

Hematopoietic Stem Cell Transplantation in Patients with Severe Acquired Aplastic Anemia: Iranian Experience

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Abstract

Introduction: Severe acquired aplastic anemia (SAA) is a rare disease and matched related hematopoietic stem cell transplantation (HSCT) is the treatment of choice especially in pediatric patients. Immunosuppressive therapy is the alternative treatment in patients who do not have a donor. We retrospectively analyzed patients who received allogeneic HSCT at our institution.

Methods: Between 1991 and 2011, 190 patients received allogeneic HSCT from HLA-matched donors (182 siblings and 8 other relatives). Median age was 20.5 years (range 1 to 50 years). The graft source was peripheral blood stem cells in majority of patients. Conditioning regimen consist with a myeloablative regimen containing cyclophosphamide with or without antithymocyte globulin. For graft-versus-host disease (GvHD) prophylaxis, we used cyclosporine with or without methotrexate at the standard doses.

Results: The median follow-up time was 30 months and 3 year overall survival and disease free survival was 82% and 75% respectively. The median time to neutrophil engraftment and median time to platelet engraftment was 12 day and 15 day respectively. Grade 3 and 4 of aGvHD occurred in 26 (23.7%) patients and chronic GvHD occurred in 46 (29.1%) of survived patients 100 days after HSCT. At time of report 82.1% of patients were alive with normal hematologic parameters. The engraftment failure rate was about 8%. The most common cause of death was GvHD.

Conclusions: However an available treatment in SAA is immunosuppressive therapy, HSCT should be seriously considered as a therapeutic option particularly if a matched sibling donor is available. The outcome of allogeneic HSCT in patients with SAA at our center was consistent with the result of other previous studies.

Keywords: Severe acquired aplastic anemia, Hematopoietic stem cell transplantation

Introduction

Severe acquired aplastic anemia (SAA) is a rare disease, defined as peripheral blood pancytopenia associated with hypocellularity of the bone marrow.(1) In most cases, bone marrow failure is thought to result from an immune-mediated mechanism which leads to T-cell activation and

release of inhibitory cytokines with subsequent destruction of hematopoietic progenitor cells.(2) The outcome of patients with SAA has improved considerably over the last decades.(3, 4) Today, immunosuppression (IS) and hematopoietic stem cell transplantation (HSCT) are the best established therapeutic options.(4-7) The choice between them

should take into account not only the immediate results but also the late complications of each treatment modality. High response rates and long-term survival have been achieved with IS but some patients require transfusional support or may develop late clonal abnormalities.(8, 9) HSCT is the elective treatment in young patients who have an HLA-identical sibling donor. An increased survival after HSCT in aplastic anemia patients was reported in recent studies.(10, 11) The aim of this study is to retrospectively analyze outcome in aplastic anemia patients who received allogeneic HSCT from human leukocyte antigen (HLA)-identical sibling donors at our center.

Materials and Methods

Patients and their donors: This study report retrospectively of 190 patients diagnosed as severe aplastic anemia who referred to our center and underwent HSCT from 1991 to 2011. Disease diagnosis was made at the referring centers where patients received their therapies. The median Karnofsky score of the whole patients was 90% (range 70 to 100%).

Donors were HLA-A-, -B-, and -DR-identical siblings in 182 patients with good performance on physical examination and normal laboratory data results. Eight patients received HSCT from other related donors (Four patients from parent and 3 patients from aunt or uncle and one patient from grandfather). The sources of progenitors were bone marrow (BMT), peripheral blood stem cell (PBSCT), or both. BMT donors underwent conventional harvest with target nucleated cell yields of 2.0×10^8 /kg recipient body weight from iliac crests of donors under general anesthesia and proper unmanipulated cells, were directly infused to the patients. PBSCT donors were treated with granulocyte colony-stimulating factor (G-CSF) 5 μ g/kg/day subcutaneously for 4 days as mobilizing. No T-cell depletion or other manipulation was carried out.

Conditioning Regimens and GvHD prophylaxis:

All patients received the conditioning regimen according to usual protocol. 173 patients conditioned by cyclophosphamide 50 mg/kg/day once daily IV, on days -5 till -2 for PBSCT or -4 till -1 for BMT plus Anti-thymocyte globulin (ATG: Thymoglobulin, Genzyme) 10 mg/kg/IV on days -5 till -3 for PBSCT or -4 till -2 for BMT and 17 other patients received cyclophosphamide alone. Patients who underwent PBSCT give rest one day before transplantation. Prophylaxis for graft versus host disease (GvHD) consist cyclosporine (1.5 mg/kg

intravenously from -3, then 3mg/kg/day intravenously from +7 in PBSCT and +11 in BMT then 12.5 mg/kg/day per oral) alone or plus a short course of methotrexate (10 mg/m² on Day +1 and 6 mg/m² on Days +3, +6, and +11). Cyclosporine started three days prior to transplantation and continued orally for 6 to 7 months post HSCT and it was discontinued in the absence of GvHD.

