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ORIGINAL ARTICLE

Unrelated cord blood transplantation for newly diagnosed patients with severe acquired aplastic anemia using a reduced-intensity conditioning: high graft rejection, but good survival

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We report a single-center experience in treating 18 consecutive patients with severe aplastic anemia (SAA) who received unrelated cord blood transplantation (CBT). The median age was 17 years (range 5–61 years). Sixteen cases received a reduced-intensity regimen composed of CY (total dose1200 mg/m^2), rabbit antithymocyte globulin (ATG, total dose 30 mg/kg) and fludarabine (FLU, total dose 120 mg/m^2). CYA and mycophenolate mofetil were used as GVHD prophylaxis. Two patients were not evaluable for engraftment because of early death on day +21 and +22. Only one of the sixteen cases achieved engraftment, but experienced secondary graft failure 3 months post transplantation. Fifteen patients experienced primary graft rejection, but all of them acquired autologous recovery. The 3-month and 6-month cumulative incidence of response was 56% and 81%, respectively. So far, 16 patients have survived for 330–1913 days (median, 750 days) after transplantation. The probability of OS at 2 years was 88.9%. Our data indicate that CBT for newly diagnosed SAA using no irradiation but FLU and ATG-based conditioning still seems to inevitably lead to the high risk of rejection, but may facilitate autologous recovery and improve survival with low risk of transplant-related mortality.

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INTRODUCTION

Acquired severe aplastic anemia (SAA) is a rare, but life-threatening, BM failure disorder, which requires immediate intervention.[1] As the first-line therapy of choice for young patients up to age 40 years with an HLA-identical sibling, allogeneic hematopoietic SCT (allo-HSCT) can produce rapid and long-lasting hematological recovery with lower risk of transplant-related mortality. Patients with SAA who lack an appropriate matched related donor usually receive antithymocyte globulin (ATG)-based immunosuppressive therapy, and those who fail it undergo transplantation from an HLA-matched unrelated donor. Although IST has remained the first-line therapy for SAA patients without a suitable donor, there was no significant improvement in survival over 10 years.[2] In addition, the treatment for failure after IST has not been satisfactory for many patients who do not have fully HLA-matched unrelated adult donors in spite of overall progress in transplantation techniques including modified conditioning regimens and high-resolution molecular HLA-typing.

Since the first successful umbilical cord blood transplantation (CBT) to treat a patient with Fanconi anemia in 1988,[3] CBT has become a promising therapeutic option for patients with nonmalignant diseases, with its unique advantages of rapid availability and significantly lower rates of acute and chronic GVHD despite broader HLA disparity.[4] Although an increasing number of successful CBT from an unrelated donor has been reported in SAA patients,[5–8] unrelated donor CBT has not yet been recommended for SAA patients because of high risk of graft failure and complications.[9] In the setting of HLA-matched related hematopoietic SCT, a fludarabine (FLU)-based conditioning regimen has been used in patients with SAA with especially high risk of graft rejection, yielding higher engraftment rates with minimal toxicity.[10–13] In order to achieve less transplant-related mortality for newly diagnosed SAA patients who lack a suitable donor, we investigated CBT with a reduced-intensity and less toxic FLU- and ATG-based conditioning regimen. Here, the outcome of 18 consecutive SAA patients who received unrelated CBT was analyzed. Most patients who rejected their grafts were autologous recovery.

PATIENTS AND METHODS

Patient and donor characteristics

18 patients (10 male and 8 female) with acquired SAA and who received CBT were included in this study. All patients or their guardians were given informed consent for transplantation. The median age was 17 years (range 5–61 years) and the median weight was 48 kg (range 16–65 kg). SAA was defined by standard criteria.[14] No patient had clonal chromosomal abnormalities. Very SAA was considered if the patient met the criteria for SAA and had a neutrophil count less than 0.2×10^9/L. Seventeen patients were newly diagnosed and treatment-naïve, and only one had a course of ATG-based immunosuppressive therapy before CBT. None of the patients had an HLA-identical sibling or a suitable unrelated donor. The median interval between diagnosis and CBT was 34 days (range, 15–195 days). All were transfusion dependent at the time of transplant, but only one of the eighteen patients received transfusions of more than 20 units before CBT.

