

Rapid Communication

Allogeneic Hematopoietic Stem Cell Transplantation in Fanconi Anemia

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Abstract

Objective: Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is the treatment of choice in patients with Fanconi Anemia (FA). The aim of our study is to evaluate the impact and benefits of allogeneic matched donor HSCT in a case of a 12 year-old girl with FA, who displayed good clinical evolution following 2 months post-transplantation.

Patients and Methods: In the pre-transplant phase, reference blood samples from the donor and recipient were collected on EDTA. The DNA from blood samples was extracted using an automated Maxwell[®] 48 RSC instrument (Promega, USA) with the Maxwell[®] RSC Whole blood DNA kit (Promega, USA). For DNA quantification, the PowerQuant System kit (Promega, USA) was used with the ABI 7500 Real-time PCR system (Applied Biosystems, USA). The amplification of the short tandem repeat markers was performed using the 24plex Investigator QS kit (Qiagen, Germany) on a ProFlex PCR System. Furthermore, the PCR products were separated and detected on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA).

Results: After 30 days of the transplantation, a Complete Chimerism (CC) was achieved with a full replacement by donor derived hematopoietic cells. After 60 days of the transplantation, the CC status was maintained with improvement of hematological findings.

Conclusion: In FA, chimerism monitoring after HSCT provides useful informations of engraftment or possibility of post-transplantation complications such as graft versus host disease.

Keywords: Fanconi Anemia (FA); Hematopoietic Stem Cell Transplantation (HSCT); Short Tandem Repeat (STR); Chimerism

Introduction

Fanconi Anemia (FA) is a rare genetic disorder, and most common congenital hematopoietic disorder presenting with bone marrow failure syndrome and increased cancer susceptibility. The disease was first documented in 1927 by the swiss physician Giulio Fanconi. The disease is caused by impaired ability to repair Deoxyribo Nucleic Acid (DNA) damage, and thus is characterized by chromosomal instability and hypersensitivity to DNA interstrand cross-linking agents such as mitomycin C [1]. The phenotype is determined by genetic mutations in at least 30 different genes. Some specific populations present founder mutations with increased carrier frequencies, including Askenazi Jewish (FANCC, BRCA2/FANCD1), northern Europeans (FANCC), Afrikaners (FANCA), Spanish Gypsies (FANCA) and others [2]. The disease usually begins with abnormal blood findings including cytopenias in childhood, but in later life, it commonly develops serious hematologic disorders including Myelodysplastic Syndrome (MDS) or hematologic malignancies such as acute myeloid leukemia (AML) [3]. Diagnosis is established in a proband who presents with increased chromosome breakage or radial forms on cytogenetic testing of lymphocytes by diepoxybutane or mitomycin C. The diagnosis can be established before or after birth, even in cases with aplastic anemia or leukemia that may or may not have the typical signs associated with the disease [4]. Because the disease is a genetic

disorder, replacement with new and healthy blood cells through Hematopoietic Stem Cell Transplantation (HSCT) is fundamentally the only curative measure [5]. It is important to estimate the success or failure of procedure and to predict recurrence of the disease after the HSCT. In that regard, evaluation of chimerism is useful measure and has been widely used after HSCT [6]. Here, we report the outcome of complete chimerism in a matched sibling donor HSCT of a 12-year-old girl.

Patients and Methods

In June 2018, a 12-year-old girl was admitted to the Clinic of Onco-hematology Timisoara due to multiple spots of hypopigmentation distributed throughout her body (head, torso, lower and upper extremities). Also, a hypochromic spot of 1.5cm was observed on her posterior torso. The laboratory analysis at the admission presented the following values: hemoglobin 7.8g/dL; thrombocytes $21 \times 10^3/\text{mm}^3$, leukocytes $2.44 \times 10^3/\text{mm}^3$, neutrophils 25.8%. The bone marrow cytology presented hypoplasia on 2 cellular lineages. She was diagnosed with Fanconi anemia. Therefore, HSCT was the only therapeutic option for cure. She was matched to a blood-related donor, younger sister. Prior to the transplantation, she was HLA-typed for HLA-A, -B, -C, DRB1, and DQB1 using high resolution typing. Prior to the HSCT, blood samples collected on anticoagulant EDTA were received from both the donor and the recipient to establish reference

genetic profiles in the Laboratory of Forensic Genetics of Victor Babes University of Medicine and Pharmacy Timisoara, Romania.

