Platelet Transfusion – the Art and Science of Compromise

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Summary

Many modern therapies depend on platelet (PLT) transfusion support. PLTs have a 4- to 7-day shelf life and are frequently in short supply. In order to optimize the inventory PLTs are often transfused to adults without regard for ABO compatibility. Hemolytic reactions are infrequent despite the presence of 'high titer' anti-A and anti-B antibodies in some of the units. Despite the low risk for hemolysis, some centers provide only ABO identical PLTs to their recipients; this practice might have other beneficial outcomes that remain to be proven. Strategies to mitigate the risk of hemolysis and the clinical and laboratory outcomes following ABO-matched and mismatched transfusions will be discussed. Although the PLTs themselves do not carry the D antigen, a small number of RBCs are also transfused with every PLT dose. The quantity of RBCs varies by the type of PLT preparation, and even a small quantity of D+ RBCs can alloimmunize a susceptible D- host. Thus PLT units are labeled as D+/-, and most transfusion services try to prevent the transfusion of D+ PLTs to D- females of childbearing age. A similar policy for patients with hematological diseases is controversial, and the elements and mechanisms of anti-D alloimmunization will be discussed.

Introduction

Platelets (PLTs) for transfusion can be collected by apheresis technology or prepared from whole blood. In the latter case, whether the PLT concentrate is prepared from the buffy coat or from platelet-rich plasma, multiple donor units are pooled together to produce a dose of PLTs that is suitable for transfusion to an adult. Without PLT therapy, many modern day treatments would not be possible. Surgery, trauma resuscitation, and many cancer therapies all depend on a constant supply of PLTs. However, owing to concerns over bacterial contamination due to their storage at room temperature, PLTs have a short shelf life compared to most other blood products. In many countries PLTs can be stored between 4 and 7 days, and, given the high demand for their use, compromises must sometimes be struck between providing what might be considered the ideal PLT product and the realities of blood bank inventory management. In many centers, PLTs are transfused to adults without regard to ABO compatibility; questions about hemolysis and other potential adverse events have been raised about this practice. Furthermore, it is also common for D- recipients to receive D+ PLTs, especially for patients with hematology-oncology diagnoses, based on the low incidence of anti-D alloimmunization. This review will take a critical look at these practices and highlight areas for future investigation to determine how safe our current assumptions and compromises surrounding PLT transfusion really are. Interested readers should consult reference [1] for an additional review of the risks and benefits of ABO incompatible PLT transfusions.

Table 1. Examples of ABO mismatches^a

Recipient ABO type	ABO-major mismatched (recipient has isohemagglutinins against ABO antigens on donor PLT)	ABO-minor mismatched (donor has isohemagglutinins against ABO antigens on recipient RBC)
A	B, AB	B, O
B	A, AB	A, O
AB	none	A, B, O
O	A, B, AB	none

^aNote that as group AB recipients lack anti-A and –B, a major mismatched PLT transfusion cannot occur, and as group O PLTs lack A or B antigens, a minor mismatched transfusion cannot occur.

Part 1: PLT Transfusion and ABO Compatibility

ABO-Minor Mismatched PLT Products and Hemolysis

In ABO-minor mismatched PLT transfusions, the plasma that accompanies the PLTs contains anti-A or anti-B antibodies (or both) which are incompatible with the recipient's red blood cells (RBCs) (table 1). Thus there exists the potential for hemolysis following the transfusion of an ABO-minor mismatched PLT unit if the titer of the incompatible antibody is sufficiently high. A recent retrospective analysis of the Medicare database in the USA revealed that in 3 out of 59,933 PLT transfusions the transfusion of ABO incompatible PLTs led to an unspecified 'adverse event', suggesting that hemolysis due to ABO-minor mismatched PLT transfusions is a rare occurrence [2]. Several investigations into the titers of donor anti-A and anti-B antibodies in both apheresis and whole blood PLT products have been performed. In one study, the authors investigated the prevalence of 'high titer' anti-A and anti-A,B antibodies in 100 group O apheresis PLTs [3]. The authors' definition of a 'high titer' IgM antibody was ≥ 64 , and ≥ 256 for IgG antibodies. Using these definitions of 'high titer' antibodies, 28% of these group O units had 'high titer' IgM anti-A or anti-A,B, while 39% had 'high titer' IgG anti-A or anti-A,B.

