

Evaluation of anti-A and anti-B alloisogglutinin titer in group O plateletpheresis donors

Abstract

Platelet (PLT) transfusions need to take into account the issues that arise due to significant amounts of ABO antigen being present on the platelets surface and anti-ABO alloisogglutinins being present in the donor's plasma. Although relatively rare, acute intravascular haemolytic transfusion reactions (AHTRs) have been caused by passive transfer of anti-A and anti-B antibodies, present in apheresis platelets (APs) of group O donors, across a minor ABO incompatibility (group A, B and AB recipients). To date, improving safety of group O PLT transfusions has focused either on establishing a safe level of alloisogglutinin titer or on reducing the volume of incompatible plasma administered. In this current study we evaluated anti-A and anti-B titers in 70 plasma samples from group O APs donors. Sixty (60) were male donors and ten (10) were female donors. Their age ranged from 20 to 58 years old (mean age: 39.8 ± 1.1). The determination of anti-A and anti-B antibodies, was performed using the method of direct agglutination, specifying IgM antibodies (Gel reagent, Ortho BioVue System). Our results showed that, anti-A titers ranged from 2 to 1024 (mean titer: 64), while anti-B titers ranged from 2 to 256 (mean titer: 32). Anti-A titers were significantly higher than anti-B ($p < 0.01$). The critical value of "high-titer" for anti-A and anti-B antibodies, based on our method and internationally accepted criteria, was defined to be at least 64. The frequency of group O APs donors, with "high-titer" anti-A and anti-B was relatively high, 55.8% and 47.2% respectively. There was no statistically significant difference between the donors, when they were divided into two age groups: over/under 40 years ($p > 0.857$ and $p > 0.861$). In conclusion, the risk of haemolysis from ABO-incompatible PLT components, due to passive transfused anti-A and/or anti-B alloisogglutinins, is small but present. Transfusion Service Personnel and Clinicians should be aware of the potential risk and they should always be alert and vigilant when it comes to ABO-incompatible platelet transfusions.

Keywords: plateletpheresis donors, platelet transfusion, alloisogglutinins

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Abbreviations: DAT, direct antiglobulin test; PPLTs, post storage, pooled plt concentrates; HTRs, haemolytic reactions; FDA, food and drug administration; CAP, college of American pathologists; TRALI, transfusion-related-acute-lung-injury; PAS, platelet additive solution

Introduction

Clinicians and Blood Service Personnel are well acquainted and experienced with RBC transfusions and practice using well established policies and strategies. To date, pre-transfusion compatibility tests performed, in Blood Banks, are still based on the interaction between antigen and antibody and subsequent agglutination of red blood cells. Tests that identify the antibody indicate the probable specificity of the antibody, but whether an antibody will destroy red blood cells bearing the corresponding antigen depends on various conditions. Thus, the concept of compatibility encompasses much more than cross matching.¹ Particularly, when it comes to PLT transfusion therapy we are dealing with issues arising from the fact that PLTs contain both significant amounts of ABO antigen on their surface, as well as anti-ABO alloisogglutinins in the donor's plasma. Although relatively rare, intravascular AHTRs have been caused by ABO antibodies after the transfusion of APs, across a minor ABO incompatibility. Additional adverse effects from ABO-incompatible plasma in PLT components include haemoglobinuria, jaundice, progressive anaemia, spontaneous coagulation, positive Direct Antiglobulin Test

