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Successful Outcome With Fludarabine-Based Conditioning Regimen for Hematopoietic Stem Cell Transplantation From Related Donor in Fanconi Anemia: A Single Center Experience From Turkey

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Background. Fanconi anemia (FA) is a heterogeneous autosomal recessive (and rarely X linked) disorder, which is characterized by congenital malformations, progressive bone marrow failure, and predisposition to malignancies. Hematopoietic stem cell transplantation (HSCT) is the only definitive treatment for the hematological manifestations in FA. ***Procedure.*** Twenty-seven patients with FA underwent HSCT using fludarabine (Flu) based regimen at our center between April 2004 and May 2014. One patient who developed acute leukemia before HSCT was excluded from the study. The remaining 26 patients were included. The median age of the patients at the time of transplantation was 9.6 years (range 5.6–17.0 years) and male/female ratio was 19/7. Donors were Human leukocyte antigen (HLA)-identical sibling in 18 patients, HLA-identical other relatives in six patients, and HLA 1-antigen mismatched sibling in two patients. Conditioning regimen consisted of Flu, cyclophosphamide, and antithymocyte globulin. ***Results.*** All patients engrafted but one developed poor graft function and underwent second HSCT. Acute graft versus host disease (GVHD) (≥grade 2) occurred in two patients (7.6%) and chronic GVHD in one patient (3.9%). Three patients developed venoocclusive disease (11.5%). Survival rate was 96.2% (25/26) at a median follow-up of 54 months (10–131 months) and all patients who survived were in good clinical condition. None of the patients developed secondary malignancy during the follow-up period. ***Conclusions.*** The present study from Turkey, a middle-income country, shows successful transplant outcome with low toxicity using Flu-based conditioning in patients with FA who underwent HSCT from HLA-related donors. Pediatr Blood Cancer 2016;63:695–700.

Key words: Fanconi anemia; fludarabine; transplantation

INTRODUCTION

Fanconi anemia (FA) is a rare autosomal recessive disorder that causes variable constitutional physical abnormalities, progressive bone marrow failure, and susceptibility to cancer, particularly acute myeloid leukemia (AML) and squamous cell carcinoma (SCC).[1–3] Fanconi cells are characterized by chromosomal instability and marked DNA hypersensitivity to cross-linking alkylating agents.[4] FA is diagnosed by detecting elevated chromosomal breakage in peripheral blood lymphocytes or fibroblasts after culture with a DNA cross-linking agent such as diepoxybutane (DEB) or mitomycin-C (MMC); in addition, complementation or molecular analyses are performed for the 16 currently known genotypes.[5,6]

Hematopoietic stem cell transplantation (HSCT) still represents the only curative treatment for bone marrow failure and the prevention/treatment of hematopoietic malignancies associated with FA.[7] Patients with FA have increased sensitivity to conventional conditioning regimens due to an underlying innate DNA repair defect.[8] Fludarabine (Flu) based conditioning regimens that are capable of intense T-cell immunosuppression have reportedly led to early, stable engraftment with minimal toxicity in patients who are unable to tolerate conventional myeloablative therapy; results have been shown in patients with several diseases.[7,9] Patients with FA are natural candidates for nonmyeloablative HSCT after Flu-containing regimens because their cells are hypersensitive to DNA cross-linking agents.[7] Herein, we report the results of 26 patients with FA who underwent HSCT using Flu-containing conditioning regimens.

MATERIALS AND METHODS

Patients

Twenty-seven patients with FA, confirmed by the presence of multiple chromosome breaks enhanced by incubation with cross-linking agents including DEB or MMC, underwent allogeneic HSCT with Flu-based conditioning regimens between April 2004 and May 2014 at our center. One patient, who developed AML before HSCT, was excluded from the study. Of the 26 patients included, one required a second HSCT due to poor graft function. Pretransplant and transplantation

