Improving Outcome of Aplastic Anaemia with HLA-Matched Sibling Donor Hematopoietic Stem Cell Transplantation: An Experience of Gujarat Cancer and Research Institute (GCRI)

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Received: 5 August 2013 / Accepted: 21 April 2014 / Published online: 8 May 2014
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Abstract Fifteen patients, with a median age of 19 years having severe aplastic anaemia (SAA) underwent human leucocyte antigen (HLA) identical sibling donor hematopoietic stem cell transplantation (HSCT) using conditioning regimens containing cyclophosphamide with antithymocyte globulin (ATG) or a combination of fludarabine and cyclophosphamide with or without ATG during December 2007 to May 2013. Cyclosporine and mini methotrexate were used as graft versus host disease (GVHD) prophylaxis. Graft source included peripheral blood stem cells in 11, bone marrow in 3 and both in 1. One patient had primary graft failure while 14 patients were engrafted with a median neutrophil and platelet engraftment time of 13.5 days. One patient had secondary graft rejection. Acute GVHD occurred in 3 patients and chronic GVHD in 4. One year death rate in engrafted patients was 14.28 %. At a mean follow-up of 21.2 months, 12 (80 %) are alive and well. One of the donors was a patient of haemophilia but the disease did not occur in the recipient. The graft was successful and the recipient is alive till date.

Keywords Aplastic anaemia · HLA-matched sibling-donor · Allogeneic bone marrow transplantation · Peripheral blood stem cell transplantation · Gujarat

Introduction

Staging of aplastic anemia is according to criteria of the International Aplastic Anemia Study Group, as follows:

- Blood—Neutrophils $<0.5 \times 10^9/L$; platelets $<20 \times 10^9/L$; reticulocytes $<1\%$ corrected (percentage of actual hematocrit [Hct] to normal Hct)
- Marrow—Severe hypocellularity; Moderate hypocellularity, with hematopoietic cells representing $<30\%$ of residual cells

Severe aplasia is defined as those including any 2 or 3 peripheral blood criteria and either marrow criterion.

Very severe aplastic anemia (VSAA) i.e. the individuals with neutrophil counts lower than $0.2 \times 10^9/L$ are less likely than others to respond to immunosuppressive therapy.

Human leucocyte antigen (HLA) matched sibling donor transplantation is therapy of choice in young patients of severe aplastic anaemia (SAA). Gujarat cancer research institute (GCRI) is one of the major hematopoietic stem cell transplant (HSCT) centres in western part of India.

Bone marrow transplantation (BMT) and immunosuppressive therapy are the main therapeutic modalities currently used to treat both children and adults, with success rates ranging from 60 to 80 %. The choice between immunosuppression or BMT is largely based on the availability of a sibling donor and on age.

For the non-responder with a matched sibling, immunosuppressive therapy delays transplantation and exposes the patient to risks of transfusions, including allosensitization and iron overload, prolonged neutropenia and infections and possibly poor performance scores. Thus early HSCT is justified in SAA.

Patients and Methods

All patients who underwent HLA identical allogeneic HSCT for aplastic anaemia at the GCRI between December 2007 and

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May 2013 were included in this study. All patients had a six antigen HLA matched sibling or family donor. Written informed consent was obtained from all patients before HSCT. Table 1 shows characteristics of patients. Table 2 shows their distribution according to sex.

High-Risk Patients

High risk patients were identified as those with any of the following risk factors—(a) Active infection (bacterial or fungal) at the time of HSCT; (b) Failed previous immunosuppressive therapy; (c) Duration disease ≥ 6 months. This is institutional classification followed at GCRI. Table 3 shows high risk patient stratification.

Transplant

All patients were nursed in HEPA-filtered rooms. The conditioning regimen used was cyclophosphamide with antithymocyte globulin (ATG) or a combination of Fludarabine and Cyclophosphamide with or without ATG. Table 4 shows the dose and duration of agents used in conditioning regimen.

Graft Source

Bone marrow was used in first 3 patients. Peripheral blood stem cells was the graft source in 11 patients. In one patient

a source combination of bone marrow and peripheral blood stem cells was used because bone marrow did not yield required amount of cells.

End Points

The parameters studied were engraftment, survival, graft failure, acute/chronic GVHD and relapse.

Engraftment

The date of neutrophil recovery was scored as the first of 3 consecutive days with ANC $0.5 \times 10^9/L$ or greater and unsupported platelet count $>20,000$ on 3 consecutive days.

