplantation. However, stem cell sources for allogeneic HCT have changed dramatically with the frequent use of peripheral blood stem cells (PBSCs) and umbilical cord blood (UCB), and no study has compared the response rates of corticosteroid therapy among stem cell sources.

To identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for patients with grade II to IV acute GVHD, a retrospective study was conducted using the national registry data on 3436 patients who received first allogeneic HCT in Japan with BM (n = 1955), PBSCs (n = 642), or UCB (n = 839).

PATIENTS AND METHODS

Patients

Clinical data for patients who received the first allogeneic HCT in Japan, achieved neutrophil engraftment (>.5 × 10^9/L), developed grade II to IV acute GVHD, and received systemic corticosteroid therapy as a first-line treatment for acute GVHD were extracted from the Transplant Registry Unified Management Program system, which is a registry of the outcomes of Japanese transplantation patients [17]. Patients who relapsed before GVHD development were excluded, as were patients who received other agents as initial therapy in addition to systemic corticosteroid therapy. This study was approved by the Data Management Committee of the Japan Society for Hematopoietic Cell Transplantation and by the ethical committee of the Nagoya University School of Medicine.

Definitions

Acute GVHD was diagnosed and graded according to established criteria [18]. Persistent nausea with histologic evidence of GVHD but no diarrhea was included as stage 1 gut GVHD. Responses of acute GVHD to corticosteroid therapy were defined as *improved* if the grade was improved without additional systemic treatment. Responses were evaluated without time limitation, and therefore were considered improved even if the GVHD was improved later than day 28 of corticosteroid therapy, although response by day 28 is proposed as the best endpoint to define need for second-line treatment [16]. Responses were also considered improved even if acute GVHD was improved and then a new immunosuppressant was added to treat chronic GVHD. Responses were defined as *stable or progressive* if the grade was unchanged or worsened after first-line corticosteroid therapy or if second-line systemic treatment for acute GVHD was added regardless of responsiveness to first-line corticosteroid therapy. Thus, all patients who received second-line treatment for acute GVHD were considered stable or progressive even if the GVHD was improved temporarily after corticosteroid therapy.

Acute myeloid leukemia in the first or second remission, acute lymphoblastic leukemia in the first remission, chronic myelogenous leukemia in the first chronic phase, and myelodysplastic syndromes with refractory anemia or refractory anemia with ringed sideroblasts were defined as *standard-risk malignancies*, and other malignant diseases were defined as *high-risk malignancies*.

BM transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-BM*, and BM transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-BM*. PBSC transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-PB*, and PBSC transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-PB*. For unrelated BM transplantation, all patient–donor pairs were HLA-typed to allele level for at least 3 loci (HLA-A, B, and DRB1) during the coordination process. BM transplantation from HLA-A, B, and DRB1 alleles 6/6 matched unrelated donors was defined as *MUD-BM*, and BM transplantation from HLA-A, B, and DRB1 alleles 5/6 or 4/6 matched unrelated donors was defined as *MMUD-BM*. UCB transplantation from serological HLA-A, B, and DR at least 4/6 matched donors was defined as *UCB*.

Based on the report by the Center for International Blood and Marrow Transplant Research [19], the 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 included in the conditioning regimen, whereas other conditioning regimens were classified as *nonmyeloablative*.

Onset of acute GVHD was classified into 3 groups: day ≤28, day ≥29, and unknown; however, acute GVHD that occurred earlier than day 4, which might be an error at the time of registration, was classified into unknown.

Endpoints

The primary endpoint of this study was to identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for grade II to IV acute GVHD. The secondary endpoints were to identify factors associated with nonrelapse mortality (NRM) after corticosteroid therapy and to evaluate the impact of response to corticosteroid therapy on the overall survival (OS) rate after corticosteroid therapy.

