Original Article

Graft Versus Host Disease in Allogeneic Stem Cell Transplantation - 3 ½ Years Experience

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Abstract

Objective: To evaluate the frequency and outcome of graft versus host disease after allogeneic stem cell transplant in haematological disorders at Armed Forces Bone Marrow Transplant Centre, Rawalpindi from July 2001 to December 2004.

Methods: Eighty-six patients with various haematological disorders namely aplastic anaemia (n=32), b-Thalassaemia (n=25), CML (n=22), ALL (n=3), AML (n=1) Fanconi's anaemia (n=2), and Gaucher's disease (n=1), underwent allogeneic stem cell transplantation. All patients received cyclosoprin, prednisolone and short course of methotrexate as GvHD prophylaxis. The patients who developed acute GvHD > grade-II or chronic extensive GvHD received steroids at a starting dose of 2 mg/kg body weight along with gradual increase in cyclosporine dosage (max dose 12.5 mg/kg).

Results: The overall incidence of acute GvHD grade-II to IV was 44.2% (n=38/86) where as the incidence of chronic extensive GvHD was 14% (n=12/86). Acute GvHD was 68% (n=17/25) in ß-Thalassaemia, 50% (n=11/22) in CML, 50% (n=2/4) in Acute Leukaemias and 25% (n=8/32) in Aplastic Anaemia. Chronic GvHD was 25% (n=1/4) in Acute Leukaemias, 18.8% (n=6/32) in Aplastic Anaemia, 18.2% (n=4/22) in CML and 4% (n=1/25) in ß-Thalassaemia. The overall survival in acute GvHD was 84.2% (n=32) where as the overall survival in chronic GvHD was 50% (n=6). The overall mortality in acute GvHD was 15.8% (n=6) and 50% in chronic GvHD (n=6).

Conclusion: The morbidity and mortality due to severe acute and chronic GvHD remains high despite standard prophylaxis against GvHD. New strategies are needed to prevent and treat GvHD (JPMA 55:423;2005).

Introduction

Allogeneic haemopoietic stem cell transplantation (HSCT) provides significant therapeutic benefits to patients with haematological disorders. Unfortunately, the benefits of the stem cell graft are limited by the significant morbidity and mortality due to graft-versus - host disease (GvHD). The acute form occurs within 100 days from HSCT, whereas chronic form develops beyond day+100.[1] Acute GvHD develops in approximately 30% to 60% of patients.[2] Acute GvHD results from an interaction of donor T lymphocytes with recipient antigens. Variety of lymphokines (TNF-a, IL-1, IL-12, IFN-a) are released, which activate both donor and recipient monouclear cells. These activated cells produce non-specific tissue destruction of target organs especially skin, gut and liver.[3]

Human acute GvHD is a distinctive clinicopathological syndrome featuring disorders of the skin, liver and gut that develop within the first 100 days after marrow transplantation.[4] Patients with acute GvHD are categorised into grades I,II,III and IV as described by Glucksberg-Seattle criteria (GSC)[5] Table 1.

Table 1. Acute Graft-Versus-Host Disease.

Grade	Organ Involvement		
	Skin Rash	Liver Bilirubin (umol/L)	Gut Fluid loss (ml/day)
I	<25% body	26 to 60	500 to 1000
II	25% to 50%	61 to 137	1000 to 1500
III	> 50% body or diffuse erythroderma	138 to 257	>1500
IV	Bullae desquamation	>257	>2500 or ileus

Chronic graft versus host disease GvHD is the most important cause of late transplants related morbidity and mortality.[6] Between 30 and 50% of patients surviving 6 months or longer after an HLA identical sibling transplant develop evidence of chronic GvHD. The major manifestations of chronic GvHD resemble several naturally occurring autoimmune disorders.[7]

Chronic GvHD is currently staged into either limited or chronic extensive disease according to the extent of organ involvement. Limited disease is associated with favorable outcome without systemic therapy, while patients with extensive disease have an unfavorable out come.[8] A number of prognostic factors are associated with poor outcome. These factors include Skin GvHD involving >50% of body surface area, thrombocytopenia (<100000/ul), and progressive onset chronic GvHD. On the basis of these factors, patients are stratified in low risk, intermediate risk, high risk and very high-risk categories.[9] Table 2.

