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CASE REPORT

Molecular Monitoring of Allogeneic Stem Cell Transplantation in Fanconi Anemia

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SUMMARY

Background: **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 allogenic matched donor HSCT in a case of a 12 year-old girl with FA, who displayed good clinical evolution following 2 months post-transplantation.**
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 Biosytems, USA).**
Results: **Thirty days post transplantation, a complete chimerism (CC) was achieved with a full replacement by donor derived hematopoietic cells. Sixty days post transplantation, the CC status was maintained with improvement of hematological findings.**
Conclusions: **In FA, chimerism monitoring after HSCT provides useful information regarding engraftment or possibility of post-transplantation complications such as graft versus host disease.**
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KEY WORDS

Fanconi anemia (FA), hematopoietic stem cell transplantation (HSCT), short tandem repeat (STR), chimerism

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INTRODUCTION

Fanconi anemia (FA) is a rare genetic disorder, and most common congenital hematopoietic disorders present 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 deoxyribonucleic 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), Africans (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 a 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.

CASE PRESENTATION

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 hypocromic spot of 1.5 cm was observed on her posterior torso. The laboratory analysis at the admission presented the following values: hemoglobin 7.8 g/dL, thrombocytes 21 x 10^3/mm^3, leukocytes 2.44 x 10^3/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 HCST, 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.

Prior to the HSCT, bone marrow cytogenetic testing was performed and revealed specific chromosomal instability and breaks. In Figure 1, the karyotype analysis of the FA patient is presented.

DNA Extraction from the biological samples

Prior to the allogenic HCST, peripheral blood samples collected on anticoagulant EDTA were 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 each sample according to manufacturer recommendations, preparing the mixed solution with a final volume of 18 µL, consisting of 10 µL of PowerQuant 2 x Master Mix, 7 µL of Amplification grade water, and 1 µL 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 20.18 ng/µL in donor blood and 34.05 ng/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 the recommendations of the manufacturer. The DNA samples's 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 25 µL. The final volume of the reaction contained 7.5 µL of Fast Reaction Mix 2.0, 2.5 µL of Primer Mix, 12.5 µL of nuclease-free water, and 2.5 µL of template DNA. The Investigator 24plex QS Kit (Qiagen, Germany) contains 22 autosomal STR markers as follows: D1S1656, D2S441, D2S1338, D3S1358, D5S 818, D7S820, D8S1179, D10S1248, D12S391, D13S3 17, 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 =

Table 1. Monitoring of genetic chimerism through STR markers on pre-HSCT, 30 and 60 days after HSCT.

STR markers	Alleles			
	Donor	Recipient (pre-HSCT)	Recipient (30 day)	Recipient (60 day)
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

Underlined indicates complete chimerism in the post-HSCT period.

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 1 µL of the amplified PCR product (DNA sample) and 1 µL of the allelic ladder (AL). They were added into the mix containing 12.5 µL of Hi-Di Formamide (Applied Biosystems, USA) and 0.5 µL 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 (Qualitype, Germany).

RESULTS

Using capillary electrophoresis, we obtained the electropherograms and the genotypes of the donor and the recipient. Both genetic profiles are shown in Table 1. Thirty days after the HSCT, a new blood sample and electropherogram were collected from the recipient. The donor's genetic profile was completely 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 day 71 after the HCST, with the following results of blood parameters: hemoglobin 11.2 g/dL; red blood cells 3.81 x 10^6/mm^3; thrombocytes 102 x 10^3/mm^3; leukocytes 7.09 x 10^3/mm^3; lymphocytes 53.64%; monocytes 15.24%; granulocytes 29.54%; eosinophils: 1.64% and basophils: 0.10%. In Figure 2, an aspirate bone marrow is presented.

Figure 1. Karyotype analysis in Fanconi anemia.

