

## ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN FANCONI ANEMIA; A SINGLE CENTRE EXPERIENCE

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### ABSTRACT

**Objective:** To determine the treatment outcome of Hematopoietic stem cell transplantation in Fanconi Anemia.

**Study Design:** Case series.

**Place and Duration of Study:** Armed Forces Bone Marrow Transplant Center, Rawalpindi, from Jan 2001 to Jun 2018.

**Methodology:** Data of all Fanconi anemia patients who had fully HLA matched bone marrow transplant during this period was analysed for variables affecting the outcome in terms of overall survival. Those fanconi anemia patients who had myelodysplastic changes or acute myeloid leukemia were excluded.

**Results:** Total 27 patients underwent fully HLA matched allogeneic bone marrow transplant for Fanconi Anemia. Mean age of patients at transplant was  $12.12 \pm 5.16$  years. All patients at transplant were in aplastic phase. Conditioning was done with fludarabine  $120\text{mg}/\text{m}^2$ , ATG  $20\text{ mg}/\text{kg}$  and Cyclophosphamide at a dose of  $20\text{-}40\text{ mg}/\text{kg}$ . Mean time to neutrophil engraftment was  $12.3 \pm 2.92$  days and for platelets  $20 \pm 10.3$  days. Major post-transplant complications were neutropenic fever in 26 (96%), hypertension in 18 (66.6%), mucositis in 12 (44.4%), azotaemia in 8 (29.6%), gut toxicity in 7 (25.9%) and haemorrhagic cystitis in 5 (18.5%) patients. Four patients (14.8%) had acute graft versus host disease while 7 (26%) patients had chronic GVHD. Overall survival at 6 months, 1, 5 and 8 years was 67%, 63%, 59% and 55% respectively. While overall survival in patients transplanted at younger age (<11 years) was 81.8% compared to 37.5% in older age group (>11years) and was statistically significant ( $p$ -value = 0.03).

**Conclusion:** Our study demonstrated survival difference in Fanconi anaemia patients when transplanted at younger age and conditioning with cyclophosphamide  $30\text{ mg}/\text{kg}$ , fludarabine  $120\text{mg}/\text{m}^2$  and thymoglobulin  $10\text{mg}/\text{kg}$  as an acceptable conditioning protocol.

**Keywords:** Fanconi anaemia, Graft versus host disease, Hematopoietic stem cell transplant.

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### INTRODUCTION

Fanconi Anemia is a rare bone marrow failure syndrome characterized by congenital malformations, progressive cytopenias with malignant predisposition<sup>1</sup>. It was first described by Swiss pediatrician Guido Fanconi in 1927. The incidence of FA is around 3 per million with a carrier frequency of 1 in 300<sup>2</sup>. It is reported in all races and ethnic groups with highest incidence in some Spanish populations, with carrier frequency of 1 in 70<sup>3</sup>. Individuals with birth defects are diagnosed at younger ages than those without birth defects. Genetic mutations in specific genes results in Fanconi anemia. Protein produced by

these genes constitutes Fanconi anemia pathway, essential for repair of damaged DNA especially Interstrand Cross Links (ICLs) resulting in build-up of ICLs when FA pathway is non-functional. The ICLs results in abnormal cell death or uncontrolled cell growth particularly in rapidly dividing cells of bone marrow and developing fetus causing congenital malformations, bone marrow failure and pre-disposition to malignancies<sup>4</sup>.

Clinical presentation of FA is heterogeneous. One third patients have no physical features suggestive of FA thus making it mandatory to screen all patients with bone marrow failure. Cardinal physical findings are absent or hypoplastic thumb, microcephaly, skin pigmentation and peculiar fanconi facies<sup>5</sup>. Majority of patients develops pancytopenia in the first decade of life

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Received: 31 Jul 2019; revised received: 01 Sep 2019; accepted: 05 Sep 2019

with cumulative incidence of BMF around 90% by 40 years of age<sup>6</sup>. It can present with MDS converting into Acute myeloid leukemia or with leukemia as initial manifestation<sup>7</sup>. There are increased chances of developing solid tumors, especially in patient who underwent allogeneic bone marrow transplant necessitating surveillance in all transplant recipients<sup>8</sup>.

