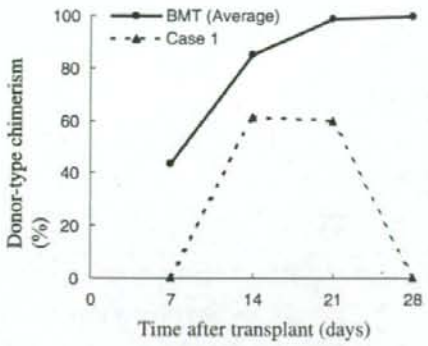
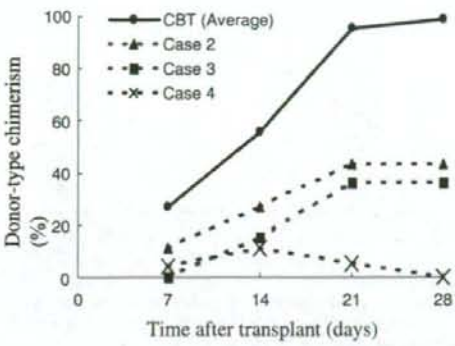


a) Graft failure after BMT



b) Graft failure after CBT

Fig. 4 Percentages of donor-type chimerism in the four patients who failed to achieve engraftment. **a** Percentage of donor-type chimerism in one patient (case 1) who failed to achieve engraftment after BMT. **b** Percentages of donor-type chimerism in three patients (cases 2, 3, and 4) who failed to achieve engraftment after CBT

RIST using fludarabine and busulfan with TBI, however, Barker et al. [2] reported all engraftment patients without relapse had complete myeloid and lymphoid donor chimerism at more than 1 month after transplantation. Furthermore, Kusumi et al. [12] reported that RIST with TBI reduced the risk of graft failure compared with RIST with antithymocyte globulin (ATG). Stable engraftment was in fact achieved in 21 (87.5%) of the 24 patients who received RIST with TBI in the present study. The high incidence of complete chimerism may be one of the factors responsible for achievement of engraftment in patients who received RIST.

Several studies have shown that stem cell sources influenced the rate of engraftment and median time to engraftment [12, 14, 19, 21, 22]. The Stem Cell Trialists's Collaborative Group [21] reported that PBSCT led to faster engraftment than did BMT (median time=14 vs 21 days). Laughlin et al. [14] reported that median time to engraftment after CBT was 27 days. In our study, median times to engraftment were 12 days after PBSCT, 16 days after BMT, and 26.5 days after CBT ($P<0.01$). When we analyzed the influence of stem cell sources on donor-type chimerism, it was clear that PBSCT led to faster complete chimerism and that CBT led to slower complete chimerism. This delay in complete chimerism after CBT may be one of the reasons for the delay in engraftment and the high incidence of graft failure in patients who received CBT compared with those in patients who received BMT or PBSCT.

Graft failure occurred in four patients, including one patient who received BMT (case 1) and three patients who received CBT (cases 2, 3, and 4). Considering our results showing that stem cell source influenced the percentage of donor-type chimerism, we evaluated each case by comparing with the average percentage of donor-type chimerism in patients with each stem cell source. A notable decrease in donor-type chimerism was observed in all four patients who failed to achieve engraftment (Fig. 4). Our study suggests that stem cell source should be taken into account for prediction of engraftment and graft failure by monitoring chimerism. In addition, our results for average percentage of donor-type chimerism in patients who achieved engraftment for each stem cell source (PBSCT=81.5% on day 7, BMT=84.8% on day 14, CBT=55.8% on day 14) may be useful for predicting engraftment and graft failure.

However, in this study, there was no significant influence of conditioning regimen within the stem cell source (for example, PBSCT with CST, RIST with and without TBI). Since one of the reasons for this might be the small number of patients in this study, further studies are required to evaluate the influence of conditioning regimen within the stem cell source.

In this study, we analyzed very early chimerism (days 7, 14, 21, 28) and evaluated correlations between conditioning regimens, stem cell sources, and donor-type chimerism. RIST with TBI led to more complete chimerism than did RIST without TBI. On day 7, the percentage of donor-type chimerism after PBSCT was significantly higher than that after BMT or CBT. On day 14, the percentage of donor-type chimerism after CBT was significantly lower than that after BMT or PBSCT. This study suggests that differences in conditioning regimens and stem cell sources should be taken into account when considering the percentage of donor-type chimerism.

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References

1. Balon J, Halaburda K, Bieniaszewska M, Reichert M, Bieniaszewski L, Piekarska A et al (2005) Early complete donor hematopoietic chimerism in peripheral blood indicates the risk of extensive graft-versus-host disease. Bone Marrow Transplant 35:1083–1088 doi:10.1038/sj.bmt.1704962
2. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE (2003) Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood 102:1915–1919 doi:10.1182/blood-2002-11-3337
3. Baron F, Sandmaier BM (2006) Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia 20:1690–1700 doi:10.1038/sj.leu.2404335
4. Champlin R, Khouri I, Shimoni A, Gajewski J, Kornblau S, Molldrem J et al (2000) Harnessing graft-versus-malignancy: non-myeloablative preparative regimens for allogeneic haematopoietic transplantation, an evolving strategy for adoptive immunotherapy. Br J Haematol 111:18–29 doi:10.1046/j.1365-2141.2000.02196.x
5. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S et al (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 94:3234–3241
6. Galimberti S, Benedetti E, Morabito F, Fazzi R, Pacini S, Andreazzoli F et al (2005) Chimerism does not influence graft-versus-myeloma and graft-versus-host disease in reduced intensity setting. Transpl Immunol 15:173–177 doi:10.1016/j.trim.2005.06.002
7. Giralt S, Thall PF, Khouri I, Wang X, Braunschweig I, Ippolitti C et al (2001) Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood 97:631–637 doi:10.1182/blood.V97.3.631
8. Gleissner B, Blau IW, Sindram A, Reinhardt R, Knauf W, Thiel E (2001) Analysis of chimerism during the early period after allogeneic peripheral stem cell transplantation. Clin Lab Haematol 23:401–406 doi:10.1046/j.1365-2257.2001.00408.x
9. Huisman C, de Weger RA, de Vries L, Tilanus MG, Verdonck LF (2007) Chimerism analysis within 6 months of allogeneic stem cell transplantation predicts relapse in acute myeloid leukemia. Bone Marrow Transplant 39:285–291 doi:10.1038/sj.bmt.1705582
10. Jaksch M, Uzunel M, Remberger M, Sundberg B, Mattsson J (2005) Molecular monitoring of T-cell chimerism early after allogeneic stem cell transplantation may predict the occurrence of acute GVHD grades II–IV. Clin Transplant 19:346–349 doi:10.1111/j.1399-0012.2005.00345.x
11. Keil F, Prinz E, Moser K, Mannhalter C, Kalhs P, Worel N et al (2003) Rapid establishment of long-term culture-initiating cells of donor origin after nonmyeloablative allogeneic hematopoietic stem-cell transplantation, and significant prognostic impact of donor T-cell chimerism on stable engraftment and progression-free survival. Transplantation 76:230–236 doi:10.1097/01.TP.0000071862.42835.76
12. Kusumi E, Kami M, Yuji K, Hamaki T, Murashige N, Hori A et al (2004) Feasibility of reduced intensity hematopoietic stem cell transplantation from an HLA-matched unrelated donor. Bone Marrow Transplant 33:697–702 doi:10.1038/sj.bmt.1704425
13. Lassaletta A, Ramirez M, Montero JM, Gonzalez-Vicent M, Balas A, Madero L et al (2005) Full donor chimerism by day 30 after allogeneic peripheral blood progenitor cell transplantation is associated with a low risk of relapse in pediatric patients with hematological malignancies. Leukemia 19:504–506
14. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE et al (2004) Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 351:2265–2275 doi:10.1056/NEJMoa041276
15. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG et al (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood 97:3390–3400 doi:10.1182/blood.V97.11.3390
16. Miura Y, Tanaka J, Toubai T, Tsutsumi Y, Kato N, Hirate D et al (2006) Analysis of donor-type chimerism in lineage-specific cell populations after allogeneic myeloablative and non-myeloablative stem cell transplantation. Bone Marrow Transplant 37:837–843 doi:10.1038/sj.bmt.1705352
17. Petersen SL, Madsen HO, Ryder LP, Svejgaard A, Masmas TN, Dickmeiss E et al (2004) Chimerism studies in HLA-identical nonmyeloablative hematopoietic stem cell transplantation point to the donor CD8(+) T-cell count on day + 14 as a predictor of acute graft-versus-host disease. Biol Blood Marrow Transplant 10:337–346 doi:10.1016/j.bbmt.2004.01.003
18. Ritchie DS, Morton J, Szer J, Roberts AW, Durrant S, Shuttleworth P et al (2003) Graft-versus-host disease, donor chimerism, and organ toxicity in stem cell transplantation after conditioning with fludarabine and melphalan. Biol Blood Marrow Transplant 9:435–442 doi:10.1016/S1083-8791(03)00128-9
19. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A et al (2004) Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med 351:2276–2285 doi:10.1056/NEJMoa041469
20. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G et al (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 91:756–763
21. Stem cell Trialists' Collaborative Group (2005) Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol 23:5074–5087
22. Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y et al (2004) Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood 104:3813–3820 doi:10.1182/blood-2004-03-1001
23. Tsutsumi Y, Tanaka J, Kato N, Zhang L, Mori A, Kobayasi R et al (2002) Analysis of mixed chimerism in patients after allogeneic stem cell transplantation using a capillary electrophoresis system. Acta Haematol 107:195–202 doi:10.1159/000058314
24. Tsutsumi Y, Tanaka J, Sugita J, Kato N, Zhang L, Yonezumi M et al (2002) Analysis of T-cell repertoire and mixed chimaerism in a patient with aplastic anaemia after allogeneic bone marrow transplantation. Br J Haematol 118:136–139 doi:10.1046/j.1365-2141.2002.03628.x
25. Valcarcel D, Martino R, Caballero D, Mateos MV, Perez-Simon JA, Canals C et al (2003) Chimerism analysis following allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning. Bone Marrow Transplant 31:387–392 doi:10.1038/sj.bmt.1703846

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ORIGINAL ARTICLE

Prognostic value of serum surfactant protein D level prior to transplant for the development of bronchiolitis obliterans syndrome and idiopathic pneumonia syndrome following allogeneic hematopoietic stem cell transplantation

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Bronchiolitis obliterans syndrome (BOS) and idiopathic pneumonia syndrome (IPS) cause high mortality and impaired survival after allogeneic hematopoietic stem-cell transplantation (allo-HSCT). Early recognition of patients at high risk of developing BOS/IPS may lead to improving the outcome of allo-HSCT. We retrospectively analyzed serum surfactant protein A, D (SP-A, -D) and Kerbs von Lungren 6 Ag (KL-6) levels before allo-HSCT in 56 patients who survived more than 90 days after allo-HSCT and compared values of these serum markers and other transplant factors in BOS/IPS patients with those in non-BOS/IPS patients. Five patients developed BOS and two developed IPS at a median interval of 303 and 117 days (range, 100–452 and 95–153) from transplantation. As a result of univariate analysis, pretransplant serum SP-D levels but not SP-A, KL-6 in BOS/IPS patients were significantly lower than those in non-BOS/IPS patients ($P=0.03$). In multivariate analysis, the patients with lower pretransplant serum SP-D level had a trend toward frequent development of BOS/IPS ($P=0.08$). Constitutive serum SP-D level before allo-HSCT may be a useful, noninvasive predictor for the development of BOS/IPS.

