Allogeneic Hematopoietic Stem Cell Transplantation from Related Donors in Fanconi Anemia

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Abstract

Introduction: Allogeneic hematopoietic cell transplantation (HSCT) is the only therapeutic modality capable of correcting the hematologic manifestations of Fanconi Anemia (FA).

The development of well tolerated, immunosuppressive conditioning regimens for FA patients undergoing HSCT has proven to be a rather challenging task for hematologists.

Methods: We analyzed the outcome of 30 FA patients (median age at HSCT was 9 years age range, 2-32 years) who underwent HSCT between 1992 and 2008 in Shariati Hospital Tehran, Iran. . Patients were transplanted from either an HLA-identical sibling or matched relative (n=29), or an HLA-partially matched relative(n=1).

Four different conditioning regimens without radiation were used .Graft versus host disease (GVHD) prevention consisted of cyclosporine with methotrexate or cyclosporine alone.

Results: The median follow-up duration for survivors was 2.7 years (ranged 1 month to 12 years). The median survival time was 8.5 months. The 5-year overall survival was 43.6% (SE=10.0%). All surviving patients had normal blood counts with full donor engraftment.

The median survival rate for patients who did or did not receive fludarabine in preparation for the allograft was not statistically significant (p-value=1.0).

Conclusion: Our study demonstrates that none of the studied variables significantly affected the survival, including sex, age, radiation-free conditioning regimens, corticosteroids before transplant, pre-transplant transfusions, acute GVHD and congenital abnormalities.

The availability of better diagnostic tools to predict clinical course of FA, and modification of the conditioning regimen should improve survival and long-term consequences of therapy for patients in the future.

Keywords: Fanconi anemia, Hematopoietic Stem Cell Transplantation

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Introduction

Fanconi anemia is an autosomal recessive disorder characterized by diverse congenital abnormalities (Growth retardation, Cafe´ au lait spots, Facial features, Thumb, radius or hips abnormality, Other genetic defects), chromosomal instability, progressive bone marrow failure, and increased risk of developing myelodysplasia, acute myeloid leukemia, and various epithelial malignancies.(1, 2)

Fanconi anemia is defined by genomic instability and cellular hypersensitivity to DNA crosslinking agents. This hypersensitivity serves as a diagnostic test (using agents such as diepoxybutane) and allows diagnosis of Fanconi anemia in cases of subtle clinical features or no detectable congenital abnormalities.(3)

The basic understanding and diagnosis of FA patients has been greatly advanced over the past decade with subtyping of FA cell lines into discrete complementation groups, each representing mutation in specific genes. To date, a total of twelve complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M) have been classified with eleven of these genes cloned and characterized.(4, 5)

Hematopoietic stem cell transplantation (HSCT) still represents the only option able to definitively cure the marrow failure associated with this disease, as well as to prevent/treat myeloid malignancies, although it does not prevent the occurrence of solid tumors.(6-8)

Hematologic abnormalities occur in virtually all patients with FA at a median age of 7 years (range,

birth to 41 years).(9, 10) Based on clinical data in the International Fanconi Anemia Registry, the cumulative incidence of bone marrow failure by age 40 years is 90%. Initial hematologic findings are most commonly pancytopenia; however, some patients present with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). The cumulative incidence of MDS or AML by age 40 is 33%.(9,10)

The use of conditioning regimens based on reduced doses of cyclophosphamide, either alone or together with limited field radiotherapy, has cured a large proportion of patients transplanted from an HLA identical sibling.(8, 11)

Results of unrelated donor (UD) HSCT have been less encouraging, mainly due to increased difficulties with engraftment and higher incidence of both acute and chronic graft-versus-host disease (GVHD).(12, 13)

However, even in the HLA identical transplant setting, FA patients remained at increased risk of severe graft-versus-host disease (GVHD) when compared to patients with severe idiopathic aplastic anemia, because the defective DNA repair machinery renders tissues more prone to become a target of the alloreactive aggression leading to GVHD.(14)

We here report the outcome of 30 FA patients transplanted in IRAN from related donors.

