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Case Report

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Persistent Antigen A after Minor ABO-Incompatible Hematopoietic Stem Cell Transplantation in Children: Two Case Reports

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Abstract
Introduction: ABO blood type changes after ABO-incompatible hematopoietic stem cell transplantation (HSCT). Most non-hematopoietic tissues retain the expression of the patient's own ABO antigens, which may adsorb from the plasma onto the donor's red blood cells (RBCs). Because of this phenomenon, a persistent patient's A and/or B antigen could be detected in the laboratory, despite 100% white cell donor chimerism. Adsorption of the patient's soluble ABO antigens on the newly formed RBCs complicates the interpretation of the patient's blood type and decision of transfusion therapy. ***Case Presentation:*** The first case report is a 6-year-old girl, A, D+, with T-cell acute lymphoblastic leukemia (ALL), transplanted with HLA-matched unrelated group O, D+ bone marrow. A second case report describes an 8-year-old girl, AB, D–, with ALL transplanted with an HLA-matched related group B, D+ bone marrow. The presence of persistent antigen A was registered in both patients more than 1 year after HSCT, despite complete donor chimerism. ***Conclusion:*** The weak expression of ABO antigens on RBCs after HSCT should be examined in detail for proper planning of transfusion therapies.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has long been established as the optimal treatment for many hematological malignancies and congenital hematologic and immunologic disorders [1]. Limitations in donor availability require that ABO-incompatible (ABOi) donors be used. In general, 30% of HSCT from HLA-matched related donors and up to 50% of those from HLA-matched unrelated donors are ABOi, which requires special serological monitoring after HSCT [1–7].

Expression of A and B antigens on red blood cells (RBCs) is controlled by genes located on chromosome 9 within the ABO locus. A biochemically and functionally related system is the H system, which contains only 1 H antigen necessary for the formation of ABO antigens. Expression of the H antigen is the function of the *FUT1* locus, located on chromosome 19. Along with these 2 systems, the Lewis system is also described. Lewis antigen expression is controlled by a gene pair (*Le/Le*) at the *FUT3* locus. The process of biosynthesis of all antigens ABO, H, and Lewis is also controlled by the *FUT2* locus, which encodes the synthesis of these antigens in secretions and determines the secretory status. *FUT1*, *FUT2*, and *FUT3* genes are all located on chromosome 19. *FUT1* and *ABO* encode specific glycosyltransferases that synthesize H, A, and B antigens in mesodermal and hematopoietic tissues. *FUT2* and *FUT3*, together with

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ABO, are responsible for H, A, B, Lea, Leb, ALeb, and BLeb antigens on ectodermal tissues such as the gut, respiratory, urinary mucosae, and exocrine secretions. These antigens could be transported to the plasma and adsorbed onto the membrane of RBCs [8–10].

In the case of allogeneic HSCT, RBCs are produced from the donor's hematopoietic stem cells, which express antigens encoded by the donor's ABO gene. As a final result, the patient's ABO blood group is changing to the donor's ABO blood group after ABOi HSCT [4, 11]. On the other hand, most non-hematopoietic tissues retain expression of the patient's own ABO antigens for life which may adsorb from the plasma onto the donor-derived RBCs. Because of that phenomenon, in some HSCT patients, a persistent patient's A and/or B antigen could be detected in the transfusion laboratory, despite 100% white cell donor chimerism [12]. Adsorption of the patient's soluble ABO antigens on the newly formed RBCs further complicates the interpretation of the patient's blood group. All these circumstances could influence the decision of transfusion therapy and the assessment of the clinical condition of the patient [8]. In the pre-transplantation phase, the patient should be transfused with the original, native ABO RhD group. The peri-transplantation phase begins with the start of conditioning and ends with the engraftment of all cell lines. During that phase, the transfusion of blood components must be compatible with both the recipient's ABO RhD group and the donor's ABO RhD group. In the case of minor ABOi HSCT, the recommended blood group for RBCs transfusion is donor's, while for transfusions of platelets, cryoprecipitate, and fresh frozen plasma (FFP), the recipient's blood group is recommended. The posttransplantation phase begins with the engraftment of all cell lines and continues until the patient remains engrafted. In that phase, the donor's ABO RhD blood group is recommended for all blood components [13]. This article presents serological and molecular findings in two children with persistent antigen A after minor ABOi HSCT and our transfusion policy.