Table 3 High risk stratification of patients

Duration of disease ≥ 6 months	6
Infection at the time of HSCT	1
Failure on ATG	3 (all 3 patients also had duration of disease ≥ 6 months)
Total	7
Deaths	3/7 (42.86 %)
Overall survival	4/7 (57.14 %)

Table 1 Patient characteristics

	Mean	Range	Median
Age in years	22.33	5–46	19
Previous transfusions	24.5	1–192	8
Diagnosis to HSCT interval in months	17.43	0.5–96	4
Mononuclear cell volume	5.09×10^8	3.77–5.5	5.1
CD34 % ($N = 9$)	0.6 %	0.27–0.76 %	0.63 %
Donor age	15	7–45	19
Neutrophil engraftment time in days	14.7	10–125	13.5
Platelet engraftment time in days	21.78	10–125	13.5
RBC units required till discharge	4.5	1–10	4
SDP units required till discharge	8.6	2.5–25	6.75
Hospitalisation duration after stem cell infusion	30	14–114	24

Table 2 Outcome of males and females

Variable	Males	Females
Death	3/12	0/3
Median survival in months till date	9.5	14
GVHD	5/12 (41 %)	2/3 (66.66 %)
Median neutrophil engraftment time in days	13 (+1 primary rejection)	14
Median platelet engraftment time in days	13	14

Table 4 Conditioning regimens, patient characteristics with incidences of GVHD, graft rejection, death and survival

		Median neutrophil engraftment time in days	Median platelet engraftment time in days	GVHD	Graft rejections	Outcome	Median follow up in months
ATG + C	Cyclophosphamide: 200 mg/kg divided in 4 days Thymoglobulin : 2.5 mg/kg/ day × 3 days	17	17	1 Acute GVHD	1 Primary rejection	1 Male (33.3 %) death, rest 2 (66.6 %) alive till date	49
ATG + C + F	Fludarabine : 30 mg/ m ² × 5 days cyclophosphamide: 120 mg/kg divided in 3 days Thymoglobulin :2.5 mg/kg/ day × 3 days	15	15	1 Acute GVHD	1 Secondary graft rejection	1 Male(33.3 %) death, rest 2(66.6 %) alive till date	24
C + F	Fludarabine : 30 mg/ m ² × 6 days cyclophosphamide: 120 mg/kg divided in 3 days	13	12	1 Acute, 4 chronic GVHD		1 Male (11.1 %) death, rest 8 (88.8 %) alive	9

Chimerism Analysis

Chimerism analysis was done using VNTRs when donor and recipient had same sex. It was done at post-HSCT day 30(day+30). In patients who had a sex mismatched transplant, chimerism was assessed with FISH for X and Y chromosomes. Repeat chimerism analysis was carried out after 1 month if initial chimerism showed a mixed picture. Split chimerism analysis was not done.

Graft Failure

Graft failure was scored as primary failure if ANC was $<0.5 \times 10^9/L$ on day 28 or secondary failure if there was neutrophil recovery followed by an otherwise unexplained fall to $<0.5 \times 10^9/L$ for 3 or more days.

Antimicrobial Prophylaxis

No antibacterial or antifungal prophylaxis was used. Acyclovir was started on day +1 for herpes prophylaxis. Sulfamethoxazole/Trimethoprim for prophylaxis against pneumocystis carinii was started once the platelet count was $>50,000/cmm$. PCR for CMV infection was checked serially starting from day +1, every week till 4 weeks followed by every 15 days. Prophylaxis with ganciclovir was given in 3 patients (day –8 to day –1). CMV infected patients were treated with ganciclovir 5 mcg/kg every 12

hourly for 21 days, followed by daily 5 days a week for 2 weeks.

GVHD Prophylaxis

GVHD prophylaxis consisted of cyclosporine (CSA) in combination with short course methotrexate (MTX). CSA was started at a dose of 5 mg/kg/day intravenously in two divided doses on day –1, and changed to the oral route once the counts had improved and the patient was able to take oral medication. Cyclosporin dose was adjusted according to drug therapeutic levels, as well as with renal status of the patient. Trough level of cyclosporine were tried to maintain at the lower level in therapeutic range to avoid hypertension and drug toxicity (200–250 ng/ml) without affecting efficacy. CSA was continued at full doses for a period of 12 months and then tapered (in the absence of GVHD) over a period of 6 months. Intravenous MTX was given initially at a dose of 15 mg/m² on day +1, and 10 mg/m² on day +3 and day +6 followed by folinic acid rescue 24 h later. Individual doses of methotrexate were omitted in the presence of severe mucositis, bleeding or jaundice. GVHD was diagnosed on clinical grounds and in case of doubt biopsy was performed. Treatment was initiated with either intravenous dexamethasone or methylprednisolone, which was converted into oral steroids once the symptoms of GVHD had improved. Chronic GVHD was treated with steroids and MMF.

