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Detection of Clinically Significant Warm Reactive Anti-A1 in a Post Red Blood Cells Transfusion Patient with Acute Lymphoblastic Leukemia

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Abstract

The purpose of this article is to report that some anti-A1 that react at 37˚C are IgG antibodies and are clinically significant, as they can cause the destruction of a proportion of A1 cells *in vivo* following the transfusion of red blood cells. Therefore, when a serum of an individual contains anti-A1, further testing of the plasma against group A1, A2, and O by referencing red blood cells and individual cells against anti-A1 lectin (Dolichos biflorus lectin) must be performed. Confirming the specificity of anti-A1 by referencing red blood cells is also important in selecting the appropriate blood for transfusion. ABO antibodies are naturally occurring and activate the complement cascade, making them more likely to cause severe transfusion reactions compared to antibodies to other RBC antigens.

Keywords

Anti-A1, Clinical Significant, Direct Antiglobulin Testing, Anti-A1 Lectine, Compatibility

1. Introduction

Sera from group B individuals appear to contain two antibody components, anti-A and anti-A1. A1 cells react with both components, whereas A2 cells react only with anti-A. Anti-A1 is present in the serum of some A2 and A2B people. Therefore, the two major subgroups of A are A1 and A2. Differences between A1

and A2 red cells include a substantially higher number of A sites on A1 than A2 red cells, while A2 red cells have substantially higher expression of H antigen than A1 cells. Anti-A1 is usually considered clinically insignificant when it is active *in vitro* at 30˚C. However, if it is active at 37˚C, it is considered clinically significant and can cause the destruction of a proportion of A1 cells *in vivo* following the transfusion of red blood cells. In this case report, the authors support other previously reported cases indicating that anti-A1 is clinically significant and can cause hemolytic transfusion reactions if detected at 37˚C. Additionally, we highlight the role of Transfusion Medicine Services team members and the available resources in collaborating to be more meticulous in detecting and investigating the presence of subgroup anti-A1 and its role in causing incompatible red blood cells to avoid undesired patient care outcomes.

2. Case Report

Eleven years old girl with acute lymphoblastic leukemia was admitted to emergency room (ER) department at King Abdullah Specialized Children's Hospital due to severe anemia as a new patient without any history. She presented with two months' history of persistence paleness, fatigability and weight loss and she received red blood cells and platelets transfusion at the private hospital. On the same admission, her initial laboratory values demonstrated pancytopenia with hemoglobin (Hgb) level of 4.3 (11.3 - 15.0) g/dL, Hematocrit (Hct) of 0.131 (0.31 - 0.45) L/L, Platelets count of 6 (150 - 400 × 10^9/L) and Low Density Hypogammaglobinemia (LDH) of 1424 (125 - 220) U/L. Type and screen sample with six units of platelets and two units of packed red blood cell requests were received in Blood Bank. Patient blood group was confirmed as A positive with negative antibody screening (**Table 1**). The two units of red blood cells were prepared by using electronic cross-match and transfused without any complications. Hematological parameters after the transfusion were: Hemoglobin (Hgb) 9.0 g/dL and Hematocrit (Hct) 0.255 L/L. The patient was transferred to the pediatric intensive care unit and subsequently experienced fluctuations in hematological values, with a tendency of further drops. On the fourth day of admission a new type and screen and direct antiglobulin test (DAT) samples were received in blood bank and blood group was found to be A positive with weak reaction with A1 cells in reverse grouping (**Table 2**). On observing a discrepancy in the reverse grouping, which was considered in the beginning Pseudo agglutination due to rouleaux effect because it dispersed by replacing the plasma with normal saline which is known as saline replacement technique and antibody screening was found to be positive (**Figure 1**). Direct antiglobulin testing has been shown to be positive, where 1+ with complement anti-Sera (anti-C3d) and Anti-Immunoglobulin (anti-IgG) respectively. In this sample, a discrepancy was encountered and as per our institution guidelines all ABO discrepancies must be investigated and resolved before the correct ABO type can be determined and the test was repeated to rule out any technical errors. So, if similar discrepant results are noted after repeating the test, record the result as Discrepant and only type O, Rh-compatible