Cord unit selection was primarily on the basis of the cryopreserved total nucleated cells (TNCs); the target TNCs had to exceed 3.5×10^7/kg. Following this, the choice was based on the best HLA compatibility, with at least four of six loci matching. Four patients received double umbilical cord

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blood units; fourteen cases received a single unit. Typing of HLA Ags was performed by DNA-based methodology: high-resolution technique was used for HLA-DRB1 loci, whereas low-resolution typing was used for HLA-A and HLA-B loci. Umbilical cord blood units were obtained from various banks in China, including the bank of Guangzhou ($n=9$), Sichuan ($n=8$) and Shandong ($n=5$). Patient characteristics, the number of nucleated cells, and CD34-positive cells of the cord blood units at the time of infusion are shown in Table 1.

Transplantation procedure

Conditioning regimen. The first two patients received a regimen that consisted of FLU (total dose 120 mg/m^2), CY (total dose 120–200 mg/kg), rabbit anti-thymocyte globulin (ATG, Fresenius, Bad Homburg, Germany, total dose 10–15 mg/kg) and methylprednisolone (total dose 1.5 g over 3 days). One patient failed to engraft and autologous recovery and another patient experienced secondary graft failure 3 months post transplantation. Considering a more intensive preparation might reduce graft rejection, but it can increase transplant-related mortality. So, the last 16 patients received an intensified immunosuppressive, but less toxic conditioning regimen that consisted of FLU (total dose 120 mg/m^2, days -6 to -3), CY (total dose 1200 mg/m^2, days -5 to -2), and ATG (total dose 30 mg/kg, days -3 to -1).

GVHD prophylaxis. All patients were given a combination of CsA (Novartis, Stein, Switzerland) and mycophenolate mofetil (MMF, Roche, Basel, Switzerland) for GVHD prophylaxis. I.v. CsA was started (3 mg/kg/day) on day -1 and continued until patients were able to take CsA orally with target trough levels of 200–250 ng/mL for at least 1 month, then CsA blood concentration was kept at about 150–250 ng/mL for at least 6 months. MMF (25 mg/kg/day, p.o. over three times) was started on day $+1$ until day $+28$ or neutrophil recovery.

Supportive care. All the patients were isolated in a room equipped with a laminar airflow system. Infection prophylaxis consisted of oral itraconazole and i.v. acyclovir. Platelet transfusions were administered for bleeding or to maintain a platelet count greater than 20×10^9/L. Packed red blood cell transfusions were administered to maintain a hematocrit greater than 0.25. Blood products were irradiated with 2500 cGy. Steroids were given for 1 week to prevent serum sickness from the foreign protein (ATG). To facilitate the recovery of neutrophils, all patients received recombinant human TPO (rhTPO, 3SBio Inc., Shenyang, China) 150 U/kg/day s.c. from day 5 to 20 and G-CSF (filgrastim) 5 μg/kg/day i.v. for 60 min from day 6 to the ANC exceeded 2.0×10^9/L for 2 consecutive days. CMV monitoring weekly was started at the time of neutrophil recovery by DNA quantitation using PCR analysis in the peripheral blood. Ganciclovir or foscarnet was used as a preemptive CMV therapy when CMV-DNA $>10^3$ copies/mL. Surveillance for bacterial, fungal and *Pneumocystis carinii* was based on clinical requirements. All patients received broad-spectrum antibiotics for neutropenic fever, and additional agents were added based on clinical status and the results of pathogen reports. In the case of persistent fever and prolonged neutropenia, an antifungal therapy was empirically added.

Chimerism analysis, assessment of outcome and statistical analysis. Patients had weekly chimerism studies following transplant on whole blood for the first month after CBT by amplification and analysis of STR polymorphisms using multiplex amplification of STR loci (PowerPlex 16; Promega, Madison, WI, USA) with analysis by capillary electrophoresis (ABI 3130 × l; Applied Biosystems, Foster City, CA, USA), then repeated on BM monthly as indicated, according to the patients' condition. Complete chimerism was reported if only donor cells were detected, mixed chimerism was defined as the detection of 5–95% donor cells, whereas graft failure was defined as no detection of donor cells. Probability of response and OS were measured. CR was defined as normal blood count for age and sex. PR was defined as independence from transfusion and an ANC greater than 0.5×10^9/L without growth factor support. Nonresponders were patients who remained transfusion dependent or died. Relapse was defined as no longer meeting criteria for PR or CR. Survival and outcome data were collected and analyzed as of 30 June 2011, with a minimum follow up of 11 months for all survivors. Actuarial survival was estimated using the Kaplan–Meier method.