Case Reports

Our first case report is a 6-year-old girl diagnosed with T-cell acute lymphoblastic leukemia. Due to the poor response to the initial therapy, she was classified in the high-risk group, and further treatment included HSCT from an HLA-matched unrelated donor.

The patient's original blood group was A, D+, Le (a–b–), and the donor's blood group was O, D+. The change of blood group after HSCT was monitored using the automatic technique (IH-500, Bio-Rad, DiaMed GmbH, Cressier FR, Switzerland) and gel card method (DiaClon ABO/D + Reverse Grouping, monoclonal antibodies, Bio-Rad, DiaMed GmbH). The patient's RBCs were tested with the anti-A_1 and anti-H (Anti-A_1/Anti-H(A_2) lectin; IMMUNODIAGNOSTIKA GmbH, Ecshelbronn Germany) manual technique/tube method. The chimerism results were based on simultaneous PCR analysis of 15 polymorphic STR gene loci and amelogenin using the AmpFISTR®Identifiler®Plus PCR Amplification Kit (Applied Biosystem). Results of the patient's ABO and D blood typing and chimerism at different times pre- and posttransplant are shown in Table 1.

Molecular typing of the *ABO* gene (from the DNA extracted from the patient's donor-derived leukocytes) proved the exclusive presence of the *ABO*O.01* allele. The RBC-FluoGene ABO kit performed on the FluoVista device (both Inno-train Diagnostik, Germany) was used for molecular ABO testing.

At the time of the last checkup (18 months posttransplant), the patient's RBCs showed (2+) agglutination with monoclonal anti-A, while the patient's plasma reacted with B RBCs (4+) shown in Figure 1. Reversed typing by the tube method showed very weak (+/–) agglutination with A RBCs and strong (4+) agglutination with B RBCs. The patient did not need an RBC transfusion. The complete blood count and basic biochemical analyses were all normal. In case there was a need for transfusions, our recommendation was O, D + RBCs, A, D+ or A, D– platelets, cryoprecipitate, and FFP.

Our second case report is an 8-year-old girl with common B-cell acute lymphoblastic leukemia. She was treated according to protocol ALLIC BFM 2009. Clinical and hematological remission of the disease was confirmed on the 33rd day. One year later, a late medullary relapse of the disease was diagnosed, and treatment was continued according to protocol ALL REZ BFM 2002. HSCT from the HLA-matched related donor (sister) was performed.

The patient's original blood type was AB, D–, Le (a+b–), and the donor's blood type was B, D+. Results of the patient's ABO and D blood typing and chimerism at different times pre- and posttransplant are shown in Table 1. Molecular ABO typing, using the same test as above, demonstrated that the patient is heterozygous with the ABO*O.01 and ABO*B alleles indicating the blood type B.

We suspected that persistent antigen-A is soluble, secreted from the patient's non-hematopoietic cells, and adsorbed onto the donor's type RBCs. To confirm our hypothesis, we performed an adsorption-elution procedure according to Misaki Y et al. [11]. A healthy donor's O, D– RBCs were mixed with the patient's plasma and stored overnight at 4°C. This mixture was washed with saline the next day and divided into two test tubes. Monoclonal anti-A was added in test tube A, and monoclonal anti-B was added in test tube B and stored overnight at 4°C. The next day, unadsorbed antibodies were removed by washing with saline. For the elution of antibodies, 1,200 µL of saline was added to both test tubes and incubated at 52°C for 10 min, then centrifuged. We performed a crossmatch test of eluates A and B with A_1, A_2, B, and O RBCs using the automatic technique (IH-500, Bio-Rad, DiaMed GmbH, Cressier FR, Switzerland) and gel card method. The eluate from test tube A showed a strong agglutination (3+) with A_1 and (2+) with A_2 RBCs, while the agglutination with B and O RBCs was negative. All agglutinations with eluate from test tube B were negative, shown in Figure 2.