Abbreviations: AAA, acquired aplastic anemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; CsA, cyclosporine A; CY, cyclophosphamide; DEB, diepoxybutane; FA, Fanconi anemia; Flu, fludarabine; GVHD, graft versus host disease; HC, hemorrhagic cystitis; HSCT, hematopoietic stem cell transplantation; LFI, limited field irradiation; MMC, mitomycin-C; SCC, squamous cell carcinoma; VOD, venoocclussive disease

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TABLE I. Pretransplant Characteristic of Patients

Number of patients	26
Number of patients with ≥3 malformations	14 (53.9%)
Pretransplant treatment	7 (26.9%)
Oxymethalone	4 (15.4%)
Oxymethalone + corticosteroid	3 (11.5%)
The number of transfusions prior to HSCT	
<5	17 (65.4%)
5–20	4 (15.4%)
>20	5 (19.2%)
Pretransplant complications	
Cytogenetic abnormalities	7 (26.9%)
Transiently abnormal liver function tests	5 (19.2%)
Pulmonary atelectasia	1 (3.9%)
Pulmonary nodule	1 (3.9%)
Decreased cardiac function	1 (3.9%)
Immune hemolysis	1 (3.9%)

HSCT, hematopoietic stem cell transplantation.

TABLE II. Transplantation Characteristics of Patients

Median age	9.6 y (5.6–17.0 y)
Gender (male/female)	19/7
Median time between diagnosis and HSCT	31 mo (10–90 mo)
Donor characteristics	
HLA-identical sibling	18 (69.2%)
HLA-identical other relative	6 (23.1%)
HLA 1-antigen mismatched sibling	2 (7.7%)
Gender (donor/recipient)	
M/M	11 (42.3%)
F/M	7 (26.9%)
F/F	4 (15.4%)
M/F	4 (15.4%)
Source of stem cells	
Bone marrow	18 (69.2%)
Peripheral blood	7 (26.9%)
Bone marrow + cord blood	1 (3.9%)
Cell dose (CD34+ cells/kg)	$4.8 \pm 7.0 \times 10^6$ (range: 0.8–34.7×10^6)
CD34+ selection	5 (19.2%)
Conditioning regimen	Fludarabine (35 mg/kg/day × 5 day) Cyclophosohamide (5–10 mg/kg/day× 4 day) ATG* (20–40 mg/kg for ATG Fresenius, 5–10 mg/kg for thymoglobulin)
GVHD prophylaxis	
CsA + MTX	20 (76.9%)
CsA**	5 (19.2%)
CsA + methylprednisolone	1 (3.9%)

*ATG Fresenius was used in 24 patients; thymoglobulin was used in two patients; **CD34+ selection was performed. y, year; mo, month; F, female; M, male; ATG, antithymocyte globuline; GVHD, graft versus host disease; CsA, cyclosporine A; MTX, methotrexate.

characteristics of the patients are summarized in Tables I and II. The median age of the patients was 9.6 years (5.6–17.0 years) at the time of transplant; 19 of the patients (73.1%) were male. The median duration from diagnosis of FA to HSCT was 31 months (10–90 months).

The extent of malformations was assessed according to the number of anatomic sites or functional systems involved (including the head, limbs, kidneys, gastrointestinal tract, urogenital tract, cardiovascular system, central nervous system, and endocrinologic system), excluding skin abnormalities, which were observed in more than 90% of the patients. Each of the major anatomic or functional systems was considered one site. The patients were categorized as having either limited (<3 sites) or extensive (≥3 sites) malformations.[10] According to this classification, 14 of our patients (53.9%) had extensive malformations.

Seven patients (26.9%) had cytogenetic abnormalities including add 6q, partial trisomy 1q, del 11q23, del q11qter, hypodiploidy, add 7q, and del 7q31. All patients received erythrocyte and/or platelet transfusions prior to HSCT. Seventeen patients received transfusions (7.5%) less than five times, four received transfusions between five and 20 times, and five patients received more than 20 transfusions prior to HSCT. Pre-HSCT oxymethalone treatment was used in seven (26.9%) cases (alone in four and with corticosteroids in three cases).