blood should be issued until the investigations are completed. Since the antibody screen was positive, antibody identification was carried out by using solid phase technique (Figure 2) where anti-Jka with dosage effect was identified. Four red blood cells units of group A positive, JKA negative were fully cross matched by using indirect antiglobuline technique and found to be incompatible. In contrast, two red blood cell units of O positive, JKA negative were cross-matched by using The Indirect Antiglobulin Crossmatch technique. Anti-A1 lectin showed mixed field reaction with the patient cells due to the history of blood transfusion of A positive red blood cells and since the patient had been recently transfused within the last three months, the phenotyping will not be reliable (Table 3). On further investigations to prove the presence of anti-A1, simultaneously, eight units of red blood cells group A positive were tested against anti-A1 lectine and six units out of them were found to be positive while the remaining two units were found to be negative. The red blood cells units which are agglutinated with Anti-A1 lectin are said to be of the subgroup A1 and those which are not agglutinated by Anti-A1 lectin fall into subgroups A2. Again, the two units that were not agglutinated by Anti-A1 lectin were compatible on serologic cross-match using the same methodology. Finally, the results are most consistent with anti-Jka and anti-A1 and patient blood group is A2. The ideal decision that was taken was to transfuse the two units of A1 negative and Jka negative red blood cells units, so the units were subjected to compatibility testing and patient successfully received the products without complications or transfusion reaction and had an appropriate increase of hemoglobin to 7.6 g/dL that was sustained during the hospitalization.

Table 1. Blood Grouping with the 1st sample.

Type and Screening Test by Solid Phase Technique							
Forward Blood Grouping				Reverse Blood Grouping		Interpretation	Antibody Screening
Anti-A	Anti-B	Anti-D	Rh Cont.	A1 Cell	B Cell	ABO/RH	3 Cells Panel
4	0	4+	0	0	3	A POS	Negative

Table 2. Blood Grouping of sample after four day of admission * after saline replacement, Weak Positive (WP).

Tube Method Blood Group						
Forward Blood Grouping				Reverse Blood Grouping		Interpretation
Anti-A	Anti-B	Anti-D	Rh Cont.	A1 Cell	B Cell	ABO/RH
4+	0	4+	0	WP/*0	3+/*3+	A POS

Table 3. Testing of patient sample with Anti-A1 Lectin.

Patient Cells with	Patient's Plasma with			
Anti-A1 Lectin	A1. Cell	A2. Cell	B. Cell	O. Cell
Mixed field reaction	0	0	3	0

CAPTURE-R READY SCREEN (3)
Master List

IMMUCORE, INC, Norcross, GA 30071 USA
US LICENSE NO:886
LOT NO: E482
EXPIRES: 2022/07/19

CELL		Rh-Hr						Kell						Duffy		Kidd		Lewis		P1	MN				Lutheran		Xga	Patient Result
	DONOR	D	C	c	E	e	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	
I	R1wR1 B7316	+	+	0	0	+	+	0	+	+	+	0	+	+	+	+	0	0	+	+	0	+	0	+	0	+	0	1+
II	R2R2 C5474	+	0	+	+	0	0	+	+	0	+	0	+	+	0	0	+	+	0	0	+	+	+	+	+	+	+	0
III	rr N4928	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	0	0	+	+	0
	Positive Control	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	3+

Figure 1. Antigram showing results of Antibody screening 3 cell panel.

CAPTURE-R READY- ID
Master List

IMMUCORE, INC, Norcross, GA 30071 USA
US LICENSE NO:886
LOT NO: ID 432
EXPIRES: 2022/07/05

CELL		Rh-Hr						Kell						Duffy		Kidd		Lewis		P1	MN				Lutheran		Xga	Patient Result
	DONOR	D	C	c	E	e	Cw	K	K'	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xg a	
1	RzR1 A4999	+	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	0	0
2	R1wR1 B2230	+	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	0
3	R2R2 C6697	+	0	+	+	0	0	0	+	0	+	0	+	+	W	+	0	0	+	+	+	+	+	+	0	+	+	WP
4	Ror D1371	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0	0	0	+	+	0
5	r'r E1161	0	+	+	0	+	0	0	+	+	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0
6	r"r F848	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	0	+	0	+	+	0
7	rr H1797	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0
8	rr G1827	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	+	+	WP
9	rr H2055	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	0	+	+	+	0	0
10	rr N3054	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	+	0
11	rr G1782	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	0	WP
12	rr H1990	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	0	+	+	0
13	rr N4483	0	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	0	0	+	0	+	0	0	+	+	0
14	R1R2 A4668	+	W	+	+	+	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	0	+	0	+	+	0
15	POSITIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	4
16	NEGATIVE CONTROL	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	0

Figure 2. Antigram showing results of antibody identification 16 cell panel.

