Unrelated Hematopoietic Stem Cell Transplantation for Children with Acute Leukemia: Experience at a Single Institution

We evaluate the outcomes in children with acute leukemia who received allogeneic hematopoietic stem cell transplantation (HCT) using unrelated donor. Fifty-six children in complete remission (CR) received HCT from unrelated donors between 2000 and 2007. Thirty-five had acute myeloid leukemia, and 21 had acute lymphoid leukemia. Stem cell sources included bone marrow in 38, peripheral blood in 4, and cord blood (CB) in 14. Four patients died before engraftment and 52 engrafted. Twenty patients developed grade II-IV acute graft-versus-host disease (GVHD) and 8 developed extensive chronic GVHD. With median follow-up of 39.1 months, event free survival and overall survival were 60.4% and 67.5%, respectively, at 5 yr. Events included relapse in 10 and treatment-related mortality (TRM) in 10. The causes of TRM included sepsis in 4, GVHD in 4 (1 acute GVHD and 3 chronic GVHD), veno-occlusive disease in 1 and fulminant hepatitis in 1. Patients transplanted with CB had event free survival of 57.1%, comparable to 63.2% for those transplanted with other than CB. In conclusion, HCT with unrelated donors is effective treatment modality for children with acute leukemia. In children with acute leukemia candidate for HCT but lack suitable sibling donor, unrelated HCT may be a possible treatment option at the adequate time of their disease.

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INTRODUCTION

Although the survival of children with acute lymphoblastic leukemia (ALL) has been dramatically improved, about 20-30% of these patients still relapse requiring more intensified treatment modality such as hematopoietic cell transplantation (HCT) to be cured (1, 2). In addition, certain group of children with ALL necessitates HCT at early remission because they have a high rate of relapse (2-5). With current intensified chemotherapy, 80-85% of children with acute myeloid leukemia (AML) achieve remission, but the long-term event free survival (EFS) remains between 35 to 50% (6-8). In pediatric AML except patients who have Down syndrome or acute promyelocytic leukemia, HCT could be performed in first complete remission (CR), especially for patients with unfavorable features if they have matched relative donors. HCT using matched relative donor has showed the better outcome compared to chemotherapy as post-remission therapy in large cohort studies (9, 10).

Although HCT is recognized as potentially curative treatment for acute leukemia as mentioned above, less than one-third of children have sibling donor (11). HCT from unrelated donors could be another option for the treatment of children with acute leukemia who need HCT for cure but lack a suitably matched relative. In general, HCT using unrelated donor has been associated with a higher graft-versus-host disease (GVHD) and treatment related mortality (TRM) (12, 13). With improvement of human-leukocyte antigen (HLA) typing methods resulting in less severe GVHD, advances in prophylaxis and treatment of infections and accumulation of experience on unrelated HCT, the outcome of unrelated donor HCT approaches those of HCT using matched sibling donor (14-17). Furthermore, the graft-versus-leukemia (GVL) effects rationalize the use of unrelated donor in hematologic malignancies with high risk of relapse (18).

This is the report of a single institution's experience on the unrelated donor HCT for 56 children with acute leukemia. We evaluated the outcomes in children with acute leukemia who received allogeneic HCT using unrelated donor to see whether the unrelated donor HCT could be an effective treatment to cure children with acute leukemia. We also report TRM and GVHD and so on.

MATERIALS AND METHODS

Between January 2000 and April 2007, 56 children with acute leukemia underwent allogeneic HCT from unrelated donors at the Asan Medical Center. The patients' medical records were retrospectively reviewed and were analyzed as of April 2008. All 56 patients were transplanted in CR. Patients and donors were typed for HLA-A, -B, and -DRB1 by serological method between 2000 and 2002. High molecular resolution typing was performed for HLA-A, -B, -C and -DRB1 since 2003. Recipient-donor histocompatibility was determined by serology for HLA-A, -B, and -DRB1 antigens.

The patients were hospitalized in laminar airflow rooms. To prevent infection, all patients received prophylactic acyclovir and itraconazloe or fluconazole. Granulocyte colony-stimulating factor (G-CSF) was administered to most patients. The myeloablative conditioning regimen was administered, and stem cells were infused through a central venous catheter on day zero. All patients received prophylactic treatment using cyclosporine with methotrexate or prednisone to prevent acute GVHD. The day of engraftment was defined as the first of 3 consecutive days on which the granulocyte count exceeded 0.5×10^9/L and the last of 7 consecutive days when the platelet count $>20 \times 10^9$/L without transfusion support. Donor engraftment was documented by short-tandem repeat polymerase chain reaction (STR-PCR), which was usually performed on day 28 after transplantation. Acute and chronic GVHD were diagnosed and graded according to standard criteria (13-15). Patients were not considered to be evaluable for acute GVHD if they died before engraftment or for chronic GVHD if they died before day 100 after transplantation.