Donor Characteristics and Sources of Stem Cells

The median age of the donors was 15.5 years (0.8–56.3 years); 15 of donors (57.7%) were males. All of the donors except one had positive Cytomegalovirus (CMV) serology. The donors were HLA-matched siblings in 18 cases, HLA-identical other relatives in six cases, and HLA 1-antigen mismatched siblings in two cases. Bone marrow was used as the source of stem cells in 18 patients, peripheral blood in seven patients, and combined bone marrow and cord blood in one patient. Twenty-one patients received unmanipulated stem cells, and five patients received CD34+ positively selected grafts. CD34+ cells were prepared using the CliniMacs one-step procedure (Miltenyi Biotech GmbH, Bergisch-Gladbach, Germany). The source of the stem cells was peripheral blood in all patients who received CD34+ positively selected grafts. The mean CD34+ cell dose was $4.8 \pm 7.0 \times 10^6$/kg ($0.8–34.7 \times 10^6$/kg).

Conditioning Regimen and Graft Versus Host Disease Prophylaxis

All patients received conditioning regimens including Flu (35 mg/m^2/day for 5 days), cyclophosphamide (CY, 5-10 mg/kg/day for 4 days), and antithymocyte globulin (ATG; total dose of 20–40 mg/kg for ATG Fresenius, 5–10 mg/kg for thymoglobulin). Different types of ATG were used depending on the availability of ATG types in our country. The dosage of CY and ATG varied depending on the donor characteristics and number of transfusions prior to HSCT. Of the 21 patients who received unmanipulated stem cells, 20 received cyclosporine A (CsA) and a short course of methotrexate (MTX) for graft versus host disease (GVHD) prophylaxis, administered at days +1, +3, and +6, at a dose of 10 mg/m^2 in all patients except one, who underwent HSCT from his grandfather (in that case, the source of stem cells was peripheral blood and the patient

received an additional dose of MTX at day +11). CsA and methylprednisolone were preferred for treating one patient. CsA was used for GVHD prophylaxis in the remaining five patients who received CD34+ positively selected grafts.

Supportive Treatment

All patients were hospitalized in single rooms with hepafilters throughout HSCT period. Patients received acyclovir, fluconazole, and trimethoprim-sulfomethaxozole for prophylaxis against herpes simplex and varicella-zoster viruses, fungal infection, and infection with *Pneumocystis jiroveci,* respectively. CMV infection was routinely monitored using real-time PCR assays. Broad-spectrum antibiotic coverage was initiated at the first evidence of fever ($T_{max} > 38°C$). Intravenous immunoglobulin was administered weekly at a standard dose of 400 mg/kg from day –1 to day +21 and depending on IgG levels thereafter. Intravenous glutamine, enoxaparin, ursodeoxycholic acid, and vitamin E were given for venoocclussive disease (VOD) prophylaxis.

Definitions

Neutrophil engraftment was defined as the first of three consecutive days with a neutrophil count above 0.5×10^9/l. Platelet engraftment was defined as a platelet count higher than 20×10^9/l with no transfusion requirements for at least 7 days. The absence of hematopoietic recovery at day 60 and autologous hematopoietic reconstruction were considered engraftment failure. Complete chimerism was defined as the presence of ≥95% donor cells; mixed chimerism was defined as the presence of 10–95% donor cells in peripheral blood. Acute and chronic GVHD were diagnosed and graded according to the Seattle criteria.[11,12] Poor graft function was diagnosed in patients with two or three cytopenic lines (Hb < 10 g/dl, neutrophil count $<1.0 \times 10^9$/l, platelet count $<30 \times 10^9$/l) for at least two consecutive weeks beyond day +14, with transfusion requirement, associated with hypoplastic–aplastic bone marrow, in the presence of complete donor chimerism and in the absence of severe GVHD and relapse.[13] VOD was diagnosed and staged according to the Seattle criteria.[14] Hemorrhagic cystitis (HC) was defined as painful hematuria with a negative urine culture for bacteria or fungus and without any other explanation, such as general bleeding diathesis or urinary tract catheterization for reasons other than HC, urinary calculi, or bladder neoplasms.[15] Organ toxicity was defined according to National Cancer Institute common toxicity criteria and mucositis according to published standards.[16]

Statistics

Statistical analysis was performed using SPSS software, version 15.0. Descriptive statistics were generated as medians and ranges for continuous variables and proportions for categorical variables. The cumulative incidence of acute and chronic GVHD, probability of overall survival, and event-free survival were calculated using Kaplan–Meier analysis. We defined overall survival as the time from HSCT to death from any cause. Loss of engraftment was considered an event and surviving patients were censored at last follow-up.

