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ORIGINAL ARTICLE

Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia

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From January 1991 to March 2007, 61 children and adolescent with acquired severe aplastic anemia received BMT in our institutions. We retrospectively compared the outcome of 30 cases of matched-sibling donor BMT (MSD-BMT) and 31 cases of unrelated donor BMT (URD-BMT). We observed one graft failure among MSD-BMT recipients and three graft failures among URD-BMT recipients, respectively. No patients in the MSD-BMT group developed grades II–IV acute GVHD compared with 11 of 30 patients (37%) in the URD-BMT group ($P<0.001$). One of 30 MSD-BMT recipients (3%) developed chronic GVHD compared with 8 of 30 URD-BMT recipients (27%) ($P=0.013$). The incidence of EBV and CMV reactivation was 11 of 20 URD-BMT recipients and 23 of 30, respectively. One patient in the URD-BMT group died of a motor accident 5.5 years after BMT. Ten-year OS was 100% in MSD-BMT recipients and 93.8% (95% CI, 81.9–100%) in URD-BMT recipients, respectively ($P=0.252$). Ten-year failure-free survival was 96.7% (95% CI, 90.2–100%) in the MSD-BMT group and 84.7% (95% CI, 70.2–99.2%) in the URD-BMT group, respectively ($P=0.161$).
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Introduction

BMT from an HLA-matched-sibling donor (MSD) is the treatment of choice for children and young adults with severe aplastic anemia (SAA). However, this approach is limited by the availability of HLA-matched donors. Immunosuppressive therapy (IST) with a combination of anti-thymocyte globulin (ATG) and CsA has been an alternative treatment for patients without a suitable related donor.[1,2] BMT from an HLA-matched unrelated donor (URD) is indicated as salvage therapy for patients who fail to respond to one or more courses of IST and patients who experience relapse of disease.[3,4]

Results from MSD-BMT for SAA patients have been excellent. The recent cohort report from the International Bone Marrow Transplantation Registry (IBMTR) showed a 5-year survival of 75% in children.[5] However, the outcome for a URD-BMT is less encouraging, mainly because of the high incidence of graft failure and acute GVHD. In a retrospective IBMTR study of patients transplanted between 1988 and 1994, the 5-year survival rate was 39% for 181 patients who received BMT from HLA-matched URD and 36% for 51 patients who received BMT from HLA-mismatched URD.[6] The development of an optimal conditioning regimen and better donor selection through high-resolution HLA typing may result in improvement of OS. In the recent report from the French Registry, the survival rate improved from 29% in 1989–1998 to 50% in 1999–2004.[7] The 5-year survival reached 78% for 14 children who transplanted from an HLA 10/10 antigen-matched donor.

Recently, Kennedy-Nasser *et al*. compared the outcome of 36 SAA children who received MSD-BMT or BMT from an alternative donor and found no difference in OS between the two groups.[8] In this study, we retrospectively compared the outcome of 61 SAA children and adolescent undergoing MSD-BMT and URD-BMT in our hospitals.

Patients and methods

Patients

Between January 1991 and March 2007, 61 patients with SAA received allogeneic BMT at Nagoya University Hospital ($n=28$) and Japanese Red Cross Nagoya First Hospital ($n=33$). Thirteen patients with an MSD received BMT as a first-line therapy. Other patients received prior therapies before referral to our hospitals. The diagnosis and assessment of disease severity were according to published

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criteria.[9] Chromosome breakage test has been used to exclude Fanconi anemia.

Patient characteristics are shown in Table 1. In the MSD-BMT group (17 boys and 13 girls), the median age at time of transplantation was 10 years (range, 0.8–18 years), and in the URD-BMT group (21 boys and 10 girls), the median age at time of transplantation was 9 years (range, 3–17 years). The median period from diagnosis to transplantation was significantly longer for URD-BMT recipients (median 56 months, range 9–144 months) than for MSD-BMT recipients (median 10 months, range 1–75 months) ($P<0.001$).