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Introduction

Late-onset noninfectious pulmonary complications (LONIPCs) after allogeneic hematopoietic stem-cell transplantation (allo-HSCT) are a critical problem. Many studies have reported that the development of LONIPCs is closely associated with the occurrence of chronic GVHD (cGVHD).[1–3] However, the pathogenesis of LONIPCs is unclear. LONIPCs are classified into bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans with organizing pneumonia and idiopathic pneumonia syndrome (IPS). BOS and IPS are difficult to cure, cause high mortality and decrease survival after allo-HSCT. The incidences of BOS and IPS reportedly vary widely from 1.7 to 26% and from 2.0 to 17% in allo-HSCT, respectively.[4,5] Early identification of patients with high risk for BOS/IPS and early treatment for them may be crucial in improving the outcome of allo-HSCT. Although many other risk factors for BOS/IPS have been reported,[4–8] it remains difficult to detect patients at high risk for BOS/IPS.

Surfactant proteins A (SP-A) and D (SP-D) are collectins mainly synthesized by alveolar type II cells. The best-known function of surfactant (mainly SP-B) is to prevent the collapse of alveoli by decreasing surface tension at the alveolar air-lipid interface. SP-A and -D play an important role as mediators in innate immunity in the lung.[9,10] SP-D has diverse innate immune functions as follows:[9] binding and agglutination of pathogens; enhancement of phagocytosis/killing of pathogens; rapid clearance of bacterial endotoxin; moderation of inflammatory response to infection and in allergy; antioxidant properties, clearance of apoptotic cells and Ag presentation. Furthermore, it has been reported that significant severe lung inflammation frequently occurs in SP-A-deficient mice given allogeneic donor BM plus spleen T cells followed by conditioning with CY and lethal irradiation.[11]

Based on these previously reported studies, we hypothesized that individual constitutive levels of surfactant protein in allo-HSCT recipients might be associated with the development of BOS/IPS. In addition, Kerbs von

Lungren 6 Ag (KL-6), which is a high-molecular-weight glycoprotein (molecular weight >1000K) classified in humans as MUC1 mucin, strongly expressed on type II alveolar pneumocytes and bronchiolar epithelial cells, and is a well-known marker for the activity of interstitial pneumonia, was also reported to increase in BOS after lung transplant.[12] The current study examined whether SP-A, -D or KL-6 in serum before transplant has a prognostic value for noninfectious severe complications, BOS/IPS after allo-HSCT.

Patients and methods

Patients

We retrospectively evaluated 56 patients with hematological malignancies who had undergone allo-HSCT between November 2001 and 2006 at our institute and survived more than 90 days after transplant. Patients who had obstructive lung disease before transplant or active pneumonia at blood sampling were excluded. The median age at transplant was 42 years old (range, 16–69) (Table 1). These patients included 31 with AML, 5 with ALL, 2 with CML, 6 with myelodysplastic syndrome (MDS), 7 with non-Hodgkin's lymphoma and 5 with adult T-cell leukemia (ATL) (Table 1). In total 25 patients (45%) received a transplant in the advanced phase of the disease. Standard disease status included MDS (refractory anemia), acute leukemia or lymphoma in the first or second remission or chronic leukemia in the first chronic phase. Advanced disease status included patients with MDS (refractory anemia with excess blasts), secondary acute leukemia, Ph chromosome-positive ALL, ATL and all patients beyond the second remission or the first chronic phase in acute and chronic leukemia or lymphoma. This study was approved by the Institutional Review Board. The concept, procedure and potential risks of the study were explained and written informed consent was obtained from all enrolled patients.

Allogeneic hematopoietic stem-cell transplantation

In total, 15 patients received allo-HSCT from HLA-identical sibling donors, 6 from HLA-mismatched sibling donors, 20 from HLA-identical unrelated donors and 15 from HLA-mismatched unrelated donors including 10 cord blood transplants (CBT) (Table 1). HLA matching (HLA-A, -B and -DR) was determined by DNA genotyping in siblings and unrelated transplants except CBT, and only serologic typing was performed in CBT. We defined HLA mismatch as the presence of at least one serological or allele mismatch between recipient and donor. As a source of allogeneic stem cell grafts, BM cells were used for 28 patients, mobilized PBSCs for 18 patients and cord blood for 10 patients.

In total, 26 patients (46%) received the following myeloablative conditioning: BU/CY ($n=10$); CY/TBI ($n=7$); Ara-C/CY/TBI ($n=8$) and other ($n=1$) (Table 1). These conditioning regimens were selected individually based on the status of the underlying disease. On the other hand, reduced-intensity conditioning was employed for 30 patients (54%) ineligible for myeloablative conditioning because of advanced age, comorbidity, organ dysfunction or prior intensive chemotherapies. The reduced-intensity conditioning included fludarabine (Flu)/BU ($n=19$); Flu/BU/TBI ($n=3$); other Flu-containing regimens ($n=3$) and total lymphoid irradiation-containing regimens ($n=5$).

Table 1 Characteristics of patients

Characteristics	*Total*
No. of patients	56
Median age at transplant (years)	42 (16–69)
Sex (male/female)	25/31
Disease	
AML	31
ALL	5
MDS	6
CML	2
NHL	7
ATL	5
Disease status	
Standard	31
Advanced	25
Donor HLA	
Matched related	15
Matched unrelated	20
Mismatched related	6
Mismatched unrelated	15 (CB 10)
Stem cell source	
BM	28
PBSC	18
CB	10
Conditioning	
Myeloablative	26
CY + TBI	7
AraC + CY + TBI	8
BU + CY	10
Other	1
Nonmyeloablative	30
Flu + BU	19
Other Flu-based regimen	6
TLI containing regimen	5
GVHD prophylaxis	
CsA + sMTX	44
CsA alone	8
CsA + MMF	1
Tacrolimus + sMTX	2
Tacrolimus alone	1

Abbreviations: ATL = adult T-cell leukemia; CB = cord blood; Flu = fludarabine; MDS = myelodysplastic syndrome; MMF = mycophenolate mofetil; NHL = non-Hodgkin's lymphoma; sMTX = short-term MTX.

Acute GVHD prophylaxis and diagnosis

As a prophylaxis for acute GVHD (aGVHD), 44 patients received both CsA and short-term MTX; 8, CsA alone; 1, CsA and mycophenolate mofetil (MMF); 1, tacrolimus alone; 2, tacrolimus and short-term MTX (Table 1). The doses of CsA were adjusted to the target of trough level from 150 to 250 ng/ml until Day 100, whereas the doses of tacrolimus were adjusted to the target of trough level from

10 to 15 ng/ml until Day 100 and then these drugs were tapered unless GVHD occurred. Intravenous administration of MTX was performed at 10 mg/m^2 on Day 1, and 7 mg/m^2 on Days 3 and 5. aGVHD was diagnosed clinically, graded according to standard criteria and confirmed by appropriate biopsies. Chronic GVHD (cGVHD) was also defined according to the standard criteria. However, since BOS is generally incorporated as part of the manifestation of extensive cGVHD, there is inevitably a major confounding between BOS and extensive type of cGVHD. Therefore, we classified cGVHD into limited or extensive type except lung manifestation.

Pulmonary function tests

Pulmonary function tests (PFTs) were undergone pre- and post transplant. Pretransplant PFTs were performed within 30 days before transplant and post transplant PFTs were performed at a median time of 355 days (range, 90–1402) after transplant. The following parameters were evaluated: forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio.

Definition of BOS and IPS

The criteria used to diagnose BOS were as follows: clinical or radiological signs and symptoms of lung disease and/or abnormalities in PFTs, and a decline in FEV_1 to less than 80% of the predicted value and FEV_1/FVC less than 70% in PFT, without evidence of infectious causes.[2,13] IPS was diagnosed with the following criteria: widespread alveolar injury (multilobular infiltrates on chest radiography or computed tomography, with symptoms and signs of pneumonia and evidence of abnormal pulmonary physiology), absence of clinical or laboratory evidence of active lower respiratory tract infection (bacterial, fungal, viral or parasitic) and absence of malignancy.[7,14]

Collection and analysis of blood samples

Serum samples from peripheral blood were collected from 56 patients before allo-HSCT. Additionally, in five BOS and one IPS patients, we also evaluated post transplant SP-D, -A and KL-6 serum levels. The serum was separated by centrifuging the blood at 3500 r.p.m. for 10 min. The serum samples were then stored at −80 °C until they were used. According to the manufacturer's recommended protocol (SRL Inc.; Tokyo, Japan), levels of SP-D and -A in serum were determined using a commercially available enzyme immunoassay, and levels of KL-6 were measured using a commercially available electro chemiluminescent immunoassay. Since the detection sensitivity limit in the SP-D assay was 17.2 ng/ml, values less than 17.2 ng/ml in SP-D (6 in 49 non-BOS/IPS patients and 2 in 7 BOS/IPS patients) were statistically evaluated as 17.2 ng/ml.

Statistics

Univariate analysis was performed by the Mann–Whitney *U*-test for metric variables and χ^2-test for categorical variables. The Mann–Whitney *U*-test was used to compare values of SP-D, -A, KL-6 levels and age between the BOS/IPS and the non-BOS/IPS groups. χ^2-test was used to compare disease status (high vs standard), donor type (HLA mismatched vs others), conditioning regimen (myeloablative vs reduced-intensity conditioning), TBI (high-dose TBI (12 Gy) vs others), BU (BU vs no BU), aGVHD (grades II–IV vs 0–I) and cGVHD (extensive type vs none and limited) in the BOS/IPS group with those in the non-BOS/IPS group. Multivariate analysis was performed with logistic analysis. Multivariate logistic analysis was conducted with covariates with *P*-values <0.10 on univariate analysis. All *P*-values were two-sided and a significance level of 0.05 was used.

Results

The median follow-up time of 56 patients was 615 days (range, 91–2040). Of 56 patients, 40 patients (71%) had aGVHD including 27 patients with grades II–IV (48%) and 12 patients with grades III–IV (21%), and 39 patients (70%) had cGVHD including 28 patients with extensive type (50%). In all patients, 2-year overall survival (OS) and event-free survival (EFS) were 70 and 66%. In total, 2-year OS and EFS in the BOS/IPS and the non-BOS/IPS patients were similar (2-year OS: 71 vs 69% and 2-year EFS: 71 vs 64%, respectively).

