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Thalassaemia

Peripheral blood stem cell transplantation in children with beta-thalassemia

MA Yesilipek, V Hazar, A Küpesiz, A Kızılörs, A Uguz and O Yegin

Akdeniz University School of Medicine, Department of Pediatric Haematology and Immunology, Antalya, Turkey

Summary:

Fifteen patients with beta-thalassemia received an allogeneic peripheral blood stem cell transplant. Median age was 3.5 years (1–15 years). Six were class I, four class II and five class III according to the Pesaro criteria. All of the donors were HLA-phenotypically identical (13 siblings and two parents). Nine patients were given BU + CY and six BU + CY plus ATG as conditioning. All patients received MTX (+1, +3, +6) and CsA (9–12 months) post transplant for GVHD prophylaxis. The median neutrophil and platelet engraftment times were day 12 and day 16, respectively. cGVHD was observed in three patients. Two patients died. Thirteen patients are well, and transfusion-independent 2–30 months after PSCT. No recurrences of thalassemia have been seen. Overall and event-free survival were 86.6%. In conclusion, we suggest that PSCT can be considered a safe and effective treatment for children with beta-thalassemia. *Bone Marrow Transplantation* (2001) **28,** 1037–1040.

Keywords: peripheral blood stem cell transplantation; beta-thalassemia; children

Stem cell transplantation is considered the only curative treatment approach for the hemoglobinopathies. Successful cure of β-thalassemia by bone marrow transplantation (BMT) was first reported by Thomas and associates in 1982.[1] Subsequently, a number of centers have explored this approach as a curative therapy for β-thalassemia. The most extensive experience with BMT in β-thalassemia was reported by Lucarelli and co-workers[2] in Italy who studied more than 800 transplanted patients. Recently, peripheral blood stem cells (PBSC) have been increasingly used in transplantation. Some authors have suggested that peripheral blood stem cell transplantation (PSCT) has some advantages as compared with bone marrow stem cell transplantation.[3,4] Diaz *et al*[5] reviewed the literature and reported that the use of cytokine-mobilized PBSC for allogeneic transplantation appears to be safe for both pediatric donors and patients, leading to rapid hematopoietic engraftment with a similar incidence of acute graft-versus-host disease (AGVHD) as is seen after BMT.

We report the PSCT experience of our department concerning 15 patients with beta-thalassemia.

Correspondence: Prof Dr MA Yesilipek, Akdeniz University School of Medicine, Department of Pediatric Haematology-Oncology, 07070, Antalya, Turkey
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Materials and methods

Fifteen patients with beta-thalassemia received an allogeneic peripheral blood stem cell transplant at Akdeniz University School of Medicine Department of Paediatric Haematology-Oncology. Patient characteristics are shown in Table 1. All of the patients were transfusion-dependent before stem cell transplantation. Fourteen patients had homozygous beta-thalassemia and one had HbS-beta-thalassemia. Female to male ratio was 9/6 and median age of the patients was 3.5 years (range 1–15 years). Liver biopsy was performed on all patients who were older than 3 years. Six of them were class I, four class II and five were class III according to the Pesaro criteria.[6] All but two patients were HbS antibody-positive. Only one patient (No. 4) was HCV antibody-positive. Thirteen of the donors were fully HLA-matched siblings and two were parents

Table 1 Pretransplant patient characteristics

Patient	*Age (year)*	*Diagnosis*	*No. of transfusions*	*Splenectomy*	*Ferritin*	*Class*
1	2	TM	28	No	2775	I
2	9.5	TM	96	No	9600	III
3	1.5	TM	10	No	1150	I
4	9	TM	96	No	5980	II
5	3.5	TM	36	No	6471	II
6	15	S/B	162	Yes	12 084	III
7	8	TM	85	Yes	2473	III
8	1	TM	6	No	714	I
9	3.5	TM	36	No	2000	II
10	12	TM	140	Yes	11 637	III
11	1.5	TM	5	No	2800	I
12	5	TM	44	No	1696	II
13	2	TM	20	No	1300	I
14	14	TM	152	Yes	5655	III
15	1	TM	7	No	805	I

TM = beta thalassemia major; S/B = HbS + beta thalassemia.