Table 1 Characteristics of patients and BMT

	MSD-BMT group	*URD-BMT group*	P
Patient number	30	31	
Median age (years) (range)	10 (0.8–18)	9 (3–17)	
Sex (M/F)	17/13	21/10	
Etiology			
Idiopathic	29	28	
Hepatitis associated	1	3	
Median time to BMT (months)	10 (1–75)	56 (9–144)	$P<0.001$
Prior therapy			
Not done	13	0	
Androgen	11	2	
CsA	2	4	
ATG + CsA	4	24[a]	
HLA disparity between donor and recipient			
Fully matched at HLA-A, -B, and -DR loci	30	13	
One locus mismatched			
HLA-A (serotype/genotype only)	0	1/1	
HLA-B (serotype/genotype only)	0	1/3	
HLA-DR (serotype/genotype only)	0	9/2	
Two loci mismatched			
HLA-DR (serotype) and HLA-DRB1 (genotype)	0	1	
Donor			
Median age (years) (range)	9 (1–17)	33 (24–49)	$P<0.001$
Sex (M/F)	15/15	15/16	
Blood type between recipient and donor			
Match	18	10	
Major	2	7	
Minor	6	13	
Bi-direction	4	1	
Infused cell dose (× 108/kg)	4.3 (2.0–5.9)	3.4 (0.8–5.9)	$P=0.009$
Preconditioning therapy			
CY(200 mg/kg) + TLI(7.5 Gy)	10	0	
CY(200 mg/kg) + ATG	13	0	
CY(3 g/m^2) + Flu (100 mg/m^2) + TLI(3 Gy)	7	0	
CY(200 mg/kg) + TBI(5 Gy) + ATG	0	16	
CY(120 mg/kg) + TBI(10 Gy) + ATG	0	15	
GVHD prophylaxis			
CsA + MTX	30	11	$P<0.001$
Tacrolimus + MTX	0	20	

Abbreviations: ATG = anti-thymocyte globilin; Flu = fludarabine; MSD-BMT = matched-sibling donor BMT; URD-BMT = unrelated donor BMT.
[a]Three of 24 patients received the immunosuppressive therapy with ATG and CsA, twice.

BM donor selection

URDs were recruited though the Japan Marrow Donor Program (JMDP) ($n=29$) or the National Marrow Donor Program in the United States (NMDP) ($n=2$). We identified donors who were serologically matched with recipients at 6/6 ($n=19$) or 5/6 ($n=12$) loci. Among 19 serologically matched pairs, molecular typing revealed that 13 pairs were fully matched, 5 pairs were one allele mismatched (HLA-A, 1; HLA-B, 3; and HLA-DRB1, 2) and 1 pair was two alleles mismatched (DRB1).

Transplantation procedure

Ex vivo T-cell depletion was not carried out in all patients. A median of 4.3×10^8 total nucleated marrow cells/kg (range, 2.0–5.9) were infused in the MSD-BMT group and a median of 3.4×10^8 total nucleated marrow cells/kg (range, 0.8–5.9) were infused in the URD-BMT group (Table 1).

Three different conditioning regimens were used for MSD-BMT recipients. Between 1991 and 1993, 10 recipients received CY (50 mg/kg for 4 days) and TLI (7.5 Gy).[10] Between 1994 and 2002, 13 recipients received CY (50 mg/kg for 4 days) and rabbit ATG (Thymoglobulin, IMTIX-SANGSTAT, Lyon, France, 2.5 mg/kg for 4 days).[11] After 2003, seven recipients received CY (750 mg/m^2 for 4 days), fludarabine (25 mg/m^2 for 4 days), and TLI (3 Gy). Two different regimens were used for URD-BMT recipients. Before 2001, 16 recipients received CY (60 mg/kg for 2 days), TBI (10 Gy), and rabbit ATG (2.5 mg/kg for 4 days).[12] After 2002, the TBI dose was reduced. Fifteen patients received CY (50 mg/kg for 4 days), TBI (5 Gy), and rabbit ATG (2.5 mg/kg for 4 days) (Table 1).