Characteristics of BOS/IPS patients

Of 56 patients, 5 (9%) developed BOS and 2 (4%) developed IPS (Table 2). Respiratory failure progressed rapidly in the two IPS patients, leading to death despite undergoing mechanical ventilation. Thus, it was difficult to perform transbronchial lung biopsy in these patients. However, in one of these patients, the autopsy was performed and the results of autopsy showed pulmonary congestion, fibrosis and diffuse alveolar damage, and did not show any evidence of viral, bacterial or fungal pulmonary infections. In the other patient, the results of bronchoalveolar lavage (BAL) examination showed no evidence of viral, bacterial and fungal pulmonary infections. Therefore, we diagnosed these patients as having IPS. The median time between the diagnosis of BOS/IPS and allo-HSCT was 303 days (range, 100–452) and 135 days (117 and 153), respectively. The median age of the BOS/IPS patients was 32 years (range, 18–67). Of seven BOS/IPS patients, there were no patients receiving allo-HSCT from matched related donors (3 matched unrelated donors, 2 one Ag-mismatched related donors, 1 one allele-mismatched unrelated donor and 1 one Ag and one allele-mismatched unrelated donor). All of the seven BOS/IPS patients had extensive type of cGVHD classified except lung manifestation. All patients received steroid-based therapies, but two IPS patients died. Among five BOS patients, BOS was stable in three patients and improved in two patients and all of the five BOS patients are still alive.

Serum SP-A, -D and KL-6 levels pre- and post transplant

In all patients, median pretransplant SP-A, -D and KL-6 levels in serum were 27.2 ng/ml (range, 6.7–83.7), 45.3 ng/ml (range, 17.2–159) and 232 U/ml (range, 104–725), respectively (Figure 1). The median pretransplant

Table 2 Characteristics of patients with BOS/IPS

No.	Sex	Age	Disease	Donor	HLA mismatch	aGVHD	cGVHD[a]	Event	Onset[b]	Therapy	Response	Pretransplant serum markers			Outcome (cause of death)
												SP-D (ng/ml)	SP-A (ng/ml)	KL-6 (U/ml)	
1	F	29	AML	Unrelated	Match	0	Extensive	BOS	307	mPSL	No change	27.6	31.9	164	1621+
2	M	57	MDS	Related	1 Ag	2	Extensive	BOS	452	mPSL	No change	26.2	26.4	246	1972+
3	M	18	NHL	Related	1 Ag	1	Extensive	BOS	303	HD-mPSL	Improved	41.2	13.8	106	897+
4	F	48	AML	Unrelated	Match	1	Extensive	BOS	100	mPSL	Improved	17.2	32.3	310	770+
5	F	32	AML	Unrelated	1 allele	2	Extensive	BOS	146	mPSL	No change	45.5	14.1	165	1944+
6	F	67	ALL	Unrelated	Match	1	Extensive	IPS	153	HD-mPSL	Progression	17.2	24.9	223	155 (IPS)
7	M	28	MDS	Unrelated	1 Ag+1 allele	2	Extensive	IPS	117	HD-mPSL + sivelestat	Progression	43.4	26.3	135	136 (IPS)

Abbreviations: aGVHD = acute GVHD; BOS = bronchiolitis obliterans syndrome; cGVHD = chronic GVHD; F = female; IPS = idiopathic pneumonia syndrome; KL-6 = Kerbs von Lungren 6 Ag; M = male; MDS = myelodysplastic syndrome; mPSL = methylprednisolone; NHL = non-Hodgkin's lymphoma; SP-A = surfactant protein A; SP-D = surfactant protein D.
[a]cGVHD of the lung was excluded.
[b]Days after transplantation.

values of SP-A, -D and KL-6 in serum were 28.1 ng/ml (range, 6.7–83.7), 54.4 ng/ml (range, 17.2–159) and 242 U/ml (range, 104–725) in 49 patients without BOS/IPS, whereas 26.4 ng/ml (range, 13.8–32.3), 27.6 ng/ml (range, 17.2–45.5) and 165 U/ml (range, 106–310) in 5 patients with BOS, and 24.9 and 26.3 ng/ml, 17.2 and 43.4 ng/ml and 223 and 135 U/ml in 2 patients with IPS, respectively (Table 2).

In five BOS patients, post transplant SP-A, -D and KL-6 levels, when BOS was still active, specifically, obstructive abnormality in the examination of spirography sustained in all 5 patients, did not significantly change from the pretransplant values (median pre- vs post transplant values of SP-D, 27.6 vs 31.3 ng/ml; SP-A, 26.4 vs 36.55 ng/ml and KL-6, 165 vs 236.5 U/ml). On the other hand, in one IPS patient, respiratory dysfunction and abnormal opacity persisted during intubation, post transplant values of SP-A, -D and KL-6 apparently became higher than the pretransplant values (pre- vs post transplant SP-D in this patient, 43.4 vs 143 ng/ml; SP-A, 26.3 vs 136 ng/ml; KL-6, 135 vs 470 U/ml).

Risk factors for developing BOS and IPS

In the univariate analysis, pretransplant SP-D levels in serum were significantly lower and the incidence of extensive cGVHD was significantly more frequent in the patients with BOS/IPS as compared with the patients without BOS/IPS ($P = 0.03$ and 0.03, respectively) (Table 3). Pretransplant KL-6 levels in serum tended to be lower in the BOS/IPS group ($P = 0.09$). Thus, three factors, pretransplant SP-D and KL-6 level in serum and extensive type of cGVHD, were employed as covariates in multivariate logistic analysis. In multivariate analysis, the serum SP-D levels in the BOS/IPS group tended to be lower than those in the non-BOS/IPS group ($P = 0.08$) (Table 4).

Discussion

The major finding in our study is that low steady-state serum concentration of SP-D before allo-HSCT was closely associated with BOS/IPS. The following risk factors for BOS, advanced recipient or donor age, preceding acute or cGVHD, PBSCT, MTX for aGVHD prevention, female donor to male recipient, BU containing or intensive conditioning, lower serum IgG levels, lower pretransplant FEV_1/FVC ratio, respiratory viral infections within 100 days post-HSCT and an episode of interstitial pneumonitis have been reported.[5] Similar risk factors, greater patient age (>40 years), myeloablative regimens, aGVHD and high-dose TBI reportedly contribute to the development of IPS.[7,8] These reports suggest that the pathogenesis both of BOS and IPS involves compositive processes including non-allo-immune reaction affecting pulmonary function in allo-HSCT such as lung toxic conditioning, aging and a history of pulmonary infection and allo-immune reaction such as acute or cGVHD after allo-HSCT. However, in our study we found no significant association between age, donor source, conditioning regimen and aGVHD. Possible explanatory factors are (1) small population size, (2) various types of conditioning regimen and (3) only patients

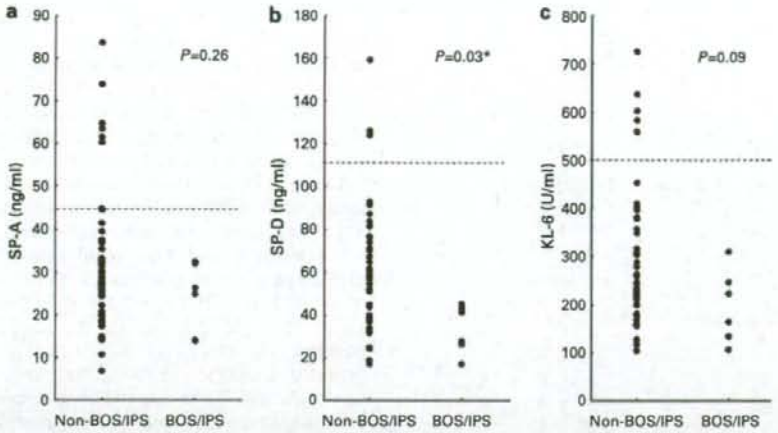

Figure 1 Distribution of serum markers (SP-A (**a**), SP-D (**b**) and KL-6 (**c**)) before transplantation for patients with/without BOS/IPS. SP-D values before SCT were significantly lower in patients with BOS/IPS than in those without BOS/IPS. In SP-A and KL-6, no statistical significance existed between patients with BOS/IPS and those without BOS/IPS. The dashed lines indicate the upper limit of the normal range for each marker. SP-A = surfactant protein A; SP-D = surfactant protein D; BOS/IPS = bronchiolitis obliterans syndrome/idiopathic pneumonia syndrome and KL-6 = Kerbs von Lungren 6 Ag.

Table 3 Comparison of risk factors between patients with and without BOS/IPS

Risk factor	*Median (range) or number*		P
	BOS/IPS	*Non-BOS/IPS*	
Age	32 (18–67)	42 (16–69)	0.96
High-risk disease	3/7	22/49	0.92
HLA mismatches	4/7	17/49	0.25
RIST	4/7	26/49	0.69
High-dose TBI containing regimen	3/7	12/49	0.30
BU containing regimen	4/7	34/49	0.52
aGVHD II–IV	3/7	27/49	0.54
cGVHD extensive (except lung)	7/7	21/49	0.03*
Serum SP-D levels before SCT (ng/ml)	27.6 (17.2–45.5)	54.4 (17.2–159)	0.03*
Serum SP-A levels before SCT (ng/ml)	26.3 (13.8–32.3)	28.1 (6.7–83.7)	0.26
Serum KL-6 levels before SCT (U/ml)	165 (106–310)	242 (104–725)	0.09

Abbreviations: BOS = bronchiolitis obliterans syndrome; IPS = idiopathic pneumonia syndrome; KL-6 = Kerbs von Lungren 6 Ag; RIST = reduced-intensity stem cell transplantation; SP-A = surfactant protein A; SP-D = surfactant protein D.
*$P<0.05$.

Table 4 Multivariate logistic analysis of risk factor for BOS/IPS

Risk factor	*Odds ratio (95% CI)*	P
Serum SP-D levels before SCT	0.95 (0.90–1.01)	0.08
Serum KL-6 levels before SCT	0.99 (0.98–1.01)	0.22
cGVHD extensive (except lung)	5.26 (0.49–56.0)	0.16

Abbreviations: cGVHD = chronic GVHD; CI = confidence interval; SP-D = surfactant protein D.

who survived more than 90 days after allo-HSCT in this study.

In clinical practice, elevated serum SP-D and KL-6 levels have been used as a marker of various lung diseases and activity of interstitial pneumonia.[10,12] Significant elevation in the serum level of SP-D and KL-6 in pulmonary inflammation is considered to be evoked by an activated secretion from the bronchiolar epithelium and/or an increase in the permeability of the lung/blood interface partly caused by the destruction of the bronchiolar epithelium and vascular endothelial cells. Also in this study, SP-A, -D and KL-6 were apparently elevated in IPS but not in BOS. Although we cannot conclude that surfactants and KL-6 increased in IPS but not in BOS based on the results in only one patient with IPS, in IPS the rapid and diffuse destruction of lung tissue may promote the release of SP-D into the circulation, while in BOS, which is a kind of chronic lung disease, the serum SP-D levels remained low. Although very little is known about the role of SP-D in chronic lung diseases, these results suggest that IPS and BOS have distinct etiologies.

