Evaluation of Anti-A and Anti-B Alloisogglutinin Titer in Group O Plateletpheresis Donors

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ABSTRACT

BACKGROUND: 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). AIM: (1) To evaluate anti-A and anti-B titers in the plasma of group O APs donors. (2)To define a critical value of “high-titer” based on our methodology. (3)To increase the awareness of medical staff on the potential risk of ABO-incompatible PLT transfusions. MATERIALS AND METHODS: In this current study we evaluated anti-A and anti-B titers in 30 plasma samples from group O APs 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 tube titration method specifying IgM antibodies. RESULT: 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, 56.6% and 43.4% respectively. CONCLUSION: In conclusion, the risk of haemolysis from ABO-incompatible PLT components, due to passive transfused anti-A and / or anti-B alloisogglutininis, 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: Platelepherisis donors; Platelet transfusion; Alloisogglutinins

INTRODUCTION
Platelet transfusions are indicated in thrombocytopenic patients with active bleeding and in patients with platelet dysfunction for whom other therapies have not been effective. Platelets may also be administered prophylactically in the thrombocytopenic patient with certain risk factors about to undergo an invasive procedure. Platelet concentrate can be obtained from fresh whole blood or via an apheresis donation. Patients should be given platelet concentrates from ABO-identical donors if possible. It has been an accepted practice for platelets to be transfused out of the ABO group as a second-line therapy when ABO-identical platelets are unavailable. Platelets bear ABH antigens both as intrinsic structures and as extrinsic ones adsorbed from the plasma. The donor plasma associated with platelet concentrate contains the naturally-occurring antibodies against A or B antigens lacking in the donor. There are two types of ABO-incompatibility. A major ABO-incompatibility occurs when donor platelets containing antigens are incompatible with antibodies present in the recipient’s plasma. In contrast, a minor ABO-incompatibility occurs when donor plasma contains antibodies against the patient’s ABO antigen. Accelerated destruction of the platelet in the recipient has occurred after a major ABO-incompatible transfusion. The transfusion of platelets containing high titers of antibodies to the antigens on the red blood cells of a minor ABO-incompatible patient can cause clinically significant hemolytic. Additional adverse effects from ABO-incompatible plasma in PLT components include haemoglobinemia, jaundice,
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progressive anaemia, spontaneous coagulation, positive Direct Antiglobulin Test (DAT) and increased osmotic fragility of the patient’s red blood cells.\(^1\) 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 37ºC. 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).\(^2\) 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.\(^3,4\) 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. Titors such as 128-256 are considered critical,\(^5-8\) but not all agree.\(^9-10\) 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 plateletpheresis donors to determine anti-A and anti-B titers by tube titration method, specifying IgM antibodies.

**AIM & 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

**MATERIAL AND METHOD**

We collected plasma samples (EDTA) from 30 prospective group O APs donors obtained from our Blood Bank Service. Donors ranged between 20 and 58 years of age (mean age: 39.8±1.1). All plasma samples were stored at 2º-8ºC and tested, within 3 days from collection, for alloisogglutinin titer. Samples were tested, in parallel, for anti-A and anti-B antibodies by tube titration 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% Surgiscreen (Bio Rad System). Serial twofold dilutions of plasma were prepared in 0.9% saline using a calibrated pipette. Specifically, one drop 2-5% suspension of A1 and B RBCs add into dilution, mix well and test by a serologic technique appropriate to the antibody. Results were read and recorded immediately after centrifugation. The highest dilution causing agglutination was assumed to represent IgM antibody titers. The result were 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.\(^5-13\)

**RESULTS**

A total of 30 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). 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
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commonly cited [5,17- 24] the critical value for “high-titer” anti-A/B antibodies, for our tube titration method, 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, 56.6% and 43.4% respectively. Table 1 shows the results of anti-A and anti-B titers by direct agglutination (IgM) using tube titration 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.

<table>
<thead>
<tr>
<th>IgM Titers</th>
<th>No of Samples Anti-A1</th>
<th>No of Samples Anti-B</th>
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<tr>
<td>Total</td>
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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. PLTs express ABO antigens on their surface and PLT components are usually suspended in the original donor plasma. It has been recently reported 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 alloisoagglutinins in the plasma that, if present in “high-titer”, have the potential to haemolysise 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. 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. 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. 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. 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. Acute HTRs occur typically with transfusion of high titer anti-A to A1 recipients. 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. Josephson CD et al. confirms the high prevalence of “high-titer” anti-A or anti-A, B in group O APs donors. Furthermore, Kiefel V 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.
patient experienced severe back and flank pain with hypertension and chest pain within the first 15 minutes 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. 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.

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. However, there is a lack of agreement as to what titer is clinically relevant and whether IgM or IgG antibody is more significant. It is generally agreed that children, neonates and regularly transfused patients should receive only ABO-compatible PLT components. 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. All components labeled as “suitable for neonatal use” are therefore negative for high-titer anti A, B. Studies have shown that documented HTRs have occurred from plasma incompatible PLT transfusions with antibody titers considered to be low (<64). As such, Fauzie et al. reported two HTRs, one of the HTRs occurred in a patient who received 390 ml 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 598 ml 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 x 10^11 PLTs and contains 50 to 60 ml of donor plasma. Therefore, a typical dose of 5 pooled random PLT units contain at least 2.8 x 10^11 PLTs and 250 to 300 ml of plasma. Similarly, an AP product contains 2.5 x 10^11 PLTs or more and 200 to 400 ml 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. In the above study, analysis of the common characteristics of adults
with HTRs, after PLT transfusions

- Patients repeatedly transfused with incompatible PLT products over days or weeks, and
- Those receiving multiple PLT transfusions in a short period of time.\textsuperscript{36}

Children and neonates are theoretically at a higher risk of hemolysis due to a smaller blood volume.\textsuperscript{37} 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 \textit{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.\textsuperscript{36} 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

- Minimizing the amount of incompatible plasma transfused can result in PLT loss and increase the number of PLTs transfused as much as 50\% and
- Numbers and viability of PLTs stored up to 5 days in 50 ml plasma and then concentrated in 10 ml plasma after centrifugation at 1500 X g for 7 minutes, 2000 X g for 10 minutes or 5000 X g for 6 minutes should be clinically acceptable.\textsuperscript{38}

Additionally, plasma reduction requires trained personnel, specialized equipment and time. Romphruk AV et al.\textsuperscript{17} 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. It has been recently shown that PLTs stored in platelet additive solution (PAS) are effective and reduce adverse events associated with PLT transfusion [39-41]. 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 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. 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
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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, 56.6% and 43.4% 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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