Table 1: Patient, donor and transplantation characteristics

Number of patients	30 (100%)
Median patient age at HSCT (range)	9 years (2–32)
Median donor age at HSCT (range)	16 years (3–37)
Patient gender	
Male	19 (63.3 %)
Female	11 (36.7 %)
Donor gender	
Male	18 (60 %)
Female	12 (40 %)
Stem cell source	
Bone marrow	20 (66.7 %)
Peripheral blood	10 (33.3 %)
Type of donor employed	
Sibling, HLA matched	27 (90 %)
Other relative, HLA matched	2 (6.6 %)
Other relative, HLA mismatch	1 (3.3 %)
Status at transplantation	
Aplastic anemia	28(93.3 %)
MDS	2 (6.7 %)
Physical abnormalities	
Yes	19 (63.3 %)
No	11 (36.7 %)
Corticosteroids before transplant	
Yes	15 (50 %)
No	15 (50 %)
Pre-transplant transfusions(blood and/or platelet)	
Yes	20 (66.7 %)
No	10 (33.3 %)

Patients and Methods

Between January 1992 and April 2008, 30 Iranian patients with FA underwent transplant from a family related donor in Shariati Hospital in Tehran, Iran. Details on the patients' and donors' characteristics are reported in Table 1.

Confirmation of the diagnosis of FA was made in all patients by documenting increased chromosomal breakage with diepoxybutane (DEB) and/or mitomycin-C.

Median age at HSCT was 9 years (range, 2-32 years). In all donor-recipient pairs, histocompatibility was determined by serology or low resolution molecular typing for HLA-A and -B antigens and by allelic typing for -DRB1. 27 patients received stem cell from healthy full human leukocyte antigen (HLA) matched siblings, one patient from his father (HLA- matched), one patient from her mother (HLA- matched) and one patient with one antigen mismatch from her father. Bone marrow or peripheral blood stem cells (PBSC) were used as the source of stem cells. Bone marrow was collected by the standard techniques from the donor on the day of infusion. In the PBSC group, donors received 10 μg/kg/day of GCSF for five consecutive days before the stem cell collection.

Patients were hospitalized in single rooms with highly efficient particulate air (HEPA) filters. Acyclovir was given as prophylaxis for herpes simplex and varicella-zoster virus, and trimethropim-sulphametoxazole was given as pneumocystis carinii prophylaxis. Administration of trimethoprim-sulfamethoxazole twice weekly was resumed after recovery from neutropenia as a preventive measure against Pneumocystis carinii infection. Tests for cytomegalovirus (CMV) PP65 antigen or CMV DNA PCR were performed weekly. Positive cases were treated by preemptive therapy for 14–21 days or until antigen tests became negative.

Fluconazole was given to prevent fungal infections. Empiric broad-spectrum antibiotics were given for febrile neutropenia. In cases of persistent fever that did not respond to antibiotic therapy within 5 days, amphotericin was added until the neutropenia resolved. Patients received a regular diet. Additional supportive measures, such as parenteral nutrition and blood component transfusions, were administered as necessary. All transfused blood products were irradiated before infusion.

Table 2:Pretransplantation Conditioning Regimens

Conditioning		Number of Patients (%)
Flu[1]	30 mg/m^2/d × 5d	
Cy[2]	10 mg/kg/d × 2 d	11 (36.7)
hATG[3]	10 mg/kg/d × 4 d	
Oral Bu[4]	0.2 mg/kg/d × 4 d	
Cy	15 mg/kg/d × 4 d	13 (43.3)
hATG	10 mg/kg/d × 3 d	
Oral Bu	0.2 mg/kg/d × 4 d	5 (16.7)
Cy	15 mg/kg/d × 4 d	
Cy	50 mg/kg/d × 4 d	1 (3.3)

[1]Flu= Fludarabine, [2]Cy= Cyclophosphamide, [3]hATG= horse Antithymocytic globulin, [4]Bu= Busulfan

Among the 30 patients who received their graft, four categories of conditioning regimens could be identified. No radiation therapy was given. They are described in Table 2. For Graft-versus host disease (GVHD) prophylaxis 16 patients received cyclosporin alone (53.3%) and 14 patients received cyclosporin and methotrexate at standard doses (46.7%).

Treatment related adverse events experienced by the patients were graded using the National Cancer Institute (NCI), Common Toxicity Criteria (CTC) (NCI, 1998). Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded according to standard criteria.(15, 16) Neutrophil engraftment was defined as a neutrophil count >500 $\times 10^3/\mu L$ for at least 3 consecutive days without growth factor support. Time to platelet engraftment was defined as the first of 3 consecutive days on which the platelet counts were >20'000 ×103/μL without transfusions for 7 days.

Primary graft failure was defined as failure to achieve a neutrophil count >500 $\times 10^3/\mu L$ by day 42. Secondary graft failure was defined as initial engraftment (neutrophils >500 $\times 10^3/\mu L$) and subsequent decline to a neutrophil count <500 ×103/μL. Documented donor engraftment was confirmed by chromosomal analysis for sex-mismatched patient/donor pairs, ABO typing in patients with red blood cell group differences and PCR targeting variable number of tandem repeats (VNTR) loci.

