Original Article

ß-Thalassaemia major: Bone Marrow versus Peripheral Blood Stem Cell Transplantation

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Abstract

Objective: To compare PBSCT with BMT in Thalassaemia patients in terms of rejection, non-rejection mortality, disease free survival and overall survival.

Methods: Fifty six patients were transplanted from September 2000 - July 2005. Twenty nine underwent BMT and 27 received PBSCT. Most patients were intensely transfused to keep minimum haemoglobin of 12 gm/dl and received desferioxamine, 24 hours infusion, before transplantation. Pesaro class I (n-20) and class II (n-20) received conditioning with standard Bu/Cy. Of class III (n-16), ALG was added to standard Bu/Cy in 9 who received PBSCT and 7, who received BM, were conditioned with Hydrea 20-30 mg / kg (day - 45 to -11), Azathioprin 2-3 mg / kg (day - 45 to day -11), Fludarabine 25 mg / kg (day -17 to -13) followed by Bu14 / Cy 200 started on day - 10. Triple immunosuppression was used for whole PBSC group and class III-BM group. For others, a GvHD prophylaxis comprised of MTX and cyclosporine only. MNC dose infused was > 4 x 10^8/kg (range 4.8-8.2) recipient weight in PBSC patients and for BM its range was 1.6 - 5.2 MNC / kg . All patients received G-CSF 5mg / kg / day, from day + 5, till ANC > 0.5x109 / l. Median age of the donor was 8.6 years. All recipients and donors were genotypically HLA matched except in one. PBSC were harvested on day 5 of G-CSF administration. Follow up ranged from 273 - 2088 days.

Results: Median age for BM and PBSC group was 5.2 and 6.9 years. Engraftment was achieved in all cases. Median time to ANC of 0.5 x 10^9/ l in BMT / PBSCT patients was 13 / 10 days (range 11-19 / 9 - 15) and for platelets of 20 x 10^9 / l it was 17 / 14 days (range 14 - 28 / 12 - 19). aGvHD (grade II - IV) was seen in 30% / 26% cases in BMT / PBSCT group. Incidence and severity of chronic GvHD was not statistically different in two groups (BM-24% & PBSC -30%). Six patients rejected the graft. Of the four who rejected the graft from class III, 3 were from PBSC group. DFS in risk classes of the two groups was not significant. Overall survival / disease free survival for the BM and PBSC group as on December 2005 was 73% / 65% and 67% / 55%.

Conclusion: This study shows that major outcomes with PBSCT are not statistically different from BMT. Rejection and disease free survival in class 3 patients who received intensified immuno-suppression and large doses of PBSC is comparable to BM group who were conditioned according to newer Lucrali protocol (JPMA 58:107;2008).

Introduction

Most thalassaemia births are now seen in developing countries where it remains a major health and socioeconomic issue for the family and the nation.[1,2] Treatment options are limited: transfusion or transplantation. Because of inadequacy of transfusion and iron chelation, most children in the developing countries die much earlier than in the west.[3]

Blood and Marrow Transplantation remains the only cure. The results of transplantation in thalassaemia have improved in last one decade and patients can now be prognostically categorized in three groups. Lucrali et al have shown that for children in class I, disease free survival (DFS) of 91% can be achieved. For class II patients the results are marginally worse with overall survival (OS) and DFS of 84 % and 80 % largely because of non rejection mortality.[4] But for children in class III, transplant related complications and rejection are high and DFS is just 60%.[5] Rejection rate of 11 to 51% have been described by different studies using different conditioning regimens for thalassaemia patients.[6]

PBSCT is an attractive option particularly in resource constraint countries where literacy rate generally remains low. Family / donor willingness and low cost remain attractive features in this context.[7] Also PBSC grafts are known to facilitate early engraftment and immune reconstitution.[7,8] With the success of reduced intensity transplant (RIT) and donor lymphocyte infusion (DLI), it is clear that lymphocytes present in the graft have the potential to eradicate the malignant clone / defective haemopoiesis.[9] In our opinion, PB grafts with more favourable subset of lymphocytes may prove to be a better

modality of transplantation in multiply transfused thalassaemic children who generally have higher tendency to reject the graft. However, the possibility of higher GvH in PBSCT group, as reported in aplastic anaemia, remains a concern.[10] Also, PBSC graft, if combined with intense immunosuppression (conditioning and aGvHD prophylaxis) specially in group III, the chances of graft rejection and non rejection mortality, at least theoretically, are likely to be reduced and may be translated into improved disease free survival. However, large scale studies using PBSC as a source of stem cells in thalassaemia are scarce.

