Original Article

Survival and Graft Versus Host Disease in First 100 Patients Undergoing Allogeneic Peripheral Blood Stem Cell Transplantation: a Single Centre Experience

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Abstract

Objective: To present the survival and evaluate the demographic characteristics as risk factors for acute and chronic graft versus host disease (GvHD) in 100 recipients of HLA identical related allogeneic peripheral blood stem cell transplantation.

Methods: Indications for transplant were non-malignant and malignant haematological disorders. Bu/Cy conditioning was given for haematological malignancies and β-Thalassaemia major, Cyclophosphamide was given in aplastic anaemia. GvHD prophylaxis was Cyclosporin and Methotrexate. The patients received a median nucleated cell dose of 7.93 10^8/kg.

Results: Of 100 recipients, 72 were males and 28 females. Median age was 13.5 years (range 1.5-44). There were 65 male and 35 female donors. Median age was 15 years (range 4-45). Grade-I aGvHD was noted in 18 (18%), Grades-II in 6 (6%), Grade-III in 3 (3%) while Grade-IV in 1 (1%) patients. Diagnosis was found to be a significant risk factor for aGvHD. Kaplan Meyer analysis showed that malignancy, aGvHD, recipients above 14 years of age, female patients and engraftment after 12 days were associated with poor outcome. Of 78 patients alive beyond 100 days, 19 (24%) developed cGvHD. Mean follow up was 466 days (range 30-1766). Median survival of this cohort of patients was 338 days (mean 479 days, 95% CI 72 - 729).

Conclusion: Incidence of acute and chronic GvHD was similar to published data. Grade of aGvHD, extent of cGvHD, female patients and haematological malignancies were associated with higher rate of aGvHD and a worse outcome (JPMA 55:469;2005).

Introduction

Graft versus Host Disease (GvHD) and infections remain a major impediment to successful transplantation and is an important determinant of long-term outcome. In a developing country setting where infections are the most common cause of morbidity, immuno-suppressed patients are at increased risk of getting opportunistic as well as community acquired infection. Acute Graft versus Host Disease (aGvHD) is an important cause of early mortality in the recipients of allogeneic blood and marrow transplantation.[1,2] aGvHD results when immuno-competent donor T cells (present in the graft) recognize host antigen as

non-self. This is followed by an afferent phase of T-Lymphocyte stimulation (antigen presentation, T-cell activation and T-cell proliferation) or efferent phase of T-cell and secondary effector cell response (cytokine secretion, cytotoxic T- cells and NK-cells) leading to host tissue damage.[3] Rate of grades 2 to 4 GvHD reported have ranged from 14% to 70% for allogeneic blood stem cell transplant recipients with HLA-identical donors; reason for this wide range is unclear. Although histo-incompatibility remains the strongest risk factor for acute GvHD within the subset of patients receiving non-T-cell-depleted marrow transplants from HLA-matched related donors, a number of other variables like diagnosis, recipient-donor sex mismatch, recipient age, female donor-male recipient pair, alloimmunized and/or parity of the donor, increased dose of total body irradiation (TBI), lower intensity of GvHD prophylaxis, and viral seropositivity of recipient or donor have been implicated in association with aGvHD.[4,5]

GvHD primarily affects skin, liver and gut. No therapy is absolutely effective and all current measures for preventing or treating aGvHD increase other risks to patient. Chronic Graft versus Host Disease (cGvHD) is the single major determinant of long term outcome and quality of life following stem cell transplantation.[1,6] Prior aGvHD, degree of HLA mismatch, source of the stem cells and age remain important determinant of cGvHD. Use of allogeneic PBSC has been shown to have a similar incidence of aGvHD and higher incidence of cGvHD as compared to bone marrow stem cells.[6-8] The condition may be limited to skin or there may be multi-organ involvement, which affect the long term outcome and quality of life.[6,7]

Apart from infections and GvHD, regimen related toxicity like mucositis, diarrhoea, hepatic veno-occlusive disease, haemorrhagic cystitis and cyclosporine induced neurotoxicity is also a major cause of morbidity and affect survival. In this paper we present our results of survival in first 100 recipients of HLA identical related allogeneic peripheral blood stem cell transplantation.