Low production of surfactant protein and secretary protein in the alveoli is considered an important pathogenesis of certain types of lung disease such as adult respiratory distress and BOS.[15–17] SP-D is synthesized by non-ciliated Clara cells as well as pulmonary type II

pneumocytes. A decrease in the serum level of Clara cell secretary protein (CC16) was observed in BOS in both lung[16] and SCT.[17] The latter study also suggests that decreased serum levels of CC16 might permit early diagnosis of BOS. It was also reported that there is a protective effect of exogenous surfactant instillation to donor lungs before retrieval on post-lung transplantation surfactant function.[18] In addition, it was observed that absence or low levels of SP-D augment inflammation by acute oxidative stress in the mice model[19] and severe lung inflammation frequently occurred in SP-A-deficient mice receiving myeloablative allogeneic BM and spleen T-cells transplant.[11] Furthermore, in mice transtracheal human SP-A treatment attenuated the manifestation of IPS probably as a result of suppressed IFN-production by allo-activated lung-infiltrating T cells, and thereby, early survival was improved.[20]

The molecular weight of SP-D and -A is smaller than KL-6.[21] SP-D leaks more easily than SP-A because most of SP-A tightly bind to surfactant lipid aggregates in alveoli but SP-D appears to be lipid free.[22] In these points, a decrease in the serum SP-D level might reflect low production of SP-D in the alveoli of chronically damaged lung more than SP-A and KL-6. However, this is just speculation because it is unclear how the serum SP-D concentration is regulated and whether steady-state serum values of SP-D exclusively reflect constitutive synthesis levels of SP-D in the lung.

It has recently been shown that the constitutional SP-D serum levels are largely determined genetically based on the twin studies.[23,24] Moreover, it has been reported that, among the nine haplotypes of the human gene coding surfactant protein-D (SFTPD), the third most common haplotype of SFTPD (allele frequency 13.53%) is associated with a low serum level of SP-D.[25]

Therefore, we speculate that patients who have a certain SFTPD polymorphism will be put at higher risk of BOS/IPS when additive allo-immunological reaction or pulmonary infection occurs to them in allo-HSCT.

This study has the following limitations: (1) This study was retrospective in a single institution, (2) consisted of heterogeneous patient characteristics, diseases, conditioning regimens and GVHD prophylaxes and (3) we did not show decreased secretion or production of surfactant protein in the alveoli of the lung. To prove a causal linkage between the level of surfactant protein secretion in the alveoli and the pathogenesis of BOS/IPS in allo-HSCT patients, we need to directly measure the surfactant protein production level in the alveoli by BAL in allo-HSCT patients. However, measurement with BAL is an invasive examination for routine practice and has artifacts related to the instillation of fluid into the airway. Therefore, it may be a better alternative to address the association between single nucleotide polymorphism in SP-D in allo-HSCT recipients and LONIPCs.

In conclusion, pretransplant serum SP-D level may be a noninvasive and useful predictive marker for the development of BOS/IPS following allo-HSCT. To establish the usefulness of constitutive serum SP-D in allo-HSCT, a prospective trial in a large number of patients is needed.

Acknowledgements

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References

1 Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG *et al.* Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. *Br J Haematol* 1998; **100**: 680–687.
2 Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y *et al.* Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. *Blood* 2003; **102**: 4236–4242.
3 Patriarca F, Skert C, Sperotto A, Damiani D, Cerno M, Geromin A *et al.* Incidence, outcome, and risk factors of late-onset noninfectious pulmonary complications after unrelated donor stem cell transplantation. *Bone Marrow Transplant* 2004; **33**: 751–758.
4 Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 425–434.
5 Yoshihara S, Yanik G, Cooke KR, Mineishi S. Bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans organizing pneumonia (BOOP), and other late-onset noninfectious pulmonary complications following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 749–759.
6 Santo Tomas LH, Loberiza Jr FR, Klein JP, Layde PM, Lipchik RJ, Rizzo JD *et al.* Risk factors for bronchiolitis obliterans in allogeneic hematopoietic stem-cell transplantation for leukemia. *Chest* 2005; **128**: 153–161.
7 Fukuda T, Hackman RC, Guthrie KA, Sandmaier BM, Boeckh M, Maris MB *et al.* Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood* 2003; **102**: 2777–2785.
8 Keates-Baleeiro J, Moore P, Koyama T, Manes B, Calder C, Frangoul H. Incidence and outcome of idiopathic pneumonia syndrome in pediatric stem cell transplant recipients. *Bone Marrow Transplant* 2006; **38**: 285–289.
9 Clark H, Reid K. The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema. *Arch Dis Child* 2003; **88**: 981–984.
10 Hartl D, Griese M. Surfactant protein D in human lung diseases. *Eur J Clin Invest* 2006; **36**: 423–435.
11 Yang S, Milla C, Panoskaltsis-Mortari A, Hawgood S, Blazar BR, Haddad IY. Surfactant protein A decreases lung injury and mortality after murine marrow transplantation. *Am J Respir Cell Mol Biol* 2002; **27**: 297–305.
12 Walter JN, Fan LL, Bag R, Zhang H, Doan M, Mallory GB *et al.* Serum KL-6 as a marker for bronchiolitis obliterans syndrome after lung transplantation. *Transplantation* 2006; **82**: 709–711.
13 Clark JG, Crawford SW, Madtes DK, Sullivan KM. Obstructive lung disease after allogeneic marrow transplantation. Clinical presentation and course. *Ann Intern Med* 1989; **111**: 368–376.
14 Wong R, Rondon G, Saliba RM, Shannon VR, Giralt SA, Champlin RE *et al.* Idiopathic pneumonia syndrome after

high-dose chemotherapy and autologous hematopoietic stem cell transplantation for high-risk breast cancer. *Bone Marrow Transplant* 2003; **31**: 1157–1163.

15 Greene KE, Wright JR, Steinberg KP, Ruzinski JT, Caldwell E, Wong WB *et al.* Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am J Respir Crit Care Med* 1999; **160**: 1843–1850.

16 Nord M, Schubert K, Cassel TN, Andersson O, Riise GC. Decreased serum and bronchoalveolar lavage levels of Clara cell secretory protein (CC16) are associated with bronchiolitis obliterans syndrome and airway neutrophilia in lung transplant recipients. *Transplantation* 2002; **73**: 1264–1269.

17 Mattsson J, Remberger M, Andersson O, Sundberg B, Nord M. Decreased serum levels of clara cell secretory protein (CC16) are associated with bronchiolitis obliterans and may permit early diagnosis in patients after allogeneic stem-cell transplantation. *Transplantation* 2005; **79**: 1411–1416.

18 Struber M, Fischer S, Niedermeyer J, Warnecke G, Gohrbandt B, Gorler A *et al.* Effects of exogenous surfactant instillation in clinical lung transplantation: a prospective, randomized trial. *J Thorac Cardiovasc Surg* 2007; **133**: 1620–1625.

19 Kierstein S, Poulain FR, Cao Y, Grous M, Mathias R, Kierstein G *et al.* Susceptibility to ozone-induced airway inflammation is associated with decreased levels of surfactant protein D. *Respir Res* 2006; **7**: 85–93.

20 Yang S, Milla C, Panoskaltsis-Mortari A, Ingbar DH, Blazar BR, Haddad IY. Human surfactant protein a suppresses T cell-dependent inflammation and attenuates the manifestations of idiopathic pneumonia syndrome in mice. *Am J Respir Cell Mol Biol* 2001; **24**: 527–536.

21 Al-Salmi QA, Walter JN, Colasurdo GN, Sockrider MM, Smith EO, Takahashi H *et al.* Serum KL-6 and surfactant proteins A and D in pediatric interstitial lung disease. *Chest* 2005; **127**: 403–407.

22 Takahashi H, Kuroki Y, Tanaka H, Saito T, Kurokawa K, Chiba H *et al.* Serum levels of surfactant proteins A and D are useful biomarkers for interstitial lung disease in patients with progressive systemic sclerosis. *Am J Respir Crit Care Med* 2000; **162**: 258–263.

23 Husby S, Herskind AM, Jensenius JC, Holmskov U. Heritability estimates for the constitutional levels of the collectins mannan-binding lectin and lung surfactant protein D. A study of unselected like-sexed mono- and dizygotic twins at the age of 6–9 years. *Immunology* 2002; **106**: 389–394.

24 Sorensen GL, Hjelmborg JB, Kyvik KO, Fenger M, Hoj A, Bendixen C *et al.* Genetic and environmental influences of surfactant protein D serum levels. *Am J Physiol Lung Cell Mol Physiol* 2006; **290**: 1010–1017.

25 Heidinger K, Konig IR, Bohnert A, Kleinsteiber A, Hilgendorff A, Gortner L *et al.* Polymorphisms in the human surfactant protein-D (SFTPD) gene: strong evidence that serum levels of surfactant protein-D (SP-D) are genetically influenced. *Immunogenetics* 2005; **57**: 1–7.

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ORIGINAL ARTICLE

Cardiac and autonomic nerve function after reduced-intensity stem cell transplantation for hematologic malignancy in patients with pre-transplant cardiac dysfunction

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Abstract Recent reports have shown that cardiomyopathy caused by hemochromatosis in severe aplastic anemia is reversible after reduced-intensity allogeneic stem-cell transplantation (RIST). We comprehensively evaluated cardiac and autonomic nerve function to determine whether cardiac dysfunction due to causes other than hemochromatosis is attenuated after RIST. In five patients with cardiac dysfunction before transplant, we analyzed the changes in cardiac and autonomic nerve function after transplant, using electrocardiography (ECG), echocardiography, radionuclide angiography (RNA), serum markers, and heart rate variability (HRV), before and up to 100 days after transplant. There was no significant improvement in cardiac function in any patient and no significant alteration in ECG, echocardiogram, RNA, or serum markers. However, on time-domain analysis of HRV, the SD of normal-to-normal RR intervals (SDNN) and the coefficient of variation of the RR interval (CVRR) decreased significantly 30 and 60 days after transplant (P=0.04 and 0.01, respectively). Similarly, on frequency-domain analysis of HRV, low and high frequency power (LF and HF) significantly and temporarily decreased (P=0.003 and 0.03, respectively). Notably, in one patient who had acute heat failure after transplantation, the values of SDNN, CVRR, r-MSSD, LF, and HF at 30 and 60 days after transplantation were the lowest of all the patients. In conclusion, this study suggests that (a) RIST is well-tolerated in patients with cardiac dysfunction, but we cannot expect improvement in cardiac dysfunction due to causes other than hemochromatosis; and (b) monitoring HRV may be useful in predicting cardiac events after RIST.

Keywords Cardiac dysfunction · Heart rate variability · Reduced intensity allogeneic stem-cell transplantation · Acute heart failure

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is recognized as a curative treatment for hemato-

logic malignancy. However, myeloablative regimens are difficult to administer in elderly or ill patients because of increased transplantation-related toxicity. Therefore, reduced-intensity conditioning regimens for allo-HSCT have been developed for these vulnerable patients, who are ineligible for myeloablative conditioning. There are, however, no reports that describe whether reduced-intensity allogeneic stem-cell transplantation (RIST) is sufficiently well tolerated in patients with single-organ comorbidity, including cardiac, pulmonary, renal, or hepatic dysfunction. Therefore, the safety of RIST for patients with impaired organ function has not been sufficiently verified. Indeed, the existence of pretransplant organ dysfunction can worsen the prognosis after transplantation whether nonmyeloablative conditioning or myeloablative conditioning is used [1, 2]. Furthermore, in RIST utilizing melphalan and fludarabine, a high incidence of severe left ventricular failure (three of 21 patients; 14%) was reported [3]. This report casts doubt on whether RIST is safe in patients with cardiac dysfunction.