Table 1. Donor/recipient characteristics and outcome of CBT for SAA

Case	*Recipient sex/age (years)*	*Disease status at CBT*	*Dx to CBT (days)*	*HLA match*	*Transplanted cell dose*		*% Donor chimerism (days tested)*	*ANC ⩾0.5 × 10^9/L*	*Response*	*Relapse (month)*	*CsA discontinued*	*Survival (days)*
					TNC (10^7/kg)	*CD34$^+$ (10^5/kg)*						
1	M/10	VSAA	60	4/6+5/6	13.02	3.88	30 (+14)	40	CR	No	Yes	1913+
2	F/16	VSAA	30	6/6+5/6	6.34	1.96	100 (+21)	12	CR	Secondary GF (+3 months)	Yes	1818+, second transplant
3	F/6	VSAA	27	5/6	8.90	2.95	18 (+21)	42	CR	No	Yes	1206+
4	F/12	VSAA	15	5/6	3.53	1.38	0	21	CR	No	Yes	1181+
5	M/8	SAA	31	5/6	5.43	4.29	38 (+14)	25	CR	No	Yes	1040+
6	M/16	VSAA	35	5/6+5/6	4.42	1.65	0	28	CR	No	Yes	1000+
7	M/53	SAA	65	4/6+5/6	6.86	2.54	39 (+14)	21	PR	Yes (+6 months)	No	814+, transfusion dependent
8	F/13	SAA	59	5/6	4.86	2.45	11 (+21)	37	CR	No		810+
9	F/20	VSAA	43	5/6	4.12	1.65	0	NA	NA		Yes	Dead; 22
10	M/20	SAA	64	6/6	3.22	1.99	0	38	CR	No	No	681+
11	F/5	VSAA	195	5/6	7.38	4.35	0	NA	NA			Dead; 21
12	M/30	VSAA	32	4/6	3.43	1.58	0	57	CR	No	Yes	463+
13	F/61	SAA	33	5/6	3.38	1.08	0	39	PR	No	No	446+
14	M/23	VSAA	70	5/6	3.39	0.71	0	57	CR	No	Yes	425+
15	M/17	VSAA	31	4/6	3.65	2.05	0	46	PR	No	No	398+
16	M/21	SAA	69	5/6	2.65	1.31	37 (+21)	20	PR	Yes (+3 months)	No	389+
17	F/7	VSAA	33	5/6	5.31	3.41	0	46	CR	No	No	354+
18	M/21	SAA	19	5/6	2.34	2.29	0	14	PR	No	No	330+

Abbreviation: CBT = cord blood transplantation; Dx = diagnosis; F = female; GF = graft failure; M = male; NA = not applicable because of early death; SAA = severe aplastic anemia; TNC = total nucleated cells; VSAA = very severe aplastic anemia.

RESULTS

Hematopoietic recovery and chimerism analysis

The median nucleated cell dose infused was 4.14×10^7 cells/kg (range 2.34–13.02) and the $CD34^+$ cell dose infused was 2.02×10^5 cells/kg (range 0.71–4.35). Two patients were not evaluable for engraftment or hematopoietic recovery because of early death on day +21 and +22 due to severe infection and intracranial hemorrhages. Both of the death cases were very SAA with prolonged duration from diagnosis to transplantation. One was a refractory case complicated by pneumonia before CBT, who had received one course of IST half a year ago. Nearly 30 days passed before the other transferred to our hospital, and she had severe septicemia before CBT. Of the remaining 16 cases, only 1 case had engraftment with complete donor chimerism of a single umbilical cord blood unit from day 21, whose ANC $> 0.5 \times 10^9$/L and platelet $> 20 \times 10^9$/L occurred on day 12 and day 31 post double CBT, respectively. However, she experienced secondary graft failure 3 months post transplantation and attained survival by successful haploidentical-related transplantation. The remaining 15 patients experienced primary graft rejection, but all of them acquired autologous myeloid recovery, and the median time to a neutrophil count of 0.5×10^9/L was 37 days (range, 14–57 days). The median time to 20×10^9/L platelets was 87 days (range, 43–180 days) for 14 cases. Up to the end of June 2011 there were two relapses after transfusion-independence, as a result of decreased blood CYA concentration and zoster virus infection. Their transfusion interval become longer (more than 3 months) after recovery of the CYA trough concentration to 200–250 ng/mL. Until last follow up, only one patient was still transfusion dependent. The median time to move out from single room with high-efficiency particulate air filtration system was 40 days (range 18–61), and the median time to discharge was 63 days (range, 39–100 days).