The screening test for FA is chromosomal breakage analysis in which cultured T-lymphocytes of patient are exposed to mitogen and DNA clastogenic agent as Diepoxybutane (DEB) or mitomycin C (MMC), while confirmatory test is genetic analysis for the underlying mutation. Fanconi screening should be performed in individuals having siblings with FA, characteristic birth defects suggestive of Fanconi, all patients with Aplastic anemia, Primary MDS or AML at a young age or cancers typical of FA at younger age<sup>9</sup>. Fanconi anemia patients require regular monitoring for bone marrow failure and malignancy with serial blood counts and periodic cytogenetic analysis. Anabolic steroids result in transient hematological response with potential risk of side effects<sup>10</sup>. Hematopoietic stem cell transplantation (HSCT) is the only curative option for BMF. It has evolved overtime with improved outcome due to transplant at younger age and reduction in drug doses particularly cyclophosphamide<sup>11</sup>.

Armed Forces Bone marrow transplant is a leading institute of Pakistan, performing bone marrow transplant for various benign and malignant hematological disorders since its establishment in 2001 including Fanconi anemia. We wanted to share our center experience of post-transplant outcomes in FA in terms of overall survival and post-HSCT acute and long-term complications in this study.

## METHODOLOGY

This case series study included all patients who underwent hematopoietic stem cell transplant for Fanconi Anemia at AFBMTC, from Jan 2001 to Jun 2018. FA patients who transformed into AML or MDS were excluded from the study. Study data included age of patients, gender, age

at diagnosis, age at transplant (age <11 years versus age >11 years), donor characteristics, source of stem cells, days to neutrophils and platelet engraftment, post-transplant acute and chronic complications, causes of mortality and overall survival. Informed consent was obtained from the participants and approval was sought from Institutional review board (IRB-007/AFBMT/Approval/2018).

Diagnosis of fanconi anemia was confirmed by chromosomal breakage analysis. Genetic mutation analysis was not performed. All patients had bone marrow failure with transfusion dependency at time of transplant. Prior to admission in transplant ward, all patients were in stable clinical condition with no active bacterial, viral or fungal infection.

All patients were treated in a single room with high-efficiency particulate air (HEPA) filters and infectious control guidelines were followed. Prophylaxis against viral, fungal and Pneumocystis jiroveci pneumonia infection was given. Blood products used were leukodepleted, irradiated and viral screened. Cytomegalovirus reactivation was monitored by weekly/fortnightly quantitative PCR from day +30 till 3 months post-transplant.

Conditioning chemotherapy comprised of Fludarabine at 120mg/m<sup>2</sup>, Thymoglobulin 10mg/kg and cyclophosphamide at 20-40 mg/kg in four equal divided doses as shown in fig-1.

Neutrophil engraftment was defined as absolute neutrophil count  $\geq 0.5 \times 10^9/L$  for 3 consecutive days while platelet engraftment as platelet count  $\geq 20 \times 10^9/L$  without platelet transfusion for 1 week. Primary graft failure was defined as failure to achieve an ANC of  $0.5 \times 10^9/L$  by day 28 and secondary graft failure as ANC  $< 0.5 \times 10^9/L$  or donor chimerism less than 10% by molecular analysis in patients who previously achieved an ANC of  $0.5 \times 10^9/L$ . Overall survival (OS) was taken as time from date of HSCT to last follow up. We graded Acute GVHD as per modified Glucksberg system<sup>12</sup> while National Institute of

Health Consensus Conference guidelines were used to classify Chronic GVHD<sup>13</sup>.

SPSS version 25 was used for calculation of frequency, percentages, mean, median and Standard deviation (SD). Survival analysis was performed with Kaplan Meier estimator. A *p*-value  $\leq 0.05$  was considered as statistically significant.

## RESULTS

A total of 27 patients with FA underwent allogeneic bone marrow transplant during this period. Twenty-four patients had congenital structural anomalies while 3 were phenotypically normal (table-I). All donors were screened for fanconi anemia and were found negative.

All patients had fully HLA matched donor. Conditioning protocol, stem cell dose and other transplant details are given in table-II.