We carried out this analysis to compare PBSCT with BMT primarily in terms of rejection rate, non rejection mortality and disease free survival and overall survival as primary end points.

Subjects and Methods

Three centers from Pakistan participated in the study, which extended from September 2000 - July 2005 and during which 56 homozygous B thalassaemia patients underwent Blood and Marrow Transplantation.

All the patients were 14 years or less. Twenty nine patients received bone marrow graft and another 27 received peripheral blood stem cells. All patients had a history of blood transfusion started before the age of 18 months. None of them had thalassaemia Intermedia phenotype. Most patients were intensely transfused before transplantation to maintain a minimum Hb of 12 gm / dl and received I / V desferioxamine, 24 hrs infusion, for 1-3 months before transplantation. Patients in each group were categorized into three classes according to Pesaro risk classification. All class II and III cases had features and laboratory data suggestive of being undertransfused and iron over loaded. Most of them had serum ferritin levels above 2400 mg / dl. All recipient / donor pairs except 23 were ABO matched. Seven and nine patients were positive for HbsAg and HepC antibody on 3rd generation ELISA. All positive cases were checked for viral load by PCR. None of the cases with histological evidence of chronic active hepatitis was included in the present study.

Informed consent was obtained from parents in all cases and patients were also explained the procedure wherever applicable.

Conditioning Regimen: Pesaro class I (n-20) and class II (n-20) received conditioning with Bu 14 / Cy 200. Of class III (n-16), Anti Lymphocyte Globulin (ATG Fresenius, France) was added to Bu14 / Cy 200 in 9 cases who received PBSC grafts. Class III patients in BM group were conditioned with Hydrea 20-30 mg / kg (day - 45 to -11), Azathioprin 2-3 mg / kg (day - 45 to day -11), Fludarabine 25 mg / kg (day -17 to -13) followed by with Bu14 / Cy 200 started on day -10. All patients received G-CSF 5mg / kg / day, from day + 5, till ANC > $1x10^9$ / l.

Grafts: None of the patient received primed marrow. All patients in PBSC group received MNC dose of greater than 4 x 10^8 / kg recipient body weight (range 4.8 - 8.2) and in BM group it ranged from 1.6 - 5.2 MNC / kg recipient weight. All patients received G-CSF 5mg / kg / day, from day + 5, till ANC > 0.5 x 10^9 / l. Early haematological recovery was defined absolute neutrophil count > 0.5 x 10^9 / l for consecutive 2 days and platelet count of 20 x 10^9 / l for consecutive 3 days. CD34 count was not done in any case.

GvHD prophylaxis and treatment: Acute and chronic GvHD were graded according to Standard. Triple immunosuppression (Cyclosporin, Methotrexate day 1,3,6 11and Prednisolone) in standard doses was used for PBSC group and class 3 BM patients. For other patients aGvHD prophylaxis comprised of MTX (day 1, 3, 6, 11) and cyclosporine only. Treatment of aGvHD (grade II - IV) comprised of high dose methyl prednisolone and / or ALG at 10 mg / kg / day. In case of CsA toxicity, it was substituted with MMF. IL-2 blocking antibodies and Immunoglobins were not used in routine for acute or chronic GvHD.

Prophylaxis against infection: Fluaconazole or amphoteracin B was given as antifungal prophylaxis to all cases. Antibacterial prophylaxix was not given to any case. Gancyclovir was not used in prophylactic setting as all patients and donors were CMV IgG positive. INH prophylaxis for tuberculosis was used in first 9 cases who received BM graft. All patients received Metronidazole and Mebendazole during pre conditioning period for amoebiasis and helmentics seen commonly in our country.