3. Materials and Methods

3.1. Type and Screen Policy

For pre-transfusion testing, a full 7 ml lavender top tube is required to carry out forward (cell), as well as reverse (plasma) grouping and antibody screening and compatibility testing. The validity of type and screen sample is 72 hours post collection. If a patient's ABO-Rh group history is unknown, the blood bank will obtain two separate types and screen samples. These samples will be submitted to the transfusion services for pre-transfusion testing and compatibility. All specimens must be tested for ABO group and Rh type to determine the patient's blood group, and also for the presence of unexpected antibodies to red blood cell antigens using the antibody screening test. The ABO and D blood groups of new

patients are determined twice, in two samples that have been drawn independently of each other, in order to establish a definitive blood group prior to blood transfusion.

3.2. Serologic Testing

Whole blood samples were collected via venipuncture into an EDTA tube (BD Vacutainer K2E EDTA, 7 mL, 10.8 mg). Samples were then centrifuged at 4800 rotation per minute (rpm) for 10 minutes to separate plasma from the RBCs at Room temperature, which can be then stored at 2˚C - 10˚C for 14 days as per AABB (Association for the Advancement of Blood & Biotherapies) guidelines. ABO forward and reverse grouping and Rh antigen phenotyping were done by the automation system on the Galileo Neo system (Immucor Inc., Norcross, GA, USA). The Galileo Neo is a microprocessor-controlled instrument designed to fully automate immunohematology *in vitro* diagnostic testing of human blood. The NEO automates test processing, result interpretation and data management functions. The NEO is designed to automate standard immunohematology assays using a microplate-based platform. The antibody screens and the initial antibody identification were processed following the manufacturer's instructions of the Capture-c® Ready-Screen® and Capture-R® Ready-ID® (Immucor Inc., Norcross, GA, USA) accordingly. Both tests were done on the same automation equipment. The secondary ABO/RhD grouping was performed using conventional tube technique (Immucor Inc., Norcross, GA, USA). Extended blood grouping was performed on patient plasma using A2 cell and O cell (Immucor Inc., Norcross, GA, USA). The patient and donor RBC phenotype used A1 Lectin (Immucor Inc., Norcross, GA, USA) then the Direct antiglobulin test (DAT) was done by gel technique (Across® Monospecific Coombs®, DiaPro®). Elution on DAT-positive sample was performed using "Acid elution" method. The eluates were subsequently tested with A_1 cell and B cell by gel technique (Across® Anti-IgG®, DiaPro®).

4. Discussion

Apart from the main known blood groups in ABO system namely A, B, AB and O that were discovered by Landsteiner, there are subtypes of A antigen that are genetically and phenotypically distinct. Nearly, 80% of individuals are A1 while 20% are A2 and this percent different from one population to another. A_1 red cells express about 5 times more A antigen than A_2 red cells, but both A1 and A2 red cell react with anti-A. As far as transfusion purposes are concerned, the A_1 and A_2 blood groups are interchangeable [1].

Anti-A1 are regularly observed by reverse grouping testing and can cause ABO discrepancies during ABO routine testing at room temperature and lead to incompatible cross-matches with A1 and A1B red cells. Anti-A1 is usually of IgM isotype, reacting best at room temperature or below, and is usually considered clinically insignificant. But if the reactivity is observed at 37˚C, therefore

Anti-A1 is considered clinically significant. In spite of that, the majority of published cases describe the absence of an anti-A1 in pre-transfusion serum [2]. A1 and A2 are major subgroups of blood group, both subgroup cells react with anti-A. However, anti-A1 lectin differentiates them as it does not react with A2 cells. It is well known that anti-A in B blood group donors generally have primary anti-A1 and occasionally anti-A2 [3].

On the other hand, in a patient who is blood group A2, an immune response provoked by Allo-immunization has been reported rarely, with one case series of two patients demonstrating an increase in titer and thermal amplitude of the normally weak naturally-occurring anti-A1 [4]. Notably, at the time of initial presentation for transfusion, the pre-transfusion blood bank workup did not demonstrate an ABO discrepancy or the presence of an anti-A1 on reverse grouping. Here, after multiple transfusions with group A1 RBC units, a cold-reactive anti-A1 alloantibody was developed and this was reported [5].

The anti-A1 has characteristics where the antibody demonstrated a wide thermal amplitude. This was seen when the reverse grouping demonstrated ABO discrepancy due to the presence of anti-A1 at room temperature or during the compatibility testing by immediate spin, 37˚C, and anti-human globulin (AHG) phase.