**Table-I: Patient demographics and disease characteristics.**

	n (%)
<b>Gender</b>	
Male	15 (55.5)
Female	12 (44.5)
<b>Age at Diagnosis (years)</b>	
Mean age	10.17 $\pm$ 4.99
Range	2-20
<b>Age at Time HSCT (years)</b>	
Median	12.12 $\pm$ 5.16
Range	4-25
Waiting time for HSCT (months)	19.80 $\pm$ 12.28
<b>Physical Features</b>	
No physical abnormality	3 (11%)
Physical abnormality present	24 (89%)
Short stature	16 (66%)
Fanconi facies	17 (70.8%)
Skeletal malformation	7 (29%)
Renal anomalies	4 (16.6%)

## Post-Transplant Complications

Major post-transplant complications were febrile neutropenia in 26 (96%), cyclosporine induced hypertension in 18 (66.6%), mucositis grade II-III in 12 (44.4%), renal impairment in 8 (29.6%), gut toxicity II-IV in 7 (25.9%) and haemorrhagic cystitis in 5 (18.5%) patients. Acute GVHD II-IV was seen in 4 (14.8%) patients involving skin and gut. Chronic Graft versus Host Disease was

found in 7 (26%) patients. Out of these 7, three (42.8%) had extensive disease affecting skin, liver, gut and lungs while 4 (57.2%) had limited chronic GVHD of skin.

## Transplant Related Mortality

Five (18.5%) patients died in early post-transplant period, 3 had primary graft failure (PGF), one had diffused alveolar haemorrhage and 1 patient died due to grade IV gut toxicity. Seven (25.95%) patients died later after discharge from transplant ward. Mortality due to other complications were respiratory infections (n=4), grade IV gut GVHD (n=1), multi-system refractory CMV disease (n=1) and one patient died of secondary acute myeloid leukaemia 4.5 years after HSCT.

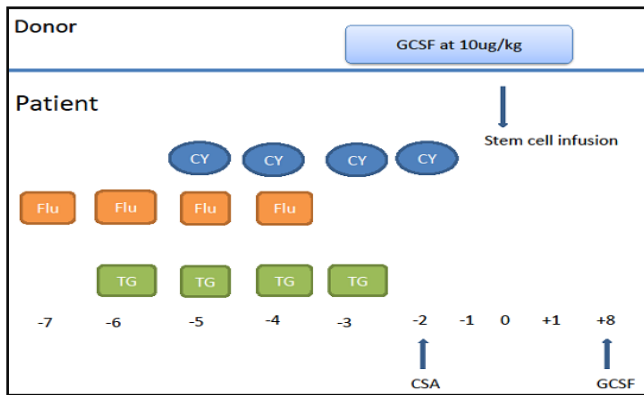
**Table-II: Transplant details.**

	n (%)
<b>Donor Relation with Patient</b>	
Sibling	23 (85.2%)
Parents	2 (7.4%)
Cousin	2 (7.4%)
<b>ABO Mismatch</b>	
No ABO mismatch	18 (66.6%)
Minor ABO mismatch	6 (22.2%)
Major ABO mismatch	3 (11.1%)
<b>Gender Mismatch</b>	
No gender mismatch	16 (59.2%)
Gender mismatch	11 (40.7%)
<b>Conditioning Protocol</b>	
Flu 120mg/m <sup>2</sup> , Cy30mg/kg, ATG20mg/kg	19 (70.4%)
Flu 120mg/m <sup>2</sup> , Cy20mg/kg, ATG20mg/kg	6 (22.2%)
Flu120mg/m <sup>2</sup> , Cy40mg/kg, ATG20mg/kg	2 (7.4%)
<b>Source of Stem Cells</b>	
Bone marrow harvest	15 (55.5%)
Peripheral stem cells Only	2 (7.4%)
BMH + Peripheral stem cells	10 (37%)
<b>Stem Cells Dose</b>	
Mean TNC $\times 10^8$ /kg	5.37 $\pm$ 2.47
Mean CD34 $\times 10^6$ /kg	7.12 $\pm$ 4.5
Mean Neutrophil engraftment (days)	12.3 $\pm$ 2.92
Mean Platelet engraftment (days)	20 $\pm$ 10.3