DNA extraction from the biological samples

Prior to the allogenic HCST, peripheral blood samples collected on anticoagulant EDTA was received from both the donor and the recipient in the Laboratory of Forensic Genetics of Victor Babes University of Medicine and Pharmacy Timisoara, Romania. The DNA extraction was performed on the automate Maxwell 48 RSC instrument (Promega, USA) using the Maxwell RSC Whole blood DNA kit (Promega, USA) for the blood samples. We performed the DNA extraction according to manufacturer recommendations.

Quantification of the extracted DNA samples

For DNA quantification of the donor and the recipient samples, the PowerQuant System kit (Promega, USA) was used. We prepared for each sample according to manufacturer recommendations, preparing the mixed solution with a final volume of 18µL, consisting of 10µL of PowerQuant 2 x Master Mix; 7µL of Amplification grade water, and 1µL of PowerQuant 20 x PrimerMix. The quantification was performed on a 7500 real time PCR system (Applied Biosystems, USA), using the HID Real-Time PCR Analysis Software v2.0.6. Final concentrations of DNA were 20.18ng/µL in donor blood, and 34.05ng/µL in recipient.

Amplification of the DNA samples

The DNA samples from the donor and the recipient were amplified for the STR markers using the Investigator 24plex QS kit (Qiagen, Germany). The analysis was done following recommendations of the manufacturers. The DNA samples' amplification was performed on a ProFlex PCR System (Applied Biosystems, USA). PCR of the blood DNA samples was carried out in a total volume of 25µL. The final volume of the reaction contained: 7.5µL of Fast Reaction Mix 2.0µL, 2.5µL of Primer Mix, 12.5µL of nuclease-free water, and 2.5µL of template DNA. The Investigator 24plex QS Kit (Qiagen, Germany) contains 22 autosomal STR markers as follows: D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, TH01, TPOX, vWA, SE33, , DYS391, and the gender-specific marker Amelogenin. The conditions for the PCR amplification were as follows:

- 3 PCR cycles: temperature (T)-98°C for 30 seconds; T=64°C for 55 seconds; T=72°C for 5 seconds.

- 27 PCR cycles: T=96 °C for 10 seconds; T=61°C for 55 seconds; T=72°C for 5 seconds. After the 30 PCR cycles were completed, a final extension (hold) at T°=68°C was performed for 2 minutes followed by a final indefinite hold at T°=10°C.

Capillary electrophoresis of the amplified DNA samples

Samples were analyzed on a 3500 Genetic Analyzer following the manufacturer's recommendations. As autosomal STR markers, we used 1µL of the amplified PCR product (DNA sample) and 1µL of the Allelic Ladder (AL). They were added into the mix containing 12.5µL of Hi-Di Formamide (Applied Biosystems, USA) and 0.5µL DNA size standard BTO (Qiagen, Germany). Gene Mapper ID-X Software version 1.4 (Applied Biosystems, USA) was used to analyze the obtained data. For the statistical calculation, we used the Genoproof-3

Chimerism testing (Qualityte, Germany).

Results

Using capillary electrophoresis, we obtained the electropherograms and the genotypes of the donor and the recipient (Table 1). Thirty days after the HSCT, a new blood sample and electropherogram were collected from the recipient. The donor's genetic profile was present on the new electropherogram, indicating a complete chimerism. After 60 days, post-transplantation, complete chimerism was maintained (Table 1). The patient was released from the hospital on 71-day after the HCST, with the following results of blood parameters: hemoglobin 11.2g/dL; red blood cells 3.81x10⁶/mm³; thrombocytes 102x10³/mm³; leukocytes 7.09x10³/mm³; lymphocytes 53.64%; monocytes 15.24%; granulocytes 29.54%; eosinophils: 1.64% and basophils: 0.10%.

Discussion

The treatment of FA has improved with various treatment strategies in HSCT [5]. Since new cytoreductive treatments including fludarabine [7,8] and T-cell depleted graft [9,10] have been introduced, survival rates in FA after HSCT have increased. However, although HSCT aids in restoring hematopoiesis in patients, the HSCT itself are accompanied by many risks especially in FA. The defective ability of DNA repair in FA may make the patients more susceptible

Table 1: Monitoring of genetic chimerism through STR markers on pre-HSCT, 30- and 60-day after HSCT. Bold line indicates complete chimerism in the post-HSCT period.