Table 2. Risk Stratification in patients with Chronic GvHD.

1.	**Low risk**	
		No risk factor
2.	**Intermediate risk**	
		> 50% Skin involvement only, OR
		Platelet count < 100000/ul
		And/OR
		Progressive type onset
3.	**High risk**	
		> 50% Skin involvement,
		And
		Platelets <100,000/ul
		OR
		Progressive type onset
4.	**Very high risk**	
		All three risk factors

Since early 1980's cyclosporine has been the cornerstone of most GvHD prophylaxis protocols. Most centers in the United States use one of two approaches, which begin immediately after marrow infusion, either a combination of cyclosporine plus a short course of methotrexate, or cyclosporine plus prednisolone. Cyclosporine is usually given for 3 to 6 months with gradual tapering if GvHD is not present. Exact dosing schedules differ from center to center, including daily duration of infusion (1,4 or 24 hours) and the use of parenteral and oral forms.[10,11] Cyclosporin toxicity remains a major problem in post transplant follow up. There is an obvious need for new immunosuppressive strategies with less toxicity for the prophylaxis and treatment of established GVHD. It is therefore necessary to immediately replace cyclosporin/steroids and methotrexate with other immunosuppressive agents in case of undesirable toxic effects of these agents.[12] Tacrolimus is 100 times more potent than cyclosporin but attention should be paid to its dosage because of narrow therapeutic index. Mycophenolate mofetil is another new immunosuppressant with selective action on activated lymphocytes and has been used in combination regimens, permits a dose reduction of cyclosporin, tacrolimus and corticosteroids without increasing the incidence of graft rejection.[13,14]

Initial treatment for acute GvHD routinely consists of intensifying the dose of corticosteroids and cyclosporin. Furthermore, steroid-resistant (SR) acute GvHD develops in 30-60% of patients, necessitating secondary intervention. Anti thymocyte globulin (ATG) is commonly used as first line therapy in this setting. Chronic GvHD remains a significant cause of late morbidity and mortality following allogenic stem cell transplantation. However, patients with chronic GvHD are very heterogeneous, making evaluation and treatment difficult. Corticosteroids remain the most effective primary treatment of this condition.[15]

In this paper we describe our experience of graft versus host disease in allogeneic stem cell transplantation during 3 ½ years of our transplant programme.

Methods

Between July 2001 and December 2004, a total number of 86 patients with various haematological disorders received allogeneic stem cell transplant from completely HLA matched siblings. The patients were transplanted with unmanipulated bone marrow/peripheral blood stem cell harvest except for depletion of red blood cells in case of ABO blood group major mismatch. Patients with Thalassaemia Major, Gaucher's Disease, Acute and Chronic Leukaemias received myeloablastive conditioning with busulphan and cyclophosphamide (Bu 14-16/Cy 120-200), whereas the patients with Aplastic Anaemia received ALG (45 mg/kg body weightt) and cyclophosphamide (200 mg/kg body weight). The patients with Fanconi Anaemia also received Fludarabine (150/m^2) and dose of cyclophosphamide was reduced to 20 mg/kg body weight.

All patients received post HSCT immunosuppression with Cyclosporin, prednisolone and short course of methotrexate. Cyclosporin (5 mg/kg/day in two divided doses) and prednisolone (0.5 mg/kg/day) was started from Day-2 onward where as short course of methotrexate in a dose of 10 mg/m2 was given on day +1,+3,+6 and +11. The IV dose of cyclosporine was reduced to 3 mg/kg/day from Day+6 and was switched over to oral cyclosporine therapy as the patients condition permitted. The oral dose of

cyclosporine at the time of switch over from IV dose was doubled. Cyclosporin dose was adjusted according to drug therapeutic levels, as well as with renal status of the patient. Trough level of cyclosporine were maintained between 200-300 ng/ml. Cyclosporin was continued for 6 months and then gradually tapered off in next 6 months (total duration 1 year). Prednisolone was gradually tapered off in 90 days. All those patients who developed cyclosporine toxicity were switched over to mycophenolate mofetil (30 mg/kg/day). All thalassaemic patients also received IV immunoglobulin 500 mg/kg on day-1 and then 250 mg/kg on day+8 and day+22 as GvHD prophylaxis.