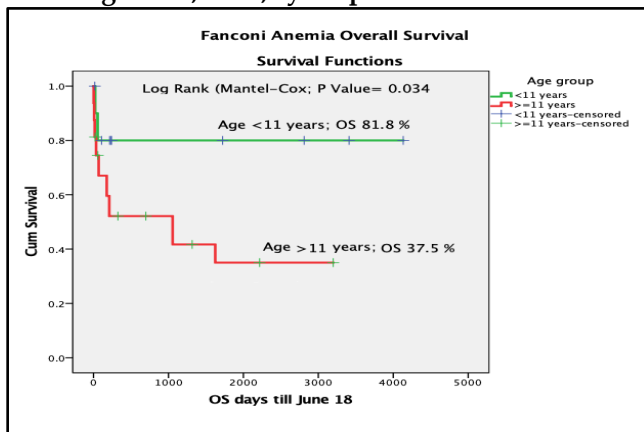
## Overall Survival

Overall survival (OS) after allogeneic bone marrow transplant at 6 months was 67% which dropped to 63% at 12 months. OS at 5 and 8 years

was 59% and 55% respectively. Overall Survival with Cyclophosphamide at 30mg/kg was 62% in comparison to 33% and 50% with Cy20mg/kg and Cy40mg/kg respectively. Although the total number of patients was very small in other two groups, 6 patients in Cy20mg/Kg and 2 in Cy40 mg /Kg. Multivariate analysis demonstrated that



**Figure-1: Conditioning regimen for Fanconi Anemia patients. CY; Cyclophosphamide, Flu; Fludarabine, TG; Thymoglobulin, G-CSF; granulocyte colony stimulating factor, CSA; cyclosporine.**



**Figure-2: Kaplan Meier survival curve in relation to age of patients at HSCT.**

patients who had transplant at younger age of less than 11 years had statistically significant superior outcome (fig-2). Patients who developed acute GVHD had inferior outcome (OS 52%) in comparison to those without GVHD (OS 78%), but this was not statistically significant ( $p$ -value 0.06). Statistically significant association was not observed when overall survival was compared with disparity in blood group or gender, waiting time for transplant and stem cell source.

**DISCUSSION**

Our study evaluated 27 fanconi anemia patients who underwent allogeneic HSCT in the last 18 years at AFBMTC. This is the largest group reported from Pakistan and provides an opportunity to analyze the outcome of HSCT practice in this rare disease. We observed congenital anomalies and malformations in 89% patients, which is close to reported frequency of 79% by Roa *et al*<sup>14</sup>.

We transplanted all patients with fully HLA matched donor. Literature review showed survival advantage in HLA matched sibling transplants over haplo-identical or matched unrelated HSCT<sup>15</sup>. Chances of finding HLA matched sibling in our population is high in comparison to western countries due to large family size and high proportion of consanguineous marriages<sup>16</sup>. Mehta *et al* observed improved outcome (OS 80% at 1 and 3 years post-HSCT) with haploidentical and matched unrelated donor transplants in fanconi anemia<sup>17</sup>. We hope that in near future we will be able to offer haploidentical transplant to those patient who do not have fully matched family donor.

We used irradiation free, fludarabine-based preparative regimen with thymoglobulin and low dose cyclophosphamide. Benajiba *et al* reported improved engraftment (100%), less conditioning toxicity (grade II mucositis in 5%), decreased incidence of both acute GVHD (15%) and chronic GVHD (10%) with better long term survival (95%) with this protocol<sup>15</sup>. However our results are in contrast to those reported by Benajiba, as we observed primary graft failure followed by infectious complication along with conditioning toxicity (gut toxicity 25.9%, mucositis 44.4%) and high early post-transplant mortality (18.5%).

In this study we used bone marrow harvest as primary source of stem cells for almost all patients and resorted to peripheral stem cells collection for completion of stem cells dose. Only two patients received peripheral stem cells. We observed no correlation with increased risk of GVHD and source of stem cells likely due to less number of patients receiving peripherals stem

cells. Kumar *et al* reported an increased risk of chronic Graft Versus Host Disease (31% vs 17%) when peripheral blood was used as source of stem cells, emphasizing the importance of considering marrow as the source of stem cells in FA patients as these patients are already at high risk of GVHD and secondary malignancies<sup>18</sup>.