Overall survival (OS) was defined as the time between transplantation and death due to any causes. EFS was defined as the time interval from transplantation to first event, either relapse or death in complete remission. TRM was defined as death from any cause other than relapse. The Kaplan-Meier method was used to summarize estimates of OS and EFS (16), and outcomes were compared by means of the exact log-rank test. The Student t test was used to compare the median age at diagnosis and HCT, the white blood cell count at diagnosis, and the number of days of engraftment. The chi-square test was used to compare the incidence of GVHD. Univariate analysis of prognostic factors was examined by using the approach of Kaplan and Meier and of Cox regression for fixed and time-dependent factors, respectively (19). All factors were considered by using defined discrete categories. Factors significant in univariate analysis were considered for inclusion in multivariate Cox regression models. P values <0.05 were considered as statistically significant. All statistical analyses were conducted with SPSS software (Statistical Package for the Social Science, version 12.0, SSPS Inc, Chicago, IL, U.S.A.).

RESULTS

Characteristics of patients at transplantation

The characteristics of patients are shown in Table 1. Of 56 patients, 35 had AML (29 CR1, 6 CR2), and 21 had ALL (10 CR1, 7 CR2, 4 CR3). Overall the median age at transplantation was 6.3 yr and 37 were male. Thirty-nine patients received HCT in CR1, 17 beyond CR1. Of 35 patients with AML, 10 had t(8;21) of whom 7 were in CR1 and 1 was CR2, 2 had t(15;17) in CR2 and none had inv (16). Of 21 patients with ALL, 4 had t(9;22), 4 had infant ALL of whom 3 had 11q23 abnormalities, 1 had very high initial white cells over 500,000/μL, 1 had secondary ALL and 11 had early bone marrow relapse. Stem cell sources included bone marrow (BM)

Table 1. Clinical characteristics of patients at the time of transplantation

Characteristics	AML	ALL
No. of patients	35	21
Age (yr) at HCT		
Median (range)	6.3 (0.6-15.4)	6.9 (1.2-18.4)
Gender (male:female)	24:11	13:8
Cytogenetics		
t(8;21)	10	0
inv(16)	0	0
t(15;17)*	2	0
Monosomy 7	2	0
Complex (>5 abnormal)	2	0
11q23 abnormalities	1	5
t(12;21)	0	1
t(9;22)	0	4
Normal	5	3
Other abnormalities	11	5
Unknown	2	3
Phase of disease		
CR1	29	10
≥CR2	6	11
Stem cell sources		
Bone marrow	27	11
Peripheral blood	0	4
Cord blood	8	6
Conditioning regimen		
Bu/Cy	26	3
TBI/Cy	0	11
Other†	9	7
GVHD prophylaxis		
Cyclosporine/methotrexate	27	15
Cyclosporine/prednisone	5	5
Cyclosporine/MMF	3	1

*, CR2 at transplantation; †, Bu/Cy/ATG (3), TBI/Cy/ATG (4), Bu/Flu/ATG (3), Cy/Flu/ATG (2), TBI/VP16 (1), Bu/Cy/VP16 (1), TBI/Cy/Flu/MMF (1), Bu/Cy/ATG/VP16 (1).
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; No., number; HCT, hematopoietic stem cell transplantation; CR, complete remission; Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation; ATG, anti-thymocyte globulin; Flu, fludarabine; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil.

in 38, peripheral blood (PB) in 4, and cord blood (CB) in 14. All of the 42 patients who underwent HCT with BM or PB were matched at HLA-A, -B, and -DRB1 antigens. In contrast, only two of 14 CB transplantation received grafts that were matched at HLA-A, -B, and -DRB1 antigens. Busulfan containing regimen was the most common conditioning for AML and total body irradiation (TBI)-based regimen for ALL. The median infused cell doses were 9.7×10^6 CD34+ cells/kg for non-CB transplantation and 1.6×10^5 CD34+ cells/kg for CB transplantation, respectively. The transplantation outcomes are shown in Table 2.