At the time of the last checkup (14 months posttransplant), the patient's RBCs showed (2+) agglutination with monoclonal anti-A and strong agglutination (3+) with monoclonal anti-B, shown in Figure 1. The patient was in good clinical condition with normal complete blood count and basic biochemical analyses. If transfusions were needed, our recommendation was B, D– RBCs and AB, D– platelets, cryoprecipitate, and FFP.

Table 1. The results of the patient's ABO/D blood typing and chimerism at different times pre- and posttransplant

Period	Anti-A	Anti-B	Anti-A,B	Anti-D	Anti-H	A_1 cells	B cells	Chimerism (%)
P_1 At Dg	3+	0	nt	3+	nt	0	3+	nt
P_1 14dPT	mf	0	nt	3+	nt	0	4+	100
P_1 2mPT	2+	0	nt	4+	nt	0	3+	100
P_1 15mPT	1+	0	2+	4+	4+	2+	4+	100
P_1 18mPT	2+	0	+/–	4+	nt	+/–	4+	nt
P_2 At Dg	4+	3+	nt	–	nt	–	–	nt
P_2 14dPT	mf	3+	nt	mf	nt	–	–	98
P_2 2mPT	1+	4+	nt	mf	nt	–	–	100
P_2 5mPT	1+	4+	3+	3+	3+	–	–	99
P_2 14mPT	2+	3+	nt	3+	nt	–	–	100

P_1, patient 1; P_2, patient 2; At Dg, at diagnosis; d, day; m, month; PT, posttransplant; mf, mixed field; nt, not tested.