All patients were available for chimerism studies weekly in the first month post CBT. The results of the 18 patients can be classified into three patterns: One patient achieved 80 and 100% donor cells on day14 and day 21, respectively. She had full trilineage recovery, but progressive pancytopenia recurred 3 months later and donor cells declined to 0%. Six cases showed 11–39% donor cells between day 14 and day 21 at one test point, but donor cells were zero at the other time points. Eleven cases including two deaths showed no donor cells at all test points (Table 1).

Other complications and infections

Toxicity from the conditioning regimen was generally well tolerated. No patients experienced overt nausea, vomiting or mucositis. No early hemorrhagic cystitis or significant organ damage occurred. No GVHD occurred in the patients who engrafted or had transient mixed chimerism. However, the patients' demands for platelet transfusions increased during ATG treatment. Three patients developed serum sickness during 12–17 days after CBT; they presented with acute onset of fever, myalgias and bilateral knee arthralgia, and resolved with prolonged steroid therapy.

All patients experienced at least one episode of febrile neutropenia. Seven patients developed bloodstream infection between day −3 and day +7, and multiple pathogens were isolated from 11 blood samples. Gram-positive bacteremia was identified in two patients and causative organisms were *Staphylococcus aureus*. Gram-negative bacteremia was observed in six cases: *Escherichia coli* ($n = 4$) and *Klebsiella pneumoniae* ($n = 2$). After changing antimicrobiotics to imipenem or meropenem in combination with amikacin or vancomycin, infectious signs and symptoms improved for most patients. One patient complicated with serum sickness, died of *E. coli* sepsis. Two patients developed bacterial pneumonia, and one of them died of severe pneumonia with cerebral hemorrhage. Fungal pneumonia was diagnosed in one patient after 3 months following CBT and responded to voriconazole and CsA withdrawal. Of five cases of CMV reactivation (documented by PCR assays) between day 35 and day 70 following transplantation, only one patient had received foscarnet as preemptive therapy; the others received i.v. Ig supplementation, and none of the patients developed symptoms of CMV disease. Varicella zoster virus infections occurred in three patients after 3 months following CBT. No EBV infection was documented.

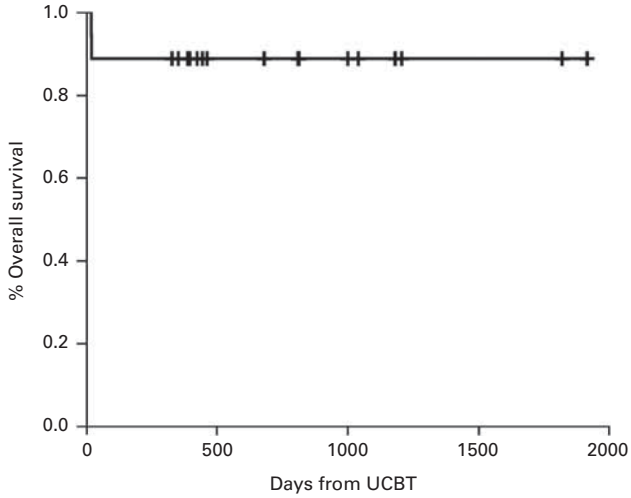

Figure 1. Kaplan–Meier estimate of OS for 18 SAA patients undergoing unrelated CBT.

Response and survival

The 3-month and 6-month cumulative incidence of response (CR and PR) was 56% and 81%, respectively. Currently, 16 patients are alive except 2 cases of early death, having survived for 330–1913 days (median, 750 days) after their transplantations. No patients developed clonal complication. The probability of survival at 2 years was 88.9% (Figure 1).

DISCUSSION

Graft failure, GVHD and regimen-related toxicities have been the major obstacles to successful hematopoietic SCT for patients with acquired SAA. SAA is traditionally associated with high rates of graft rejection. With the additional negative impact of limited cell dose and immature phenotypes of T cells in CB, the current standard of unrelated CBT for SAA patients is one of the last treatment modalities. Indeed, most of the reported experience of CBT in SAA is limited and less favorable.[15,16] In the present study, although our first two patients received high dose CY, ATG and FLU as a conditioning regimen, they still experienced primary and secondary graft failure, respectively. More intensive preparation before CBT may be needed for engraftment, but transplant-related mortality could be increased accordingly. Based on the following considerations, we performed CBT for SAA patients and deliberately chose specifically immunosuppressants targeting the T-cell population, including FLU, ATG and low-dose CY in order to maximize efficacy and minimize toxicity whilst accepting a high risk of graft rejection. First, most of our patients were newly diagnosed and do not take the risk of CBT-related mortality. Secondly, most SAA is the result of T-cell immune-mediated destruction of hematopoietic stem and progenitor cells,[17] and the primary treatment goal of SAA is to achieve functioning hematopoiesis, which ultimately protects patients from being at risk of dying of infectious complications or bleeding. Thirdly, the infused CB cells may have a role on hematopoietic recovery.