Acute and chronic GvHD were diagnosed according to standard clinical and histopathological criteria. The tissue biopsies were done in all cases who developed Grade-II-IV aGvHD and chronic local/extensive GvHD. Patients who developed acute GvHD >grade-II or extensive chronic GvHD received methyl prednisolone at a starting dose of 2 mg/kg/day and the cyclosporine dose was increased upto 12.5 mg/kg/day and subsequently adjusted according to the renal status of the patient and therapeutic levels of cyclosporine. Patients with steroid resistant GvHD received IL-2 receptor antibody, and IV human immunoglobulins.

Results

A total of 86 patients with various haematological disorders underwent allogeneic HSCT. These disorders included Aplastic Anaemia (n=32), Thalassaemia Major (n=25), Chronic Myeloid Leukaemia (n=22), Acute Lymphoblastic Leukaemia (n=3), Acute Myeloid Leukaemia (n=1), Fanconi's Aanaemia (n=2) and Gaucher's disease (n=1). The median duration of follow up was 421 days (range 8-1226 days). The median age of recipients was 14 years (ranged 1.4 - 54 years). Median age of donors was 15.5 years (range 2-54 years). Six male recipients were transplanted with an HLA - identical female donor potentially sensitised by pregnancy. All recipients and donors were positive for Cytomegalovirus.

Out of 86 patients who received allogeneic HSCT for various haematological disorders, 38 (44.2%) developed acute GvHD and 12 (14%) developed chronic GvHD. The severity of acute GvHD varied from grade-II to grade-IV, with 22 patients (25.6%) having grade-II GvHD. Grade-III GvHD was encountered in 11 (12.8%) patients and 5 (6.0%) had grade-IV GvHD. The organ involvement and frequency/severity of acute GvHD, Thalassaemia, CML, Aplastic Anaemia and Acute Leukaemias are shown in Table 3.

The incidence of grade-II to IV GvHD was 68% (17/25) in and Thalassaemia Major, 50% (11/22) in CML, 25% (8/32) in Aplastic Anaemia and 50% (2/4) in Acute Leukaemias. The overall incidence of chronic extensive GvHD was 14% (12/86). Chronic GvHD was 4% (1/25) in

Table 3. Organ Involvement in Graft Versus Host Disease.

	Acute GvHD 44.2% (n=38)			Chronic extensive GvHD 14% (n=12)	
	Grade-II 25.6% (n=22)	**Grade-III** 12.8% (n=11)	**Grade-IV** 6% (n=5)		
ß -Thal				**ß-Thal**	**1**
Skin	6	3	1		
Intestine	2	2			
Liver		2	1		
CML				**CML**	**4**
Skin	3	4			
Intestine	1		2		
Liver	1				
Aplastic Anaemia				**Aplastic anaemia**	**6**
Skin	5		1		
Intestine	2				
Liver					
Acute Leukaemia				**Acute Leukaemia (ALL)**	**1**
Skin	2 (ALL)				
Intestine					
Liver					

Thalassaemia Major, 18% (4/22) in CML, 19% (6/32) in Aplastic Anaemia and 25% (1/4) in Acute Leukaemias. None of the patients who were transplanted for Fanconis Anaemia and Gauchers Disease developed acute or chronic GvHD.

Twelve patients (14%) of acute (n=6) and chronic GvHD (n=6) died directly or indirectly due to infective complications associated with GvHD. The common infective complications were CMV infection (n=3), disseminated aspergillosis (n=1), hepatitis B viral infection (n=1), Pseudomonas septicaemia (n=1) and Clostridium difficile colitis (n=1).