After the transfusion of patient with A2 subgroup and JKA negative RBCs, the patient had an appropriate increase of hemoglobin to approximately 8 (11.3 - 15.0) g/dl and most of incompatible post transfusion cells were clear out and antibody and DAT were found to be negative. In another study, they found that it is most likely the naturally occurring anti-A1 was responsible for acute hemolytic transfusion reaction due to the presence of a warm reactive anti-A1 [6]. This is one rare case in which a subject with an anti-A1 was detected in Elution which might be indicated that this antibody is IgG class that can involve in developing hemolytic transfusion reaction. In general, in our case we did not phenotype the A1 cells reagent for JKA to prove that the reaction of Eluate with A1 was definitely due to detection of anti-A1 in the elution. Currently, A1 cells and B cells are not part of a routine eluate panel, though one could consider adding them to routine testing methods [7]. Similarly, another reported case where the anti-A1 antibody in the serum was shown to be of IgG class and IgG was demonstrated on and eluted from sensitized, transfused red blood cells [8]. As part of investigation for Hemolytic Transfusion Reactions and if the patient is non-group O, then the eluate should be tested with group O reagent cells as well as group A and group B cells [9].

As per our institution's policy for compatibility testing and the selection of blood when an Anti-A1 is in the serum of an individual with A2 or A2B, we use the Type and Screen strategy, in which ABO confirmation of patients with a definitive blood group is performed by forward grouping and reverse grouping. If a patient is with anti-A1, usually we conduct a full investigation where the patient's cells are tested against anti-A1 lectin reagent and patient's plasma is tested against A1cells, A2 cells, and O cells to exclude the presence of another unex-

pected cold antibody. When blood transfusions are required, we provide A2 or O RBC and compatibility testing is performed at an antiglobulin phase. According to the AABB standard Anti-A1 is considered clinically significant if reactivity is observed at 37˚C. Group A2 patients with an anti-A1 that is reactive at 37˚C should be transfused with group O or A2 red cells only and group A2B patients should receive group O, A2, A2B, or B red cells [10]. Likewise, another report described a blood group A2 patient who developed an anti-A1 causing clinically significant hemolysis after Hematopoietic Progenitor Cell (HPC) transplant from an A1 donor [11].

Finally, Full investigation should be carried out if the Anti-A1 is suspected as it is one of the causes of ABO discrepancies, it can develop hemolytic transfusion reactions and its clinical manifestations have also been reported in hemopoietin stem cells and organ transplantation [12]. Development of anti-A1 antibodies after allogeneic stem cell transplantation and organ transplantation has also been reported [13].

If an individual's serum contains anti-A1 that is reactive at 37˚C, further subgrouping for A2 or A2B is required to select the appropriate blood for transfusion. In addition to routine tests, an antibody screening panel should be done to confirm the specificity of anti-A1 [14].

5. Conclusion

As illustrated above, some anti-A1 antibodies are IgG antibodies that cause discrepancies with reverse grouping and with immediate spin crossmatch. Therefore, any discrepancies in blood grouping should be taken into account and investigated to confirm anti-A1 antibody specificity and solve the discrepancy. It is extremely important in the blood bank to record initial discrepant results for proper transfusion management planning. In one case we encountered, an irregular antibody was reacting with reagent red cells. Proceeding without confirming the test would have led to undesired results and adverse effects. We encourage the implementation of universal unambiguous procedures for patients with blood group A or AB and ABO discrepancy in reverse grouping, and the proper steps to solve the discrepancy, exclude the presence of anti-A1, and provide safe blood transfusion. Blood transfusion services should have clear procedures for solving ABO discrepancies. Additionally, blood bank medical technologists should be competent with a high level of training, able to recognize the nature of ABO discrepancy, and use available technologies and resources to solve it. Therefore, we conclude that detecting anti-A1 is essential for confirming the valid blood group and subsequent blood transfusion management. Our case highlighted that anti-A1 may cause severe hemolytic anemia as it can react at 37˚C. Consequently, it is necessary to include anti-A1 lectin in blood group testing protocols when there is a discrepancy in the result. Hemolysis from anti-A1 tends to be the exception rather than the rule. These rare cases may impact the care of patients with ABO discrepancies who may be developing an anti-A1 that is reactive at 37˚C.

Patient Consent

The authors certify that they have obtained all appropriate patient consent forms.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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