

ABO serology in a case of persistent weak A in a recipient following a group O–matched unrelated bone marrow transplant

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HLA-matched hematopoietic stem cell transplantation (HSCT) from red blood cell (RBC)-incompatible donors is not uncommon. The engraftment process following ABO-incompatible allogeneic HSCT results in the transition from patient blood group to donor blood group in the recipient. In contrast, most non-hematopoietic tissues retain expression of the patient's original blood group for life, and these antigens may adsorb from the plasma onto the donor-derived RBCs. Correct serologic interpretation of the ABO blood group during this engraftment process can be difficult. We present the serologic findings of a 15-year-old girl of Maori descent, who was diagnosed with acute myeloid leukemia and transplanted with an HLA-matched unrelated group O, D+ bone marrow. Despite engraftment, her RBCs showed persistence of weak A. This case report showcases the importance of awareness and correct serologic interpretation of weak persistence of recipient ABH substance on the patient's RBCs for clinical decision-making, blood component support, and patient well-being. *Immunohematology* 2017;33:99–104.

Key Words: ABO, transplant, serology, monoclonal, antibody, recipient ABH substance

HLA-matched hematopoietic stem cell transplantation (HSCT) from red blood cell (RBC)-incompatible donors is not uncommon.¹ ABO-incompatible HSCT is of particular importance because of the consequences of acute hemolysis and the complexity of blood component selection.

ABO antigens and the closely related H, secretor, and Lewis histo-blood group carbohydrates are synthesized by specific glycosyltransferases encoded by *ABO*, *FUT1*, *FUT2*, and *FUT3*, respectively. These enzymes incorporate monosaccharide units into precursor oligosaccharide chains, modifying them and creating new antigenic specificities.² *FUT1* and *ABO* encode enzymes that synthesize H, A, and B in mesodermal and hematopoietic tissues that are therefore intrinsic to the RBC. *FUT2* and *FUT3*, together with *ABO*, are responsible for H, A, B, Le^a, Le^b, ALe^b, and BLE^b carbohydrates on ectodermal tissues such as the gut, respiratory and urinary mucosae, and exocrine secretions, which are transported to the plasma and adsorbed onto the RBC membrane.^{2–4}

The engraftment process following ABO-incompatible allogeneic HSCT results in the transition from patient blood

group to donor blood group in the recipient. In contrast, most non-hematopoietic tissues retain expression of the patient's original blood group for life, and these antigens may adsorb from the plasma onto the donor-derived RBCs.⁵

The correct serologic interpretation of the recipient's ABO blood group during this engraftment process can be difficult because of serologic discrepancies caused by ABO incompatibility between the recipient and donor during engraftment, transfusion of blood components with differing ABO group to the recipient, administration of high-dose intravenous immunoglobulin producing elevated levels of ABO isoagglutinins, engraftment failure, and relapse. In addition, adsorption of recipient antigens onto donor RBCs adds another level of complexity to the interpretation of the ABO group, and this may have implications for blood component support and clinical decision-making.

We present the serologic findings of a case of persistent weak A in a recipient following a group O HLA-matched unrelated bone marrow transplant.

Case Report

A 15-year-old girl of Maori descent was diagnosed with acute myeloid leukemia in 2014. Her RBCs typed as group A, D+, Le(a–b–). A total of 46 units of group A, D+ RBCs and 64 units of group A and 2 units of group O apheresis platelets were transfused during the first 5 months following diagnosis. The patient was then transplanted with an HLA-matched unrelated group O, D+ bone marrow. A further 7 units of RBCs, all group O, D+, and 18 group A and 4 group O units of apheresis platelets were transfused post-transplant, within a 1-month period. There were no units of RBCs or platelets transfused during the following 27 months.

Engraftment was confirmed by day 24 post-transplant with short tandem repeat chimerism studies (lymphocytes) showing 99 percent chimerism (Table 1).