Patients and Methods

Patients

Between October 1999 and July 2004 (1766 days), 100 patients received a myeloablative preparative regimen and an unmanipulated blood stem cell graft from an HLA-matched related donor. All patients had non-malignant or malignant haematologic disorders. All patients had a normal serum creatinine concentration, a cardiac ejection fraction of more than 60%; a normal pulmonary function test and liver-function tests were less than twice the upper limit of normal. Written informed consent was obtained from each patient or their parents and all protocols were approved by the Ethics Committee of the Bismillah Taqee Institute of Health Sciences and Blood Diseases Centre, Karachi.

Donors

All donors were HLA identical sibling except a haploidentical mother and a 5 out of 6 antigens matched brother and one haplotype matched mother. HLA typing was carried out by lymphocytotoxicity method. Informed consent was obtained from donors or their parents/guardians if they were young. Donors were required to have normal Karnofsky index, normal serum chemical values, normal blood counts, and negative results on serologic testing for the human immunodeficiency virus, no evidence for chronic active hepatitis secondary to hepatitis B and C; pre-menopausal female donors were required to have a negative result on a pregnancy test. Subcutaneous Filgrastim (rhG-CSF) at a dose of 10 μg per kilogram was given once daily for four days.

Stem Cell Harvesting

Peripheral blood stem cells were collected using Haemonetics MCS+ cell separator on 5th day. One and a half to two blood volumes of the donor were processed. The target mononuclear cell (MNC) dose was $5x10^8$ cells per kilogram of the recipient's body weight, but the actual cell dose infused depended on the result of aphaeresis and processing. Median aphaeresis time with this machine was 250 minutes (180-300 minutes) depending upon the volume and rate of blood flow from the donor. The unmanipulated collected cells were infused on the same day.

GvHD Prophylaxis and Treatment

For GvHD prophylaxis, oral Cyclosporin 5-8 mg/kg/day in two divided doses were started on day-5 so that therapeutic levels (900-1200 ng/ml) were achieved prior to transplant. The dose was adjusted depending on whole blood Cyclosporin C2 level. A short course of oral Methotrexate 10 mg/kg was given on day +1, +3, +6 and +11 while Cyclosporine was continued for 12 months. Acute GvHD was treated with Methylprednisolone at a dose of 5-10 mg/kg/day I/V for 3-5 days. Its dose was adjusted depending upon the response. Steroid refractory patients received IV rabbit ATG-Fresenius 5 mg/kg/day and continued till clinical response. Chronic GvHD was treated with oral prednisolone and Cyclosporin. Mycophenolate mofetil, in a dose of 1-2.0 g/day, was given to patients who developed Cyclosporin neurotoxicity. Patients were observed prospectively for development of acute GvHD. The diagnosis of GvHD was based on clinical evidence with histological confirmation where needed. aGvHD was graded according to the consensus criteria.[8]

Leukodepleted blood and platelets were given as required. The day of neutrophil engraftment was defined as the first of three consecutive days on which the patient's absolute neutrophil count was above $0.5x10^9$/l while for platelets; the engraftment was defined as the first of seven

consecutive days on which the platelet count was above $20x10^9$/l per cubic millimetre without platelet transfusion.

Conditioning Regimen

Conditioning regimen consisted of Cyclophosphamide 50 mg/kg once daily intravenously for 4 days (total dose 200 mg/kg) for all cases; Busulphan 4.0 mg/kg orally in divided doses daily for 4 days (total dose 16 mg/kg) was added to all thalassaemia and leukaemia patients. Mesna was given as gm/gm of Cyclophosphamide in divided doses for 5 days. For emesis control intravenous Tropisetron 5 mg/day was given during the period of conditioning. For patients receiving Anti-Thymocyte Globulin (ATG-Fresenius), 5 mg/kg/day for 4 days was given. In Fanconi's anaemia patients, cyclophosphamide 10 mg/kg once daily intravenously for 4 days was given (total dose 40 mg/kg).