For prophylaxis of GVHD, all 30 MSD-BMT recipients and 11 of 31 URD-BMT recipients received CsA (3 mg/kg, continuous infusion from day −1) and short-term methotrexate (15 mg/m^2 at day 1 and 10 mg/m^2 at days 3, 6, and 11 after marrow infusion) according to the Seattle protocol.[13] The whole blood concentration of CsA was maintained at 200–400 ng/ml. Twenty of 31 URD-BMT recipients received tacrolimus (0.02 mg/kg, continuous infusion from day –1) and short-term methotrexate. The whole blood concentration of tacrolimus was maintained at 5–15 ng/ml. After CsA or tacrolimus was changed to oral intake, the trough level of CsA was maintained at 100–200 ng/ml and that of tacrolimus at 5–10 ng/ml. CsA and tacrolimus were continued at least 6 months for MSD-BMT recipients and 1 year for URD-BMT recipients, and then tapered. Acute and chronic GVHD were graded according to standard criteria.[14,15]

All patients received bacterial, fungal, and viral prophylaxis during the transplantation period according to the institutional standard care and G-CSF from day 5 to the time of neutrophil count greater than 1.0×10^9/l, as described earlier.[12] Monitoring of CMV was done weekly using an antigenemia assay in all recipients. EBV DNA was

monitored weekly using real-time PCR in 20 recipients who received URD-BMT after 2002. EBV reactivation was defined as viral genome load $>1\times10^{2.5}$ copies/μg DNA of peripheral mononuclear cell or viral genome load >20 000 copies/ml of the whole blood without any symptoms.[16] Pre-emptive therapy of rituximab was used in patients with EBV reactivation after 2004.

Definition of engraftment and chimerism analysis

The day of neutrophil engraftment was defined as the first of 3 days with an absolute neutrophil count greater than 0.5×10^9/l after the post-transplantation nadir. The day of platelet engraftment was defined as the first of 3 consecutive days with a platelet count greater than 20×10^9/l without platelet transfusion. Results of the chimerism test were available in 36 of 60 evaluable patients. Chimerism was determined on peripheral blood or BM mononuclear cells using fluorescence *in situ* hybridization probes for sex-mismatched pairs or short tandem repeats of polymorphic DNA sequences for sex-matched pairs.

Statistical methods

Survival time was calculated from the date of transplantation to the date of final patient follow-up in March 2008. Failure-free survival (FFS) was defined as survival without treatment failure. Death, graft rejection, relapse, and requirement of salvage therapies were considered treatment failures. The median days to engraftment and the probabilities of OS and FFS were estimated from the time of transplantation according to the Kaplan–Meier product-limit method. The log-rank test was used to compare these variables between the MSD-BMT and URD-BMT groups. The incidence of engraftment, acute and chronic GVHD, and viral infection were compared by Fisher's exact probability test between the MSD-BMT and URD-BMT groups. The non-parametric *U*-test was used to compare the following variables groups: patient and donor age, disease duration, infused cell number, observation time, and the median days to engraftment.

Results

Engraftment and chimerism studies

Neutrophil engraftment was achieved in 60 of 61 recipients (98%). Median days to engraftment were 15 days (range, 11–36 days) in the MSD-BMT group and 17 days (range, 11–31 days) in the URD-BMT group ($P=0.018$) (Table 2). Platelet engraftment was achieved in 59 of 61 recipients (97%). Median days to engraftment were 23 days (range, 14–37 days) in the MSD-BMT group and 33 days (range, 13–120 days) in the URD-BMT group ($P<0.001$) (Table 2).

One URD-BMT recipient failed to achieve neutrophil engraftment. The infused number of CD34+/CD45+ cells was only 3.8×10^5/kg in this case. The patient was rescued by haploidentical transplantation from her HLA 2 loci-mismatched mother. One case and two cases of late graft failure were observed in MSD-BMT group and URD-BMT group, respectively. In one MSD-BMT recipient, mixed chimerism (donor and recipient cell ratio; 76:22) was found at day 100 after BMT. Pancytopenia progressed to late graft failure at 5 months after BMT and continued to be transfusion-dependent over 1 year. Donor-type hematologic recovery was observed from 20 months after BMT spontaneously without donor lymphocyte infusion. Two URD-BMT patients developed severe pancytopenia 3 and 7 months after URD-BMT, respectively, and remained transfusion dependent. Curiously, repeated BM examinations showed complete donor chimerism with normal cytogenetic study in these patients.