Five months post-transplant, routine blood grouping (BioVue column agglutination technology [CAT]; Ortho

Table 1. Results of patient ABO and D blood typing by CAT (BioVue, Ortho Clinical Diagnostics, Bridgend, UK; agglutination grade 0–4+) and short tandem repeat lymphocytes chimerism studies at diagnosis and at different time points post-transplant

	Time Point	Anti-A (MH04, 3D3)	Anti-B (NB10.5A5, NB1.19)	Anti-A,B (MH04, 3D3, NB10.5A5, NB1.19)	Anti-D	Anti-H	A ₁ Cells	B Cells	STC (%)
Post-Transplant	At diagnosis	4+	0	nt	4+	nt	0	4+	nt
	Day 9	mf	0	nt	4+	nt	0	3+	nt
	Day 24	nt	nt	nt	nt	nt	nt	nt	99
	3 months	nt	nt	nt	nt	nt	nt	nt	99
	5 months	1+	0	nt	4+	nt	0	3+	99
	8 months	nt	nt	nt	nt	nt	nt	nt	98
	9 months	2+	0	nt	4+	nt	0	3+	nt
	28 months	1+	0	2+	4+	4+	0	2+	nt

mf = mixed field; nt = not tested; STC = short tandem repeat lymphocytes chimerism.

Clinical Diagnostics, Bridgend, UK) showed weak reactivity of patient RBCs with monoclonal anti-A; anti-B was detectable in the reverse group. These weak reactions persisted throughout the 28-month post-transplant review period. At the time of last review, the full blood count and renal and liver function indices were all normal.

Materials and Methods

Patient Blood Typing

ABO and D typings were performed using commercially available reagents according to the manufacturer's protocols by conventional tube test (CTT), as well as by CAT (BioVue CAT, Ortho Clinical Diagnostics, and Bio-Rad CAT, Bio-Rad Laboratories, DiaMed GmbH, Cressier, Switzerland). The patient's RBCs were also tested using a lectin (Anti-H lectin, *Ulex europaeus*; Seqirus, Victoria, Australia) and monoclonal antibodies (Epiclone Anti-Le^a and Anti-Le^b; Seqirus). The ABO antisera clones are shown in Table 2. Patient and control RBCs (Securacell; Seqirus) were treated with papain (Papain Solution; Seqirus), and the forward ABO typing was tested by Bio-Rad CAT (DiaClon ABO/Rh for Newborns; Bio-Rad Laboratories).

Papain-treated and -untreated patient RBCs were also tested against two group O plasma samples with known anti-A titers of 256 and 512, respectively, and read at antiglobulin phase (Anti-IgG-C3d; Bio-Rad CAT, Bio-Rad Laboratories).

Adsorption-Elution Studies

Adsorption-elution studies were undertaken to confirm the presence of A using one volume of papain-treated patient RBCs incubated at room temperature for 1 hour with 1 volume of anti-A containing Birma-1 (Gamma-Clone Anti-A; Immucor, Norcross, GA), anti-B using B9 (Epiclone Anti-B;

Seqirus), and a blended anti-A,B using 4E7, B9, and ES15 (Epiclone Anti-A,B; Seqirus).⁶ A rapid acid elution was then performed in accordance with the manufacturer's instructions (DiaCidel; Bio-Rad Laboratories). The eluate and last wash were incubated for 10 minutes at room temperature with commercial A₁, B, and O cells (Seqirus) using the CAT card (ID-card NaCl, enzyme test, and cold agglutinins; Bio-Rad Laboratories). Controls were run using A₁ and O RBCs (Seqirus).

All agglutination reactions, negative to positive, were graded as 0 to 4+, respectively.

Results

ABO and D Typings

Table 1 shows the results of the patient's ABO and D typings by CAT (BioVue) at different time points. At diagnosis, the patient's RBCs typed as group A, D+, with strong agglutination (4+) with monoclonal anti-A (BioVue CAT). Nine days post-transplant, mixed-field agglutination with anti-A (BioVue CAT) was observed, consistent with the recent transfusion of 2 group O, D+ RBC units.