Supportive care: All patients were kept in hepa filtered rooms with facilities of reverse barrier nursing and low bacterial diet. All blood products were leukodepleted and irradiated. Bedside filtration was used additionally.

Donor characteristics: Median age of the donor was 8.6 years. All recipients and donors were genotypically HLA matched except in one case where mother was the phenotypic match. Two donors were HepC antibody positive but negative for RNA. PBSC Donors were primed with G-CSF, 10 mcg / kg / day for 4 days. Peripheral blood stem cells were harvested on day 5 using Haemonetics MCS+ cell separator.

Descriptive statistics was used to analyze categorical data in percentage and numerical data in terms of median. Chi square test was applied to see any association between two groups to see major outcomes using SPSS version 13.0.

Results

Follow up ranged from 273 - 2088 days (median 990 days). Pre-transplant characteristics showed no significant difference in two groups (Table 1). Overall 23 transplants were group mismatch and 24 were across the sex.

Engraftment was achieved in all cases. PBSC group showed early recovery: Median time to ANC of 0.5 x 10^9 / l in BMT / PBSCT patients was 13 / 10 days (range 11-19 / 9 - 15) and for untransfused platelets of 20 x 10^9 / l it was 17 / 14 days (range 14 - 28 / 12 - 19). Major post transplant non infective complication was aGvHD. Acute GvHD (grade II - IV) was seen in 30% and 26% cases in BMT and PBSCT group. Incidence of chronic GvHD was not statistically different in two groups (BM-24% and PBSC - 30%). Chronic extensive GvH was seen in 3 cases in each group. Six patients (10%) rejected the graft. Rejection rate is not statistically different in two groups: 11% in BMT group and 15% in PBSCT group. 3 / 6 patients who rejected were from class III-PBSC group. Outcomes according to risk class are shown in Table 2. Non rejection mortality remains high in our hands: So far we have lost 17 patients. Most deaths were seen in first 100 days (10 cases). Sepsis followed by aGvH remained the major cause of death. Overall survival / disease free survival for the BMT and PBSCT group as on December 31st was 73% / 65% and 67% / 55% respectively.

We have not attempted to evaluate the influence of variables / co variables on the the major outcomes.

Table 1. Patient pre-transplant characteristics (n = 56).

Patient variables	Total	BM group (n=29)	PBSC group (n=27)
Age (years)			
Median (range)	6.4 (1-14)	5.2 (1-14)	6.9 (1.6 - 14)
Sex			
Male : Female	2.3 : 1	2.8 : 1	2 : 1
Pesaro class			
I	20	12	08
II	20	10	10
III	16	07	09
HepBsAg +ive	03	--	03
HepCAb +ive	06	03	03
HIV (Elisa)	--	--	--
Bilirubin-median (mg/dl)	0.6 (0 - 1.6)	0.4 (0 - 1.2)	0.7 (0.2 - 1.6)
ALT - median (U/L)	21 (8-124)	19 (8-102)	27 (11-124)
Chronic persistent hepatitis	03	01	02
Serum Ferritin (ng / ml)	1522 (1220-6890)	2240 (1220-5840)	2710 (1405-6890)
Major ABO mismatch	09	04	05
Minor ABO mismatch	14	05	09
Female donor to male patien	13	04	09
Male donor to female patient	11	05	06

Table 2. Major Outcomes according to risk class.

	BM group	PBSC group	Total
	n (%)	n (%)	n (%)
Class I	**n = 12**	**n = 8**	**n = 20**
Overall survival	09 (75)	06 (75)	15 (75)
Disease free survival	09 (75)	06 (75)	15 (75)
Rejection	-- --	-- --	-- --
Mortality	03 (25)	02 (25)	05 (25)
Class II	**n = 10**	**n = 10**	**n = 20**
Overall survival	07 (70)	06 (60)	13 (65)
Disease free survival	06 (60)	06 (60)	12 (60)
Rejection	01 (10)	01 (10)	02 (10)
Mortality	03 (30)	04 (40)	07 (35)
Class III	**n = 7**	**n = 9**	**n = 16**
Overall survival	05 (72)	06 (66)	11 (68)
Disease free survival	04 (58)	03 (34)	07 (44)
Rejection	01 (14)	03 (34)	04 (25)
Mortality	02 (28)	03 (34)	05 (31)

Table 3. Major outcomes in relation to source of stem cells.