Chimerism studies were performed for 36 recipients. Fourteen of 15 MSD-BMT recipients (93%), and 21 of 21 (100%) URD-BMT recipients achieved complete donor chimerism (Table 2).

Table 2 Outcome of transplantation

	MSD-BMT group	*URD-BMT group*	P
Patient number	30	31	
Neutrophil engraftment	30 (100%)	30 (97%)	
Median days of neutrophil engraftment (range)	15 (11–36)	17 (11–31)	$P=0.018$
Platelet engraftment	30 (100%)	29 (94%)	
Median days of platelet engraftment (range)	23 (14–37)	33 (13–120)	$P<0.001$
Chimerism analysis			
Evaluable patient	15	21	
Complete donor type	14 (93%)	21 (100%)	$P=0.417$
Mixed chimerism	1	0	
Acute GVHD			
Evaluable patient	30	30	
II–IV	0	11 (37%)	$P<0.001$
III–IV	0	6 (20%)	$P=0.012$
Chronic GVHD			
Evaluable patient	30	30	
Limited/extensive	1/0 (3%)	6/2 (27%)	$P=0.013$
CMV			
Evaluable patient	30	30	
Reactivation	5 (17%)	23 (77%)	$P<0.001$
EBV			
Evaluable patient	ND	20	
Reactivation	ND	11 (55%)	
Development to LPD	0	2 (10%)	
Rituximab therapy	0	5	
Late graft failure	1 (3%)	2 (6%)	
Median observation time (month)	118 (25–206)	67 (12–173)	$P=0.004$
Alive	30	30	

Abbreviation: LPD = lymphproliferative disorder.

GVHD

No recipient in the MSD-BMT group developed grades II–IV acute GVHD. In contrast, 11 of 30 evaluable URD-BMT recipients developed grades II–IV acute GVHD ($P<0.001$) (Table 2). Although 10 of 11 patients with acute GVHD responded to standard steroid therapy

(methylprednisolone; 2 mg/kg), 1 recipient developed steroid-refractory acute GVHD of the gut and required second-line therapies with anti-TNF-α receptor antibody (infliximab) and anti-CD25 antibody (basiliximab). The patient responded to the second-line therapy. Limited type chronic GVHD was observed in only 1 of 30 MSD-BMT recipients (3%). In contrast, 8 of 30 URD-BMT recipients (27%) developed chronic GVHD (6 limited type and 2 extensive type) ($P = 0.013$).

Viral infection

CMV reactivation was observed in 5 of 30 MSD-BMT recipients (17%) and 23 of 30 URD-BMT recipients (77%) ($P < 0.001$) (Table 2). Empiric therapy with ganciclovir was immediately started when CMV antigenemia tests were positive. As a result, only one patient in MSD-BMT group suffered from CMV enteritis. Eleven of 20 evaluable URD-BMT recipients (55%) developed EBV reactivation (Table 2). Two of these 20 patients presented with fever and cervical lymph node swelling. On the basis of findings of a lymph node biopsy, they were diagnosed as EBV-associated lymphoproliferative disorder (EBV-LPD). Before the introduction of rituximab for treatment of EBV reactivation or EBV-LPD, we reduced the dose of immunosuppressant when the patients were diagnosed as having EBV reactivation or EBV-LPD. After 2004, five patients were treated with a course of rituximab, anti-CD20 antibody. The EBV genome load became undetectable 2 weeks after the use of rituximab in all patients. Both patients with EBV-LPD were cured with the reduction of the immunosuppressant dose and rituximab. When gross hematuria was observed and hemorrhagic cystitis was suspected, BK virus DNA in urine samples was examined. BK virus DNA was identified in three patients. The symptoms disappeared within 2 weeks without any therapy in two patients. Cidorfovir was administered to one patient with long-standing severe cystitis.