By day 24 post-transplant, engraftment was confirmed with the short tandem repeat chimerism studies (lymphocytes) showing 99 percent donor detectable (Table 2).

Five months post-transplant, the patient's RBCs showed weak agglutination (1+) with A sera (monoclonal Anti-A; BioVue CAT, Ortho Clinical Diagnostics) while the patient's plasma reacted only with B cells (Seqirus) (3+). There was no evidence of mixed-field agglutination at this time. The last transfusion had occurred 4 months prior.

The patient's blood type was reviewed at 28 months post-transplant, at which point the weak reactivity (1+) of the patient's RBCs with monoclonal reagent (monoclonal

Table 2. Antisera clones for each of the ABO monoclonal antibodies used

	Anti-A	Anti-B	Anti-A,B
CAT (BioVue)	MH04, 3D3	NB10.5A5, NB1.19	MH04, 3D3, NB10.5A5, NB1.19
CAT (Bio-Rad)	LM297/628 (LA-2)	LM306/686 (LB-2)	ES131 (ES-15), BIRMA-1, ES-4
CTT (Gammaclone, Immucor)	Birma-1		
CTT (Epiclone, Seqirus)	4E7, 8F2	B9	4E7, B9, ES15

Highlighting indicates the directly agglutinating clones.

Anti-A; BioVue CAT, Ortho Clinical Diagnostics) remained (Fig. 1A). Agglutination of the patient’s RBCs with MH04, 3D3, NB10.5A5, NB1.19 clones (monoclonal anti-A,B; BioVue CAT, Ortho Clinical Diagnostics), although still weak, was stronger (2+) than that observed with the MH04, 3D3 clones (monoclonal Anti-A; BioVue CAT, Ortho Clinical Diagnostics) (Fig. 1). In contrast, there was no agglutination with anti-A LM297/628 (LA-2) clones (DiaClon ABO/Rh for Newborns; Bio-Rad CAT, Bio-Rad Laboratories) and very weak agglutination (<1+) with anti-A,B ES131 (ES-15), BIRMA-1, ES-4 clones (DiaClon ABO/Rh for Newborns; Bio-Rad CAT, Bio-Rad Laboratories) (Fig. 2A). Reactivity, following papain treatment of the patient’s RBCs, was observed with anti-A (2+ to 3+) and enhanced with anti-A,B (3+ to 4+) (both DiaClon

ABO/Rh for Newborns; Bio-Rad CAT, Bio-Rad Laboratories) (Fig. 2B). Papain-treated commercial A₁, A₂, A_{wk}, B, and O RBCs (Seqirus) were included as controls for the Bio-Rad CAT (Bio-Rad Laboratories); there was no false-positive or false-negative agglutination.

When CTT typing was performed, there was no agglutination using two different A sera (4E7, 8F2, Epiclone Anti-A, Seqirus, and Birma-1 Gamma-Clone Anti-A, Immucor), although weak agglutination (1+) was observed with AB clones 4E7, B9, and ES15 (Epiclone Anti-A,B; Seqirus).

Group O plasma with an anti-A titer of 512 agglutinated both untreated (2+) and papain-treated (3+ to 4+) patient RBCs. In contrast, the group O plasma with an anti-A titer of 256 only agglutinated papain-treated (2+) patient RBCs and not the untreated sample.

Adsorption-Elution Studies

BIRMA-1 (Gamma-Clone Anti-A; Immucor) and 4E7, B9, and ES15 (Epiclone Anti-A,B; Seqirus) were detectable in the eluate with A₁ cells following adsorption-elution studies (4+), and the absence of mixed-field reactivity confirmed the presence of A only on the patient’s RBCs. Eluates prepared from anti-A- or anti-A,B-adsorbed RBCs were not reactive with commercial B or O cells (Seqirus). Monoclonal anti-B was not detectable in the eluate, and there were no antibodies demonstrable in the final wash.