Statistical Analysis

Descriptive variables were analysed using mean, median, range. Disease -free survival (DFS) was calculated as survival in the absence of death or relapse. Overall survival (OS) included all patients who were alive on the last evaluation. The probability of OS were estimated using Cox proportional hazards regression analysis.

GVHD

- Acute GVHD—disease presenting within the first 100 days of hematopoietic cell transplantation (HCT) with rash, jaundice, diarrhoea or fever.
- Chronic GVHD—onsets of the disease after first 100 days with scleroderma like presentation.
- Overlap syndrome—in which diagnostic or distinctive features of chronic GVHD and acute GVHD appear together.

Results

There were 15 patients (12 male and 3 female) including 4 Paediatric (age <14) patients, with a median age of 19 years (range: 5–46). Table 1 shows the characteristics of all treated patients. Median time from diagnosis to HSCT was 4 months (range: 0.5–96) while median number of transfusions before HSCT was 8 (range 1–192).

Table 3 shows that at the time of HSCT seven patients (46.66 %) were considered as high risk. Risk factors included active infection in 1, duration of disease ≥ 6 months and hence more transfusions in 6, failed immunosuppression (ATG or CSA) in 3 and no one had a second BMT. All three deaths were in this subgroup. There were 3 (42.86 %) deaths and 57.14 % overall survival at the time of analysis in high risk patients. All three deaths were in high risk males. Both the females in high risk group are alive and well.

Engraftment

The graft source was peripheral blood stem cells (PBSC) in 11 (73.33 %) patients and bone marrow in 3 (20 %). One had combined source of both bone marrow and PBSC as bone marrow did not yield the required amount of cells. The median cell dose was 5.1×10^8 total nucleated cells/kg or MNC/Kg (range: 3.77–5.5). CD 34 count was available only in 9 patients and in percentages. Mean CD34 was 0.6 %. It is equivalent to $>2 \times 10^6$ /kg.

Fourteen (93.33 %) engrafted and one patient (6.66 %) had primary graft rejection. The median time to ANC $500/\text{mm}^3$ was 13.5 days (range: 10–20), PLC unsupported

20,000/mm³ was 13.5 days (range: 10–125). Post-transplant Chimerism data were available for 14 evaluable patients. The day + 30 chimerism showed 100 % donor chimerism in 11 patients (73.33 %), two patients showed 99 % donor chimerism while 1 female showed mixed chimerism (95 % XY). This female who showed mixed chimerism on day + 30 later got 100 % donor chimerism. But at a follow up of 5 years she was again noticed to have 100 % recipient chimerism with normal blood counts. This patient was not counted in rejection because she maintained the cure of aplastic anemia.

Cyclosporine Prophylaxis

Cyclosporine was started in injectable form in 14 patients and in oral form in 1. After a median duration of 13 days all patients receiving injectable cyclosporine also were shifted to oral cyclosporine except one who was shifted to oral Tacrolimus. Two patients receiving cyclosporine had cyclosporine toxicity hence were shifted to mycophenolate mofetil (MMF) before discharge.

GVHD

No patient developed veno-occlusive disease of the liver. Cyclosporine in combination with short course MTX was used in all 15 patients (100 %) was used for GVHD prophylaxis. Of the 14 patients who were evaluable for GVHD, 3 (29 %) developed acute GVHD. All had grade II GVHD. All patients responded to a combination of cyclosporine and steroids. One patient later expired owing secondary graft rejection and infection.

Four patients (28.57 %) developed limited chronic GVHD. All improved with a combination of steroids and MMF.

Of the 3 patients receiving bone marrow source cells 1 had acute graft versus host disease (GVHD) which was manageable with treatment, 1 had primary graft rejection, hence died due to infection and the 3rd patient is uneventfully well. Of the 11 patients having PBSC source, 2 had acute GVHD. Out of these 1 later died due to secondary graft rejection and infection. 4 had chronic GVHD. Of these 1 died due to orbital cellulitis. One patient whose graft source was from both bone marrow and PBSC did not develop any GVHD and is uneventful till date.

Survival and Deaths

Hundred day transplant-related mortality was nil (0 %), 6 month mortality was 2/15 (13 %) and 1 year mortality was 3/15 (20 %). At a median follow-up of 14 (range 3–65) months, 12 (80 %) are alive and well. Among the 9 patients who did not have any high-risk features at the time of HSCT, the overall survival was 100 %.