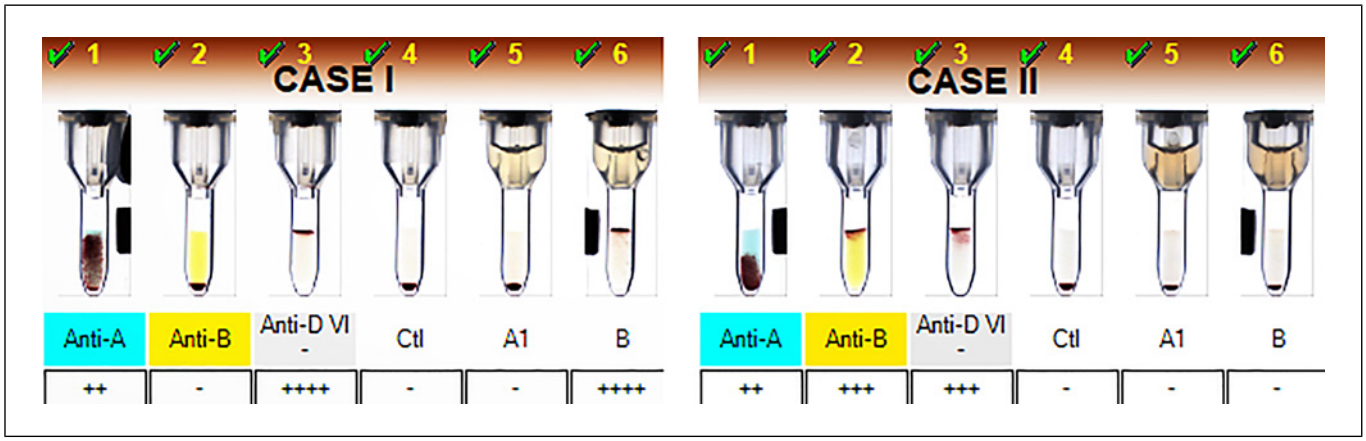

Fig. 1. The results of ABO/D typing 18 months posttransplant (case I) and 14 months posttransplant (case II) using the automatic technique (IH-500, Bio-Rad, DiaMed GmbH, Cressier FR, Switzerland) and gel card method (DiaClon ABO/D + Reverse Grouping, monoclonal antibodies, Bio-Rad, DiaMed GmbH) showing (2+) agglutination with monoclonal anti-A. Ctl, control.

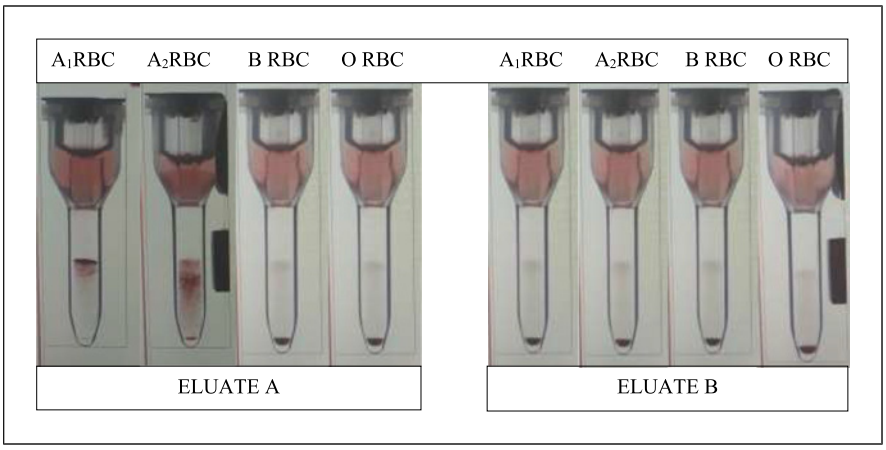

Fig. 2. The results of crossmatch testing of eluate A and eluate B with A_1, A_2, B, and O RBCs (case II). A crossmatch test of eluates A and B with A_1, A_2, B, and O RBCs using the automatic technique (IH-500, Bio-Rad, DiaMed GmbH, Cressier FR, Switzerland) and gel card method showing a strong agglutination (3+) with A_1 and (2+) with A_2 RBCs and negative with B and O RBCs from the test tube A. All agglutinations with eluate from test tube B are negative.

Discussion

Regular monitoring of the ABO blood type after ABOi HSCT as well as correct serological interpretation of results is an important part of control and transplantation outcomes. New automated techniques in immunoserology enable early detection of persistent recipient's ABO antigens, while flow cytometry can quantify them [11, 14]. On the other hand, the recipient's soluble antigen can be proven by using a simple adsorption-elution method [11]. Molecular ABO typing can prove that the donor's RBCs have been engrafted, which can indirectly also indicate that it is a case of soluble patient's ABO antigens with the appropriate endorsement of the serological data.

The literature data in the field of immunoserological changes after ABOi HSCT are scarce. Most of the articles are based on case reports from the adult population. De Vooght et al. described 2 case reports of persistent recipient's ABO antigens in young adults [12]. Their results of ABO blood typing 6 months after HSCT showed a weak agglutination with anti-A (1+/0.5+). White cell donor chimerism was 100% in both patients, and they were in good clinical condition. The main problem was the patient's concern that their original stem cells were still present in the circulation and that the disease would relapse [12]. Therefore, it is more important that physicians correctly interpret the results and explain them in detail to the patient or their parents.