In contrast, three recent reports have shown marked improvement in cardiac function after transplant in patients with severe aplastic anemia (AA) and cardiomyopathy in secondary hemochromatosis, despite the underlying mechanism being unknown [4–6]. Conversely, the reversibility of cardiac dysfunction due to causes other than hemochromatosis has not been investigated. Hence, the primary objective of this study was to evaluate how cardiac dysfunction due to causes other than hemochromatosis changes after RIST.

Heart rate variability (HRV), which is generally recognized as an index of sympathovagal balance and autonomic cardiovascular control, has been investigated in various diseases that precipitate sudden death. Recently accumulated evidence has shown that a decrease in RR interval variability is strongly associated with sudden death and/or cardiac events after a myocardial infarction [7–10]. Furthermore, the usefulness of HRV as a clinical tool has been explored in numerous other conditions such as congestive heart failure, vasovagal syncope, hypertrophic cardiomyopathy, obstructive sleep apnea, diabetic neuropathy, and various neurological conditions [11–16]. In this study, we comprehensively analyzed the changes in cardiac function after RIST, assessed by various methods including electrocardiography (ECG), echocardiography, radionuclide angiography (RNA), natriuretic peptides and troponin T, and autonomic nerve function on 24-h Holter ECG for HRV.

Materials and methods

Patients

We prospectively enrolled five consecutive patients with pretransplant cardiac dysfunction from a group of 116 patients who had undergone allo-HSCT between September 2003 and August 2007 at our institute. In this study, the patients who were eligible for this study were those who had a left ventricular ejection fraction (LVEF) of under 50% on pretransplant radionuclide angiography, had ever been noted to have an LVEF of under 50% on echocardiography, or had a history of heart failure prior to transplant. The median age at transplantation was 42 years old (range 37–55). Pretransplant LVEFs were less than 50% in two patients, two patients had previously had an LVEF of under 50%, and the remaining patient had a history of heart failure prior to transplantation and an LVEF of under 50% at pretransplant assessment. No patient received mediastinal radiation therapy before transplantation nor did any patient have a history of hypertension, diabetes mellitus, and/or ischemic heart disease. The median level of serum ferritin prior to transplant was 189.6 ng/ml (range 58.3–1837.9), and there were no patients who were diagnosed as having cardiac hemochromatosis on echocardiography. We therefore strongly suspected anthracycline treatment as the cause of cardiac dysfunction in all five patients. As in a previous report [17], we calculated the cumulative anthracycline dose using the following ratios: daunorubicin 0.5, pirarubicin 0.8, mitoxantrone 3.4, idarubicin 1.6, and epirubicin 0.6, with cardiotoxicity of doxorubicin considered to be 1.0. These patients included three patients with non-Hodgkin's lymphoma (intravascular lymphoma, mantle cell lymphoma, and follicular lymphoma) who where refractory to chemotherapy, one patient with acute myeloid leukemia in her second complete remission (CR), and one patient with acute lymphoblastic leukemia in his first CR (Table 1). This study was approved by the Institutional Review Board. The concept, procedure, and potential risks of the study were explained, and written informed consent was obtained from all enrolled patients.

Allogeneic HSCT

Three patients received allo-HSCT from HLA-identical unrelated donors, and two patients from HLA-mismatched cord blood (Table 1). HLA matching (HLA-A, -B, and -DR) was determined by DNA genotyping in three unrelated BMTs, and only serologic typing was performed for the two cord blood transplants (CBT). Three patients received transplants from donors with matched blood types, one patient received a transplant with a major blood type mismatch, and one patient received a transplant with a minor blood type mismatch.

Reduced-intensity conditioning was employed for all five patients. Of the three patients from HLA-identical unrelated donors, two patients received reduced-intensity conditioning including fludarabine/oral busulfan (fludarabine 30 mg/m^2/day, on days −7 to −2 and oral busulfan 4 mg/kg/day, on days −4 and −3), and the remaining patient

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t1.1 **Table 1** Patients characteristics

t1.2	No.	Sex	Age	Disease	Disease status	Source	HLA Mismatch (A, B, DR)	aGVHD prophylaxis	Eligibility	Cumulative dose of anthracycline (mg/m^2)
t1.3	1	F	39	AML	CR2	uBM	Allele match	CsA + sMTX	LVEF 37%	250
t1.4	2	F	37	IVL	Ref	uBM	Allele match	CsA + sMTX	LVEF 46%, a history of AHF	380
t1.5	3	F	55	FL	Ref	CB	2 Ag	CsA alone	a history of decreased LVEF in 45%	391.8
t1.6	4	M	42	ALL	CR1	uBM	Allele match	CsA + sMTX	LVEF 48%	240
t1.7	5	F	46	MCL	Ref	CB	2 Ag	CsA + sMTX	a history of decreased LVEF in 39%	150

t1.8 We calculated the cumulative dose of anthracycline as daunorubicin 0.5, pirarubicin 0.8, mitoxantrone 3.4, idarubicin 1.6, epirubicin 0.6, when the intensity of the cardiotoxicity of doxorubicin was considered to be 1.0
IVL intravascular B cell lymphoma, *FL* follicular lymphoma, *MCL* mantle cell lymphoma, *Ref* chemo-refractory, *uBM* unrelated bone marrow, *Ag* antigen, *CB* cord blood, *CsA* cyclosporine, *sMTX* short-term methotrexate, *LVEF* left ventricular ejection fraction

received fludarabine/intravenous busulfan (fludarabine 30 mg/m^2/day, on days −9 to −4 and intravenous busulfan 1.6 mg/kg/day, on days −9 and −5). In contrast, the two patients who underwent CBT had fludarabine/intravenous busulfan/total body irradiation (fludarabine 30 mg/m^2/day, on days −9 to −4, intravenous busulfan 1.6 mg/kg/day, on days −9 and −5, and total body irradiation 4 Gy divided into two fractions/day, on days −3, −2, or −1).

As prophylaxis for acute graft-versus-host disease (aGVHD), four patients received both cyclosporine A (CsA) and short-term methotrexate (MTX), and one patient who received cord blood was given CsA alone. Intravenous MTX was administered on day 1 (10 mg/m^2), and on days 3 and 5 (7 mg/m^2). The doses of CsA were adjusted to a target trough level between 150 and 250 ng/ml until day 100, except where disease progression or drug toxicity occurred, and then tapered unless GVHD occurred. aGVHD was diagnosed clinically, graded according to standard criteria and confirmed by appropriate biopsies. Chronic GVHD (cGVHD) was also defined according to the standard criteria. In CBT, pre-engraftment immune reactions (PIR) were defined according to the report of Kishi et al. [18].

The evaluation of cardiac and autonomic nerve function

We evaluated cardiac function with 12-lead electrocardiograms (ECG), echocardiography, radionuclide angiography (RNA), serum markers, and autonomic nerve function with heart rate variability assessed on 24-h ECG monitoring (within the 100 days before transplant) and posttransplantation [on days 30 (±4), 60 (±6), and 100 (±12)].

The 12-lead ECG

Twelve-lead ECG was performed at rest, and the QTc interval was measured automatically using a novel recording system (FDX-6521, Fukuda Denshi, Tokyo, Japan). In brief, QT intervals were measured for 15 s at 25 mm/s. QTc intervals were then calculated by correcting the QT interval using the Bazett formula [19].

Echocardiography

Two-dimensional echocardiographic examinations were performed using a Power Vision 6000 (Toshiba, Tokyo, Japan). We assessed the following variables: (1) posterior wall (PW) and interventricular septal wall (IVS) for evaluation of left ventricular hypertrophy, (2) left ventricular end-diastolic dimension (LVDd) and end-systolic dimension (LVDs) for evaluation of left ventricular dilatation, (3) early peak flow velocity/atrial peak flow velocity (E/A) and deceleration time (DcT) for evaluation of left ventricular diastolic function. During the examination, the gain setting was optimized to a level just below background noise, and the transducer frequency was set to 2.5 or 3.5 MHz.

RNA

Radionuclide angiography was performed with the gated-equilibrium technique. Blood cells were targeted with 99 mTc-pertechnetate, facilitated by pyrophosphate and injected intravenously. ECG-gated gamma camera scanning was performed using a VertexPlus (ADAC /Philips, CA, USA) from two directions; the left anterior oblique and anterior views were obtained in order to image the left ventricle separately from the right ventricle, and the left atrium and 64×64 matrix was used. Acquisition time was 180 s, and ECG-gated images were acquired with 20 frames per cardiac cycle. LVEF and peak-filling rate (PFR) were calculated as parameters of left ventricular systolic and diastolic function.

Serum markers (natriuretic peptide measurement)

We employed human atrial natriuretic peptide (hANP) and brain natriuretic peptide (BNP) as parameters of cardiac function. Blood samples for natriuretic peptide measurement were drawn from the patients into chilled tubes containing aprotinin and 1 mg of ethylenediamine tetraacetic acid (EDTA)/ml. The tubes were immediately placed on ice and centrifuged at 4°C, and the separated plasma was stored at −80°C before assaying. According to the manufacturer's recommended protocol (SRL, Tokyo, Japan), hANP and BNP were measured with a commercially available immunoradiometric assay. The normal values of hANP and BNP are less than or equal to 40 and 20 pg/ml, respectively.

Heart rate variability

Data obtained from the 24-h ambulatory ECG recordings were analyzed with RR data analysis software (MemCalc/CHIRAM version 1, Suwa Trust, Tokyo, Japan). We employed markers including the coefficient of variance of the RR interval (CVRR), the SD of the NN interval (SDNN), and the squares of the differences between adjacent normal-to-normal RR intervals (r-MSSD) in time domain analysis and the low-frequency (LF) area, high-frequency (HF) area, and LF/HF ratio in frequency domain analysis.

Other parameters

In addition, we evaluated parameters that possibly influence HRV, such as systolic and diastolic blood pressure, heart rate, body temperature, CsA trough, and hemoglobin to assess which parameters affected HRV in this study. For analysis, we used the averaged values of each parameter on the three preceding days that were closest to the day when the HRV evaluations were conducted.

The diagnosis of acute heart failure

Clinical criteria for acute heart failure were established by referring to those used in the Framingham study [20]. We diagnosed acute heart failure when a minimum of two major criteria or when one major and two minor criteria were present concurrently. Major criteria included orthopnea, pulmonary congestion, pulmonary rales, a gallop rhythm, and jugular venous distention. Minor criteria included tachycardia (rate>120/min), shortness of breath, ankle edema, and hepatomegaly.

Statistical analysis

The Friedman test was used for detecting changes pre- and posttransplantation (30, 60, and 100 days after transplant) in all serum markers, parameters on echocardiography, RNA, ECG and HRV, systolic and diastolic blood pressure, heart rate, body temperature, CsA trough level, hemoglobin, and C-reactive protein. All P values were two-sided, and a significance level of 0.05 was used.

Results

Table 1 shows the five patients' characteristics. The median follow-up time was 324 days (range 127–1517). Engraftment was achieved in all five patients (neutrophil engraftment occurred in the three patients with unrelated BMT 13, 17, and 21 days after transplantation; in the two patients with CBT, it occurred 27 and 31 days after transplant, respectively). No patient died within 100 days of the transplant, although in one patient (no. 2), the disease relapsed within 100 days, as she had disease recurrence in the central nervous system at day 42. In this case, therefore, CsA was tapered and stopped by day 60. Although salvage chemotherapy and donor lymphocyte infusion were performed on days 65 and 76, respectively, she died from disease progression on day 127 (Table 2). An angiotensin-receptor antagonist was administered from before transplantation in one patient (no. 5), but angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and diuretics were not administered before transplantation in any patient.