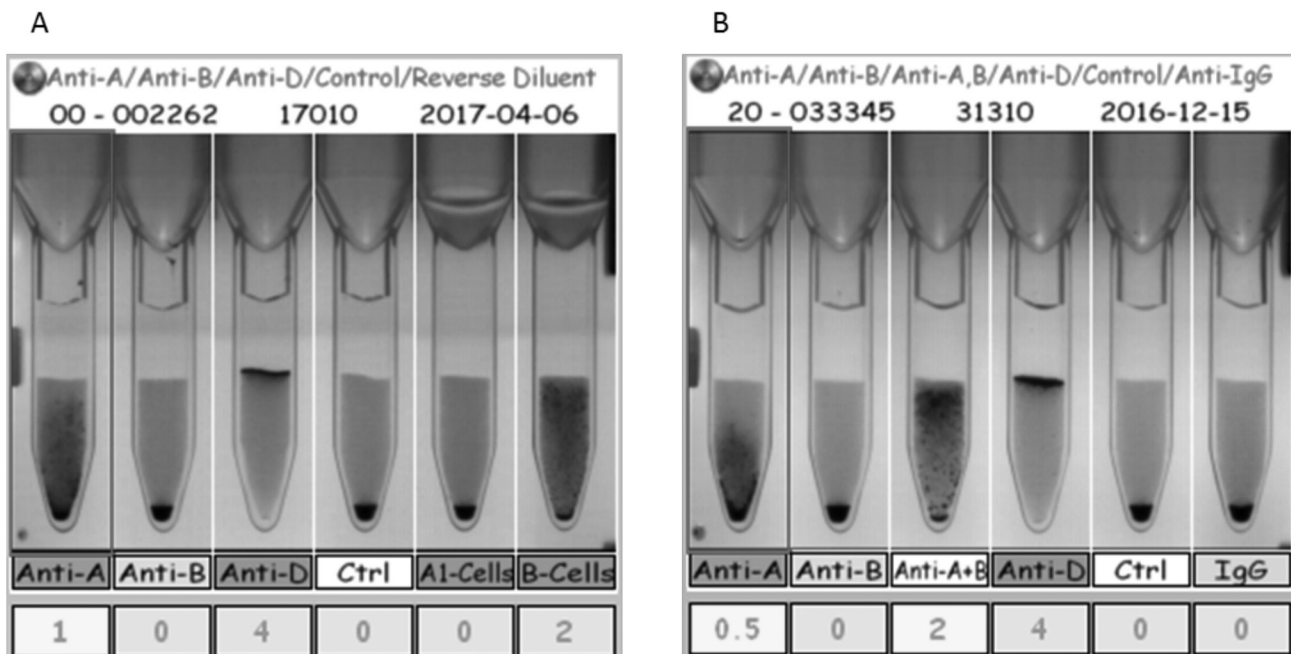


Fig. 1 Results of patient's ABO typing, forward and reverse, and D typing using CAT (BioVue; Ortho Clinical Diagnostics, Bridgend, UK) showing a weak reaction with monoclonal anti-A (A) and the comparative difference in reaction strength with monoclonal anti-A,B (B). ctrl = control.