STR markers	Alleles			
	Donor	Recipient (pre-HSCT)	Recipient (30 days)	Recipient (60 days)
THO1	6;7	6;7	6;7	6;7
D3S1358	15;17	15;18	15;17	15;17
vWA	15;18	15;18	15;18	15;18
D2S11	29;29	28;29	29;29	29;29
TPOX	8;11	8;11	8;11	8;11
DYS391	-	-	-	-
D1S1656	16;17.3	15;16.3	16;17.3	16;17.3
D12S391	18;19	18;19	18;19	18;19
SE33	22.2;27.2	18;30.2	22.2;27.2	22.2;27.2
D10S1248	13;14	13;14	13;14	13;14
D22S1338	16;17	15;16	16;17	16;17
D19S433	15;15.2	14;15	15;15.2	15;15.2
D8S1179	13;14	13;15	13;14	13;14
D2S1338	16;17	16;17	16;17	16;17
D2S441	11.3;11.3	17;23	11.3;11.3	11.3;11.3
D18S51	15;21	13;15	15;21	15;21
FGA	20;21	20;21	20;21	20;21
D16S539	11;12	11;12	11;12	11;12
CSF1PO	10;12	10;12	10;12	10;12
D13S317	8;12	8;12	8;12	8;12
D5S818	11;12	11;11	11;12	11;12
D7S820	8;9	9;10	8;9	8;9
AMELOGENIN	XX	XX	XX	XX

to chemotherapeutic agent or irradiation, and increase the risk of acute or chronic Graft-Versus-Host Disease (GVHD) and following tumors such as oral squamous cell carcinoma [11-13].

Chimerism analysis as a post-transplantation monitoring is a well-established measure. Among them, a measurement of Short Tandem Repeats (STR) using Polymerase Chain Reaction (PCR) is a most sensitive and a standard method to estimate the chimerism in HSCT [14]. Monitoring the chimeric status of patients following bone marrow transplantation is very important in establishing post-transplantation plan. Many physicians predict graft failure or disease recurrence after allogeneic HSCT by checking chimeric status even prior to any detectable morphologic recurrence [6]. Complete chimerism (CC) indicates that impaired bone marrow cells are fully replaced with healthy donor's cells, which has been accepted as the ultimate goal in early and late stage of HSCT. However, the CC status has been also known to be associated with a higher risk of GVHD [15] after HSCT. In addition, recent strategy with reduced-intensity regimens to avoid post-transplantation complication increases the rate of Mixed Chimerism (MC) in HSCT. Some studies demonstrated that MC was also considered acceptable as a tolerant and disease-free status, especially in non-malignant hematopoietic diseases [16,17]. Nonetheless, most studies have still demonstrated that the CC was associated with the higher survival rate than the MC [15]. Eventually, it is necessary to determine the reasonable goal of chimerism and the treatment protocols with appropriate intensity to achieve a balance between graft success and GVHD or post-transplantation tumor in non-malignant hematologic diseases [18].

Our patient showed complete chimerism on 30-day after HSCT and maintained complete status until 60-day, meaning that she was in complete clinical and morphological remission. Nonetheless, a patient like her who received a matched sibling-donor HSCT with reaching early CC should be carefully monitored whether to develop acute or chronic GVHD and other following complications during post-transplantation follow-up [19,20]. Finally, given recent success of gene therapy in trials to treat genetic disorders such as adrenoleukodystrophy, Wiskott-Aldrich syndrome, and metachromatic leukodystrophy, it is possible that gene therapy will emerge as a new treatment modality for with FA. This possibility is further supported by the recent development of refinements in HSC culture and transduction for FA [21].

Conclusions

In FA, the monitoring of chimerism after HSCT provides useful informations such as graft failure and possibility of GVHD. The CC status indicates successful engraftment and the better prognosis than the MC, while it is associated with post-transplantation complications including acute or chronic GVHD. Therefore, it is important to predict graft failure or post-transplantation complication, and to prepare possible therapeutic options through chimerism analysis. Above all, we expect that further study would determine the proper goal of chimerism and develop treatment protocol to avoid both graft failure and post-transplantation complication in non-malignant hematopoietic diseases such as FA.

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