(Table 2). Conditioning regimens consisted of busulphan (BU) 16 mg/kg and cyclophosphamide (CY) 200 mg/kg totally in the class I and II patients. All class III patients received 14 mg/kg BU and 160 mg/kg CY as conditioning. ATG 10 mg/kg was added to the conditioning regimen in the last six patients. G-CSF (5 μg/kg s.c.) was given to donors for peripheral blood stem cell mobilization for 5 days. In older children and parents, antecubital veins were used if appropriate for stem cell apheresis. Femoral dual lumen catheters were used for harvesting in other child donors. Local anesthesia and sedation were given. We did not use central lines in any donors. Median apheresis time was 140 min (90–230 min, depending on volume and access). Peripheral blood stem cell apheresis was performed on the fifth day, but continued on days 6 and 7 if necessary. The median apheresis count was one (range 1–3) (Table 2). For GVHD prophylaxis, all patients received MTX (10 mg/m^2 on day +1 and 8 mg/m^2 on days +3, +6, i.v.) and CsA 3 mg/kg on day −1, then 2 mg/kg i.v. for 2–3 weeks and switched to the oral form if patient status was suitable. The dose was regulated depending on serum levels, for 9–12 months post transplant.

All of the recipients received 5 μg/kg G-CSF, s.c., starting on day +1. Successful engraftment was defined as the first consecutive 3 days of the absolute neutrophil count exceeding 0.5×10^9/l for neutrophil engraftment, and the first of 7 consecutive days with an untransfused platelet count exceeding 20×10^9/l for platelets. GVHD was graded as previously described by EBMT.[7]

Results

Engraftment was achieved in all patients. The median neutrophil and platelet engraftment times were day 12 (9–14 days) and day 16 (12–48 days), respectively.

Two patients died. One, aged 18 months, died on day +15 from head trauma and intracranial hemorrhage. The other patient died on day +25 of pulmonary hemorrhage. Their platelet counts were 14×10^9/l and 27×10^9/l, respectively. GVHD was graded according to standard criteria. Acute GVHD was observed in four patients, 26.6%, (grade I in two patients and grades II and IV each in one patient). They were successfully treated with steroids. In the follow-up period, three patients showed limited chronic graft-versus-host disease (cGVHD). Moderate veno-occlusive disease (VOD) occurred in two patients. Chelation therapy was restarted in the twelth month post transplant.

All of the 13 patients are well, and transfusion-independent 2–30 months after PSCT. The most recent Hb levels of the patients were within normal limits (Table 3).

No recurrences of thalassemia have been seen. Overall and event-free survival are 86.6%.

Discussion

Beta-thalassemia major is one of the most severe genetic hemoglobinopathies and has a very high incidence in the west and south of Turkey.[8] Although regular transfusion and chelation therapy protocols have improved the quality of life of thalassemic patients recently, allogeneic stem cell transplantation is the only available procedure which may definitively cure the disease.

In 1982, Thomas *et al*[1] in Seattle, successfully cured the first thalassemia major patient by bone marrow transplantation. At the time, BMT was considered an alternative treatment for hemoglobinopathy patients. Lucarelli *et al*[2] from Pesaro reported the results of more than 800 successfully transplanted patients.

Recent studies have shown that PSCT results in rapid hematological and immunological recovery without excessive aGVHD compared with BMT.[9] This results in a reduced transfusion requirement before hematological recovery, earlier discharge, and lower transplantation costs. Data from the literature suggest that PSCT appears to be safe for pediatric donors, yields sufficient progenitor stem cells and results in prompt engraftment.[5,10–14] The median neutrophil engraftment day was 12, and all patients achieved engraftment before day 14 in our series. Mobilization of PBSC and collection by leukapheresis has practical advan-

Table 2 Donor characteristics

Donors	*Relationship*	*Age (years)*	*Weight (kg)*	*Gender patient/donor*	*Mobilization adverse effects*	*No. of aphereses*	*Apheresis complication*
1	Sibling	6	24	F/M	—	2	—
2	Sibling	14	42	M/M	—	1	—
3	Sibling	6	49	M/F	—	1	—
4	Sibling	12	31	F/M	—	1	—
5	Sibling	2	11	F/M	—	2	—
6	Sibling	16	60	F/M	—	2	Thrombocytopenia
7	Sibling	6	23	M/F	—	2	—
8	Sibling	4	15	F/M	—	1	—
9	Sibling	5	17	M/F	—	2	Thrombocytopenia
10	Sibling	6	22	M/M	—	3	—
11	Sibling	4	16	M/F	—	1	—
12	Mother	38	57	F/F	Bone pain	1	—
13	Father	36	80	F/M	Fever, bone pain	1	—
14	Sibling	12	54	F/M	—	1	—
15	Sibling	7	24	F/M	—	1	—