Three patients with grade-III acute GvHD developed CMV infection (n=2) and pseudomonas septicaemia (n=1) in early post transplant phase (20-30 days). One patient with acute GvHD grade-II, developed chronic GvHD as a sequelae and also acquired disseminated fungal infection (Aspergillosis) at 8 months post transplant. One case with acute GvHD Grade-III also developed chronic GvHD of skin and liver which later was complicated by CMV pneumonia at 7 months post transplant. Two patients had acute grade-IV GvHD of the intestine. Out of these two, one patient also developed Clostridium difficile infection 2 months post transplant whereas in the other patient GvHD progressed into chronic phase and he developed fatal Hepatitis B viral infection six months post transplant. Two patients had fatal acute grade-IV GvHD of skin and liver. Three patients developed extensive chronic GvHD involving skin and lungs at 6 months, 8 months, and 1 year post transplant.

The overall survival in patients with acute GvHD was 84.2% and 50% in patients with chronic GvHD (Table-4). The maximum follow up period among surviving patients without chronic GvHD at the time of analysis was two years.

Table 4. GvHD - Incidence and Outcome.

Total No. of Patients		**86**
	Acute GvHD	**Chronic GvHD**
Incidence	44.2% (n=38)	14% (n=12)
Survival	84.2% (n=32)	50% (n=6)
Mortality	15.8% (n=6)	50% (n=6)
	Causes of mortality	
Direct		**Direct**
GvHD Grade-IV intestine (n=2)		Extensive chronic GvHD (n=3)
Complicated by infections		**Complicated by infections**
CMV Infection (n=2)		CMV Infection (n=1)
Pseudomonas septicemia (n=1)		Disseminated aspergillosis (n=1)
Clostridium difficile infection (n=1)		Fulminant Hepatitis (n=1)

Discussion

Allogeneic stem cell transplantation (SCT) has significant therapeutic benefit for many patients with haematopoeitic disorders. Unfortunately the benefits of SCT are limited by significant morbidity and mortality related to graft versus host disease. Acute GvHD remains one of the major complications of allogeneic SCT and a major determinant of outcome. Two basic approaches for GvHD prophylaxis after stem cell transplantation are either treatment of the recipient with pharmacologic agents (Cyclosporine, Prednisolone, Methotrexate, ALG) or in vitro purging of donor T lymphocytes from the marrow. With Cyclosporin therapy, the prevalence of nephropathy can be as high as 75%. Hypertension has been reported to occur in more than 90% of allo-BMT recipients receiving cyclosporin for prevention of GVHD and neurotoxicity has been reported to occur in 3-5% of such patients. Moreover long-term use of glucocorticoid increases the risk of hyperglycaemia, infections, vascular necrosis of bone and osteoporosis. The therapeutic index of methotrexate is extremely low and occurrence of hyperbilirubinemia, bone marrow suppression and mucositis is common. Therefore these agents should be immediately replaced with other immunosuppressive agents with less undesirable toxic effects.[12]

Despite state of art prophylaxis, acute GvHD develops in about 50% of all HLA identical transplants. When the donor and recipient are unrelated or histo incompatible, the incidence of acute GvHD is much higher (40% to 90%) and in nearly 100% of non identical transplants.[16]

In a prospective randomized trial, methotrexate combined with prednisolone was compared to a combination of cyclosporin and prednisolone. The incidence of acute GvHD (grade-II-IV) was 28% in patients treated with cyclosporin and prednisolone as compared to 47% of those who received methotrexate and prednisolone.[17] In our study overall incidence of grade-II-IV acute GvHD was 44.2%. However the incidence was high in patients with ß-Thalassaemia (68%).

This was most likely related to frequent blood transfusions from the relatives after the diagnosis of disease. However the incidence was quite low in aplastic anaemia (25%). The low incidence of acute GvHD in aplastic anaemia seems to be related to younger age of the patients and early transplant after diagnosis (within 3-6 month).