RESULTS

Engraftment and Chimerism. Engraftment was achieved in all 26 patients. The mean days of neutrophil and platelet engraftments were 14.5 ± 2.9 (9–19) and 26.0 ± 6.8 (16–41), respectively. Nineteen (73.1%) of the 26 patients achieved full donor chimerism. Of the remaining seven patients, four (15.4%) achieved 90–95% donor chimerism and only three patients (11.5%) had achieved <90% donor chimerism at last visit. After hematologic recovery with full donor chimerism, one patient who received a CD34+ positively selected graft developed poor graft function. This patient underwent a second HSCT. All patients except one with poor graft function had normal hematological values.

GVHD. Acute GVHD (grade 2) developed in two (cumulative incidence; 7.7%) patients. Both patients improved after the addition of methylprednisolone to ongoing CsA. One patient (cumulative incidence; 3.9%) developed mild chronic GVHD without preceding acute GVHD and was treated with CsA and short course methylprednisolone.

Infectious Complications and Regimen-Related Toxicities. Twenty-one patients (80.8%) had febrile episodes. CMV PCR positivity was shown in six patients (23.1%), bacteremia was detected in two patients (7.7%), and other infections were documented in two patients (7.7%; skin infection in one patient and herpes labialis in the other patient). VOD developed in three patients (11.5%), two with mild form and one with moderate form; all three patients recovered. Mucositis (≥grade 3) was detected in 14 patients (53.9%) and resolved after neutrophil engraftment. Five patients (19.2%) developed HC. Hepatotoxicity (>grade 2) developed in seven patients (26.9%); six (23.1%) with grade 3 and one (3.9%) with grade 4 toxicity. Six patients developed renal toxicity; three had grade 1 toxicity (11.5%) and three had grade 2 (11.5%) toxicity.

Outcome. Twenty-five of the 26 (96.2%) patients are still alive. All surviving patients recovered with normal hematopoietic function and normal peripheral blood counts. The median time of follow-up was 54 months (10–131 months). One of the patients developed poor graft function with full donor chimerism. A second HSCT was performed 5 months after the first HSCT, but hematologic recovery was not observed and the patient died due to poor graft function and bacteremia 90 months after the first HSCT. None of the patients developed myelodysplastic syndrome, acute leukemia, or solid malignancy within the follow-up period. Five-year overall survival and event-free survival were calculated as 96%.

DISCUSSION

Patients with FA are very sensitive to conventional conditioning protocols.[17] Both busulfan and CY used in classical conditioning regimens are alkylating agents and they put these patients at risk for increased HSCT-related toxicities. The use of high-dose CY and ionizing radiation for conditioning regimens for patients with FA frequently resulted in excessive organ toxicity and death in the early posttransplant period.[4] For this reason, reduced doses of CY and limited field irradiation (LFI) have been used widely as a standard conditioning regimen for patients with FA, with 5-year survival rates approaching 75% after HSCT from HLA-identical siblings.[18–20] Unfortunately, long-term follow-up studies have indicated that approximately