Engraftment

Fifty-two of the 56 patients achieved a sustained granulocyte count greater than 0.5×10^9/L at a median of 16 days (10-48) post transplantation. Three patients, 2 CB transplantation and 1 non-CB transplantation, died before day 28 without recovery of granulocyte count greater than 0.5×10^9/L. One patient who received CB transplantation died on day 110 of sepsis with a granulocyte count $<0.5 \times 10^9$/L. Fifty of the 56 patients achieved a sustained platelet count greater than 20×10^9/L at a median of 33 days (16-171) post transplantation. CB transplantation had significantly slower neutrophil and platelet engraftment than transplantation using BM or PB ($P=0.002$, <0.001, respectively; Fig. 1).

Acute and chronic GVHD

Twenty of 52 evaluable patients developed grades II-IV acute GVHD. Two had grade IV acute GVHD and all those died of complications related to GVHD. The cumulative incidence of acute GVHD grade II-IV in evaluable patients at day 100 post-transplantation was 40.3%. Both univariate and multivariate analyses revealed that early engraftment before 15 days post transplantation was associated with higher incidence of acute GVHD grade II or above ($P=0.004$, Table 3). Chronic GVHD was developed in 23 of 49 evaluable patients including clinically extensive chronic GVHD in 8

Table 2. Outcomes after transplantation

Outcomes	AML	ALL
Median day to engraftment		
Neutrophil (range)	16 (10-48)	17 (11-43)
Platelet (range)	33 (20-171)	31 (16-147)
GVHD		
Acute (evaluable)	(34)	(18)
0-1	20	12
2-4	14	6
Chronic (evaluable)	(31)	(18)
Limited	9	6
Clinical extensive	3	5
Treatment-related mortality	5	5
Relapse	8	2

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; GVHD, graft-versus-host disease.

Table 3. Risk factors for acute GVHD (Grade II-IV)

Factors	Patients	Incidence of aGVHD	Univariate P value	Multivariate P value
Disease			>0.5	
ALL	18	36.2%		
AML	34	43.9%		
Phase of disease			0.36	
CR1/2	49	38.6%		
CR3	3	66.7%		
Stem cell source			0.3	
BM/PB	41	44.0%		
CB	11	27.3%		
Engraftment			<0.001	0.004
≤15 days	22	63.3%		
>15 days	30	24.4%		

aGVHD, acute graft-versus-host disease; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CR, complete remission; BM, bone marrow; PB, peripheral blood; CB, cord blood.
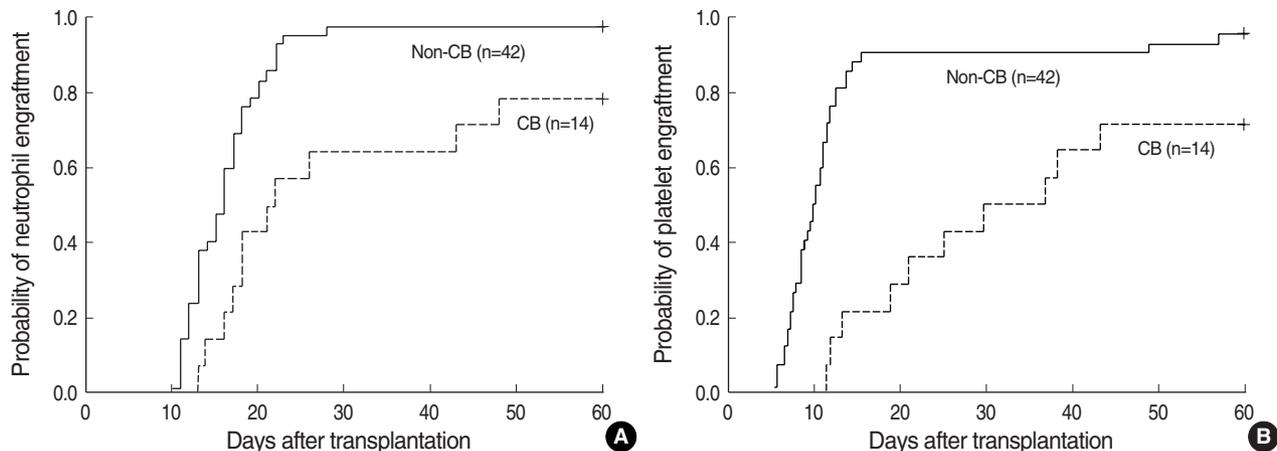

Fig. 1. Engraftment rate of neutrophils (A) and platelets (B) according to stem cell sources.

patients.