(DAT) and increased osmotic fragility of the patient's red blood cells.² Improving safety of group O PLTs has focused on defining a safe level of antibody titer or by reducing the volume of incompatible plasma administrated. Levels of A and B antibodies appear to be influenced mainly by environmental factors and anti-A and anti-B molecules may be IgM, IgG or IgA. Some sera contain all three classes while non-stimulated individuals are predominantly IgM. Changes in the characteristics of anti-A or anti-B occur as a result of further immunization with pregnancy or by incompatible transfusions. They are serologically detectable through increases in titers, agglutinin avidity and haemolytic activity and have greater activity at 37°C. Sera from group O people contain two separable antibodies, anti-A and anti-B and a cross reacting antibody called anti-A,B (mostly IgG).³ From another point of view the risk of "high-titer" units is considered low with group O, post storage, pooled PLT concentrates (PPLTs). Moreover, the majority of laboratories internationally do not include a method to limit the risk of haemolysis when PLTs containing ABO-incompatible plasma must be transfused.^{4,5} On the other hand, various studies report that a potential risk does exist when ABO-incompatible PLT units, containing "high-titer" anti-A and anti-B antibodies, are transfused. The risk is even greater when group O PLT components are transfused out-of group. Titers such as 128-250 are considered critical,⁶⁻⁹ but not all agree.¹⁰⁻¹² It is necessary to establish a "golden standard" method for the determination of antibody titers in order to be able to differentiate accurately between "high-titer" donors from

the other donors. In this current study we tested samples from group O plateletapheresis donors to determine anti-A and anti-B titers by direct agglutination gel method, specifying IgM antibodies.

Objectives

- To evaluate anti-A and anti-B titers in the plasma of group O APs donors
- To define a critical value of "high-titer" based on our methodology
- To increase the awareness of medical staff on the potential risk of ABO-incompatible PLT transfusions

Materials and methods

We collected plasma samples (EDTA) from 70 prospective group O APs donors obtained from our Blood Bank Service. 60 were male donors and 10 female donors. Donors ranged between 20 and 58 years of age (mean age: 39.8 ± 1.1). All plasma samples were stored at $2^{\circ}\text{--}8^{\circ}\text{C}$ and tested, within 3 days from collection, for alloisogglutinin titer. Samples were tested, in parallel, for anti-A and anti-B antibodies by direct agglutination using the manual gel method. Prior to initiating plasma dilutions a screening test was performed, to exclude the presence of any unexpected blood group antibodies using the 0.8% Surgiscrreen (Ortho Biovue System). Serial twofold dilutions of plasma were prepared in 0.9% saline using a calibrated pipette. Specifically, 10 μl of 3% commercially prepared A₁ and B RBCs (Ortho Biovue System) and 40 μl of neat or diluted plasma were added to buffered gel cards (Reverse Diluent Cassette, Ortho Biovue System), and within half an hour, centrifuged (3400rpm) for 5 min using the Ortho Biovue System centrifuge. Results were read and recorded immediately after centrifugation. The highest dilution causing agglutination was assumed to represent IgM antibody titers. The result was interpreted as the reciprocal of the highest dilution at which macroscopic agglutination (1+) was observed. Thus, anti-A and anti-B titers were determined and, according to international citations for our methodology, "high-titers" were defined as at least 64. The borderline value necessary for a titer to be characterized as high, depends on the serological method used and it can be adjusted to reflect the protocol of each Blood Bank Service.⁶⁻¹⁶

Statistical analysis

The descriptive statistics method was used for the analysis of the results (anti-A/B titers, age). Due to the small number of females (10/70-14.3%) in the donor population, gender correlations were not attempted. Titers were compared for significance using Pearson's correlation coefficient on the SPSS statistics computer programme.

Results

A total of 70 samples from group O APs donors were tested for anti-A and anti-B antibody titers. The donors were 20 to 58 years old (mean age: 39.8 ± 1.1). Men were prevalent (60/70-85.7%) in the donor population, females were the minority (10/70-14.3%). The screening test for unexpected blood group antibodies was negative for all samples. Anti-A titers ranged from 2 to 1024 (mean titer: 64), Anti-B titers ranged from 2 to 256 (mean titer: 32). Anti-A titers were significantly higher than anti-B ($p < 0.01$). Based on what is most commonly cited^{5,17-24} the critical value for "high-titer" anti-A/B antibodies, for our method (direct agglutinin (IgM) by gel), was defined to be at least 64. The prevalence of group O APs donors

with "high-titer" anti-A and anti-B, in our study is relatively high, 55.8% and 47.2% respectively. Table 1 shows the results of anti-A and anti-B titers by direct agglutination (IgM) using the manual gel method. The above results are also demonstrated in Figure 1 and Figure 2 as the relative percentage in the form of histograms. There was no significant difference between donors when they were divided into two age groups: over and under 40 years ($p > 0.857$ and $p > 0.861$), (Table 1) (Figure 1 & 2).