Disease Free Survival (DFS) was defined as the time between transplantation and the earliest occurrence of graft failure or death from any cause. Overall Survival (OS) was measured by the time interval between the date of transplantation and the date of death for any cause or the date of the last follow-up for survivors.

Engraftment, GVHD, and survival were the primary outcomes of interest.

According to some articles that the use of Fludarabine was associated with improved engraftment and survival, we have compared both group of patients (using or not using Fludarabine).

Results

The study included 30 patients undergoing a HSCT for FA .Graft failure occurred in 5 patients; two had primary graft failure, while three children experienced secondary graft failure. All these 5 patients (four given HSCT from an HLA-identical sibling and one from a partially matched father) were died.

Median times to neutrophil and platelet engraftment were 10 days (range: 7-41 days) and 17 days (range: 10-79days), respectively. 1-year disease free survival rate was 75.5% (SE=9.6%). (Figure 1)

Twenty patients (66.7%) developed aGVHD. 13 patients (43.3 %) had involvement of their skin and 12 patients (40 %) had gastorintestinal involvement. Eight of 28 patients (28.5%) that were alive on day +100 developed cGVHD (Table 3). All patients with chronic GVHD had previously acute GVHD.

The median follow-up duration for survivors was 2.7 years (ranged 1 month to 12 years). The median survival time was 8.5 months. The 5-year overall survival was 43.6% (SE=10.0%). 2 of 3 patients, transplanted from another relative (not sibling) died.

The main cause of death was GVHD in 4 cases (13.3%). Other causes of death were: secondary graft failure in 3 cases (10%), primary graft failure in 2 cases (6.7%), and infection in 3 cases (10%). ARDS (adult respiratory distress syndrome), brain hemorrhage, TTP (Thrombotic thrombocytopenic purpura) and lymphoblastic lymphoma were the cause of death in 4 other patients. Causes of death according to conditioning regimens described in Table 4.

The median survival rate for patients who received a fludarabine-containing conditioning regimen was 8.5 months, and 35.2 months in survivors of nonfludarabine conditioning regimens. However, the observed difference in both groups was not statistically significant (p-value=1.0). (Figure 2)

Table 3: Acute and chronic GVHD*

GVHD	Number of Patients (%)
Acute GVHD	20 (66.7)
Grade I	5 (16.7)
Grade II	5 (16.7)
Grade III	6 (20.0)
Grade IV	4 (13.3)
Chronic GVHD	8 (26.7)
Limited	3 (10.0)
Extensive	5 (16.7)

*GVHD=graft-versus-host disease

Table 4: Causes of death according to conditioning regimens

Conditioning regime	No. of patient	No. of death	Cause of death (No. of patient)
Flu * Cy** hATG***	11	6	Primary graft failure (2) secondary graft failure (2) GvHD (1) New malignancy (1)
Oral Bu**** Cy	13	5	GvHD (3) Infection (1) ARDS †(1)
Oral Bu Cy hATG	5	4	Infection (2) Brain hemorrhage (1) TTP ‡ (1)
Cy	1	1	Secondary graft failure (1)
Total	30	16	

* Flu = Fludarabine, ** Cy = Cyclophosphamide, ***hATG = horse Antithymocytic globulin, ****Bu= Busulfan, GVHD=graft-versus-host disease, †ARDS= adult respiratory distress syndrome, ‡ TTP= Thrombotic thrombocytopenic purpura

The occurrence of acute GVHD was statistically higher in recipients of a fludarabine- regimen containing. The probability of acute GVHD after a fludarabine-containing regimen was 45.5% and for nonfludarabine-containing regimen was 26.6% (P=0.06). There were no statistically significant differences in risks of chronic GVHD by conditioning regimen (P=0.36).

Comparing survival rate between male and female and between patients under 12 years and older was not statistically significant (p-value=0.74, p-value=0.34).

The survival rate between patients who did or did not receive Pre-transplant transfusions (blood and/or platelet) was not statistically significant (p-value=0.22).

There was no statistically significant differences in survival rate by receiving corticosteroids before transplant (P=0.51).

Discussion

The development of well tolerated, immunosuppressive preparative regimens for patients undergoing HSCT has proven to be a rather challenging task for hematologists. This is even more relevant in patients with FA whose underlying increased chromosomal fragility makes them extremely susceptible to chemotherapy and radiation toxicity.(17-19) This report summarizes our long-term experience without radiation containing therapies to serve as a baseline for comparison for newer treatments.