Statistical Analysis

Univariate and multivariate logistic regression analyses were used to identify factors associated with the response to corticosteroid therapy. The probability of NRM after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated on the basis of cumulative incidence curves in which relapse was treated as a competing event [20]. The probability of OS after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated according to the Kaplan-Meier method [21]. The groups were compared using the log-rank test. Competing risk regression analysis was used to identify factors associated with NRM after corticosteroid therapy. The adjusted probability of OS after corticosteroid therapy was estimated using the Cox proportional hazards model, with consideration of other significant clinical variables in the final multivariate models [22]. *P* values were 2 sided, and $P < .05$ was considered significant. The following covariates were considered for the multivariate models: patient age, patient sex, sex mismatch between patient and donor, disease, stem cell source, cytomegalovirus serostatus, preconditioning, GVHD prophylaxis, in vivo T cell depletion, year of transplantation, onset of acute GVHD, grade of acute GVHD, organ involvement of acute GVHD, and response to systemic corticosteroid therapy (improved or stable/progressive). The data were analyzed by STATA version 12 statistical software (StataCorp, TX).

RESULTS

Patient, Transplantation, and GVHD Characteristics

A total of 3436 patients met the inclusion criteria. Patient and transplantation characteristics are shown in Table 1. Patient age at transplantation ranged from 0 to 82 years (median, 40 years); the number of patients age <18, 18 to 49, and ≥50 years was 672, 1626, and 1138, respectively. Stem cell sources were BM (n = 1955), PBSC (n = 642), and UCB (n = 839). All UCB transplantation was performed with a single unit. In vivo T cell depletion was performed in 168 (5%) patients by either antithymocyte globulin or antilymphocyte globulin. No other drugs, such as alemtuzumab, were used for in vivo T cell depletion, nor was ex vivo T cell depletion used in any patients. The year of transplantation ranged from 1984 to 2009; the majority of cases (94%) were performed in 2000 or later.

Characteristics of acute GVHD cases are shown in Table 2. The numbers of patients who developed acute GVHD at day ≤28 and day ≥29 were 2344 and 994, respectively. Of 3436 patients who received systemic corticosteroid therapy as the first-line treatment for grade II to IV acute GVHD, 2190 (63.7%) showed improvement of acute GVHD.

Factors Associated with Improvement of GVHD by Corticosteroid Therapy

MUD-BM, HLA-mismatched stem cell source other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement of acute GVHD, including gut, were significantly associated with a lower probability of improvement by corticosteroid therapy (Table 3). On the other hand, adult patient (ages 18 to 49 years) and UCB were significantly associated with a higher probability of improvement by corticosteroid therapy (Table 3). Although some factors, such as disease, cytomegalovirus serostatus, and preconditioning, were significant for corticosteroid response in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between

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ORIGINAL ARTICLE

Recent decrease in non-relapse mortality due to GVHD and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia

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]

Although recent improvements have been indicated in the outcome after allogeneic hematopoietic cell transplantation (allo-HCT), little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We compared the incidence and causes of non-relapse mortality (NRM) after allo-HCT in non-remission among three consecutive four-year periods using a nationwide transplant outcome registry database. A total of 3308 patients with acute leukemia in non-remission were analyzed. The risk of NRM decreased over the three periods, and the hazard ratios (HRs) in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.86 (95% CI, 0.70–1.06; $P=0.16$) and 0.65 (95% CI, 0.53–0.80; $P<0.01$), respectively. A significant decrease in the HR for overall mortality was also observed in 2005–2008 (HR 0.85; 95% CI, 0.75–0.97; $P=0.02$). We found that a decrease in the incidences of death due to GVHD and infection contributed to the reduction in NRM, to which high-resolution donor-recipient HLA matching and other improvements may have contributed. As none of the subgroups showed improved survival without a reduction in NRM, the effective prevention of transplant-related complications appears to be necessary for improving outcomes after allo-HCT in non-remission.

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Keywords: allogeneic hematopoietic cell transplantation; acute leukemia; non-remission; non-relapse mortality; GVHD

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is recognized as a potentially curative therapy for patients with high-risk hematologic malignancies, which can lower the risk of relapse. However, treatment-related mortality, which may offset the benefit of a reduced risk of relapse, has long been a major problem. Several changes have been made in modalities of allo-HCT, including patient-donor HLA matching, conditioning regimens, immunosuppressive therapy, and the prophylaxis, diagnosis and treatment of GVHD and infection. As a result, the risk of non-relapse mortality (NRM) after allo-HCT has decreased over the past few decades.[1–6]