Of the five patients, none developed acute heart failure early after the conditioning chemotherapy. Pre-engraftment immune reactions (PIR) occurred in the two patients who had CBT, on days 8 and 13, but neither developed acute heart failure during the period of evaluation (Table 2). Three patients had aGVHD (grades I, III, and IV, respectively). The two patients with grade III and IV aGVHD were given high-dose steroids. Diuretics were appropriately given to patients with body weight gain during PIR or aGVHD. Three patients had sepsis, two cases of which were severe. One patient (no. 3) experienced prolonged MRSA sepsis and another patient (no. 4) experienced septic shock due to catheter infection. Two patients died from disease progression. One patient (no. 4) developed acute heart failure that might partly have been caused by pneumonia and drug-induced acute renal failure 147 days after transplantation. In this patient, acute progressive dyspnea, bilateral pleural effusions, cardiomegaly, and leg edema appeared soon after pneumonia and drug-induced acute renal failure. We diagnosed her as having acute heart failure (New York Heart Association functional class IV) and performed endotracheal intubation, administered catecholamines and diuretics, and discontinued or reduced the dose of drugs thought to be nephrotoxic. Thereafter, she recovered rapidly, and she was extubated 156 days posttransplant.

t2.1 **Table 2** Outcomes

t2.2 No.	Neutrophil engraftment	aGVHD	aGVHD	Severe infection	AHF	Outcome (Cause of death)
t2.3 1	Day 21		Limit			Alive 1,517+
t2.4 2	Day 17		NA			Dead 127 (relapse)
t2.5 3	Day 31	IV (day 13, skin and gut)	Extensive	Prolonged MRSA sepsis >2 months from day 56	+Day 147	Alive 461+
t2.6 4	Day 13	III (day 29, gut)	Limit	Septic shock at day 61		Alive 324+
t2.7 5	Day 27	I (day 49, gut)	Limit			Dead 170 (relapse)

t2.8 *aGVHD* acute graft-versus host disease, *cGVHD* chronic graft-versus-host disease, *AHF* acute heart failure after transplantation, *MRSA* methicillin-resistant *Staphylococcus aureus*

Table 3 shows the posttransplantation changes in cardiac parameters in all five patients. All parameters for RNA, echocardiography, natriuretic peptides, and ECG showed no significant changes at any of the evaluation points. On the other hand, some parameters in the analysis of HRV changed significantly (Fig. 1). SDNN and CVRR significantly decreased at 30 to 60 days after transplantation and then recovered (P=0.04 and 0.01, respectively). Moreover, LF and HF also temporarily decreased (P=0.003 and 0.03, respectively). In particular, the patients with acute heart failure had the lowest values of SDNN, CVRR, r-MSSD, LF, and HF at 30 to 60 days after transplant (Fig. 1, dashed line). LF/HF did not change significantly.

Table 4 shows changes in the parameters that might possibly have influenced HRV, including systolic and diastolic blood pressure, heart rate, body temperature, CsA trough levels, and hemoglobin. On analysis, diastolic blood pressure and body temperature showed a statistically significant change (P=0.03 and 0.04, respectively).

Discussion

In this study, we observed no significant improvement of impaired cardiac function, but in contrast, we did detect a temporary, marked decrease in HRV soon after SCT.

As mentioned earlier, there have been three Japanese reports of the use of RIST for severe AA in patients with impaired cardiac function [4–6]. Before HSCT, LVEFs in these three cases were 20–40%. In all three cases, cardiomyopathy was caused by secondary hemochromatosis attributable to heavy transfusions. Although the mechanism was not clarified, LVEFs in all three cases dramatically recovered to a normal level, a few months to years after transplant. In one case, prompt improvement in cardiac function was seen with persistent iron deposition and without normalization of hemoglobin level [4]. These reports demonstrate that cardiomyopathy is reversible in patients with secondary hemochromatosis and severe AA. It is therefore interesting to determine whether cardiac

t3.1 **Table 3** Posttransplant changes of cardiac parameters

t3.2	Baseline	Day 30	Day 60	Day 100	P
t3.3 Radionuclide ventriculography					
t3.4 LVEF (%)	48 (37–64)	51 (41–59)	45 (38–55)	47 (39–58)	0.39
t3.5 PFR (EDV/s)	2.0 (1.3–3.1)	1.8 (1.6–3.2)	1.5 (1.2–3.2)	1.9 (1.3–2.7)	0.56
t3.6 Echocardiography					
t3.7 IVS (mm)	7.7 (6.8–9.3)	10.0 (8.0–11.0)	8.9 (7.7–12.8)	8.6 (6.8–10.7)	0.08
t3.8 PW (mm)	9.6 (8.3–11.2)	10.7 (8.0–11.8)	10.7 (9.5–12.2)	10.8 (8.0–13.2)	0.42
t3.9 LVDd (mm)	45 (37–58)	44 (40–56)	47 (32–53)	46 (37–54)	0.42
t3.10 LVDs (mm)	34 (28–44)	34 (27–42)	35 (24–43)	33 (31–42)	0.90
t3.11 E/A	0.91 (0.72–1.17)	1.24 (0.84–2.24)	1.10 (0.85–2.08)	0.87 (0.55–1.15)	0.83
t3.12 DcT (ms)	185.5 (160–220)	196 (124–212)	234 (96–296)	186 (172–296)	0.62
t3.13 Natriuretic peptides					
t3.14 hANP (ng/ml)	15 (12–40)	25 (12–119)	35 (10–98)	29 (10–66)	0.80
t3.15 BNP (ng/ml)	36 (5–100)	68 (5–183)	21 (9–180)	35 (2–107)	0.61
t3.16 Electrocardiography					
t3.17 QTc (ms)	435 (422–490)	427 (408–478)	422 (408–457)	415 (405–421)	0.14

t3.18 *LVEF* left ventricular ejection fraction, *PFR* peak filling rate, *IVS* intraventricular septal wall, *PW* posterior wall, *LVDd* left ventricular end-diastolic dimension, *LVDs* left ventricular end-systolic dimension, *E/A* early peak flow velocity/atrial peak flow velocity, *DcT* deceleration time, *hANP* human atrial natriuretic peptide, *BNP* brain natriuretic peptide

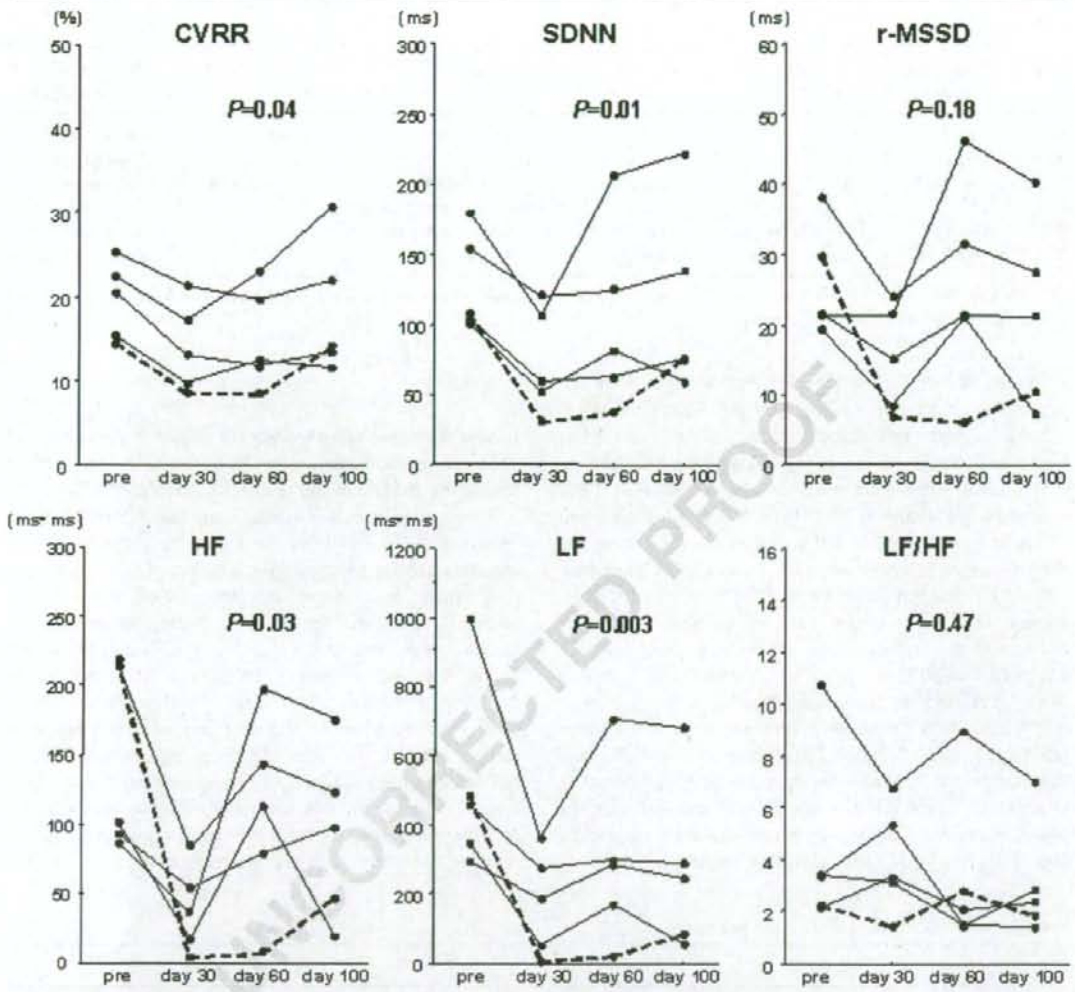

Fig. 1 Changes in HRV indicators during the first 100 days after allogeneic stem cell transplantation. The value of SDNN in the time-domain analysis and the values of HF and LF in the frequency-domain analysis significantly and temporarily decreased 30 days after transplantation. *SDNN* the standard deviation of all normal beats, *r-MSSD* the square root of the mean of the sum of squared differences between adjacent normal-to-normal intervals, *HF* high frequency power, *LF* low frequency power

dysfunction caused by other etiologies such as chemotherapy can similarly be restored. However, no significant improvement was observed in any patient in this study, suggesting that it is likely that the reversibility of cardiac function depends on the etiology of the cardiac dysfunction.

In all five patients, there were no significant changes in cardiac parameters such as left ventricular systolic and diastolic function on echocardiography and RNA, serum troponin T (data not shown), BNP and hANP, and QTc on ECG, throughout the monitoring period. One patient had acute heart failure after the completion of all assessments, but she recovered with appropriate monitoring and therapy. These results suggest that RIST is relatively well-tolerated in patients with cardiac dysfunction, provided management is adequate.