Cell dose and HLA compatibility are more important for engraftment and survival in CBT for patients with nonmalignant diseases compared with patients transplanted with malignant disorders. The current recommended lowest dose is 3.5×10^7 NC/kg at infusion with less than two HLA disparities.[18] In order to get a higher cell dose, several small successful results were reported by the use of double CBT for SAA patients.[19–21] However, Chan *et al*.[16] noted the graft characteristics (HLA match, TNCs/kg, $CD34^+$ cells/kg) of the non-engrafting patients were similar to those who were engrafted when they received the lowest dose of 3.5×10^7/kg pre-thaw. Yoshimi *et al*.[15] also found that the number of infused TNC and $CD34^+$ cells had no impact on the engraftment and survival with the minimum of 2×10^7/kg TNCs infused. Lately, Yamamoto *et al*.[22] have reported promising results of engraftment and survival in CBT for SAA patients using a reduced intensity conditioning regimen of 125 mg/m^2 FLU, 80 mg/m^2 melphalan and 4 Gy fractionated TBI with a median infused TNC 2.5×10^7/kg. In our series, the minimum TNC at infusion was 2.34×10^7/kg, all patients who had no multiple transfusion exposure before transplant experienced engraft failure. The conditioning regimen must be considered as a major cause of graft failure. Compared with Yamamoto's regimen, both added FLU to the regimens, which was identified as a favorable factor for engraftment and survival in SAA. CY and melphalan is also comparable, but the CY dose was significantly reduced in our study. The main discrepancy may be the use of ATG or TBI in two groups. ATG has been a key drug to facilitate engraftment and reduced GVHD in SAA patients who received unrelated donor transplantation.[23] but long-surviving ATG might lead to an *in vivo* depletion of CB T-cells that are critical for the engraftment. In contrast, low-dose TBI may efficiently deplete recipient T cells without affecting donor cells, which was also confirmed in a previous feasibility study using alternative donors.[24] Furthermore, the patients with anti-HLA antibodies that specifically correspond to mismatched Ag in umbilical cord blood graft may be reduced engraftment rate,[22,25] but we could not detect anti-HLA antibodies due to limited conditions. In addition, all our evaluable patients were newly diagnosed and treatment-naïve, the patients' immune systems have not been suppressed by previous immunosuppressive therapy and thus may help lead to graft failure.

Combination ATG and CsA is the most effective IST. The reported time to response varies between studies as do the results of different methods to calculate response intervals and to classify response. The best reported result of trilineage hematologic recovery was 50% after one course of the standard IST, the median time to transfusion independence was 96 days.[26] Our 15 patients attained myeloid recovery at the median time of 37 days (range, 14–57 days) except for 2 early deaths. Faster neutrophil recovery may be correlated with powerful immunosuppression before and after CBT, consisting of FLU, low-dose CY, ATG, CsA and MMF. Furthermore, we suggest that the cells of infused grafts may be helpful for autologous recovery but still have no experimental evidence. Indeed, there has been growing evidence that cord blood T cells could respond to allo-Ags from extremely early time point post-transplant.[27] We also detected 11–39% donor cells between day 14 and day 21 in six patients post CBT. However we could not measure T-cell chimerism in this study and did not find that the transient mixed chimerism could accelerate autologous myeloid recovery. Whether the infused cord blood T cells act against host T cells is still worthy of further study.

The toxicity of the conditioning regimen was mild and tolerable; infection was major cause of death. It is confirmed that SAA patients who respond to combination ATG/CsA have excellent survival.[28] In our cohort, OS at 2 years was 88.9%, the high incidence of autologous recovery in these patients means that long-term survival may be achieved.

In summary, cord blood may not be the first option in SAA patients lacking a HLA-matched family donor due to a high risk of graft failure. Our data indicate that CBT for newly diagnosed SAA using no irradiation but FLU and ATG-based conditioning still seems to inevitably lead to the high risk of rejection, but may facilitate autologous recovery and improve survival with a low risk of transplant-related mortality. However, as in our study the duration of follow up was limited and the number of patients was very small, the rate of disease relapse and late complication should be further evaluated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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