Our patients had neutrophil and platelet engraftment at 12 and 20 days respectively. Similar results (neutrophil engraftment at 13 days, platelet engraftment at 20 days) were reported by Ayas from King Faisal Specialist Hospital and Research Center (KFSHRC) in KSA<sup>19</sup>.

In our study 11 (40.8%) patients had Graft versus host disease. More patients had chronic GVHD involving skin, gut, liver and lungs as compared to acute GVHD (26% Vs 14.8%). Although there is improvement in HLA typing in recent times but the incidence of GVHD has not decreased in patients with FA, contributing to high morbidity and mortality. Guardiola *et al* reported high incidence of GVHD in FA patients as compared to similar cohort of Aplastic anemia patients (Cumulative incidence of grades II-IV Acute GVHD in patients  $\leq 12$  years of age was 72% in FA versus 13% in non-FA patients)<sup>20</sup>. Patients in our study who developed GVHD had inferior overall survival (78% vs 52%,  $p$ -value 0.06). European Blood and Marrow Transplantation (EBMT) group also reported GVHD as the leading cause of post-transplant mortality in FA (35%) followed by infections (27%) and secondary malignancy (10%)<sup>21</sup>.

Major factors contributing to mortality in our study were primary graft failure, conditioning toxicity, opportunistic infections and GVHD. Macmillan *et al* reported opportunistic infections (13%) and conditioning toxicity (10%) as leading cause of death<sup>22</sup>. One patient in our study died due to secondary malignancy (Acute Myeloid leukemia) at four years post BMT. The long-term overall survival in fanconi anemia post-transplant patients is a diversely affected by secondary malignancies especially solid tumors<sup>8</sup>. To fully

ascertain long term risk of malignancy, our cohort of patients need long term follow-up.

We observed superior post-transplant outcome and better long-term overall survival (OS) in patients who were transplanted before the age of 11 years (81.8% vs 37.5% with  $p$ -value  $< 0.03$ ). Mehta *et al* reported excellent outcomes when HSCT was performed before 10 years of age (OS 92.3% in  $< 10$  years vs 63.2% in  $> 10$  years;  $p < 0.02$ )<sup>17</sup>. Ayas *et al* demonstrated poor post-transplant outcome at older age (OS 78% in  $\leq 14$  years of age versus 34% in  $\geq 14$  years,  $p < 0.001$ ) and in FA patients with myelodysplastic or leukemic transformation (5 years OS 67% versus 43%,  $p < 0.03$ )<sup>23</sup>. We transplanted all patients in aplastic phase without any features of MDS or acute leukemia. So we cannot comment on poor outcome of those with MDS or leukemia. Thus in this study the only statistically significant difference with superior outcome was early age at time of transplant.

#### LIMITATION OF STUDY

Limitations of study are small cohort of patients, retrospective nature of study, lack of genetic mutation analysis and changes in supportive care during the study period. Since Fanconi anemia is a rare disorder so the number of cases at one particular center will always remain low. In order to formulate better HSCT strategy, there is need to have a greater number of patients. It is suggested that all centers in Pakistan offering bone marrow transplant analyze their data together under unified transplant registry.

#### Disclosure

Abstract of this study was presented as free paper at Pakistan Society of Hematology (PSH) conference held at Aga Khan University Hospital Karachi in March 2019. It was also displayed as poster at 45<sup>th</sup> Annual Meeting of the European Society for Blood and Marrow Transplantation (EBMT).

#### CONCLUSION

We observed significant survival difference in Fanconi anaemia patients when they were transplanted at younger age of less than 11 years.

Moreover low dose Cyclophosphamide at 30 mg/kg with Thymoglobulin 10mg/kg and Fludarabine 120mg/m<sup>2</sup> was well tolerated and acceptable conditioning protocol. GVHD adversely affected long term survival necessitating better preventive and treatment strategies. Long-term follow-up is mandatory in these patients due to increased risk of malignancy.

### CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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