Outcome of Related and Unrelated Cord-Blood Transplantation in children at Hematopoietic Stem Cell Transplantation Research Center of Shariati Hospital

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Abstract
Introduction: In 1989, the first successful umbilical cord blood transplantations (UCBTs) was reported in a boy with fanconi's anemia, using umbilical cord (UCB) of his HLA matched sister. Cord blood transplantation is a good substitute for bone marrow or peripheral blood transplantation especially for children and small body size adults.
Methods: Between 1998 and 2007, 14 children(10 boys and 4 girls) with non-malignant(9 Beta-Thalassemia Major, 3 SCID,1 Hurler) and malignant(1 AML) diseases were given an allogeneic CB transplant from sibling (10 cases) or unrelated(4 cases). In the majority of cases, busulfan and cyclophosphamide were given in various dosage in conditioning regimen GVHD prophylaxis consisted mainly of (CsA) alone (in 9 cases), the combination of CsA and methotrexate (in 4 cases) and methotrexate alone (in 1 case).
Results: In thalassemic patients 89% are alive but 55% with disease. Graft failure occurred in 4 thalassemic and 1 AML patients. Only 2 cases experienced GVHD (1 acute GVHD grade2 and 1limited chronic GVHD). All three cases of SCID that transplanted very late after many infectious complications are dead.
Conclusion: This study demonstrated that CBT is curative in some thalassemic patients but in future, we can improve results of CBT with use of ATG, Alemtuzumab, Thiotepa, G-CSF, double cord, TNC dose of 4×10^7/kg and elimination of methotrexate for GVHD prophylaxis in HLA- match sibling.
Keywords: Cord blood transplantation, Children, Thalassemia

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Introduction
In 1989, the first successful umbilical cord blood transplantations (UCBTs) was reported in a boy with fanconi's anemia, using umbilical cord (UCB) of his HLA matched sister.(1)
Two differences between UCBT and Bone marrow transplantation (BMT)/ PBSCT Specify the former from the latter in relation to the practical approach. First, the number of nucleated cells contained within each UCB unit approaches only a tenth that represented within a typical bone marrow allograft and 1/100 compared to peripheral blood allografts.
Second, UCBT is associated with higher immune tolerance than does BMT, thereby permitting a more liberal HLA matching.(2)
Many patients with severe non-malignant and malignant diseases are not eligible for conventional HSCT mainly because they have no HLA identical sibling donor able to donate bone marrow. For such patients, an alternative option is cord blood transplantation from an unrelated or related donor.(3)

Patients and methods
Between 1998 and 2007, 14 children (10 boys and 4 girls) with non-malignant (9Beta-thalassemia major, 3 SCID, 1 Hurler), and malignant disease (1 AML) were given an allogeneic CB transplant from a sibling (10 Cases) or unrelated (4 cases).
Information on these patients was collected through computerized data recordings and inpatient and outpatient records.
Prior to transplantation, all thalassemia patients were assigned to 1 of 3 classes of risk according to the criteria proposed by Lucarelli et al.(4)
In the majority of patients, CB was collected from Shariati Hospital cord blood bank and only in three patients from foreign cord blood bank (1 from Italy,

1 from Czech, 1 from Germany).
One patient (number 3 in table 1) transplanted at day of collection without cryopreservation of cord blood progenitors.

HLA typing and donor/ recipient matching

Low- resolution serologic typing was used for HLA-A and HLA-B and Low-resolution PCR (polymerase chain reaction) typing was used for the DRB1 antigens in all donor / recipients pairs.

Transplantation regimen and GVHD prophylaxis

Data regarding conditioning regimen are given in table 1.
GVHD prophylaxis consisted mainly of cyclosporine (CsA) alone (9 cases), the combination of CsA and methotrexate (MTX) (4 Cases) and MTX alone (one case).
Antithymocyte globulin (ATG) was associated with BU and CY in two cases (number 4 and 9 in table 1).

Supportive therapy

Empirical broad spectrum antibiotic therapy was started when children became febrile, and antifungal was used in the presence of clinical evidence of fungal infection or fever persisting after 3 to 5 days of antibiotics.
Cytomegalovirus (CMV) serologic status was studied before and after transplantation in all children. Prophylaxis of herpes virus and CMV infection consisted mainly of acyclovir. As prophylaxis for pneumocystis jirovecci (carinii) pneumonia, patients usually received oral cotrimoxazole, starting at 35 days after transplantation. Recipients with SCID were in full coverage of antibiotic, antifungal, antiviral and IvIg before and after transplantation.
Acute and chronic GVHD were graded according to previously published criteria.(5)
Established GVHD was usually treated with steroids.

End points

Hematopoietic and lymphoid engraftment was documented by FISH (one case) and PCR [VNTRs (veriable number tandem repeats) in one case and STRs (short tandem repeats in 11 cases)].
Full donor engraftment was defined as the presence of more than 95% the donor cells.
Myeloid engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count was 0.5×10^9/L or higher with evidence of donor hematopoiesis, and platelet engraftment as the time to reach sustained platelet count of 20×10^9/L or higher in the absence of platelet transfusions for 7 consecutive days.

Results

Neutrophil and platelet engraftment

Graft failure occurred in 4 thalassemic and one AML patients.
Two patients underwent a second allorganic BMT from the same donor of CB cells (table 1), that one patient sustained donor engraftment.
One patient underwent a second allogeneic PBSCT from mother because parents not allowed BMT 8 months later from same donor.
Information on chimerism was available in all patients but one.
Mixed chimerism, defined as the presence of more than 5% of recipient cells, was documented in the early (i.e., the first 3-4 months) posttransplantation period in 4 thalassemic patients and 2 SCID patients.
Two thalassemic patients have a stable mixed chimerism after discontinuation of any immuno-suppressive treatment with independence to regular transfusion.