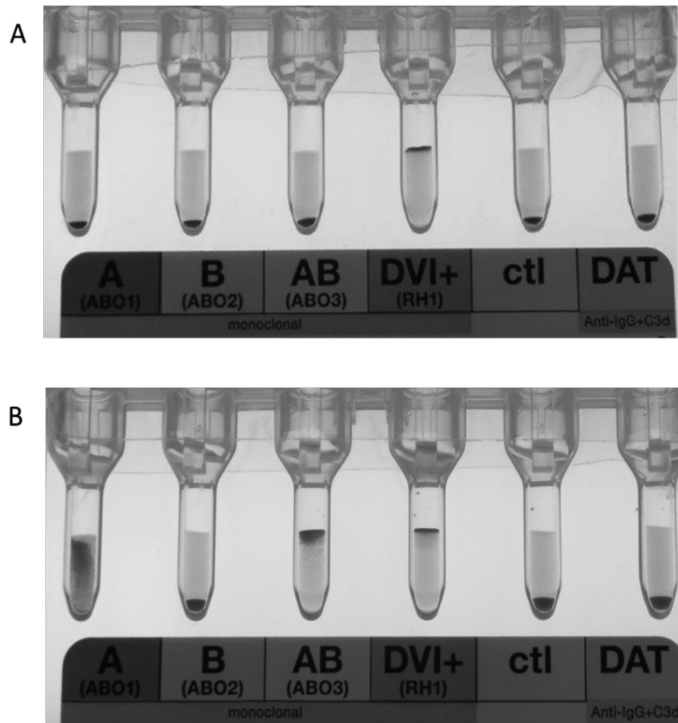


Fig. 2 Results of ABO forward type, D type, and direct antiglobulin test (DAT) with untreated patient's red blood cells (RBCs) using CAT (Bio-Rad Laboratories, DiaMed GmbH, Cressier, Switzerland) (A) compared with papain-treated patient RBCs (B). ctl = control.

Discussion

A compromise in ABO compatibility is often required to satisfy the most important predictor of allogeneic matched unrelated HSCT outcome—namely, the HLA match between donor and recipient.⁷ As a consequence, difficulties can arise in ABO interpretation.

One area of ABO-incompatible HSCT that has received minimal attention, for which there is limited serologic information, is that of the weak persistence of recipient ABH substance.^{5,8–12}

In the case we describe, the patient's RBCs consistently showed weak agglutination with A and A,B sera (monoclonal Anti-A and Anti-A,B; BioVue CAT; Ortho Clinical Diagnostics) up to 28 months after the group O transplant; the reaction strength with anti-A,B was stronger than that with anti-A. Although other anti-A,B clones were also reactive by different methods, other monoclonal A reagents were not. This pattern of reactivity is similar to that described for the ABO subgroup A_x , where very low levels of A are agglutinated by the majority of group O sera, but by only a few group B sera.¹³ This phenomenon was thought to be a result of the higher

association constant found with immune anti-A,B in group O sera compared with anti-A in group B sera.¹⁴

Two clones, ES-15 and MH04, which were present in the antisera showing persistent weak agglutination with the patient's RBCs post-transplant, have been reported to detect low levels of A expression.^{15,16} As an antisera, clone ES-15 has been reported to agglutinate 95 percent of A_x examples¹⁵; the BIRMA-1 clone agglutinated only 60 percent.¹⁷ MH04, although able to detect weak subgroups of A including A_x , has been associated with false-positive reactions in 1 percent of group B donors. These donors, termed B(A), were shown to exhibit high levels of B-gene–specified transferase activity resulting in the transfer of small amounts of *N*-acetyl-D-galactosamine (A-active structures) to H-active structures on group B RBCs.¹⁶ As a consequence of this finding, recommendations were made that monoclonal A reagents should not detect the so-called B(A) phenomenon. It was considered desirable, but not essential, for the monoclonal A reagents to react with RBCs expressing very low levels of A, including A_x and A_h , but essential for monoclonal anti-A,B reagents to do so.¹⁸

It is interesting to speculate further as to why the reaction strength with A,B sera was stronger than with the A sera (BioClone Anti-A and Anti-A,B; BioVue CAT; Ortho Clinical Diagnostics) when the clones for A,B sera (which include MH04) were a blend of the A and B clones. This difference in reaction strength does not fit the concept of a higher association constant as described for human anti-A,B in group O sera.¹⁴ Perhaps the potency of MH04 has simply been reduced to overcome the B(A) phenomenon.