In our first case report, after we had detected a weak agglutination with monoclonal anti-A, 6 months after HSCT, we decided to perform molecular ABO typing, which confirmed the change of the blood type into the donor's. This confirmation made it much easier to understand this phenomenon. It also reduced the parent's concern about the occurrence of the engraftment. We observed a similar situation with the second case report, which was more complex because, in addition to minor ABOi, there was also major RhD incompatibility. Molecular ABO typing, 6 months after HSCT, confirmed that the blood type was B, and immunoserology testing additionally confirmed the D+ phenotype (donor's). Using the adsorption-elution method, we proved that this persistent antigen-A is soluble, which is consistent with the results of other authors [8, 11].

Some authors investigated the adsorption mechanism of the soluble patient's antigens onto the donor's RBCs. Hult AK et al. [14] prepared in vitro experiments to investigate the plasma-to-cell transfer of ABO antigens, cell-to-cell transfer of ABO antigens, and the detection of A and B glycosyltransferase activity in plasma. They used blood samples from 11 patients transplanted with HSCs from ABOi donors, and 15 non-transplanted patients of blood group A, B, or AB were transfused with O RBCs units. To investigate the functional activity of A and B glycosyltransferases, they added the substrate uridine diphosphate N-acetylgalactosamine for A-glycosyltransferase, uridine diphosphate galactose for B-glycosyltransferase, and $MnCl_2$ to group O RBCs, then mixed with group A_1, A_2, or B plasma and incubated at 37°C. After 4 h of incubation with an appropriate substrate, A and B antigens were readily detectable by flow cytometry [14]. Based on their research, it can be concluded that glycosyltransferases play a crucial role in the mechanism of ABO antigens binding from plasma to O RBCs.

In both cases that we describe, the patient's RBCs showed stronger agglutination with anti-A, B serum (human antibodies) than with anti-A serum (monoclonal antibodies). These results are probably due to the higher association constant found with immune anti-A, B in group O serum compared with anti-A in group B serum [8]. Testing with anti-A, B serum showed that the presence of persistent antigen A is similar to subgroup A_x [15]. Due to this fact, at one point, we thought that the donor for our first patient was subgroup A_x, but we rejected that hypothesis after molecular ABO typing. Despite the fact that the molecular typing proved blood group change, our transfusion policy was based on serological results, and the recommendation was the same as for the peri-transplantation phase.

There is no literature data that explains the relationship between persistent antigen A and relapse rate. The data are mostly based on case reports, and there is no clinical study that investigated the prediction of higher relapse risk in patients with persistent antigens. In their clinical practice, De Vooght et al. [12] had 5 patients with persistent antigens for 2 years. None of their patients show any signs of engraftment failure or relapse of the disease, with a follow-up time ranging from 1 to 8 years.

Our case reports have some limitations. First and foremost, we did not perform antigen quantification by flow cytometry. In our first case, the adsorption-elution method was not performed and we have not proven that the antigen is soluble. We also did not perform the *FUT2* genotyping to determine secretory status. Considering that there is no literature data that explains the relationship between persistent antigen A and relapse rate, future multicenter studies could be focused on such research.

In conclusion, we suggest that any weak expression of ABO antigens after HSCT should be examined in detail due to proper planning of transfusion therapies. Automated techniques in transfusion laboratories significantly contribute to the early detection of persistent ABO antigens after allogeneic HSCT. ABO molecular typing determines whether it is a persistent antigen, graft rejection, or disease relapse. The adsorption-elution method is a simple and inexpensive method that can ultimately prove soluble ABO antigens.

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Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of the Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić," Belgrade, approval number 8/68/23. The entire research was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Biljana Andrić contributed to the conception and design of the manuscript, material preparation, data collection, and analysis and drafting of the manuscript. Zorica Radonjić contributed to the conception and design of the manuscript. Olivera Šerbić, Dragana Vujić, Željko Zečević, Marija Simić, Borko Gobeljić, Snežana Jovanović-Srzentić, and Ivana Radović commented and made critical revision on previous versions of the manuscript and read and approved the final version of the manuscript for publication.

Data Availability Statement

Data could be obtained from the corresponding author upon request.

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