Acute and chronic GVHD

Only 1 of the 14 patients experienced grade 2 acute GVHD, that had been transplanted from an HLA-disparate donor (table 1).
Limited chronic GVHD was diagnosed in one of the 14 patients. Other details about results are shown in table 1.

Discussion

Cord blood transplantation (CBT) is a good substitute for bone marrow or peripheral blood transplantation, especially for children and small body size adults.
Rate of cord blood transplantation increasing in the world (6) and its indications extended to many malignant and nonmalignant disorders.
It seems that is as effective as other sources for thalassemia (7).
The most important factor for successful cord blood transplantation is timing of transplantation and risk factors of patients.
Many patients in other studies transplanted in more advanced stage of disease and after many failures of previous treatments.
For example in our study all of severe combined deficiencies transplanted very late after many infectious complications which reduce chance of successful transplantation dramatically.

Table 1: characteristics of cord blood transplanted patients

	Age at CBT	Diagnosis	Clinical status at CBT	HLA	Conditioning	GVHD Prophylaxis	MNC infused $\times 10^7$/kg	Chimerism % of donor cells	Method of detection	Outcome and survival	Other	Source of cord
1	3y	Thal	Pesaro class I	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA	2.08 Washed	+55 (2nd transplant) 100%	STR	Alive without disease	Retransplanted from same donor 5y later	Iran
2	9y	Thal	Pesaro class I	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA	1.26 Washed	0% 1st transplant 10% 2nd transplant	STR	Dead	Retransplanted from same donor 3y later	Iran
3	10y	Thal	Pesaro class I	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA	1.3	3y 99%	FISH	Alive with disease		Iran
4	5y	Thal	Pesaro class II	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA +MTX	2.43	1 mo 10%	STR	Alive with disease	ATG	Iran
5	3y	Thal	Pesaro class II	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA +MTX	2.34	4 mo 5%	STR	Alive with disease		Iran
6	6y	Thal	Pesaro class II	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA	1.01	3 mo Zero%	VNTR	Alive with disease		Iran
7	1y	SCID	Infection Prophylaxis	Unrelated mismatched 5/6	Bu(2mg/kg) Cy(20mg/kg)	CsA	2.73	1y 20%	STR	Dead	Chronic GVHD Limited	Iran
8	4y6mo	Thal	Pesaro class II	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA +MTX	2.08	1.5y 10%	STR	Alive without disease		Germany
9	8mo	Hurler Syn	Cardiomyopathy	Unrelated mismatched 4/6	Bu(16mg/kg) Cy(200mg/kg)	CsA	5.84	1 mo 100%	STR	+46 dead	ATG AcuteGVHD	Iran
10	13y	AML	In partial remission	Sib Matched	Bu(16mg/kg) Cy(120mg/kg)	CsA	1.3	70% 2nd transplant	STR	+51 dead	Retransplanted in +48 from mother	Iran
11	1y	SCID	Infection prophylaxis	Unrelated matched	Flu(100mg/m^2) Cy(20mg/kg)	CsA	3.82	+14d 65%	STR	Dead		Czech
12	21mo	SCID	Infection prophylaxis	Unrelated mismatched 4/6	Bu(2mg/kg) Cy(20mg/kg)	MTX	1.49	-	-	Dead +19		Italy
13	6y	Thal	Pesaro class I	Sib Matched	Bu(14mg/kg) Cy(200mg/kg)	CsA +MTX	2.9	5mo Zero%	STR	Alive With disease		Iran
14	14y	Thal	Pesaro class II	Sib mismatched 5/6	Bu(16mg/kg) Cy(200mg/kg)	CsA	1.12	3mo 20%	STR	Alive without disease		Iran

CBT: Cord blood transplantation, Thal: Thalassemia, MNC: Mononuclear cell, CsA: Cyclosporine A, MTX: Methotrexate, Bu: Busulphan, Cy: Cytarabine, Flu: Fludarabine, ATG: Anti-thyroglobulin, AML: Acute myelogenous leukemia, SCID: Severe combined immunodeficiency,

Also one patient with acute leukemia transplanted after several relapse and in persistent leukemia state.

For improvement of results we should consider patients for cord blood transplantation earlier and also it seems wise to adhere to current recommendations of a minimum recommended TNC dose of 4.0 $\times 10^7$/kg for CBT for haemoglobinopathies.(3)

We can use double cord blood units in larger recipients or when cell dose is inadequate.(8)

Another approach for improving the outcome of CBT is via addition of ATG, alemtuzumab (campath-1H) or thiotepa, all of which are associated with low rates of graft rejection (9-11). We used ATG in 2 patients only.

In other studies the use of granulocyte colony – stimulating factor (5-10 μg/kg/d) on day 1 after transplantation and on each day thereafter until the neutrophil count remained $>1.0\times10^9$/l for 3 consecutive days may also have contributed to speedy engraftment especially in HLA mis-matched CBT.(12, 13)

We use methotrexate (MTX) for GVHD prophylaxis in five patients (one SCID and four thalassemia) that mostly resulted in unfavorable outcome and confirm previously reported data on the unfavorable impact of MTX in CB transplant recipients (11, 14)

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