Table 1 Anti-A and anti-B titers in O group plateletapheresis donors

IgM titers	No of samples	
	Anti-A	Anti-B
2	2	1
4	-	2
8	3	3
16	6	12
32	20	19
64	23	16
128	10	11
256	3	6
512	2	-
1024	1	-
Total	70	70

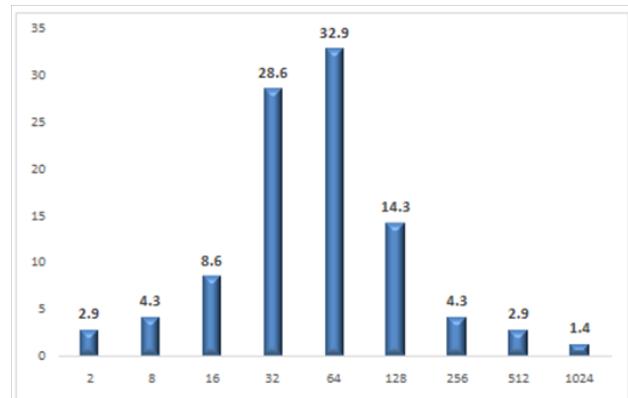


Figure 1 Anti-A titers in O group plateletapheresis donors.

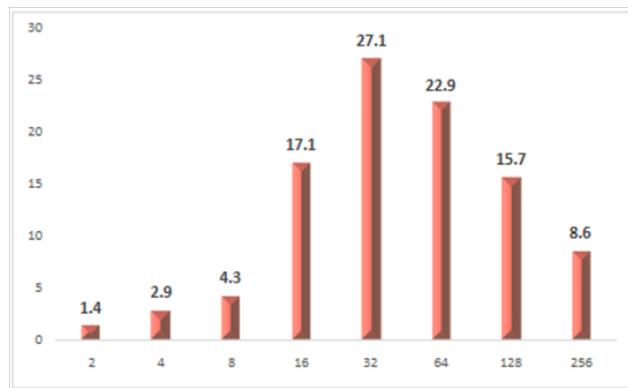


Figure 2 Anti-B titers in O group plateletapheresis donors.