Prophylaxis and treatment of infection

All patients were kept in isolation rooms with facilities of HePa air filtration unit and reverse barrier nursing. All patients got anti-helminth, anti-malarial and anti-amoebic drugs prophylactically prior to conditioning therapy. No patient received antibiotics for gut decontamination or prophylactic antibacterial antibiotics during neutropenic period. Ganciclovir was not given prophylactically as all donors and recipients were CMV IgG positive. Empirical antibiotics included Ceftriaxone and Amikacin for febrile neutropenia, oral Itraconazole for antifungal prophylaxis while oral acyclovir was used for antiviral prophylaxis according to the institutional antibiotic policy for the prophylaxis and treatment of bacterial, fungal, and viral infections. All patients received injection G-CSF 5 mcg/kg/day subcutaneous starting on day +4 till ANC rose to $1.5x10^9$/l.

Estimates of the incidence of aGvHD and treatment-related mortality were calculated by the method of Kaplan and Meier. Comparisons of categorical data were by chi-square test, and associations of factors with GVHD rates were analyzed using paired t test. Data was analyzed with SPSS version 10.0 statistical software package.

Results

The patients and donors characteristics are shown in table 1.

Table 1. Patients and Donors Characteristics.

Patient Variables	Numbers	Donor Variables	Numbers
All Patients	100	All donors	100
Patients median age years (Range)	13.5 (1.5 – 44)	Median Age (range)	15 (4-45)
Age < 14 years	55	Sex	
		Male	65
		Female	35
Male : Female	72 : 28	HLA matched	
		Sibling	97
		Parents	2
		Cousin	1
Disease		Multiparous	3
Aplastic Anaemia	55		
β-thalassaemia major	20		
Haematological malignancies	22		
DBA	1		
Fanconi's Anaemia	2		
Conditioning therapy		Transfused (Donors less than 20 kg required blood transfusion because of removal of up to 150 ml of PBSC product)	8
Bu/Cy	45		
Cy	50		
Cy/ATG/Flu	5		
GvHD prophylaxis		Sex mismatch	35
CSA / Mtx	78		
CSA/Mtx/Prednisolone	22		
Median FU in the surviving patients	466 days (30 – 1766 days)	Donor / Patient sex	
		Male / male	43
		Female / female	12
		Male / female	17
		Female / male	28
		MNC dose infused	7.45 SD 3.34

DBA: Diamond Blackfan Anaemia; Bu/Cy: Busulphan/Cyclophosphamide; Cy: Cyclophsphamide; ATG: Anti-thymocyte Globulin; GvHD: Graft versus Host Disease; CSA: Cyclsporin A; Mtx: Methotrexate; HLA: Human Leucocyte Antigen; PBSC: Peripheral Blood Stem Cell; MNC: Mononuclear Cell.

Engraftment

Peripheral blood stem cells were collected from 100 donors, in 40 of them, a single aphaeresis procedure was sufficient while 60 donors required two aphaeresis sessions. Engraftment was achieved in all patients; median time to absolute neutrophil count of >0.5 x 10^9/l was 10 days (range 8-12 days) and platelet count of >20 x 10^9/l was 14 days (12-17 days).

Blood Transfusion

Twenty-two patients had a major blood group mismatch while 18 patients had a minor mismatched transplant. Mean blood transfusion requirement was 4 units/patient (range 2-6 bags) while the mean platelet transfusion requirement was 6 transfusions/patient (range 4 - 8 transfusions; 1 unit of platelet per 10 kg body weight per transfusion). Seventeen patients showed platelet refractoriness; seven of them had associated VOD or aGvHD while remaining were heavily transfused thalassaemic or aplastic anaemia patients.

Fate of Graft Failure or Rejection

All 3 patients (3%) with primary graft failure received a second dose of stem cell from bone marrow and engrafted well. Of 2 patients who had secondary graft failure died secondary to VOD and multi-organ failure before engraftment. Of 7 patients (7%) who rejected their grafts, 5 received a second graft from peripheral blood stem cell from the same donor 9 months to 14 months after the first transplant; two accepted the graft and are well, one rejected the second graft as well while the remaining two died secondary to fungal infections.