Fig. 1 Event free survival plotted against time in months from HSCT

Fig. 2 Overall survival plotted against time in months from HSCT

We compared engraftment time with different conditioning regimens (Table 4). It was higher with high dose cyclophosphamide (17 days) and reduced to up to 12 to 13 days with use of fludarabine with reduced dose of cyclophosphamide.

All 4 (100 %) paediatric patients are alive and disease free at the time of evaluation while only 8 of 11 (72.72 %) adults are alive and disease free.

Three deaths were reported within first year. One year death rate was 20 %. Cause of death was infection in all.

Figures 1, 2 represents the graph showing Kaplan–Meier analysis of event free survival and overall survival of patients undergoing HSCT for aplastic anemia versus time. The trend indicated that at 3 years both the event free survival and overall survival plateau to 60 %. The events being graft rejection with recurrence or persistence of aplastic anemia and severe GVHD or septicaemia leading to death.

Infections and Secondary Cancers

Infections reported in first month were bacterial ($n = 2$, 13 %) and fungal ($n = 1$, 7 %). During 1–6 months CMV

($n = 6$, 40 % cases) was most common. All 6 were treated with ganciclovir. Three patients were given prophylactic ganciclovir support along with conditioning regimen. One of them still had CMV infection. One patient had BK polyoma virus infection and 2 had septicemic deaths. One patient had death due to orbital cellulitis at 10th month due to fungal aetiology. No secondary cancers were noted in the study duration.

Discussion

Though immunosuppressive therapy is most commonly used therapy for SAA, allogeneic HSCT is treatment of choice in patients with age <40 years age when sibling donor is available as it is curative [1].

We have analysed the data of 15 consecutive patients undergoing matched sibling donor HSCT at our institution and their follow up till the time of analysis. At a median follow up of 14 months overall survival was 80 %. In this the event free survival was calculated by counting the events of graft rejection with recurrence or persistence of aplastic anemia (1/15) and severe GVHD (1/15) or

Table 5 Summary of idiopathic SAA patients undergoing matched sibling donor H5C1 at GCRI from 2007 to 10 June 2013 with their outcomes OI date

Age years/ sex	Graft source	Conditioning regimen	Date of transplant	Successful engraftment	GVHD acute/chronic	Secondary- graft rejection	Infections during month			Final status: till date and specific issue if any
							0–1	1–6	>6	
18/F	BM	ATG + C	19/12/ 2007	Y	Acute GVHD-skin, liver	N	Bacterial pneumonia	CMV		Alive & well
35/M	PBSC	ATG + C	02/05/ 2009	Y	N	N	Mucour			Alive & well
28/M	BM	ATG + C	12/10/ 2009	N	Primary graft rejection	NA		Septicemia		Death due to -septicemia on <i>P</i> 114
27/M	PBSC	ATG + C + F	19/03/ 2010	Y	N	N				Alive & well
15/M	PBSC	ATG + C + F	18/05/ 2011	Y	Acute GVHD, Secondary graft rejection–no response to DLI	Y		CMV, septicemia		Death due to septicemia on <i>P</i> 123
13/M	BM	ATG + C + F	28/05/ 2011		N	N				Alive & well
19/M	PBSC	C + F	11/07/ 2011	Y	Chronic GVHD–skin, eye	N		CMV		Alive & well
41/M	PBSC	C + F	16/02/ 2012	Y	Chronic GVHD–skin, GIT	N	Bacterial pneumonia	BK polyoma virus		Alive & well
12/F	PBSC	C + F	13/04/ 2012	Y	Acute GVHD–skin, eye	N		CMV		Alive & well, delayed platelet recovery- up to-60 days
16/M	PBSC	C + F	07/07/ 2012	Y	Chronic GVHD skin, liver	N		CMV	Orbital cellulitis, fungal etiology	Death due to orbital cellulitis due to fungal etiology on <i>P</i> 305.
10/M	PBSC	C + F	21/08/ 2012	Y	N	N				Alive & well, Red cell aplasia- responded to IVIG, steroids
46/M	PBSC	C + F	30/08/ 2012	Y	Chronic GVHD GIT, liver	N		CMV		Alive & well
19/F	PBSC	C + F	12/09/ 2012	Y	N	N				Alive & well
5/M	PBSC	C + F	06/02/ 2013	Y	N	N				Alive & well, unexplained anemia in 1st month - responded to steroids
31/M	Bm + PBSC	C + F	11/03/ 2013	Y	N	N				Alive & well