Discussion

PLT transfusions are indicated for the prevention and treatment of hemorrhage in patients with thrombocytopenia or PLT function defects. PLTs may also be administered prophylactically to the thrombocytopenic patient with certain risk factors before undergoing an invasive procedure.^{22,25} PLTs express ABO antigens on their surface and PLT components are usually suspended in the original donor plasma.²⁶ It has been recently reported²⁷ that a group O PLT donor would have no A or B antigen expression on his or her platelets but anti-A, anti-B and anti-A,B alloisogglutinins in the plasma that, if present in "high-titer", have the potential to haemolyse the red cells of a non-group O recipient (minor ABO-incompatibility). In ABO "major-incompatibility" PLT transfusions (e.g. O recipient receives A, B or AB PLT product) anti-A/anti-B in the recipient may reduce PLT increment.²⁸ Approximately 10-40% of patients today in the United States (US) receive plasma incompatible PLT transfusions, but haemolytic reactions (HTRs) remain a rare event.^{7,29} These reactions are likely rare due to the dilution of the incompatible ABO antibodies in the recipients plasma volume and/or the neutralization of the anti-A or anti-B antibodies by the recipients soluble or endothelial based A and B antigens.³⁰ The true incidence of HTRs associated with PLT transfusions is unknown. Estimates range widely from 1:2000-1:46147,³¹ but reports of significant haemolysis following such transfusions are well established in the literature.³²⁻³⁴ Unfortunately, fatal haemolytic episodes have been observed, as in the case of an A Rh D (+) patient transfused with a dry-platelet unit from a group O AP donor.³⁵ The hemovigilance System in England (SHOT 2006, 2008) has reported that PLTs account for 20% (9/44) of acute HTRs overall,³⁶ and 33% (3/9) of HTRs in children.³⁷ One particular case concerns a child who received a PLT transfusion from her mother with a fatal outcome.³⁸ In the US, the frequency of published HTRs due to PLTs, containing ABO-incompatible plasma, is low (25 cases during a 30year period). However, 6 deaths reported to the US Food and Drug Administration (FDA), in a 10year retrospective study, were attributed to the use of PLTs with ABO-incompatible plasma. The College of American Pathologists (CAP) Transfusion Medicine Proficiency Testing Survey revealed that 83% of North American laboratories have a policy to prevent HTRs from ABO-minor mismatched PLT transfusions.³⁹ The AABB Standards require that the transfusion service shall have a policy concerning transfusion of components containing significant amounts of incompatible ABO antibodies or unexpected red cell antibodies without providing a definition of what value constitutes a "high-titer" antibody.⁴⁰ In one small study, 82% of patients receiving incompatible PLTs developed a positive DAT while none of the patients receiving type identical PLTs developed positivity.⁴¹ Acute HTRs occur typically with transfusion of high titer anti-A to A, recipients.³⁹ This problem has been particularly apparent in small children receiving SDPs which contain large volumes of incompatible plasma due to their relatively smaller blood volume.⁴² Josephson et al.¹⁷ confirms the high prevalence of "high-titer" anti-A or anti-A, B in group O APs donors. Furthermore, Kiefel¹⁸ notes that usually PLT concentrates from group O donors were implicated in severe HTRs. While most reported cases of HTRs due to incompatible plasma involve group O donors, a recent case report identified a group A, AP donor with a "high-titer" anti-B which caused an HTR in two different group B recipients.¹³ The first patient experienced severe back and flank pain with hypertension and chest pain within the first 15minutes of platelet transfusion while the second patient had a syncopal episode after completing the platelet transfusion. The post-transfusion DATs were both positive and evaluates from both

recipients contained anti-B. Further investigation of the donor demonstrated an anti-B titer of 16384.¹³ Thus, clinicians should be aware of the risk of HTR when transfusing large volumes of incompatible ABO PLTs (particularly group O PLT components and particularly to children) to out-of-group recipients. On the other hand, transfusion of only ABO-compatible PLTs is not always feasible due to the limited availability at a time of urgent need, the limited shelf-life of PLTs (5 days), and the fact that group O PLT donors outnumber the other donors. In addition, patients who have developed refractory thrombocytopenia may need HLA-compatible PLTs. Not infrequently, it is difficult to identify donors who are both HLA-compatible and ABO-compatible. Because these patients often require multiple PLT transfusions, some PLT donations collected will be HLA-compatible but not ABO-compatible.²⁰ Strategies to reduce the risk of PLT associated HTRs include screening donor plasma for "high-titer" antibodies, volume reduction – substitution, washed PLTs and setting a maximum volume of incompatible plasma to be transfused to a patient in a defined period of time. Various methods of determining titers include tube saline agglutination with or without indirect antiglobulin testing, micro-column agglutination, automated microplate technology and in vitro haemolysis assays. Critical titers, depending on the method used, include the following: greater than 1:16 for *in vitro* haemolysis assays, greater than 1:64 to 1:100 for immunoglobulin IgM, and greater than 1:256 to 1:400 for IgG.²¹ However, there is a lack of agreement as to what titer is clinically relevant and whether IgM or IgG antibody is more significant.^{17,31,39} In Greece, the policy regarding the use of ABO-incompatible PLTs is determined by the medical director of each transfusion facility. It is generally agreed that children, neonates and regularly transfused patients should receive only ABO-compatible PLT components. This proposal is not only adopted by the Hellenic Coordinating Haemovigilance Centre (SKAE) but it is also recommended as follows: male-only plasma transfusion implementation for preventing transfusion-related-acute-lung-injury (TRALI) and ABO-compatible PLT transfusions for preventing HTRs.^{43,44} Until today, 2 cases of HTRs from ABO-incompatible PLT transfusions have been reported to the SKAE. One of the cases was attributed to the transfusion of group O SDPs to a group A recipient.^{43,44} The guidelines for the Blood Transfusion Services in the United Kingdom²³ state that "each Blood Establishment should have a testing and issuing policy to avoid the use of "high-titer" anti-A and/or anti-B in instances where a significant adverse clinical reaction is likely". It is recommended that a saline agglutination test should give a negative result of a dilution of 1:128 or an equivalent dilution by other techniques. All components (RBCs, PLTs and FFP) of all ABO groups, which are found to be negative for "high-titer", anti A, B are labeled as "NEG: HT". This includes pooled PLTs if all constituent donations are negative. Currently procedures now identify approximately 10% of all donations as "high-titer". The specification for neonatal components include the requirement for donations to be high-titer anti A, B negative.²³ All components labeled as "suitable for neonatal use" are therefore negative for high-titer anti A, B. After adopting this national uniform approach only eight cases of possible haemolysis in the UK reported to SHOT during an eight year period.⁴⁵ Quillen et al.⁹ has proposed a critical titer of 250 by gel, which classified 25% of group O PLTs as high-titer. Quillen et al.⁹ note that since starting a universal screening, they have not had a single haemolytic transfusion reaction to a PLT product, and they note that they had an incidence of 1 in 2460 prior to implementing universal screening. In another equivalent review, Cooling et al.⁵ propose a critical titer of 128-200 by gel. Of course, other factors in addition to the anti-A and anti-B titer are believed to