Until the early 1990's, survival of FA patients with hematological complications undergoing HSCT was poor due to severe radiation related toxicity and acute GVHD.(17, 20) It has been suggested that the use of irradiation as part of the conditioning regimen is a significant risk factor for secondary malignancy among patients undergoing HSCT for non-Fanconi aplastic anemia. This risk could be higher in patients with FA because of their chromosomal instability and defective DNA repair mechanisms.(21- 23)

Based on concerns regarding the long-term effect of irradiation in FA patients, the transplant teams have eliminated radiation from the preparative regimen. Owing to its immunosuppressive properties, the fact that it is nonmyeloablative,(24-26) and its lessened tendency to cause late malignancies compared to radiation- based regimens,(27-29) Cyclophosphamide (CY) has been a preferred conditioning regimen for patients with aplastic anemia (AA) and FA treated by marrow allografts. Initially, FA patients were conditioned with the typical AA regimen of 200 mg CY/kg,(30); however, it was recognized soon that patients with FA experienced more serious CY-related toxicities than patients with other types of AA,(31,32) probably from their poor ability for DNA repair after exposure to an alkylating agent. Transplant teams, therefore, began CY dose reduction in FA patients undergoing marrow allografts.(11, 20, 33, 34)

Fludarabine monophosphate, a purine analog-inhibiting adenosine deaminase, has been demonstrated to display a potent immune-suppressive effect and has been used successfully before allogeneic HSCT in patients who are not eligible for conventional myeloablative conditioning, because regimens including this drug are well tolerated and have limited extra-medullary toxicity,(35) an advantage in patients with DNA breakage susceptibility. Based on some reports,(36, 37) it seems that Fludarabine -based protocols may be particularly suitable for improving dramatically the outcome of FA patients in need of HSCT, and appeared to be much better tolerated.

We believe that the use of non radiation-containing regimens should be considered for FA patients to lessen the potential risk of secondary malignancy. Our study demonstrates that none of the studied variables significantly affected the survival, including sex, age, radiation-free conditioning regimens, corticosteroids before transplant, pre-transplant transfusions, acute GVHD and congenital abnormalities. The availability of better diagnostic tools to predict clinical course of FA, and modification of the conditioning regimen should

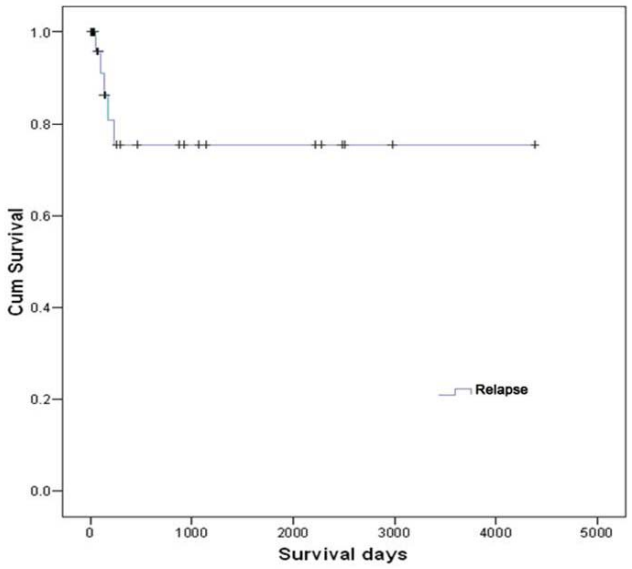

Figure 1: Disease free survival rate of patients

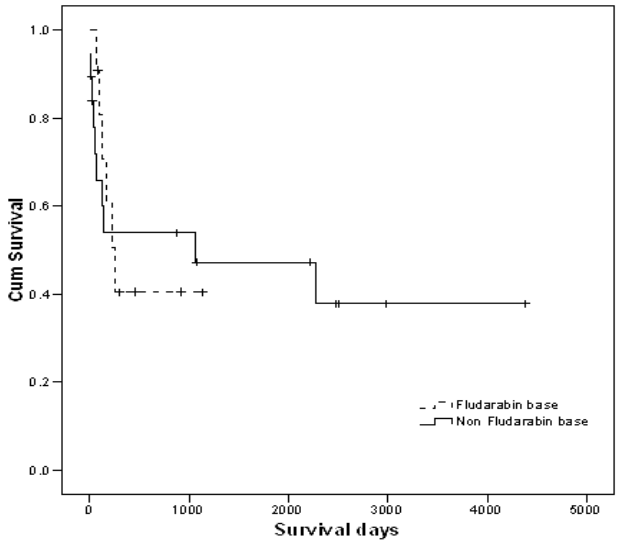

Figure 2: Overall survival rate of patients received fludarabin base or non fludarabin base conditioning regime

improve survival and long-term consequences of therapy for patients in the future.

However, larger cohorts of patients should be evaluated for longer follow-up periods to define the optimal regimen for patients with Fanconi anemia in need of HSCT.

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