Acute GVHD

All grades of acute GvHD were seen in 29 (29%) patients (95% CI, 24% to 37%). Grade II-IV aGvHD was seen in 10 (10%) cases, grade II in 6, grade III in 3 and grade IV in 1, (95% CI, 8% to 20%). Out of 28 female patients, 18 (64.2%) developed aGvHD compared to 11 of 72 males (15.2%). Median time of occurrence of aGvHD was 12 days range (day +7 to +42). Eleven out of 55 patients (20%) less than 14 years and 18 out of 45 patients (40%) above 14 years developed aGvHD. Skin was the most common (26 patients) and the first organ involved followed by the gut aGvHD (5 patients). Acute GvHD was seen in 10 out of 55 (18%) patients with aplastic anaemia, 7 out of 20 (35%) patients with β-thalassaemia, 11 of 22 (50%) patients with haematological malignancies and 1 of the 2 patients with Fanconi's anaemia. One patient with Diamond Blackfan Anaemia did not show any sign of aGvHD. All three patients who had multiparous donors developed aGvHD. Patients with grade II aGvHD responded to intravenous methylprednisolone 10 mg/kg/day in two divided doses while for grade III and grade IV aGvHD ATG-Fresenius was also added. Grade III aGvHD improved in one case while the remaining three in grade III and one grade IV did not.

Risk factors and aGvHD

Survival and aGvHD analysis is shown in table 2. Patient and donor demographics as well as characteristics of the grafts were evaluated as potential risk factors for grades 2 to 4 and grades 3 to 4 GVHD. Only primary disease was found to be a significant risk factor for aGvHD (p 0.002). Donor sex, female donor for male recipient, donor parity and mononuclear cell dose (MNC) were not found to be risk factors for aGvHD (p 0.66). Higher rates of aGvHD occurred in patients who were older than 14 years or who had malignancy. Donor parity was correlation with the risk of grades 2 to 4 GVHD. Of all 29 patients who developed any grade of aGvHD, 21 (72%) had skin involvement alone, 5 (17%) had gut involvement and 3 (11%) had liver involvement.

Chronic GvHD

Of 78 patients alive at 100 days, 19 (24.3%) developed cGvHD. In three cases it was de-novo and in the rest of the cases it was continuation of aGvHD. In 4 out of 19 cases, it was of extensive nature. Three of four patients with extensive disease died on 747, 1455 and 1701 days post transplant respectively.

Non Infectious - non GvHD Morbidity

During first 100 days after transplant, mucositis and diarrhoea developed in 2 and 82 patients respectively. Complications related to cyclosporine A were hypertension (76 patients), seizures (11 patients), blindness lasting for 20 minutes to 72 hours (5 patients) and gingival hypertrophy (8 patients). Cyclosporine level was monitored two hours after taking the dose (C2) twice a week in majority and level was kept between 900-1200 ng/ml. Cyclosporine was replaced with Mycophenolate mofetil (MMF) in those who developed seizures or blindness. Mild to moderate hepatic VOD developed in 14 cases; 3 were severe. Jaundice was seen in 19 cases. Early haemorrhagic cystitis (during conditioning and first 7 days after) developed in 11 cases while late haemorrhagic cystitis (occurred after day +21) in 7 patients. Graft failure requiring second dose of stem cell was noted in 3 cases. After 100 days of transplant, cyclosporine induced hypertrichosis, tremors, hypertension gingival hypertrophy were seen in more than 50% patients. Graft rejection occurred in 6 cases. Relapse of primary disease occurred in 9 cases.

Infectious Morbidity

During first 100 days, ninety-five patients developed 107 episodes of febrile neutropenia. Seven patients developed oropharyngeal thrush. Two patients developed CMV colitis; one progressed pneumonitis. Herpes stomatitis occurred in 5

Table 2. Survival and aGvHD Association.