contribute to the associated risk of haemolysis. Studies have shown that documented HTRs have occurred from plasma incompatible PLT transfusions with antibody titers considered to be low (<64).⁴⁶⁻⁴⁷ As such, Fauzie et al.⁴⁷ reported two HTRs, one of the HTRs occurred in a patient who received 390ml of group O APs with an anti-A titer of only 32 and severe back pain was the only transfusion symptom reported by this patient. In contrast, the other patient had cyanosis, dyspnea and haemoglobinuria after receiving 598ml of group O APs with an anti-A titer of 256/512 (IgM/IgG). Another variable is the amount of volume of incompatible plasma transfused. This varies according to the PLT product transfused. Each random-donor PLT unit contains at least 5.5×10^{10} PLTs and contains 50 to 60 ml of donor plasma.⁴⁸ Therefore, a typical dose of 5 pooled random PLT units contain at least 2.8×10^{11} PLTs and 250 to 300ml of plasma. Similarly, an AP product contains 2.5×10^{11} PLTs or more and 200 to 400ml of plasma. If that single AP donor has a high antibody titer, then subsequent haemolysis may be a concern. However, the pooling of multiple random-donor PLT units should dilute out any antibodies present in the plasma of a single "high-titer" random donor. Therefore, pooled random donor PLT products generally carry a lower risk of haemolysis.⁴⁹

In the above study, analysis of the common characteristics of adults with HTRs, after PLT transfusions pinpoints

- i. Patients repeatedly transfused with incompatible PLT products over days or weeks, and
- ii. Those receiving multiple PLT transfusions in a short period of time.⁴⁹

Children and neonates are theoretically at a higher risk of hemolysis due to a smaller blood volume.⁵⁰ Specifically, premature infants and neonatal patients that require PLT transfusions may develop circulatory overload when administered a 50 ml unit of PLT concentrate. The above study also evaluated the influence of centrifugation and resuspension (steps used to reduce the volume of stored PLT concentrates) on PLT properties by *in vitro* methods and by determining post-transfusion increments in neonatal patients. PLT morphology, mean PLT volume, hypotonic stress response, synergistic aggregation, and PLT factor 3 activities were not affected by the processing steps. The centrifugation and resuspension steps did not cause an enhanced discharge of lactate dehydrogenase from PLTs. These results indicate that the volume of stored PLT concentrates can be reduced in a manner which maintains PLT properties.⁴⁹ Others, tried to determine the best procedure for concentrating PLTs in a smaller volume after storage, they studied PLT loss after concentration at various centrifugation g forces for various times.