AML and ALL account for the largest proportion of diseases indicated for allo-HCT. Furthermore, a substantial number of patients with AML or ALL receive allo-HCT in non-remission. Despite the fact that high-risk acute leukemia is definitely indicated for allo-HCT, patients with non-remission leukemia carry various factors that lead to a higher risk of treatment-related toxicity, including comorbidities due to prior chemotherapy and intensified conditioning regimens in need of an antitumor effect,[7–11] and a deteriorated general condition due to refractory disease. Although prior studies have shown improvements in the outcome after allo-HCT,[1–5] little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We recently reported changes in the incidence and causes of NRM after allo-HCT in remission in Japan.[6] Using the same nationwide transplant outcome registry database, we compared the incidence and causes of NRM in patients with AML or ALL in non-remission in three consecutive four-year periods.

SUBJECTS AND METHODS

Data source

Clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, to which 267 institutions/departments contributed. The clinical data were consecutively collected through Transplant Registry Unified Management Program as described previously.[12] This study was

[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]Division of Hematology, 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]Division of Hematology, Department of Internal Medicine, 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 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
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approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated data on patients aged between 16 and 70 years who had AML or ALL and who received their first allo-HCT in non-remission between 1997 and 2008. Non-remission status was defined as any percentage of blasts in the peripheral blood, or a BM aspirate containing >5% blasts at the time of transplant. We compared the incidence of NRM after allo-HCT in three consecutive four-year 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 the detection of recurrent disease after allo-HCT. In 154 patients who died without a confirmed hematological remission within 30 days from allo-HCT, the cause of death was defined as NRM. In 293 patients who died without a confirmed hematological remission after 31 days or later after allo-HCT, the cause of death was defined as refractory disease. A separate analysis that excluded these 447 patients who died without a confirmed remission was performed. We also changed the cutoffs from 30 days to 60 days or 90 days. Analyses were performed on the basis of patient's age (16–49 years and 50–70 years), disease (AML and ALL) and donor source (HLA-matched/1-Ag-mismatched related, unrelated BM and unrelated cord blood (CB)). In this study, matching of unrelated BM between recipient and donor were determined based on serum typing. In 2003, Japan Marrow Donor Program nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, Japan Marrow Donor Program 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 C-locus. In the era considered by this study, only BM was used from unrelated volunteer donors in Japan. Conditioning regimens were classified as indicated by Giralt *et al.*[13] The causes of death other than recurrent disease were obtained from the database and the incidences of mortality associated with GVHD, infection or organ failure were compared over the three time periods. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized. The 447 patients who died without a confirmed hematological remission were excluded from the analyses regarding the causes of death.

Statistical analysis

Data were retrospectively reviewed and analyzed as of March 2012. Among the three time periods, patient characteristics were compared using the χ^2-test. The primary endpoint of the study was NRM after allo-HCT. Probabilities of NRM were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM. The Pepe and Mori test was used to evaluate the differences between groups. For the 337 patients (10%) who were known to have relapsed but whose date of relapse was unavailable, midpoint imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The incidence of NRM was estimated as the probability at 2 years from allo-HCT. Multivariate analyses were performed for NRM and relapse using competing risk regression by the method of Fine and Gray, and for survival using a Cox proportional hazard regression model. The analyses were performed separately among younger patients aged 16–49 years and older patients aged 50–70 years. In the multivariate analyses, we considered the following factors as covariates: the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL), 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-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduce-intensity conditioning (RIC)). Multivariate analyses were also performed separately for patients who received related allo-HCT, patients who received unrelated BMT (UBMT), and patients who received UCBT. 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 SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