SAA severe aplastic anemia, HSCT hematopoietic stem cell transplantation, F female, M male, BM bone marrow, PBSC peripheral blood stem cells, ATG antithymocyte globulin, C cyclophosphamide, F fludarabine, Y yes, N no, GVHD graft versus host disease, DLI donor lymphocyte infusion, GIT gastrointestinal tract, NA not applicable, CMV cytomegalovirus, P post stem cell infusion day, IVIG intravenous immunoglobulin

septicaemia (1/15) leading to death. The event free survival was 80 %. In adult patients (age >14) the overall survival and event free survival was 75 % (9/12). Four patients (100 %) who were in paediatric age group (<14 year) and one patient (100 %) who was >40 years are successfully engrafted and alive till date without any accountable event (OS and EFS 100 %). This is in accordance with the best outcomes reported worldwide [1].

Major mortality was documented in first year and mortality decreased later. Similar pattern of mortality was noticed in other studies [2].

One of the major obstacles of BMT in aplastic anaemia is graft rejection. We experienced graft rejection rate of 13 %. This was positively correlated with the high risk features. All 3 deaths occurred in the age group between 15 and 30 years (28, 15, 16 years). The reported outcomes are good in children and poor as the age advances [1]. Though all deaths were in male sex, there was unequal sex distribution in the study sample (male 12 vs female 3).

High risk males had 60 % (3/5) deaths at the time of analysis and all deaths were in 100 days to 1 year interval. Out of the two males who are alive and well, one has completed 22 months of follow up while second has completed 4 months. Out of two high risk females who are alive and well, one has completed 65 months of follow up while another has completed 14 months.

Table 3 shows that females had better outcome than males in survival but there is unequal distribution of sex in study sample. Females had more incidence of GVHD as compared to males. But there was no difference in engraftment time of neutrophils and platelets.

Initially the conditioning regimen consisted of only cyclophosphamide. This regimen has almost 35 % risk of graft rejection [3]. The conditioning regimen as well as transfusion practice was modified subsequently to eliminate graft rejections. The practice of using only irradiated blood products significantly lowered graft rejection [4]. Our institute now uses strictly irradiated blood products for transfusion in the patients who are candidates for HSCT.

A higher stem cell dose, and the incorporation of total body irradiation to cyclophosphamide conditioning, reduced the incidence of graft rejection, but was associated with high transplant-related mortality (TRM) due to more GVHD. We used a median stem cell dose of $5.1 (3.77\text{--}5.5) \times 10^8$ mononuclear cells/kg. Also we did not use total body irradiation [1, 5].

The addition of ATG to cyclophosphamide for conditioning resulted in infrequent graft rejections as well as improved overall survival [6]. We used this regimen for conditioning in 3 patients. Out of these one (33 %) died due to primary graft rejection and septicaemia, other two (66.6 %) are alive and well. Median neutrophil and platelet engraftment time in these

patients was 17 days. They have completed a median follow up of 49 months (Table 4).

Fludarabine- and cyclophosphamide-containing reduced intensity conditioning (RIC) regimens with or without ATG decreased the rejection and improved the results in Indian patients undergoing allogeneic stem cell transplantation for SAA [7]. We used this conditioning regimen in 12 patients and we had similar results. With this advancement we achieved decrease in engraftment time by 2–5 days (12–15 days as compared to 17 days) than high dose cyclophosphamide conditioning regimen (Table 4).

Table 4 represents the improved timing of engraftment as per the conditioning regimens. The median neutrophil and platelet engraftment time in Cyclophosphamide and ATG group was 17 days, which improved to 15 days when reduced intensity conditioning was used with addition of fludarabine to these drugs. The patients who were given fludarabine with cyclophosphamide without ATG also showed the improved median neutrophil engraftment time of 13 days and improved median platelet engraftment time of 12 days. Thus the addition of fludarabine (with or without antithymocyte globulin) to conditioning regimen of reduced dose cyclophosphamide showed 2–5 day decrease in median time of neutrophil and platelet engraftment (Table 4).

GVHD as a complication of BMT is positively correlated with increasing age of the patient. Grafts depleted of T cells decrease the risk of GVHD but may increase the risk of graft rejection. In our experience, out of all 4 paediatric patients (<14 years), only one experienced GVHD. While 6 out of 10 (60 %) engrafted adults had some form of GVHD.

The incorporation of cyclosporine (CSA) along with methotrexate has substantially reduced the incidence of GVHD [8]. We used the same in all these patients. Overall 7 out of 14 patients (50 %) had some form of GVHD.

Table 5 shows the summary of all 15 patients of SAA undergoing HSCT at GCRI.

Conflict of interest None.

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