Post-transplantation supportive measures: All patients were hospitalized in isolated room with positive HEPA system. Nutritional support was provided by hyperalimentation.

Acyclovir prophylaxis was given before transplantation.

Trimethoprim/sulfamethoxazole and fluconazole were used to decontaminate the gastrointestinal tract and provide anti pneumocystis jiroveci prophylaxis.

Blood component therapy was performed after gamma-irradiation according to clinical symptoms and signs to keep the platelet count above $10,000 \times 10^3/\mu$ L and the hemoglobin concentration above 8.0 g/dL.

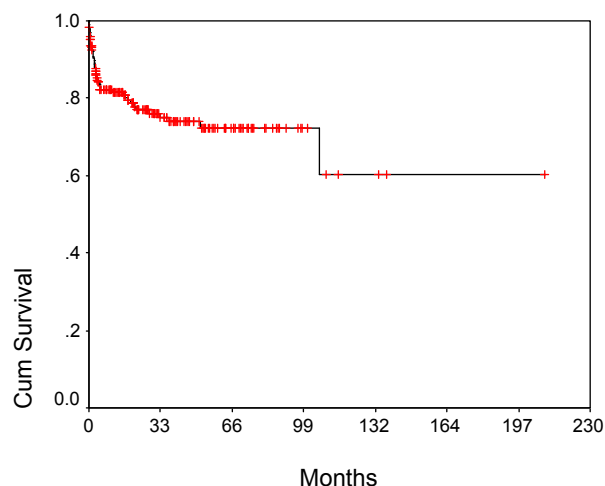


Figure- 1. Three year disease free survival after hematopoietic stem transplantation.

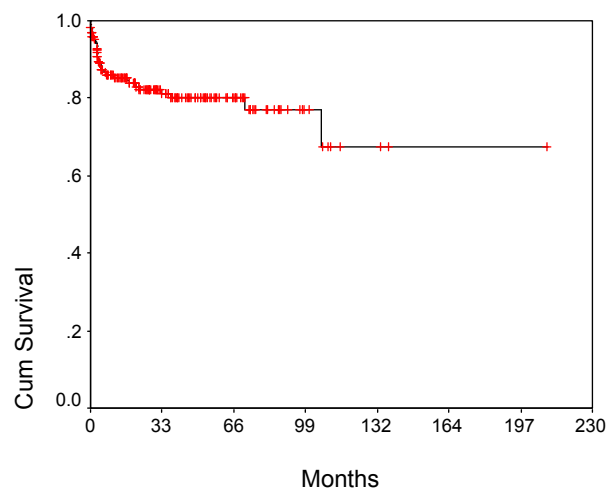


Figure- 2. Three year overall survival after hematopoietic stem transplantation.

Table- 1. Patients characteristics.

Sex:	
Male	129 (67.9%)
Female	61 (32.1%)
Age at transplantation:	
Median	20.5 years
Range	1-50 years
Source of progenitor:	
Bone marrow	50 (26.3%)
PBSC	136 (73.7%)
Donor recipient gender:	
Male to male	84 (44.2%)
Male to female	45 (23.7%)
Female to female	32 (16.4%)
Female to male	29 (15.7%)
Acute GVHD:	
Grade I	33 (35.8%)
Grade II	33 (35.8%)
Grade III	21 (22.8%)
Grade IV	5 (5.6%)
Chronic GVHD:	
Limited	33 (71.7%)
Extensive	13 (28.3%)
Mild	31 (67.4%)
Moderate	13 (28.3%)
Severe	2 (4.3%)

Table- 2. Number of progenitors infused. (N: number of patients that data are available for. n: number of cells. MNC:mono nuclear cells.)

	N	n
WBC $\times 10^8$ /kg (range)	139	9.37 (2.1-18)
MNC $\times 10^8$ /kg (range)	136	7.48 (1.07-10.9)
CD3 $\times 10^6$ /kg (range)	63	267.7 (19-825)
CD34 $\times 10^6$ /kg	63	4.05 (1-62)

All patients tested for cytomegalovirus (CMV) PP65 antigen or CMV DNA PCR were performed twice weekly and positive cases were treated by preemptive therapy for 21 days or until antigen tests became negative.

Patients were followed up routinely in post HSCT clinic after discharge, weekly during first month, every two weeks until day +100 and thereafter per need.

Definitions: The day of neutrophil engraftment was defined as the first of three consecutive days on which the patient's ANC was above $500 \times 10^3/\mu\text{L}$. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above $20,000 \times 10^3/\mu\text{L}$ without the need for platelet transfusion.

Acute and chronic GvHD was assessment and graded according to standard criteria.(12, 13) Chronic GvHD was defined as GvHD present after day 90 in surviving patients and was considered to be progressive when it appeared as a continuation of previous acute GvHD.

Relapse was indicated by becoming transfusion dependent again after transplantation.