Factor	No:	Alive	% alive	Variables	Numbers	P-value
Diagnosis:				**Patients developing aGvHD**	29	0.01
Aplastic anaemia	55	40	72.7			
β-thalassaemia major	20	13	65			
Malignancies	22	9	42			
DBA	1	1	100			
Fanconi's anaemia	2	0	0			
aGvHD				**Age**		
Yes	29	13	44.83	Less than 14 years	12/55 (25%)	0.66
No	71	55	77.46	More than 14 years	17/44 (44%)	
Grades of aGvHD				**Patients' Sex**		
Grade I	18	10	52.6	Male	11/72 (18%)	0.13
Grade II	6	4	66.66	Female	18/28 (64%)	
Grade IIII	3	1	25			
Grade IV	1	0	0			
Patient age				**Donor / Patient sex**		
<14 years	55	41	74.5	Male / male	10/43 (23%)	0.63
>14 years	45	25	55.5	Female / female	6 /12 (60%)	
				Male / female	10/17 (55%)	
				Female / male	3 / 28 (20%)	
Patient Sex				**Disease Association**		
Male	72	45	72.58	Aplastic anaemia	10/55 (18%)	0.001
Female	28	13	55.4	β-Thalassaemia major	7/20 (35%)	
				Haematological malignancies	11/22 (50%)	
				DBA	0 / 1 (0%)	
				Fanconi's anaemia	1/2 (50%)	
Preparative regimen						
Cyclo	50	38	76			
Bu/Cy	45	25	56			
Cy/ATG	5	2	40			
Donor Sex						
Male	65	42	70			
Female	35	25	62.5			
cGvHD						
Extensive	4	1	25%			
Localized	15	12	80%			
Engraftment (5 death before day 10)						
Early (<12 days)	79	59	74.68			
Late (>12 days)	16	7	43.75			

aGvHD: acute Graft versus Host Disease; cGvHD: Chronic Graft versus Host Disease.

patients; central venous catheter related infections were seen in 5 patients. Two patients passed Ascaris lumbricoides in stool during neutropenic period. This may be the late effect of anti-helminth therapy prior to conditioning. After 100 days of transplant, pulmonary tuberculosis developed in 3 and TB meningitis in one patient. None of them were on anti-TB prophylaxis. Plasmodium falciparum was the cause of fever in 6 patients, Plasmodium vivax in 2 cases. Brain abscess due to Actinomycoses spp. developed in one case, Herpes Zoster infection of skin in 2, Herpes stomatitis in 5, Herpes encephalitis in 1, Mucor mycosis in 1, Oro-pharyngeal thrush in 8 and vaginal thrush in 2 cases. Urinary tract, upper respiratory and lower respiratory tract infections due to common pathogens requiring hospitalization occurred in 6, 8 and 12 respectively. Pneumocystis carinii pneumonia was encountered in 2 cases.

Survival

Survival of patients in three major diagnoses is shown in figure. Sixty-three patients are alive following allogeneic peripheral blood stem cell transplantation for haematological disorders. Survival analysis showed that

Table 3. Causes of Death.

Causes	No.	Day since transplant
aGvHD	6	<100
Sepsis	5	<100
Hepatic VOD	3	<30
cGvHD	3	747, 1455 and 1701
CMV Pneumonitis	1	42
Cerebral Malaria	1	109
TB Meningitis	1	837
Liver failure	1	330
Respiratory arrest	1	<100
Fungal infection	3	Day 10, 21 and day 363
Multi-organ failure	3	<30
Relapse	3	210, 376 and 722
Undetermined cause	4	>100
ICH	2	<23
Herpes Encephalitis	1	192

VOD: Veno-Occlusive Disease; CMV: Cyctomegalovirus; ICH: Intracranial Haemorrhage

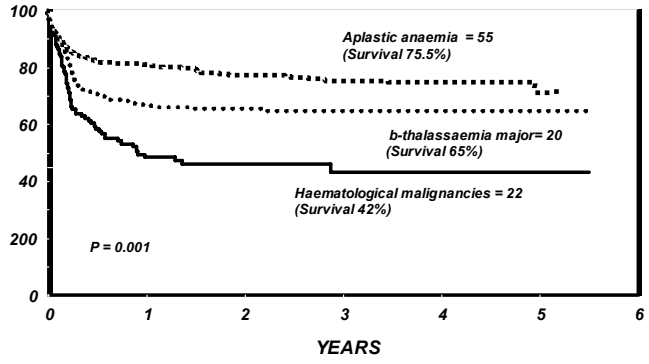

Figure. Survival after Allogeneic Peripheral Blood Stem Cell Transplant Severe Aplastic Anemia, β-Thalassaemia Major and Haematological Malignancies 1999-2004.