	BMT group	PBSCT group	Total
	n (%)	n (%)	n (%)
Overall survival	21 (73)	18 (67)	39 (69)
Disease free survival	19 (65)	15 (55)	34 (58)
Rejection	02 (07)	04 (15)	06 (10)
Mortality	08 (27)	09 (33)	17 (31)

Discussion

There has been a boom of PBSCT in last 15 years.[7,8] However, we found no randomized trials or large case control studies on the use of PBSCT in thalassaemia patients published in English literature. There are, however, few reports where a small percentage of patients received PBSC or cord blood, though no comparison to bone marrow transplantation is described.

Rejection and non rejection mortality remains a major concern in thalassaemic patients and indirectly effects DFS and OS.[5,6,11] To us PBSC grafts looked an attractive option in multiply transfused thalassaemic patients because of early recovery and less chance of rejection / recurrence of disease. Also, we used relatively high dose of mononuclear cells (> 4 x 10^8) per kg recipient weight in PBSCT group to boost the engraftment and likely reduction of graft failure / disease recurrence as has been reported in the literature in other diseases.[12,13] Contrary to our expectation, however, PBSC group as a whole showed no significant difference compared to BM group in terms of rejection or disease free survival (Table 3). Lucrali et al have reported DFS of 91% and 80 % in class 1 and 2 patients.[4-6] Data from Hong Kong showed EFS of 82%.[14]

Lawson et al, using Bu 14 and Cy 200 in 55 thalassaemia patients report DFS of 81% and rejection rate of 13.2%.[15] Data from Taiwan showed DFS of 44% with graft rejection being the major cause of failure.[16] Low OS in our hands is due to unacceptably high non rejection mortality. This appears primarily due to high transplant related causes: 10 / 17 patients died before day 100 mainly because of sepsis, aGvH, and VOD. We relate this to our early transplant experience as all the participating centers were newly emerging centers.

We saw early recovery in PBSC group which is well known for PB grafts.[8,17] However, unlike aplastic anemia, incidence and severity of GvH is no higher in PBSC group.[12] We attribute this to triple immuno-prophylaxis for whole PBSC group and intensified conditioning which class 3 patients of PBSC group recieved.

Conditioning has been a matter of interest in high risk thalassaemics (Lukralli class3, age less than 17) and the advantage of using ALG in thalassaemia patients remains controversial.[5,6,18] In this study class 3 patients in PBSC group were given ALG (along with triple immuno-prophylaxis) to intensify immunosuppression so that chances of rejection are further reduced. Pesaro group earlier reported no detectable influence of addition of ALG on probabilities of survival or relapse in high risk thalassaemia patients. However, another group from Hong Kong who used ALG to reduce the chances of rejection gives five year event free survival of 82%.[14] ALG has also been used successfully in multiple transfused aplastic anaemia patients to reduce the chances of rejection.[18,19] The newer approach taken up by Pesaro group for class 3 patients appears promising as DFS increased from 58% to 85% with a reduction from 30% to 8% in the probability of return of thalassaemic clone.[20] It is interesting to note that the rejection, mortality and DFS of class 3 PBSC patients is not statistically different from class 3 BM group who were conditioned according to newer Lucrali protocol. We assume this to be due to intensified conditioning with ALG and relatively higher dose of stem cells which class 3-PBSC group patients received. However our sample size was small and larger studies are required to establish the role of PBSCT in class 3 thalassaemia patients.

This study showed that PBSCT as a whole confers no advantage in terms of rejection or disease free survival compared to BMT. However outcomes of class III PBSC patients who received ALG, triple immuno-prophylaxis and higher dose of peripheral blood stem cells appears comparable to the newer transplant strategy adopted by Lucarelli for class III patients who received BMT.

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