Several immunosuppressive agents have been used to treat established acute GvHD. Among agents used are high doses of steroids, cyclosporin, mycophenolate mofetil, tacrolimus, ATG, various types of monoclonal antibodies against T cells, psoralen with ultraviolet light (PUVA), IL-2 receptor antibodies and thalidomide.[14] Antithymocyte globulin is accepted as a treatment option for steroid refractory acute GvHD and improvement have been observed in 30% of patients with steroid resistant GvHD. MMF has been used as salvage therapy with 29% complete or partial response in patients with grade-III-IV acute GvHD resistant to steroids and ATG.[12,15] In our patients we used high dose steroid therapy and interleukin-II receptor antibodies to treat aGvHD.

The transplant related mortality, defined as death without relapse has been found to be significantly high in patients with acute GvHD (grade-II or more). The morbidity and mortality associated with acute GvHD correlate with the severity of the organ involvement. The mortality as a direct or indirect consequence of acute GvHD may be as high as 50%. EBMT working party analysis shows 25% mortality in patients with grade 0-I acute GvHD. The mortality related to Grade-II-IV GvHD ranged from 65% to 93%. Old age of the patient and HLA mismatch transplant increase the risk of mortality.[18] In our study mortality related to acute GvHD (grade-II-IV) was 15.8%. The low mortality rate in our study was related to young age of the patients who received allograft from young sibling donors and low incidence of grade-IV acute GvHD.

Chronic GvHD develops in 35 to 50% of long-term survivors of HLA identical transplants. The incidence of chronic GvHD is 49% in HLA-non identical related transplants and 64% in unrelated transplants. The median time of onset of chronic GvHD is 3-4 months, but typical clinical manifestations can occur as early as 70 days or as late as 15 months post transplant.[19] Its incidence reported by Kausu et al is 39% whereas Wagner et al and Lougran et al have reported 40% and 69% incidence of chronic GvHD respectively.[20] In our study the overall incidence of chronic GvHD was 14%. However the incidence of chronic GvHD in our study was higher in Leukaemias (18.2%) and aplastic anaemia (20%) as compared to ß-Thalassaemia (4%). The low incidence of chronic GvHD in our series seems to be related to low

incidence of severe acute GvHD (Grade-III and Grade-IV).

The incidence of transplant related mortality due to chronic GvHD depends upon the progressive type, onset, extensive stage of GvHD and thrombocytopenia (<100x109/L).[6] The accumulative incidence of transplant related mortality due to chronic GvHD has been reported to be 7-8%.[20] Prolonged immunosuppression to treat severe chronic GvHD results in a potential increase in the risk of opportunistic fatal infection.[21,22] In our study mortality related to chronic GvHD was 50%. The major causes of mortality were either direct chronic extensive GvHD or indirectly complicated by fatal infections resulting from prolonged immunosuppression. One patient developed steroid resistant chronic GvHD which even did not respond to interleukin-II receptor antibody and human intravenous immunoglobulin therapy.

A recent analysis of 4174 HLA identical sibling transplants showed that early and long term outcome is influenced by severity of acute GvHD and at 3 years survival was 74, 64, 37 and 10% respectively for patients with grade-I, II, III and IV acute GvHD respectively.[23] The overall survival in acute GvHD was higher (84.2%) in our patients because of the fact that only two patients had grade-IV acute GvHD. Whereas the low overall survival of 50% in our patients with chronic GvHD was because of prolonged immuosuppression resulting in fatal infections.

Conclusion

The incidence of chronic GvHD was lower in this study, however the incidence of acute GvHD was similar to other studies. Whereas the mortality rate was low in acute GvHD as compared to transplant related mortality associated with chronic GvHD. The high mortality due to chronic GvHD was because of prolonged immunosuppression resulting in fatal infective complications. More effective approaches are needed to prevent GvHD after allogeneic haematopoitic stem cell transplantation.

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