OS and FFS

All patients have survived to date at a median follow-up of 118 months (range, 25–206 months) in the MSD-BMT group and 67 months (range, 12–173 months) in the URD-BMT group (Table 2). Death was observed in one URD-BMT recipient because of a motor accident 5.5 years after BMT. Therefore, 5-year OS was 100% in MSD-BMT recipients and 93.8% (95% CI, 81.9–100%) in URD-BMT recipients, respectively ($P = 0.252$) (Figure 1). Treatment failure included one late graft failure in MSD-BMT group and one primary graft failures, two late graft failures, and one death (motor car accident) in URD-BMT group, respectively. Therefore, 5-year FFS was 96.7% (95% CI, 90.2–100%) in MSD-BMT recipients and 84.7% (95% CI, 70.2–99.2%) in URD-BMT recipients ($P = 0.161$) (Figure 2).

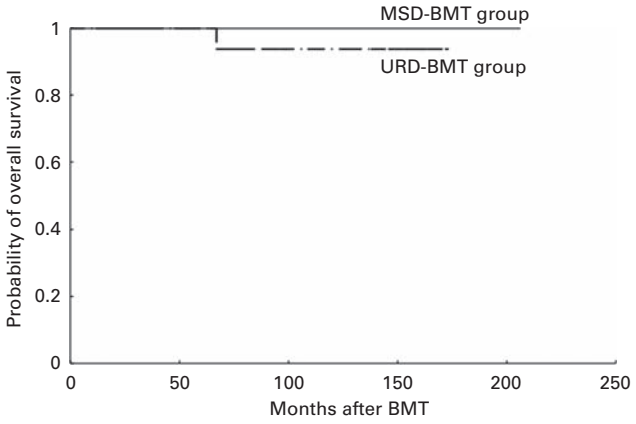

Figure 1 Kaplan–Meier estimates of OS after MSD-BMT and URD-BMT, respectively (100% vs 93.8%) ($P = 0.252$).

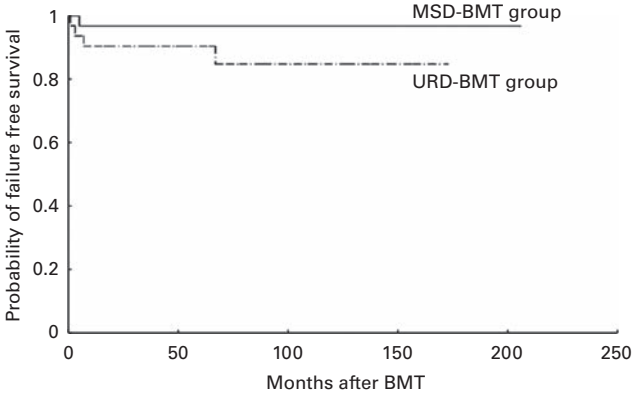

Figure 2 Kaplan–Meier estimates of FFS after MSD-BMT and URD-BMT, respectively (96.7% vs 84.7%) ($P = 0.161$).

Discussion

Previously reported outcomes of URD-BMT for SAA have been less favorable than those of MSD-BMT.[4,6,17] URD-BMT has been indicated in pediatric patients who have failed an initial course of IST. Recent improvements in outcomes of URD-BMT for SAA are impressive. In the large retrospective study reported from the European group for Blood and Marrow Transplantation (EBMT), 5-year OS increased from 32 ± 8% before 1998 to 57 ± 8% after 1998. The reason for such an improvement is speculated to be due to better HLA matching using high-resolution technology.[18] The excellent outcomes are especially prominent in children. An OS of 100% has been achieved in several small series.[12,19] Recently, Kennedy-Nasser *et al*.[8] reported that the 4-year OS for MSD recipients was 93% versus 89% for alternative donors recipients at a median follow-up of 52 months.

Our study also found no difference in OS and FFS between MSD-BT and URD-BMT. The longer disease duration before transplantation and HLA disparity between recipients and donors are major drawbacks for survival in SAA recipients transplanted from URD.[4] In this study, the time from diagnosis to transplantation was much longer in URD-BMT recipients than in MSD-BMT recipients (56 months versus 10 months). In addition, 12 serologically mismatched pairs were included in the URD-BMT group. It is remarkable that such drawbacks in the URD-BMT group did not reduce OS in our study.