malignancy, aGvHD, recipients above 14 years of age, female patients and engraftment after 12 days were associated with poor outcome (p 0.001). Mean follow up was 466 days (range 30-1766). Median survival of this cohort of patients was 338 days (mean 479 days, 95% CI 72 - 729). Procedure related mortality was seen in 22 patients. Another 15 patients died during a follow up of 1766 days. Causes of death are given in table 3.

Discussion

This is the first report on the outcome and graft versus host disease in first 100 patients undergoing allogeneic peripheral blood stem cell transplantation from a single centre in Pakistan. Allogeneic stem cell transplantation remains an important curative therapy for several haematological disorders. GvHD is the main cause of death and the leading contributing factor for morbidity and impaired quality of life after transplantation.[9] Numerous studies have documented a clear correlation between GvHD and transplant related mortality (TRM). In our series, all grades of aGvHD was seen in 29 (34%) cases while 11 (14%) of our cases developed aGvHD of grade II-IV. The reported incidence of grade II-IV aGvHD is 14%-70% in HLA matched siblings and the reason for this wide variation remains unclear.[1,10] In our hands the figure for aGvHD and grade II-IV GvHD are on the lower side of the reported literature. Possible explanations are that almost half of our cases had aplastic anaemia and majority were transplanted within 2-3 months of diagnosis, use of standard GvHD prophylaxis with CsA and MTX for low risk and addition of steroids in heavily transfused patients and children.[5,11] Our results suggest that age over 14 years was associated with a high incidence of aGvHD in agreement to other studies. We found a higher incidence of aGvHD in female patients and no effect of female donor sex on aGvHD. Przepiorka et al and Schmitz et al noted a trend for increasing risk of acute GVHD with increasing age of patient, but it was not significant in either analysis.[5,9] Another interesting finding of ours is that female recipients (64.2%) encountered aGvHD compared to males (15%). In our evaluation, primary disease had significant correlation with aGvHD. Most of our thalassaemic patients were above the age of 5 years, poorly iron chelated and had hepatomegaly. High risk patients fell in Lucarelli class 2 or 3 who are more likely to develop graft rejection and aGvHD.[12] Most of our leukaemia cases were transplanted in relapse setting or they were having accelerated or blast phase of Chronic Myeloid Leukaemia (CML). These groups of patients are well known to have higher immune mediated complications and higher treatment related mortality.[13] With improvement in GvHD prophylaxis, however, others have also reported that widely accepted demographic risk factors for acute GvHD may no longer apply for HLA-identical marrow transplant recipients. The incidence of chronic GvHD varies between 20 to 60% in most studies.[14] cGvHD was seen in 24% of our cases. In 66% of cases it was continuation of aGvHD. No association of age could be established cGvHD. However leukaemia cases developed significantly more cGvHD than patients transplanted for benign disorders as has been reported in the literature.[14,15]

Regimen related toxicity was similar to that of published data. Complications related to cyclosporine A were significant and associated with morbidity despite its level was in desired therapeutic range. Graft failure and graft rejection rates were also similar to that reported in published data. Febrile neutropenia, spectrum of fungal, viral infections during hospitalization and opportunistic and community acquired infection were as expected in a transplant setting. Important differences from the Western data are high incidence of Malaria, TB and parasitic infestations. Strategies have to be adopted to tackle these unique infections in a country like Pakistan.

Conclusion

Procedure related mortality of 22% was noted in our setting. Five year survival in all patients is estimated to be 63%. Incidence of acute and chronic GvHD was similar to published data. Only primary disease was found to be a significant risk factor for aGvHD. Malignancy, aGvHD, recipients above 14 years of age, female patients and engraftment after 12 days were associated with poor outcome.

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