40% of patients with FA develop a secondary malignancy subsequent to these protocols.[8] Additionally, these regimens have not been immunosuppressive enough to reduce graft rejection in patients who were heavily transfused.[21] For these reasons, several groups have investigated conditioning regimens that do not contain irradiation.[9,16,22–24] Elimination of radiation from the conditioning regimen may minimize the impact of HSCT on the risk of cancer and reduce the known effects of radiation on growth and development.[22] Various groups have attempted to eliminate the need for irradiation in the preparative regimens for patients with FA by using CY in doses ranging from 100 to 200 mg/kg with or without ATG.[25] Although engraftment rates were comparable to standard CY + LFI regimens, significant toxicity and markedly increased risk of death was observed in patients who received CY at doses ≥100 mg/kg.[25–28] These data indicate that there is a need for novel conditioning regimens for patients with FA to reduce the risk of toxicity, GVHD, and malignancy while achieving at least similar engraftment rates.[8]

Flu, a fluorinated purine antimetabolite that is not a cross-linking agent, is an attractive alternative.[25] Unlike DNA cross-linking agents, such as irradiation and CY, Flu does not affect the chromosomal integrity of the FA cell.[3] There has been a recent focus on reducing the toxicities of transplantation in patients with FA, and regimens incorporating Flu monophosphate have been the most successful in this regard.[29] Flu-based protocols have provided the double advantage of potent immunosuppression, which reduces the incidence of graft rejection, and minimal regimen-related toxicities.[21] It seemed that Flu-based protocols were particularly suitable for dramatically improving the outcome of patients with FA in need of HSCT.[17] The favorable effects of Flu in patients with FA has been described in reports from several centers.[9,30–34] A CIBMTR study also confirmed improved outcomes with the use of Flu-containing regimens.[3] Bitan et al. reported that no patients had major toxicities associated with a Flu-based conditioning regimen and that initial engraftment was seen in all patients, though one patient had a secondary graft rejection on day +50.[17] Locatelli et al. showed that the use of Flu improved neutrophil recovery, decreased 100-day transplant-related mortality, and improved 3-year adjusted overall survival rates (52% and 13% in patients who did and did not receive Flu, respectively).[7] Ertem et al. reported that in contrast to the busulfan-based regimens, patients who received Flu-based regimens had minimal toxicity and engrafted with stable and full donor chimerism.[8] Using a Flu-based regimen, Tan et al. demonstrated consistent and prompt primary engraftment with minimal toxicity in their series of 11 patients, which included two patients with poor renal function at time of HSCT. Donor-sustained engraftment was achieved in all patients with sibling donors.[25]

A more recent retrospective, multicenter study by the European Society for Blood and Marrow Transplantation (EBMT) reported on the outcomes of 795 patients with FA who underwent HSCT; patients who were transplanted before 10 years of age were found to have lower risk for both chronic GVHD and secondary cancers and in turn, better-long term overall survival rates.[9] Additionally, the dramatic finding in this largest series of patients was that the use of Flu-based conditioning regimens was associated with better engraftment, lower rates of acute GVHD, and ultimately better overall survival rates.[9] Our study is consistent with these findings, as engraftment was achieved without major toxicity in all patients; additionally, all patients except one survived and achieved normal hematopoiesis and acceptably high donor chimerism.

Acute or chronic GVHD is one of the most important indicators of poor prognosis. Studies have demonstrated that GVHD itself increases the risk of SCC in addition to the predilection of patients with FA for malignancy.[35,36] The incidence of GVHD in our patients is low compared to the incidence of 50–60% observed in patients who underwent HSCT with conditioning regimens that included CY or CY + irradiation.[37] Guardiola et al. evaluated the outcomes of FA and acquired aplastic anemia (AAA) patients after receiving irradiation plus CY as a conditioning regimen and found that patients with FA had a higher risk of acute GVHD (62%) than AAA (42%).[36] In a recent study of 94 patients with FA who underwent HSCT, the risks of both acute and chronic GVHD were found to be higher among patients who received irradiation compared to those who did not.[38] On the other hand, the retrospective EBMT study reported the general risk for grade 2–4 acute GVHD and chronic GVHD as 32% and 14%, respectively.[9] By using CsA in addition to a Flu + ATG-based regimen, Bitan et al. observed mild acute GVHD (grade II) in two of seven patients.[17] In their series, one patient developed limited de novo chronic GVHD.[17] George et al. reported that only two patients had acute GVHD (grade 2) and that chronic GVHD developed in one patient when using a Flu-based regimen.[21] Our results are consistent with these prior studies, as the incidence of GVHD decreased with Flu-based conditioning (acute GVHD in two patients, chronic GVHD in one patient). ATG remains in the circulation for several days, causing partial in vivo donor T-cell depletion (TCD) and possibly providing additional protection from GVHD.[32] Several studies have reported that ATG-containing conditioning regimens resulted in a major reduction in severe acute GVHD, leading to a dramatic decrease in early transplant-related morbidity and mortality in patients with FA who were transplanted from HLA-identical siblings.[39–41]