Treatment-related mortality

Ten patients died of causes not related to recurrent leukemia. The causes of TRM included sepsis in 4, GVHD in 4 (1 acute GVHD and 2 chronic GVHD), veno-occlusive disease in 1 and fulminant hepatitis in 1. One patient who had late graft failure is alive in CR at 2 yr post-transplantation. The Kaplan-Meier estimates of TRM at 100 days, 1 yr and 2 yr were 3.6%, 15%, and 19.5%, respectively. Both univariate and multivariate analyses revealed that phase of disease beyond CR1/2 was associated with higher TRM (P=0.04, Table 4).

Relapse

Eight patients with AML and 2 with ALL relapsed at a

median of 6.4 (2.7-58.2) months post-transplantation. Eight of 10 patients who relapsed died of progressive disease. One patient who had extramedullary relapse at 2.8 months post-transplantation received donor lymphocyte infusion and he survives 38.4 months post-transplantation in remission. The estimated relapse rate at 5 yr was 24.9%. Although none of 7 patients who have greater than or equal to grade III acute GVHD relapsed, acute GVHD over grade II was not associated with decreased relapse rate (0% vs. 28.6%; P=0.21).

Overall and event free survival

With a median follow-up of 39.1 months (12.7-101.1), the estimates of EFS and OS for all patients were 60.4% (ALL 65.9%, AML 58.4%) and 67.5% (ALL 62.9%, AML 69.9%), respectively (Fig. 2). Patients transplanted with CB had EFS of 57.1%, which was comparable to 63.2% for those transplanted with stem cells other than CB. There was no significant difference of EFS according to phase of disease, stem cell sources, and acute or chronic GVHD.

DISCUSSION

Although HCT is a curative therapy for pediatric acute leukemia including relapsed ALL, ALL with very high risk of relapse at diagnosis and AML except certain group of patient who had Down syndrome or acute promyelocytic leukemia, only a limited number of children have a matched sibling donors (11). The major obstacles for unrelated donor HCT are high treatment related morbidity or mortality as well as high incidence of GVHD. Balduzzi et al. (12) reported the Seattle's experience on unrelated donor bone marrow transplantation for 88 children mostly with hematologic malignancies. Acute GVHD developed in more than 80% of evaluable patients and chronic GVHD in more than 60%. Twenty-five (28%) of 88 patients died in remission and most
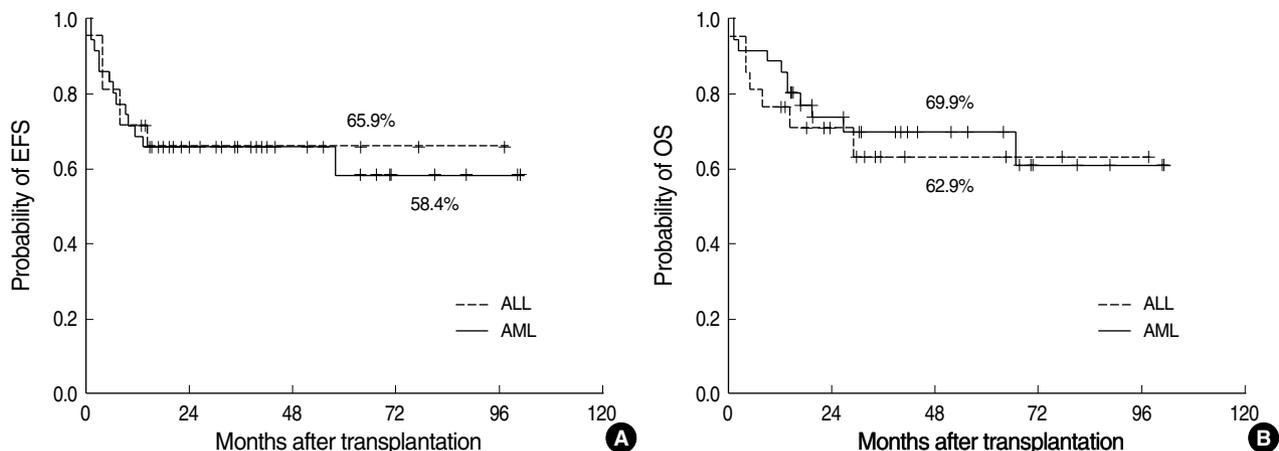
Table 4. Risk factors for TRM