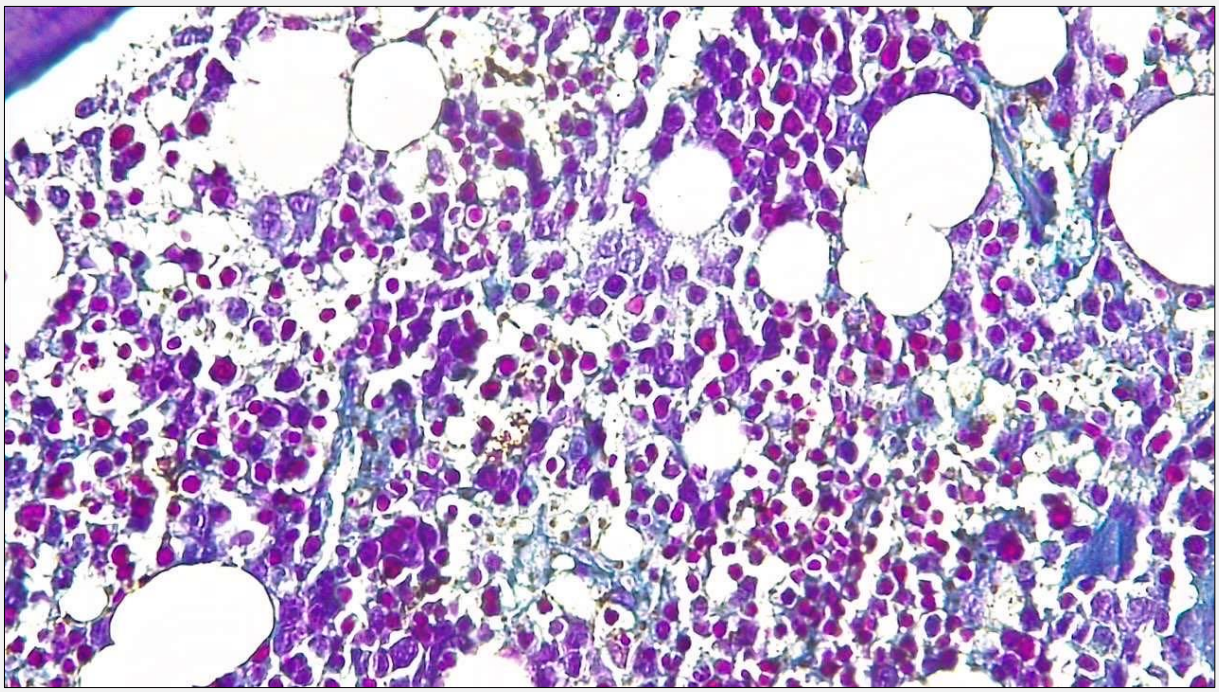

Figure 2. Bone marrow aspirate in Fanconi anemia.

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 to chemotherapeutic agents 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 standard method to estimate the chimerism in HSCT [14]. Monitoring the chimeric status of patients following bone marrow transplantation is very important in establishing a post-transplantation plan. Many physicians predict graft failure or disease recurrence after allogenic 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 also been known to be associated with a higher risk of GVHD [15] after HSCT. In addition, a 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 to be 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 day 30 after HSCT and maintained complete status until day 60, 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 she develops acute or chronic GVHD and other following complications during post-transplantation follow-up [19,20].

Finally, given the 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 may emerge as a new treatment modality for patients with FA. This possibility is further supported by the recent development of refinements in HSC culture and transduction for FA [21].

CONCLUSION

In FA, the monitoring of chimerism after HSCT provides useful information regarding graft failure and possibility of GVHD. The CC status indicates successful engraftment and has a better prognosis than 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 a develop treatment protocol to avoid both graft failure and post-transplantation complication in non-malignant hematopoietic diseases such as FA.

Declaration of Interest:
All authors declare they have no conflict of interest.