However, the incidence of post-transplant complications was much higher in URD-BMT recipients than in MSD-

BMT recipients. Acute and chronic GVHD remains a major obstacle in URD-BMT recipients. In addition, CMV and EBV reactivation were also serious problem in URD-BMT recipients. Use of ATG in conditioning regimens is a significant risk factor for development of EBV-LPD.[16,20] Moreover, a recent study has shown that SAA patients who have received more courses of IST with ATG before BMT have higher risk of EBV-LPD.[21] In this study, half of recipients transplanted from URD experienced reactivation of EBV. Three of 31 URD-BMT recipients had received two courses of ATG before transplantation but only one of three had EBV reactivation after transplantation. After the introduction of the intensive monitoring of EBV-DNA levels and early use of rituximab, we could reduce the incidence of EBV-LPD even in patients conditioned with ATG-containing regimens. Intensive viral monitoring and new agents such as rituximab are mandatory for URD-BMT.

On the basis of the encouraging results in URD-BMT, several investigators have argued against the standard guideline and insisted that BMT could be considered the first-line treatment for SAA children if a genetically matched URD is available.[22] Although OS and FFS were comparable, the severity and incidence of post-transplant complications were considerably different between MSD-BMT recipients and URD-BMT recipients. Therefore, we still consider that URD-BMT should be indicated as second-line treatment for non-responders to initial IST.

In this study, we used three different conditioning regimens for MSD-BMT recipients. The first conditioning regimen consisted of CY and TLI. TLI was replaced by ATG after the report that use of irradiation increased the incidence of acute GVHD and secondary malignancy.[23] Fortunately, we did not experience any secondary malignant neoplasms. One reason for the absence of second malignancies may be the low incidence of chronic GVHD in our series. The cooperative study of Japan Childhood Aplastic Anemia Study Group revealed that the preceding IST increased the incidence of graft rejection in children with SAA who underwent MSD-BMT.[24] Fludarabine-based conditioning regimens have been reported to guarantee a durable engraftment and excellent survival in SAA patients of high risk of graft rejection.[25] After 2003, we used a combination of CY, fludarabine, and low-dose TLI as a conditioning regimen for patients at high risk of graft rejection, and CY combined with ATG for other patients. However, one patient who had 15 months of disease duration experienced late graft failure although the CY, fludarabine, and TLI regimen were used.

The optimal conditioning regimen for URD-BMT remains undetermined. In the retrospective analysis by JMDP, a conditioning regimen that included CY, ATG, and TBI resulted in the most encouraging result.[17] NMDP conducted a prospective study to determine the optimal dose of TBI, combined with CY and ATG in patients with SAA.[26] Best results were achieved with use of 2 Gy of TBI, and higher dose of TBI was associated with increased pulmonary toxicity. An analysis of the JMDP data indicated that two of three patients who underwent conditioning with CY (120 mg/kg) plus low-dose TBI (5–8 Gy) failed to achieve engraftment and none of the three patients is alive.[17] In contrast, 10 patients who received conditioning with CY (200 mg/kg) plus low-dose TBI (2–5 Gy) achieved engraftment, and 9 remain alive. In the JMDP study, the fatal lung injury was rare. On the basis of these data, we changed our conditioning regimen from CY (120 mg/kg), ATG, and TBI (10 Gy) to CY (200 mg/kg), ATG, and TBI (5 Gy) since 2002. Considering that three cases of graft failure were observed in the conditioning regimen with 5 Gy TBI and the toxicity of our TBI-containing regimen was tolerable, an increase in the incidence of graft failure is a concern if the TBI dose is reduced further. Although recent studies showed that TBI might be partially replaced by fludarabine and/or alemtuzumab, longer follow-up is necessary to discuss the usefulness of the conditioning regimen for SAA.[25,27–29]

Conflict of interest

The authors declare no conflict of interest.

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