Mixed chimerism early after HSCT has been reported in approximately 14–44% of patients with FA who were transplanted from HLA-identical sibling donors using low dose CY + LFI.[18,42] Tan et al. reported that, excluding two patients who required a second HSCT, all other patients eventually achieved complete donor chimerism; additionally, the Flu + CY + ATG preparative regimen was sufficient for donor sustained engraftment and an estimated 2-year survival rate of 100% in 11 patients.[25] In our study, 19 patients (73.1%) had full donor chimerism, four patients (15.4%) had donor chimerism between 90 and 95%, and only three patients (11.5%) had donor chimerism below 90%.

The EBMT has reported that mortality rates were higher in patients with three or more congenital abnormalities after unrelated donor HSCT.[10] Fourteen patients (53.9%) in our study had extensive malformations, and all patients survived except one, who had limited malformation. There is a high probability of survival after an HLA-matched sibling transplant; androgens are generally avoided when an HLA-matched sibling is available.[22] In our study, seven patients received androgens before transplantation and all of these patients survived. Higher mortality in patients receiving more than 20 pretransplant transfusions has previously been reported.[18,39] In the present study,

the number of the patients who received more than 20 transfusions before transplantation was five (19.2%) and all of these patients survived. Neither HLA alloimmunization due to multiple transfusions of blood products nor previous androgen treatment seemed to affect engraftment in our patients.

In a recent study, the outcomes of patients with FA who underwent HSCT after developing acute leukemia or advanced myelodysplastic syndrome were discussed, and the use of chemotherapy prior to HSCT was found to have no additional impact on the final outcome of the patient, except for the patients with biallelic BRCA2 mutations.[43] None of our patients had overt leukemia, although seven (29%) had cytogenetics suggestive of myelodysplastic syndrome. None of the patients had advanced myelodysplastic syndrome based on WHO classifications.[43]

Current data show that, in cases of FA, HSCT from HLA-identical sibling donors is associated with an excellent outcome. An IBMTR study of 209 patients transplanted from HLA-matched siblings reported 3-year survival rates of 81% in patients <10 years of age (n = 109) and 69% in older patients (n = 100).[44] Another study from the CIBMTR, which evaluated 148 patients with FA who underwent HSCT from HLA-matched siblings, reported 5-year survival probability of 78% after irradiation-containing regimens and 81% after nonirradiation-containing regimens.[22] A 2013 EBMT study evaluating a subgroup of 211 patients with FA who underwent HSCT from HLA-identical siblings reported a 5-year survival rate of 76%.[9] Although survival rates improved with the addition of Flu to the conditioning regimen and TCD of the stem cell source, data from alternative donor HSCT studies suggest lower survival rates, ranging between 52 and 68%.[9, 45] In our study of 26 patients, 18 were transplanted from HLA-matched siblings, which we believe greatly contributed to the successful outcomes.

There are some restrictions to our study. First, we were not able to use complementation group analysis for the diagnosis of FA. Second, the observation time of surviving patients was too short to estimate the risk of developing secondary malignancies reliably.

In the present study, we achieved successful outcomes using Flu-based conditioning regimens in patients with FA undergoing HSCT. The novel aspect of this study is that these results were achieved in a middle-income country. We also showed very low complication rates, including acute and chronic GVHD and VOD. No patients developed secondary malignancy during the follow-up period, although a longer follow-up is required to confirm this evaluation.

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