References:

1. Kee Y, D'Andrea AD. Molecular pathogenesis and clinical management of Fanconi anemia. J Clin Invest 2012;122:3799-806. (PMID: 23114602)

2. Kottemann MC, Smogorzewska A. Fanconi anemia and the repair of Watson and Crick DNA crosslinks. Nature 2013;493:356-63. (PMID: 23325218)

3. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood 1994;84: 1650-5. (PMID: 8068955)

4. Auerbach AD. Fanconi anaemia and its diagnosis. Mutat Res 2009;668:4-10. (PMID: 19622403)

5. Ebens CL, Macmillan ML, Wagner JE. Hematopoietic cell transplantation in Fanconi anemia: current evidence, challenges and recommendations. Expert Rev Hematol 2017;10:81-97. (PMID: 27929686)

6. Zimmerman C, Shenoy S. Chimerism in the realm of hematopoietic stem cell transplantation for non-malignant disorders-a perspective. Front Immunol 2020;11:1791. (PMID: 32903736)

7. Aker M, Varadi G, Slavin S, Nagler A. Fludarabine-based protocol for human umbilical cord blood transplantation in children with Fanconi anemia. J Pediatr Hematol Oncol 1999;21:237-9. (PMID: 10363858)

8. Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A, Macmillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatr Blood Cancer 2006;46:630-6. (PMID: 16078221)

9. Hertenstein B, Arseniev L, Novotny J, Ganser A. A comparative review of methods for T cell depletion in the prophylaxis of graft-versus-host disease. BioDrugs 1998;9:105-23. (PMID: 18020549)

10. Chaleff S, Otto M, Barfield RC, et al. A large-scale method for the selective depletion of alphabeta T lymphocytes from PBSC for allogeneic transplantation. Cytotherapy 2007;9:746-54. (PMID: 17917892)

11. Khan FM, Sy S, Louie P, et al. Genomic instability after allogeneic hematopoietic cell transplantation is frequent in oral mucosa, particularly in patients with a history of chronic graft-versus-host disease, and rare in nasal mucosa. Blood 2010;116:1803-6. (PMID: 20548092)

12. Deeg HJ, Socié G, Schoch G, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood 1996; 87:386-92. (PMID: 8547667)

13. Yabe M, Morio T, Tabuchi K, et al. Long-term outcome in patients with Fanconi anemia who received hematopoietic stem cell transplantation: a retrospective nationwide analysis. Int J Hematol 2021;113:134-44. (PMID: 32949371)

14. Dumache R, Enache A, Barbarii L, et al. Chimerism Monitoring by Short Tandem Repeat (STR) Markers in Allogeneic Stem Cell Transplantation. Clin Lab 2018;64:1535-43. (PMID: 30274026)

15. Faraci M, Bagnasco F, Leoni M, et al. Evaluation of Chimerism Dynamics after Allogeneic Hematopoietic Stem Cell Transplantation in Children with Nonmalignant Diseases. Biol Blood Marrow Transplant 2018;24:1088-93. (PMID: 29292059)

16. Park M, Koh KN, Seo JJ, Im HJ. Clinical implications of chimerism after allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. Korean J Hematol 2011; 46:258-64. (PMID: 22259632)

17. Lejman M, Zawitkowska J, Zaucha-Prażmo A, et al. Influence of mixed chimerism on outcome in children with anaemia after haematopoietic stem cell transplantation. In Vivo 2019;33:2051-7. (PMID: 31662537)

18. Tuysuz G, Guler E, Ozel D, Kupesiz A. Results of Allogenic Hematopoietic Stem Cell Transplantation in Fanconi Anemia Caused by Bone Marrow Failure: Single-Regimen, Single-Center Experience of 14 Years. Biol Blood Marrow Transplant 2019;25: 2017-23. (PMID: 31199982)

19. Remberger M, Afram G, Sundin M, et al. High incidence of severe chronic GvHD after HSCT with sibling donors. A single center analysis. Bone Marrow Transplant 2016;51:1518-21. (PMID: 27272449)

20. Peffault de Latour R, Porcher R, et al. Allogeneic hematopoietic stem cell transplantation in Fanconi anemia: the European Group for Blood and Marrow Transplantation experience. Blood 2013; 122:4279-86. (PMID: 24144640)

21. Verhoeyen E, Roman-Rodriguez FJ, Cosset FL, Levy C, Rio P. Gene Therapy in Fanconi Anemia: A Matter of Time, Safety and Gene Transfer Tool Efficiency. Curr Gene Ther 2017;16:297-308. (PMID: 28067165)