Although monoclonal antibody specificity is important in the detection of persistent weak A expression, we cannot exclude the role of macromolecular potentiators, present in the one CAT system (BioVue) to enhance the antigen–antibody reactions. In the ABO setting, papain treatment of RBCs is also known to enhance antigen–antibody reactions through removal of glycoproteins from the surface layer, with resultant reduction of steric repulsion and a 40 percent decrease in surface charge density.^{19,20} In the case we describe, papain treatment of the patient's RBCs confirmed the presence of A, enabling direct agglutination with a monoclonal anti-A that had not been agglutinable.

Garratty et al.¹² studied the RBCs from an A_1 patient expressing weak A reactivity 5 months after receiving a group O bone marrow transplant using flow cytometry and found that there was a single right-shifted population. In contrast, chimeric or mixed-field RBCs showed two distinct populations. We, too, found no evidence of mixed-field agglutination either

by CTT or CAT, consistent with weak recipient A substance adsorbed onto the RBCs from the plasma. The concept that group O RBCs can acquire weak A or B was initially described in 1962, when Renton and Hancock²¹ observed that group O RBCs, which had been emergently transfused into group A or B recipients, produced “loose stringy” agglutination when reacted with group O sera. It was later found that the amount of A in lipid fractions isolated from the serum of group A donors was highest from group A₁ Le(a-b-) secretors.²² Needs et al.⁸ also ascertained the importance of secretor status when they observed “small quantities” of recipient ABH substance on post-transplant RBCs when the recipient was a secretor but not when the recipient was a non-secretor.

It is known that 80 percent of Le(a-b-) individuals and all Le(a-b+) individuals are ABH secretors, and all Le(a+b-) individuals are ABH non-secretors.²³ It is of interest that Henry et al.²⁴ found that glycolipid fractions prepared from the plasma and RBCs of selected Polynesian samples (including Maoris) of RBC Le(a-b-), Le(a+b-), and Le(a+b+) phenotypes were found to have Le^b glycolipids, suggesting that the recessive non-secretor gene is absent or rare in a Polynesian-derived gene pool. In our case the patient's RBCs typed as Le(a-b-). Although we recognize that the Lewis phenotype can be misleading in certain physiological and disease states,²⁵ it is likely that our patient was a secretor, and this assumption is consistent with the persistent presence of recipient A on the patient's RBCs.

One limitation of our study is that we did not measure the level of soluble recipient ABH in the patient's plasma. In a recent study by Hult et al.,⁵ flow cytometric analysis confirmed the major role of A/B adsorption from secretor plasma; an additional, secretor-independent mechanism for A/B acquisition was also indicated.

Available evidence suggests that persistence of weak recipient ABH substance is not associated with relapse.¹⁰ In the experience of De Vooght et al.,¹⁰ patients expressed concern that when this phenomenon was exhibited, it represented an unsuccessful transplant or relapse that then created unnecessary anxiety that affected the patient's quality of life.

The decision to switch a patient's blood group following ABO-incompatible HSCT is complex and varies across institutions.⁷ Major or minor ABO mismatches, engraftment, relapse, and transfusion dependence all affect the decision. In addition, adsorption of recipient antigens onto donor RBCs adds another level of complexity to the interpretation of the recipient's ABO group.

The phenomenon of recipient weak ABH substance on the patient's RBCs may affect the choice of ABO group for blood

component transfusion support in patients undergoing HSCT. For RBC transfusions, the ABO group of the components should be compatible with both the recipient and donor following HSCT, and then the components should be switched to the donor group following engraftment. It is now recommended, however, that platelet and plasma components remain ABO-compatible with both the recipient and donor for life.^{5,26}

In conclusion, the awareness and correct serologic interpretation of recipient weak ABH substance on a patient's RBCs is important for clinical decision-making, blood component support, and patient well-being.

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