Table 3 Engraftment and patient follow-up

Patient	*MNC ×10⁸*	*CD34⁺ ×10⁶*	*Engraftment PNL/PLT*	*AGVHD*	*cGVHD*	*Complication*	*Last Hb g/dl*	*Follow-up (months)*
1	11.20	—	11/13	—	—	—	14.2	Alive +30
2	6.20	—	14/20	—	—	VOD	12.5	Alive +21
3	6.00	—	12/–	—	—	Intracranial hemorrhage	—	Died
4	7.40	1.10	12/12	—	—	Bacteremia	11.0	Alive +19
5	8.60	17.28	12/15	—	—	—	12.3	Alive +18
6	9.45	2.20	9/30	Grade IV	Limited	Hemolysis, ARF, hypertension	14.4	Alive +17
7	9.32	2.73	11/19	—	—	HUS	10.0	Alive +16
8	10.30	5.16	13/16	Grade I	—	—	11.7	Alive +15
9	8.20	9.05	13/17	Grade II	Limited	CMV reactivation	10.3	Alive +13
10	5.58	1.17	14/48	—	—	—	10.7	Alive +11
11	8.20	8.03	13/–	—	—	Pulmonary hemorrhage	—	Died
12	5.30	4.04	12/14	Grade I	Limited	Bacteremia	12.6	Alive +8
13	6.75	29.44	12/14	—	—	Hypertension	9.8	Alive +5
14	6.10	8.33	12/15	—	—	CMV reactivation, arthritis	9.5	Alive +2
15	6.78	3.20	12/15	—	—	CMV reactivation	8.6	Alive +2

tages for the donor over the traditional marrow harvest, including the avoidance of general anesthesia, blood loss and invasive surgery. There are some reports that children can tolerate mobilization at the same G-CSF doses better than adults.[5,10,11] Cavallaro *et al*[15] reported that administration of G-CSF to normal donors for the purpose of mobilizing PBSC appears safe and without any obvious adverse effects. Although bone pain, fever, fatigue, transient cytopenia and elevation of serum alkaline phosphatase and transaminase have been reported as G-CSF-related side-effects in adult patients, no complaints were made by pediatric donors.[5,10,11,16] On the other hand, recent reports also suggest that a lower G-CSF dose was sufficient to increase circulating progenitor cells in children.[17] Levine *et al*[11] reported successful and safe cytokine-mobilized allogeneic peripheral blood stem cell transplants with 14 child donors in which the youngest donor was 8 years old. In our donor group, which received 5 μg/kg G-CSF s.c. for mobilization, the median age was 6 years and the youngest was 2. We did not observe any toxicity before or after apheresis. However, two donors who were parents, suffered bone pain during mobilization. After stem cell apheresis, mild thrombocytopenia was noted in the two other donors (140×10^9/l and 142×10^9/l) on whom two aphereses had been performed (Table 2). We have been following them up for possible adverse events.

It is well known that GVHD plays an important role in the outcome of stem cell transplants. The higher incidence of cGVHD reported is considered a risk factor for PSCT, occurring in up to 75% of patients at 1 year.[11] However, we have observed limited cGVHD in only three patients. The low cGVHD incidence in our patients might be due to two-drug GVHD prophylaxis and the ATG used in the conditioning regimen of our patients.

No patient experienced engraftment failure or recurrence of thalassemia. Lucarelli *et al*[18] reported that rejection is inversely related to transfusion burden and that the latest recurrence of thalassemia occurred on day +548 after transplantation. Recurrence never occurred in patients more than 2 years from transplant. Units transfused were 5–162 in our patient group (Table 1). Since our median follow-up was only 12 months we are unable to come to any conclusions on this subject.

Two patients died of hemorrhage in the thrombocytopenic period. Thirteen patients are alive without transfusion and free of thalassemia after 2–30 months follow-up.

We conclude that PBSC is an acceptable and effective source of stem cells in hemoglobinopathies, with rapid neutrophil and platelet engraftment. However, longer follow-up and larger studies are needed to define any adverse effects in child donors.

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