Table 1. Patient characteristics according to the time period of transplant

Characteristics	*1997–2000* N(%)	*2001–2004* N(%)	*2005–2008* N(%)	P-*value*
Total number of patients	637	1165	1506	
Gender				*0.064*
Male	355(56)	674(58)	793(53)	
Female	281(44)	489(42)	505(34)	
Age (years)				*<0.001*
16–29	249(39)	277(24)	265(18)	
30–39	139(22)	240(21)	278(18)	
40–49	157(25)	246(21)	304(20)	
50–59	83(13)	296(25)	430(29)	
60–70	9(1)	106(9)	229(15)	
Donor source				*<0.001*
HLA-matched sibling	248(39)	380(33)	365(24)	
Related others	94(15)	165(14)	196(13)	
Matched-unrelated BM	213(33)	288(25)	461(31)	
Mismatched-unrelated BM	34(5)	83(7)	85(6)	
Unrelated CB	23(4)	176(15)	286(19)	
Others	25(4)	73(6)	113(8)	
Disease type				*<0.001*
AML	388(61)	840(72)	1209(80)	
ALL	249(39)	325(28)	297(20)	
Ph-positive ALL	66(10)	75(6)	48(3)	
Conditioning				*<0.001*
Myeloablative	504(79)	668(57)	837(56)	
Reduced-intensity	14(2)	290(25)	426(28)	
Not categorized	119(19)	207(18)	243(16)	
GVHD prophylaxis				*<0.001*
Cyclosporin-based	472(74)	679(58)	618(41)	
Tacrolimus-based	150(24)	423(36)	806(54)	
Disease status at HCT				*<0.001*
No treatment	20(3)	43(4)	115(8)	
Primary induction failure	148(23)	292(25)	576(38)	
First relapse	154(24)	372(32)	485(32)	
⩾Second relapse	55(9)	159(14)	157(10)	
Non-remission/no detailed data	260(41)	299(26)	173(11)	

Abbreviation: CB = cord blood.

RESULTS

Patients

A total of 3308 patients with a median age of 42 years and a median follow-up of 27 months (range, 0–150) was analyzed. The characteristics of the patients and transplantation procedures according to the time period are shown in Table 1. The number of allo-HCT procedures increased over time. The number and proportion of patients aged 50–70 years, allo-HCT from an unrelated CB donor and the use of a RIC regimen increased over the three periods. Most of the myeloablative regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: 1997–2000, $n=109$, 44%; 2001–2004, $n=231$, 62%; 2005–2008, $n=426$, 78%, CB: $n=5$, 22%; $n=50$, 28%; $n=174$, 61%). The proportion of allo-HCT given for ALL in non-remission decreased over the three periods with decreasing proportions of both Ph-positive ALL and Ph-negative

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ORIGINAL ARTICLE

A case–control study of bronchiolitis obliterans syndrome following allogeneic hematopoietic stem cell transplantation

Hideki Nakasone,[1]* Junya Kanda,[1] Shingo Yano,[2] Yoshiko Atsuta,[3] Hiroatsu Ago,[4] Takahiro Fukuda,[5] Kazuhiko Kakihana,[6] Tatsuya Adachi,[7] Toshiaki Yujiri,[8] Shuichi Taniguchi,[9] Jun Taguchi,[10] Yasuo Morishima,[11] Tokiko Nagamura,[12] Hisashi Sakamaki,[6] Takehiko Mori[13] and Makoto Murata[14] on behalf of GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation

1 Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan
2 Division of Clinical Oncology and Hematology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan
3 Department of HSCT Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan
4 Department of Hematology and Oncology, Shimane Prefectural Central Hospital, Shimane, Japan
5 Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan
6 Hematology Division, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan
7 Department of Hematology, Meitetsu Hospital, Nagoya, Japan
8 Third Department of Internal Medicine, Yamaguchi University School of Medicine, Yamaguchi, Japan
9 Department of Hematology, Toranomon Hospital, Tokyo, Japan
10 Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
11 Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan
12 Department of Cell Processing and Transfusion, Institute of Medical Science, University of Tokyo, Tokyo, Japan
13 Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan
14 Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Keywords

ABO-mismatch, allogeneic hematopoietic stem cell transplantation, bronchiolitis obliterans syndrome, cord blood, graft-versus-host disease.

Correspondence
Hideki Nakasone MD, PhD, Division of Hematology, Saitama Medical Centre, Jichi Medical University, 1-847, Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan.
Tel.: +81 48 647 2111;
fax: +81 48 648 5188;
e-mail: nakasone-tky@umin.ac.jp

*Present address: Division of Blood and Marrow Transplantation, Stanford University School of Medicine, 269 West Campus Drive, CCSR 2210, Stanford, CA, USA.

Conflicts of interest
The authors report no potential competing conflicts of interest.