To prevent the development of acute heart failure in patients with impaired left ventricular function present before transplantation, we need to pay attention to the following points: (1) careful control of fluid intake and output by monitoring body weight and urine output, or checking for cardiomegaly and dilatation of the vena cava on chest X-ray and echocardiography; (2) careful monitoring of

t4.1 **Table 4** Posttransplant changes of possible parameters[a] influencing HRV

t4.2		Baseline	Day 30	Day 60	Day 100	*P*
t4.3	Systolic blood pressure (mmHg)	101 (97–117)	116 (92–137)	109 (91–136)	111 (97–118)	0.35
t4.4	Diastolic blood pressure (mmHg)	60 (57–64)	76 (62–87)	68 (67–84)	69 (68–74)	0.03
t4.5	Heart rate (/min)	79 (76–93)	89 (76–120)	85 (72–109)	86 (84–88)	0.08
t4.6	Body temperature (°C)	36.5 (36.2–36.8)	37.2 (36.8–38.2)	36.8 (36.7–38.1)	37.2 (37–37.5)	0.04
t4.7	CsA trough (ng/ml)	0	227 (193–277)	237 (0–328)	160 (0–378)	0.07
t4.8	Hemoglobin (g/dl)	8.8 (8.3–14.3)	9.9 (7.7–14.2)	9.4 (8–12.1)	10.2 (7.9–10.9)	0.55
t4.9	C-reactive protein (mg/dl)	0.1 (0.01–0.21)	0.2 (0.1–2.34)	0.12 (0.1–1.99)	0.25 (0.03–2.38)	0.23

t4.10 *CsA* cyclosporine

[a] Each data is the five patients' median value of the 3 days average data on or about the day HRV went

decline in kidney function due to renal toxicity caused by calcineurin inhibitors, antibiotics, antifungal drugs, or antiviral drugs and prompt adjustment of the dose of these drugs; and (3) transfusing at a slower rate than usual.

One study reported that prophylactic treatment with the ACE inhibitor enalapril not only prevented deterioration in left ventricular dysfunction prior to transplant, but also brought about an increase in LVEF in all patients [21]. Another study also demonstrated that early treatment with enalapril might prevent late cardiotoxicity due to high-dose chemotherapy in a randomized trial in 114 high-risk patients, identified by an increased troponin I value [22]. In our study, instead of receiving an ACE inhibitor, one patient (no. 5) started to receive an angiotensin receptor blocker before transplantation; she had no cardiac dysfunction after transplantation. Although the effectiveness of ACE inhibitors or angiotensin receptor blockers has not been sufficiently validated for preventing cardiac events in patients with pretransplant cardiac dysfunction, it may be useful to consider prophylactic administration of an ACE inhibitor or angiotensin receptor blocker.

In this case series, almost all patients had a temporary decrease in HRV posttransplant, and the patient with acute heart failure had an especially marked decrease prior to the onset of acute heart failure (Fig. 1, dashed line). Previous studies have reported a correlation between HRV and proinflammatory cytokines [23, 24], CsA [25], blood pressure [26], heart rate [27], anemia [28], and C-reactive protein [29, 30] as possible factors that influence changes in HRV after transplantation. In fact, the significant elevation of body temperature and diastolic blood pressure might have influenced the decrease in HRV. On time-domain analysis of HRV, a number of authors have identified a decreased SDNN value as a univariate risk factor for all-cause mortality, expressed as a dichotomized variable in the patients with chronic heart failure [31]. This review notes that an SDNN value of less than 44 ms was determined statistically to be the optimum cutoff point [32], and the value of 44 ms is similar to the value of <50 ms that best predicts mortality in myocardial infarction patients [7]. The predictive value of SDNN can be explained by the speculation that a reduction in the SDNN reflects the summed influence of abnormalities in sympathetic, parasympathetic and rennin–angiotensin activities, abnormal chemoreceptor function, changes in respiratory pattern, and physical inactivity in congestive heart failure [33].

Conversely, among the indicators used in frequency-domain analysis of HRV, a reduced LF value is reportedly highly predictive of sudden cardiac death in chronic heart failure [31]. Despite high levels of sympathetic activation, decreases in the LF value were often observed in patients with chronic heart failure, which may be secondary to abnormalities in central autonomic regulation and impairment of beta adrenergic receptor sensitivity [33]. Furthermore, a reduced LF value was reported as a marker of sympathetic overactivity, and it was concluded that a reduced short-term LF value during controlled breathing (<13 ms^2) is a powerful predictor of sudden death in patients with chronic heart failure [34]. In our case with acute heat failure, the LF value as well as the SDNN value temporarily but dramatically decreased to less than 44 ms and 13 ms^2, respectively, before the occurrence of acute heat failure, indicating that decreases in SDNN and/or LF values might be associated with the development of acute heat failure after transplantation. HF and r-MSSD are generally considered to be predominantly determined by the parasympathetic nervous system. In a rat study, it was reported that CsA induced a decrease in SDNN and r-MSSD values [25]. Therefore, in our study, CsA treatment after transplantation might play a role in the significant decrease in the HF value and the mild decrease in r-MSSD, by its suppression of parasympathetic activity.

This study has the following limitations: (1) the population is small and from a single institution; (2) lack of clarity about the causes for the decrease in HRV; and (3) we do not know the lower limit of cardiac function in patients still eligible for RIST. However, to our knowledge, there has been no prior report of comprehensive analyses of cardiac and autonomic nervous system function in patients with impaired cardiac function who underwent allo-HSCT.

In conclusion, RIST is relatively well tolerated in patients with pretransplant cardiac dysfunction, but apparent improvement of cardiac function cannot be expected except in cardiomyopathy induced by secondary hemochromatosis. We could not determine whether changes in autonomic function are associated with the development of cardiac events after transplant. Additional studies of a larger cohort are therefore required to confirm the usefulness of HRV for prediction of cardiac events after transplantation.

References

1. Sorror ML, Giralt S, Sandmaier BM, De Lima M, Shahjahan M, Maloney DG, Deeg HJ, Appelbaum FR, Storer B, Storb R (2007) Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first remission: combined FHCRC and MDACC experiences. Blood 110:4606–4613 doi:10.1182/blood-2007-06-096966
2. Sorror M, Storer B, Sandmaier BM, Maloney DG, Chauncey TR, Langston A, Maziarz RT, Pulsipher M, McSweeney PA, Storb R (2008) Hematopoietic cell transplantation-comorbidity index and Karnofsky performance status are independent predictors of morbidity and mortality after allogeneic nonmyeloablative hematopoietic cell transplantation. Cancer 112:1992–2001
3. Ritchie DS, Seymour JF, Roberts AW, Szer J, Grigg AP (2001) Acute left ventricular failure following melphalan and fludarabine conditioning. Bone Marrow Transplant 28:101–103 doi:10.1038/sj.bmt.1703098
4. Kunisaki Y, Takase K, Miyamoto T, Fukata M, Nonami A, Kamezaki K, Kaji Y, Gondo H, Harada M, Nagafuji K (2007) Marked improvement of cardiac function early after non-myeloablative BMT in a heavily transfused patient with severe aplastic anemia and heart failure. Bone Marrow Transplant 40:593–595 doi:10.1038/sj.bmt.1705764
5. Nishio M, Endo T, Nakao S, Sato N, Koike T (2008) Reversible cardiomyopathy due to secondary hemochromatosis with multitransfusions for severe aplastic anemia after successful nonmyeloablative stem cell transplantation. Int J Cardiol 127:400–401
6. Abe Y, Matsushima T, Tachikawa Y, Nagasawa E, Nishimura J, Nawata H, Muta K (2005) Fludarabine-based conditioning used in successful bone marrow transplantation from an unrelated donor in a heavily transfused patient with severe aplastic anemia. Int J Hematol 81:81–82 doi:10.1532/IJH97.04134
7. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ (1987) Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 59:256–262 doi:10.1016/0002-9149(87)90795-8
8. Bigger JT Jr, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN (1992) Frequency domain measures of heart period variability and mortality after myocardial infarction. Circulation 85:164–171
9. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ (1998) Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (autonomic tone and reflexes after myocardial infarction). Investigators. Lancet 351:478–484 doi:10.1016/S0140-6736(97)11144-8
10. Frenneaux MP (2004) Autonomic changes in patients with heart failure and in post-myocardial infarction patients. Heart 90:1248–1255 doi:10.1136/hrt.2003.026146
11. Guzzetti S, Cogliati C, Turiel M, Crema C, Lombardi F, Malliani A (1995) Sympathetic predominance followed by functional denervation in the progression of chronic heart failure. Eur Heart J 16:1100–1107
12. Furlan R, Piazza S, Dell'Orto S, Barbic F, Bianchi A, Mainardi L, Cerutti S, Pagani M, Malliani A (1998) Cardiac autonomic patterns preceding occasional vasovagal reactions in healthy humans. Circulation 98:1756–1761
13. Ajiki K, Murakawa Y, Yanagisawa-Miwa A, Usui M, Yamashita T, Oikawa N, Inoue H (1993) Autonomic nervous system activity in idiopathic dilated cardiomyopathy and in hypertrophic cardiomyopathy. Am J Cardiol 71:1316–1320 doi:10.1016/0002-9149(93)90547-P
14. Narkiewicz K, Montano N, Cogliati C, van de Borne PJ, Dyken ME, Somers VK (1998) Altered cardiovascular variability in obstructive sleep apnea. Circulation 98:1071–1077
15. Bernardi L, Ricordi L, Lazzari P, Soldà P, Calciati A, Ferrari MR, Vandea I, Finardi G, Fratino P (1992) Impaired circadian modulation of sympathovagal activity in diabetes. A possible explanation for altered temporal onset of cardiovascular disease. Circulation 86:1443–1452
16. Guzzetti S, Cogliati C, Broggi C, Carozzi C, Caldiroli D, Lombardi F, Malliani A (1994) Influences of neural mechanisms on heart period and arterial pressure variabilities in quadriplegic patients. Am J Physiol 266:1112–1120
17. Herait P, Poutignat N, Marty M, Bugat R (1992) Early assessment of a new anticancer drug analogue–are the historical comparisons obsolete? The French experience with pirarubicin. Eur J Cancer 28:1670–1676 doi:10.1016/0959-8049(92)90066-B
18. Kishi Y, Kami M, Miyakoshi S, Kanda Y, Murashige N, Teshima T, Kusumi E, Hara S, Matsumura T, Yuji K, Masuoka K, Wake A, Morinaga S, Kanemaru M, Hayashi T, Tanaka Y, Taniguchi S, Tokyo Stem Cell Transplant Consortium (2005) Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. Transplantation 80:34–40 doi:10.1097/01.TP.0000163289.20406.86
19. Bazett HC (1920) An analysis of the time-relations of electrocardiograms. Heart 7:353–370
20. McKee PA, Castelli WP, McNamara PM, Kannel WB (1971) The natural history of congestive heart failure: the Framingham study. N Engl J Med 285:1441–1446
21. Kakavas PW, Ghalie R, Parrillo JE, Kaizer H, Barron JT (1995) Angiotensin converting enzyme inhibitors in bone marrow transplant recipients with depressed left ventricular function. Bone Marrow Transplant 156:859–861
22. Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, Martinelli G, Veglia F, Fiorentini C, Cipolla CM (2006) Prevention of high-dose chemotherapy-induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. Circulation 11423:2474–2481 doi:10.1161/CIRCULATIONAHA.106.635144
23. Straburzynska-Migaj E, Ochotny R, Wachowiak-Baszynska A, Straburzynska-Lupa A, Lesniewska K, Wiktorowicz K, Cieslinski A (2005) Cytokines and heart rate variability in patients with chronic heart failure. Kardiol Pol 63:478–485, discussion 486-7
24. Kunz-Ebrecht SR, Mohamed-Ali V, Feldman PJ, Kirschbaum C, Steptoe A (2003) Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav Immun 17:373–383 doi:10.1016/S0889-1591(03)00029-1
25. Omar AG, El-Mas MM (2004) Time-domain evaluation of cyclosporine interaction with hemodynamic variability in rats. Cardiovasc Drugs Ther 18:461–468 doi:10.1007/s10557-004-6223-1
26. Park SB, Lee BC, Jeong KS (2007) Standardized tests of heart rate variability for autonomic function tests in healthy Koreans. Int J Neurosci 117:1707–1717 doi:10.1080/00207450601050097
27. Agelink MW, Malessa R, Baumann B, Majewski T, Akila F, Zeit T, Ziegler D (2001) Standardized tests of heart rate variability:

normal ranges obtained from 309 healthy humans, and effects of age, gender, and heart rate. Clin Auton Res 11:99–108 doi:10.1007/BF02322053