The disease free survival (DFS) was defined as the time between HSCT and relapse, death or to the last contact in patients without relapse or death event. Overall survival (OS) was measured from the time of HSCT to the time of death, or last contact in surviving patients.

The main focus of this retrospective study was the outcome of patients, evaluate DFS, OS, frequency of acute and chronic GvHD, time to engraftment, probability of relapse and finally cause of death.

Statistical analysis: Descriptive statistics were frequency and percentages for Categorical variables while Median \pm range was used for continuous variables. The DFS and OS were estimated using the Kaplan-Meier method.

Results

Patients and donors characteristics: Totally 190 patients include 129 male (67.9%) and 61 female (32.1%) assessed in this study who undergoing HSCT during 1991 until 2011. The median age was 20.5 (range 1-50) years at the time of HSCT. Table 1 summarizes the characteristics of our patients and donors. All patients tested for negative Fanconi's Anemia. The median time from diagnosis to HSCT was 1 month (range: 5 month–16 year). All patients were multitransfused before transplantation but the data about number of transfusions prior to HSCT was unavailable. Seventy four patients (39%) were sex-matched with their donors. One hundred and ten (58%) of donors were male and 79 (42%) of them were female.

Infused cells and engraftment: Among 190 patients, 137 of patients underwent PBSC and 50 patients underwent BMSC transplantation and 3 patients received stem cell from both PBSC and BMSC. Patients received cell dose, with median WBC of 9.4×10^8 (range: 2.1- 18×10^8) and MNC of 7.5×10^8 (range: 1- 10.9×10^8) respectively according to standard procedure. Table- 2 summarizes data on number of infused cells in PBSC and BMSC. Median time to granulocyte engraftment was 12 (range: 3-34) days and median day of platelet engraftment was 15 (range: 10-43) days. Fifteen patients had not neutrophil engraftment and 26 patients had not platelet engraftment during 100 days after transplantation. The number of patients who had not both ANC and platelet engraftment was 14 patients.

GvHD: Acute GvHD (aGvHD) occur in 92 of patients (48.5%) which were grade I or II in most cases. The most involved sites were skin. Grade 3

and 4 of aGvHD was seen in 26 patients (23.7%). The cumulative incidence of chronic GvHD (cGvHD) for those who survived more than 100 days was 46 (29.1%) which among them, 33 exceeds 30 months (range: 1 month-10 years). Among patient at the end of follow-up 156 (82.1%) of them were alive with normal hematologic parameters and full donor chimerism. The most common cause of death was GvHD that occurred in 6 patients and the other cause include bacterial and viral sepsis, cytomegalovirus (CMV), interstitial pneumonitis, relapse, organ failure, intracranial hemorrhage and accidental death. Five (2.6%) of recipients suffered a relapse and all of them died. In this study disease-free survival (DFS) at 3 year was 75% (Figure- 1) and overall survival (OS) at 3 year was 82% (Figure- 2).

Discussion

HSCT has been used in the treatment of aplastic anemia since 1970s using cyclophosphamide-based conditioning regimens,(3, 14) and is the choice curative treatment in patients with SAA.(4, 15) First studies reported a survival of 50% in these patients,(16-18) and there was a high incidence of graft failure and GvHD,(19) while recent studies demonstrate a survival of 70- 90%.(10, 11, 20, 21) In the present study we report our good results in patients with SAA who underwent HSCT that was in agreement of previous studies. Although, development in HSCT techniques such as changes in conditioning regimens and early transplantation have improved the outcomes in SAA patients,(22, 23) these improvements may be mainly because of developments in supportive care particularly GvHD prophylaxis.

One of the major problems after transplantations in SAA is graft failure with a rate of approximately 15%.(24) Graft failure rate in our study was about 8%. Locasciulli et al showed an association between improved survival and younger age, transplantation performed after 1996, a matched sibling donor, a short diagnosis-to-transplantation interval, and no irradiation in the preparative regimen.(25)

There is no indication for using total-body-irradiation regimens in HLA-identical sibling HSCT in aplastic anemia patients. TBI-based regimen may increase the risk of acute GvHD and interstitial pneumonitis.(26) We used cyclophosphamide and ATG as preparative regimen and our results were comparable with previous studies that used this conditioning regimen. Storb et al reported a survival of 90% in SAA patients who

patients (21%) had limited and 13 patients (8.2%) had extensive involvement (Table- 1).

Survival and Relapse: The median follow-up time of the patients who remaining alive till this report received cyclophosphamide and ATG as pre-transplantation conditioning regimen.(27) Kahl et al in a study of 81 patients showed a long-term survival of 88% using cyclophosphamide plus ATG as conditioning regimen.(28) Champlin et al found no statistical differences in survival, graft failure, or GvHD between patients who received cyclophosphamide and ATG or cyclophosphamide alone.(29)

HSCT should be seriously considered as a therapeutic option particularly if a matched sibling donor is available. The outcome of allogeneic HSCT in patients with SAA at our center was consistent with the result of other studies.

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