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Summary

Bronchiolitis obliterans syndrome (BOS) is a significant complication after allogeneic hematopoietic stem cell transplantation (HSCT). However, the pathogenesis and risks for the development of BOS have remained unclear. Therefore, a case–control study was conducted to investigate the risk factors for the development of BOS, which included the largest number of BOS cases; 196 patients with BOS were identified and compared with 1960 control recipients. The following were identified as significantly higher risk factors for the development of BOS: female recipients (OR 1.47, $P = 0.019$), ABO-mismatch HSCT (minor mismatch, OR 1.67, $P = 0.015$; major mismatch, OR 1.73, $P = 0.012$; bidirectional mismatch, OR 1.96, $P = 0.018$), busulfan+cyclophosphamide-based myeloablative conditioning (OR 1.74, $P = 0.016$), and acute graft-versus-host disease (GVHD) involving the skin (OR 1.55, $P = 0.011$). On the other hand, the risk for the development of BOS was significantly lower in patients receiving cord blood transplantation (OR 0.26, $P = 0.0011$). With respect to other target organs of chronic GVHD, ocular involvement was significantly associated with BOS (OR 2.53, $P < 0.001$). Prospective studies are required to elucidate the risk factors for the development of BOS, and future investigations should focus on finding a prophylactic approach against BOS based on these findings.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) plays a crucial role as a curative treatment for hematological diseases. However, HSCT recipients experience various adverse complications, including graft-versus-host disease (GVHD). Bronchiolitis obliterans syndrome (BOS) is one of the significant late complications following HSCT, and it is known to represent lung involvement of chronic GVHD (cGVHD). BOS is characterized by breathing difficulty and dry cough without fever, and by airway obstruction not responsive to bronchodilator therapy that may become irreversible in advanced stages of disease [1–7]. The pathological findings of BOS show bronchiolitis involving the small airway and fibrinous obliteration of the lumina of the respiratory bronchioles [3,8]. The cumulative incidence of BOS is thought to range from 2% to 10% [3,4]. BOS usually presents after the first 100 days following HSCT, and ~80% of cases present between 6 and 12 months after HSCT [3,4]. The International Bone Marrow Transplantation Registry (IBMTR) reported that BOS presented at a median of 431 days after HSCT (range: 65–2444 days) [9].

Several groups have investigated the risk factors for the development of BOS, including peripheral blood stem cell transplantation (PBSCT), busulfan (BU)-based conditioning, and the development of GVHD [9–13]. However, the results were controversial. One of the reasons for the controversy is the small number of patients with BOS, as almost all of these studies included less than 20 patients with BOS. To the best of our knowledge, there have been just two reports that included more than 50 patients with BOS by IBMTR (76 patients with BOS among 6275 HSCT recipients from HLA-identical siblings) or the Kanto Study Group for Cell Therapy (KSGCT, 57 patients with BOS among 2087 recipients). However, no study has included over 100 patients with BOS [9,13]. Both IBMTR and KSGCT reported that PBSCT and GVHD were associated with the development of BOS. However, it remains unclear whether other alternative donor sources, such as cord blood transplantation (CBT), and other possible factors, such as ABO-mismatch, affect the development of BOS.

Bronchiolitis obliterans syndrome is well known to impair the recipients' quality of life dramatically and to be associated with worse survival rates [1,3,4,6,13]. However, an effective treatment has yet to be established [1,3,4,6,13]. Therefore, it is important to elucidate the risks for the development of BOS and to establish a prophylactic approach against it. Thus, a large case–control study that included about 200 patients with BOS was performed using the Japanese transplant outcome registry database, and the risk factors were identified.

Patients and methods

Patient selection

Patients with BOS and control recipients were selected from the cohort of adult recipients (16 years or older) who received their 1st allogeneic HSCT between January 1990 and December 2009 and survived without disease relapse for at least 180 days after HSCT, reported to the Japan transplant outcome registry database and confirmed by the Transplant Registry Unified Management Program in 2010 [14]. The BOS patients were defined as adult recipients who experienced BOS by their last follow-up. The control recipients were defined as adult recipients in whom BOS was not apparently diagnosed up to their last follow-up. Using a computerized selection procedure, 10 controls, which were matched according to years of HSCT (every 5 years), were chosen for each case, because there might be changes in the clinical practices related to HSCT according to the years of HSCT. In addition, information on age, sex, and survival status at the end of follow-up was required. 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