Factors	Patients	TRM @ 5yr	Univariate P value	Multivariate P value
Disease			0.38	
ALL	21	26.0%		
AML	35	15.6%		
Phase of disease			0.003	0.04
CR1/2	52	14.3%		
CR3	4	75.0%		
Stem cell source			0.17	
BM/PB	42	15.8%		
CB	14	30.7%		
Acute GVHD			0.022	0.09
Grade 0/I	32	3.1%		
Grade II-IV	20	28.1%		

TRM, treatment-related mortality; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CR, complete remission; BM, bone marrow; PB, peripheral blood; CB, cord blood; GVHD, graft-versus-host disease.

Fig. 2. Probability of 5 yr event free survival (EFS) (A) and overall survial (OS) (B) according to disease.

of the death were due to infections associated with GVHD. Davies et al. (13) also reported high TRM of 53% in patients receiving matched marrow and 65% in recipients of mismatched marrow. In that study, TRM at day 100 was 36%. With better supportive care including strategy to infectious diseases and prophylaxis for GVHD as well as improvement of HLA typing methods resulting in low graft failure and less severe GVHD, the survival of unrelated donor HCT approaches those of HCT using matched sibling donor. Furthermore, the GVL effects of unrelated donor HCT can provide a therapeutic advantage in hematologic malignancies with high risk of relapse after transplantation. In the present study, the graft failure rate including early death, the incidence of grades II-IV acute GVHD and extensive chronic GVHD were 7.1%, 40.3%, and 16.3%, respectively, and the TRM at 100 days was 3.7% which are quite comparable to those of matched sibling donor transplantation (14, 15). The multivariate analysis revealed that early engraftment before 15 days post transplantation was associated with higher incidence of acute GVHD and phase of disease beyond CR1/2 was associated with higher TRM.

In childhood ALL, chemotherapy provides excellent outcome and HCT is not usually indicated in first remission. But some patients with very high-risk features such as age less than 12 months at presentation or certain type of cytogenetic abnormalities require transplantation in first remission (3, 4, 20-22). Although HCT using matched sibling donor had showed uniformly satisfactory survival, the outcome for unrelated HCT has been variable ranging 40% to 70%. In our study, among 10 patients with ALL who received unrelated HCT in CR1, only 2 died of complications related to transplantation at 0.3 months post-transplantation but all others are alive in remission with a median follow-up of 29 months. Relapsed ALL is another indication of HCT, especially for patients with early marrow relapse (23). In this study, among 11 patients who received HCT at relapse, 2 died of disease progression, 3 died of causes related to treatment, and 6 survive in remission with a median follow-up of 29 months. Our study showed that unrelated HCT could be considered in high-risk or relapsed childhood ALL when they lack matched sibling donor although our study have some limitations of small number of patients, short follow-up period and heterogeneous patients.

While there are some controversies for the best post-remission treatment for childhood AML, several reports revealed the therapeutic advantage of HCT compared to chemotherapy (9). HCT could be performed in first remission, especially for patients with unfavorable features if the patient has available matched sibling donor. Given the poor prognosis in childhood AML when treated with chemotherapy alone, HCT using alternative donors could be a possible treatment option for patients who do not have matched sibling donor. We have a policy to perform HCT in childhood AML without Down syndrome or t(15;17) who achieved CR if they

have any available donor including unrelated source. In our study the EFS for children who received in CR1 was 59.1% and for children in CR2 was 50.0%.

Recent studies of HCT using umbilical cord blood report promising results in childhood acute leukemia (24, 25). We found that the outcome was comparable in patients receiving cord blood or stem cells other than cord blood.

As acute GVHD and TRM are still major obstacles for unrelated donor HCT, the more comprehensive approach and advance in donor selection, prophylaxis and treatment of GVHD and supportive care including infections are still required to improve the outcome in unrelated setting. In addition, To further clarify the role of unrelated HCT for children with acute leukemia, prospective nation-wide study including larger number of patients is warranted.

In conclusion, our study suggests that the unrelated HCT for children with acute leukemia is an effective treatment modality, and for the patients who need HCT but lack suitable sibling donor, HCT using unrelated donor including cord blood may be a possible option in the adequate time of disease.

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