Their results showed that

- i. Minimizing the amount of incompatible plasma transfused can result in PLT loss and increase the number of PLTs transfused as much as 50% and
- ii. Numbers and viability of PLTs stored up to 5days in 50ml plasma and then concentrated in 10ml plasma after centrifugation at 1500Xg for 7 minutes, 2000Xg for 10minutes or 5000Xg for 6minutes should be clinically acceptable.⁵¹

Additionally, plasma reduction requires trained personnel, specialized equipment and time. Controversially, Cid et al.²⁷ remarks that PLT manipulation results in activation after centrifugation. Romphruk et al.²⁰ demonstrated that volume reducing group O APs

followed by resuspension in group AB plasma reduced the anti-A and anti-B titers to <8 thereby creating a universal donor. Karafin et al.³¹ managed to lower the risk of HTRs in adults, by limiting the volume of transfused plasma incompatible apheresis products to 600 cc in a 24hour period. It has been recently shown that PLTs stored in platelet additive solution (PAS) are effective and reduce adverse events associated with PLT transfusion.⁵²⁻⁵⁴ As PAS effectively reduces the plasma from a PLT product, it may also reduce the incidence of haemolysis due to incompatible plasma. It is not yet known whether PAS PLTs will be a cost-effective strategy for the reduction of HTRs. The vital question, as expressed in the study of Daniel-Johnson et al.¹⁹ is when an AP donor can be considered "safe". The decision to screen all PLT donors regardless of historical information was based on the concern that some donors may develop higher-titers over time, with pregnancies, immunizations, or ingestion of live bacteria such as those in certain yogurt products and pro-biotic formulations. Also, some donors who were classified as below the cut-off on one donation were classified as "high-titer" on subsequent donations. In order to increase the awareness of medical staff regarding the potential risk of HTRs, when ABO-incompatible PLT products are transfused, the following key messages and recommendations are stated below. These emerge from the previously cited studies.^{9,13,18,23,31,42} The risk of HTRs from ABO-incompatible PLT transfusions is due to passive high-titer anti-A and/or anti-B from the donor's plasma. HTRs are typically attributed to transfusions of high-titer anti-A to A₁ recipients. Infants and small children may be at greater risk. Incompatible SDPs represent greater risk than random PLTs, if ABO antibody titers are high. Titers change overtime so, a single screening of a donor is not safe. Transfusions of ABO-identical PLTs are preferred if possible. HLA/HpA compatibility is preferred over ABO compatibility when HLA class-I and HPA antibodies exist. Incompatible ABO-PLT transfusions to neonates/children are to be avoided. Transfusing group O PLTs to non group O recipients should be avoided. Use of group A PLTs for group B patients and vice versa is preferred. All PLT units (especially group O SDPs) need to be screened for a cut-off dilution (e.g. saline agglutination negative dilution of 1:128). Units need to be labeled accordingly. PLT components with high-titers anti-A/B must be transfused to ABO-identical recipients or group O recipients. If there is a significant concern about infusing incompatible plasma, volume-reduced, volume substituted, washed PLTs or additive solution PLTs may be considered. Do not transfuse to a patient more than 600 ml of incompatible plasma per day. Clinicians must be aware of the risk of HTRs from ABO-incompatible PLT transfusions, they must observe patients and report accordingly

Conclusion

The risk of haemolysis, from ABO-incompatible PLT components transfusions, due to passively transfused anti-A and/or anti-B alloisogglutinins is small but present. Transfusion Service Personnel and Clinicians should be aware of the potential risk and they need to always be alert and vigilant. The critical value for "high-titer" anti-A/B antibodies, per our method, was defined to be at least 64. Data from our study show that group O AP donors have significantly high-titers of anti-A and anti-B antibodies, 55.8% and 47.2% respectively. Lack of standardization of performing titers and lack of international consensus on what constitutes a critical titer is still an issue.

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Conflict of interest

The author declares no conflict of interest.

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