28. Gehi A, Ix J, Shlipak M, Pipkin SS, Whooley MA (2005) Relation of anemia to low heart rate variability in patients with coronary heart disease (from the Heart and Soul study). Am J Cardiol 95:1474–1477 doi:10.1016/j.amjcard.2005.02.017
29. Psychari SN, Apostolou TS, Iliodromitis EK, Kourakos P, Liakos G, Kremastinos DT (2007) Inverse relation of C-reactive protein levels to heart rate variability in patients after acute myocardial infarction. Hellenic J Cardiol 48:64–71
30. Kon H, Nagano M, Tanaka F, Satoh K, Segawa T, Nakamura M (2006) Association of decreased variation of R-R interval and elevated serum C-reactive protein level in a general population in Japan. Int Heart J 47:867–876 doi:10.1536/ihj.47.867
31. Sandercock GR, Brodie DA (2006) The role of heart rate variability in prognosis for different modes of death in chronic heart failure. Pacing Clin Electrophysiol 29:892–904 doi:10.1111/j.1540-8159.2006.00457.x
32. Aronson D, Mittleman MA, Burger AJ (2004) Measures of heart period variability as predictors of mortality in hospitalized patients with decompensated congestive heart failure. Am J Cardiol 93:59–63 doi:10.1016/j.amjcard.2003.09.013
33. Galinier M, Pathak A, Fourcade J, Androdias C, Curnier D, Varnous S, Boveda S, Massabuau P, Fauvel M, Senard JM, Bounhoure JP (2000) Depressed low frequency power of heart rate variability as an independent predictor of sudden death in chronic heart failure. Eur Heart J 21:475–482 doi:10.1053/euhj.1999.1875
34. La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, Ferrari R, Franchini M, Gnemmi M, Opasich C, Riccardi PG, Traversi E, Cobelli F (2003) Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. Circulation 107:565–570 doi:10.1161/01.CIR.0000047275.25795.17

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LETTER TO THE EDITOR

Long-term survival after HLA-haploidentical SCT from noninherited maternal antigen-mismatched family donors: impact of chronic GVHD

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Allo-SCT from an HLA-haploidentical family donor has been the treatment of choice in patients with high-risk hematologic malignancies, who are expected to have a better prognosis with SCT but lack immediate access to a conventional stem cell source.[1,2] With intent to minimize the risk of severe GVHD, most haploidentical SCT protocols employ *ex vivo* or *in vivo* T-cell depletion, albeit at the expense of an increased risk of infection or relapse as a result of poor post-transplant immune reconstitution. To develop an alternative strategy to perform haploidentical SCT that confers improved immune recovery and acceptable risk of GVHD, we have explored the feasibility of T-cell-replete SCT from family donors mismatched for noninherited maternal HLA antigens (NIMA); NIMA-mismatched donor selection is based on the hypothesis that the detection of long-term maternal or fetal microchimerism in the donor's peripheral circulation is associated with immunological hyporesponsiveness against NIMA (in the case of NIMA mismatch in the graft-vs-host direction) or against inherited paternal HLA antigens (IPA) (in the case of NIMA mismatch in the host-vs-graft direction).[3] According to this scenario, we and other groups showed that T-cell-replete HLA-haploidentical SCT from a NIMA-mismatched family donor is feasible in selected patients with poor-risk hematologic malignancies.[3,4] However, late complications and long-term outcomes in patients undergoing such transplantations have been so far largely unknown. Therefore, we retrospectively studied the severity of chronic GVHD, requirement for immunosupressive treatment, and status of primary disease in long-term survivors who received T-cell-replete NIMA-mismatched haploidentical SCT.

We collected data on 16 consecutive patients who had survived more than 3 years after NIMA-mismatched SCT performed between January 2001 and July 2004 at 11 institutions that participated in our previous nationwide study (Table 1).[3] At the time of SCT, they had a median age of 19 years (range, 2–56) and received BM ($n=5$) or G-CSF-mobilized peripheral blood ($n=11$) as treatment for acute myeloid leukemia ($n=6$), acute lymphoblastic leukemia ($n=3$), chronic myeloid leukemia ($n=4$) and other B-cell neoplasms ($n=3$); 6 patients had a chemosensitive disease and 10 had a refractory disease. Early outcomes of 14 of these transplantations have been described elsewhere.[3–5] All patients received tacrolimus-based GVHD prophylaxis after myeloablative ($n=10$) or reduced-intensity conditioning ($n=6$). The type of donor was NIMA-mismatched sibling in 9 cases, mother in 6 and daughter in 1; all patient-donor pairs had two or three serologic mismatches at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host direction. The presence of long-term maternal or fetal microchimerism was detected in all donors through NIMA- or IPA-specific nested PCR as described earlier.[3] Karnofsky score was employed to record the performance status for patients who were 16 years or older, whereas Lansky score was applied for those who were younger than 16 years of age. Organ-specific symptoms related to chronic GVHD and their severity were diagnosed and evaluated by consensus criteria proposed by the National Institutes of Health Chronic GVHD Diagnosis and Staging Working Group.[6]

At a median follow-up of 56 months (range, 38–74), 13 (81%) of 16 patients were alive and free of their primary disease. One patient was alive with relapsed disease, and two died from pulmonary complications at 51 and 52 months after transplantation in continuous remission. Fifteen (94%) patients developed classical chronic GVHD; type of onset was *de novo* in five, quiescent in six and progressive in four. According to the National Institutes of Health Clinical Scoring Systems the affected organs scored two or greater, which included lungs ($n=5$), skin ($n=4$), eyes ($n=3$), liver ($n=3$) and joints/fascia ($n=2$) (Table 2). Overall severity of chronic GVHD among these patients was classified as mild in three (20%) cases, moderate in seven (47%) and severe in five (33%). It is noted that immunosuppressive agents were successfully withdrawn from eight (50%) patients with *de novo* or quiescent onset of disease at a median of 19 months (range, 3–46) after transplantation, although two of four patients who experienced severe chronic GVHD (score 3) of lung eventually succumbed to bronchiolitis obliterans. Karnofsky or Lansky performance score at the time of last follow-up among the 14 surviving patients was 100% in 6 (43%), 80–90% in 5 (36%), 70% in 2 (14%) and less than 70% in 1 (7%).

In the present study, we found that substantial proportion of long-term survivors after NIMA-mismatched haploidentical SCT could discontinue administration of immunosuppressive agents despite the frequent occurrence of moderate-to-severe chronic GVHD. This paradoxical observation contrasts sharply with the conventional assumption that the establishment of robust tolerance across multiple HLA disparities is hardly possible in the setting of marrow or peripheral blood SCTs without employing T-cell depletion. However, clinical significance of NIMA-mismatched donor selection has

Table 1 Characteristics of long-term survivors after NIMA-mismatched haploidentical SCT

Median age at transplant, years (range)	19 (2–56)
Sex	
Male	10
Female	6
Diagnosis	
Acute leukemia[a]	9
Chronic myeloid leukemia	4
Diffuse large B-cell lymphoma	1
Plasma cell myeloma	2
Disease status	
CR or chronic phase	6
Chemorefractory or blastic phase	10
Donor type	
NIMA-mismatched sibling	9
Mother	6
Daughter	1
No. of mismatched HLA antigens[b]	
Two antigens	10
Three antigens	6
Stem cell source	
BM	5
Peripheral blood	11
Conditioning	
Myeloablative	10
Reduced-intensity	6
GVHD prophylaxis	
Tacrolimus alone	1
Tacrolimus + MTX	13
Tacrolimus + MTX + corticosteroids	2
Acute GVHD	
None or grade 1	9
Grade 2	6
Grade 3	1

[a]One patient had secondary acute myeloid leukemia developed after treatment for acute lymphoblastic leukemia.
[b]The number of serologic mismatch at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host vector was shown.

been controversial because the mechanisms underlying the tolerogenic effect against NIMA or IPA have not been fully elucidated.[7] Using murine BM transplant models, Matsuoka *et al.*[8] showed that transplant from donors exposed to NIMA *in utero*, but not from those exposed to IPA, reduced the morbidity and mortality associated with GVHD in an antigen-specific and a $CD4^+CD25^+$ T cells-dependent manner. In contrast, Opiela *et al.*[9] showed that murine neonates exposed to low levels of NIMA can develop vigorous *in vivo* cytotoxic rather than tolerogenic responses against NIMA, which might explain the inconsistent severity of GVHD after NIMA-mismatched SCTs in humans. Intriguingly, Stern *et al.*[10] recently reported that recipients of T cell-depleted haploidentical SCT using mother as the donor had the better overall survival than those using father as the donor, implying a mechanism by which previous exposure to paternally derived antigens in maternal donors would positively affect transplant outcomes.

In conclusion, our observations in this study suggested that long-term survival without continuous immunosuppressive treatment is possible after T-cell-replete HLA-haploidentical SCT from a microchimeric NIMA-mismatched donor. Although the small number of patients limits the interpretation of our results, further studies are warranted to compare late sequelae after HLA-haploidentical SCTs with various protocols in larger cohorts.

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Table 2 Clinical scoring of organ-specific symptoms related to chronic GVHD in long-term survivors after NIMA-mismatched haploidentical transplantation[a]

Involved organ site[b]	*No. of evaluable patients*	*Score 0*	*Score 1*	*Score 2*	*Score 3*
PS	14[c]	6 (43%)	7 (50%)	1 (7%)	0
Skin	16	5 (31%)	7 (44%)	2 (13%)	2 (13%)
Mouth	16	11 (69%)	5 (31%)	0	0
Eyes	16	10 (63%)	3 (19%)	3 (19%)	0
GI tract	16	14 (88%)	2 (13%)	0	0
Liver	16	10 (63%)	3 (19%)	3 (19%)	0
Lungs	16	9 (56%)	2 (13%)	1 (6%)	4 (25%)
Joints and fascia	16	12 (75%)	2 (13%)	2 (13%)	0

Abbreviations: GI = gastrointestinal; PS = performance status.
[a]Scoring was based on the worst symptoms associated with chronic GVHD.
[b]Symptoms involving female genital tract were not reported.
[c]Two patients succumbed to pulmonary complications were excluded.