BOS was reported based on clinical obstructive dysfunctions and radiological assessment with/without histological examinations [2,5,7]. Standard risk diseases were defined as follows: acute leukemia in the 1st and 2nd complete remission, chronic myelogenous leukemia in the 1st and 2nd chronic phase, lymphoma and multiple myeloma in complete and partial remission, adult T cell leukemia in complete remission, myelodysplastic syndromes, myeloproliferative neoplasms, benign hematological diseases, and congenital disorders. All other diseases were classified as high-risk. Because PBSCT from unrelated donors was not available in Japan during the evaluation period, the types of HSCT were categorized into seven groups: HLA-matched related bone marrow transplantation (MRD-BMT), HLA-mismatched related BMT (MMRD-BMT), HLA-matched related PBSCT (MRD-PBSCT), HLA-mismatched related PBSCT (MMRD-PBSCT), HLA-matched unrelated BMT (MUD-BMT), HLA-mismatched unrelated BMT (MMUD-BMT), and unrelated CBT. MMRD or MMUD was defined as a related or unrelated donor when at least HLA 1 antigen mismatch was detected at serological levels of HLA-A, B, or DR. Regimens were classified into myeloablative (MAC) and reduced intensity conditioning (RIC) based on the report by Giralt *et al.* [15]. Briefly, conditionings including total body irradiation (TBI) >8 Gy, melphalan $\geq$ 140 mg/m^2, or oral BU $\geq$ 9 mg/kg (iv BU $\geq$ 7.2 mg/kg) were classified

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 × 10^6/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. 00:000–000, 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

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*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

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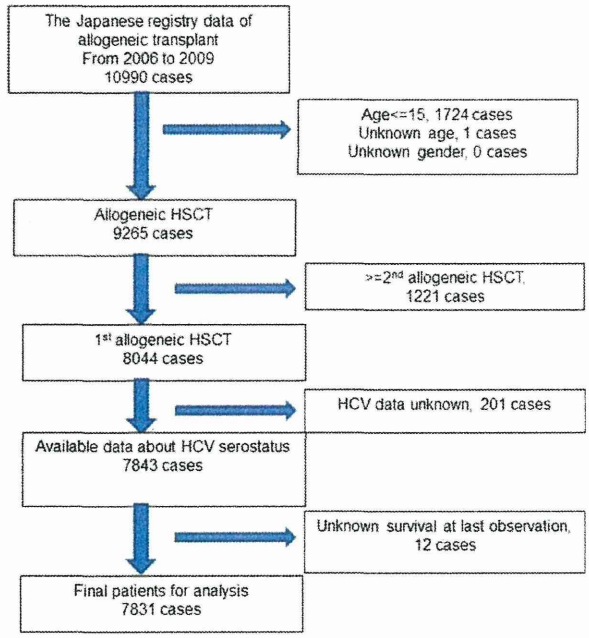

Figure 1. Scheme of patient selection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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

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.[3-12] 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-

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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-

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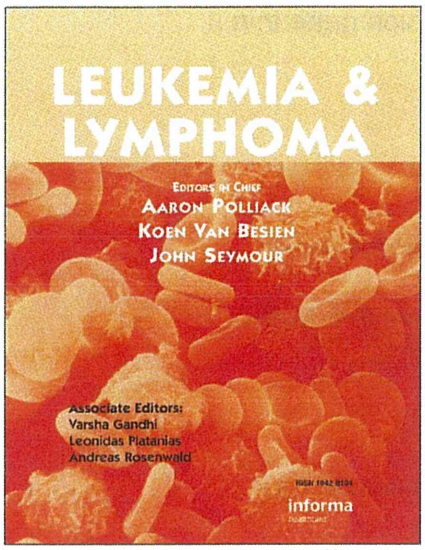

Quantitative PCR detection of CEP110-FGFR1 fusion gene in a patient with 8p11 syndrome

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

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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.

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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

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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

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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	<0.003	1		
	Non-CR		2.54	1.17-5.51	<0.02
FAB classification	M2	0.075	1		
	other than M2		3.61	1.59–8.21	<0.003
Number of HLA disparity	0	0.061		NA	
	1				
	2				
Source of the graft	BM or PBSC	0.076		NA	
	CB				

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.

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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.

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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
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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.