Another study revealed that 60% of group O pooled PLT units, which consisted of 4–5 individual units, harbored an anti-A and/or anti-B titer of ≥ 64 by the gel technique [4]. Using this technique, the mean anti-A titer was 64 and the mean anti-B titer was 32. The titers of anti-A,B were not reported. Because hemolysis has been reported in ABO-minor mismatched PLT transfusions, laboratories might consider documenting the anti-A or anti-B titer in the unit or labeling the unit simply as 'high titer' based on the local threshold for this designation.

A retrospective analysis at one institution over a 3-month period found that 15% of the transfused apheresis PLTs were ABO-minor mismatched with the recipient [5]. The authors designated units with anti-A or anti-B IgG titers of ≥ 512 as 'high titer'. While none of the group A or B apheresis PLTs transfused to ABO-minor mismatched recipients had titers high enough to exceed this threshold, 9.7% of the group O apheresis PLTs transfused to group A, B or AB recipients contained antibodies that met or exceeded their definition of 'high

titer'. Had 'high titer' been defined in this study as ≥ 256 , 26% of group O apheresis PLTs would have been labeled as such which is similar to the 39% of 'high titer' group O apheresis PLTs described above [3]. Using an IgG titer of ≥ 64 , approximately half of the group O apheresis PLTs in this study [5] would have been labeled 'high titer', a figure that is similar to a previous report of the prevalence of 'high titer' pooled group O PLT units [4]. In contrast, Quillen et al. [6] found that half of their group O apheresis PLTs would have been considered 'high titer' at a screening dilution of 1:150, while 25% of these donations are handled as 'high titer' PLTs with their current threshold of 1:250.

In spite of the relatively high occurrence of 'high titer' antibodies, acute hemolytic transfusion reactions (HTR) in recipients of ABO-minor mismatched PLTs are uncommon. The low incidence of hemolysis is possibly due to the dilution of the donor anti-A or anti-B antibodies in the recipient's plasma volume and/or the neutralization of the anti-A or anti-B antibodies by the recipient's soluble or endothelial based A and B antigen(s) [7]. The exact incidence of HTRs due to ABO-minor mismatched PLT transfusions is unknown but has been reported to range from 1:9,000 to 1:100 [8–10]. A 4-year retrospective study found that the incidence of HTRs, defined as the presence of clinical signs and symptoms of hemolysis with supportive laboratory documentation of a hemolytic event, following the transfusion of ABO-minor mismatched apheresis PLTs was 2 out of 3,816 (0.05%) [11]. In a separate study from the same institution, the authors investigated all febrile non-hemolytic transfusion reactions (FNHTR) that were reported to the blood bank during a 3-month period to more accurately estimate the incidence of hemolysis caused by ABO-minor mismatched PLT transfusions and to determine the antibody titer at which hemolysis occurred [5]. A FNHTR was defined as an increase in 1 °C to a level at or above 38.0 °C and/or chills during or temporally associated with a PLT transfusion; isolated fever or chills are symptoms that can be associated with acute hemolysis; thus the authors investigated all FNHTRs to increase their sensitivity of identifying a HTR, i.e., in case a hemolytic reaction had been incorrectly diagnosed as a FNHTR. Although no HTRs were reported to the blood bank during the 3-month study period, they found that the post-PLT transfusion direct antiglobulin test (DAT) was positive in 2/4

patients who experienced a FNHTR. In one case, 2 group A PLT units were transfused to a group B recipient. While the post-transfusion DAT was positive, the eluate was non-reactive and the titers of anti-B in the transfused PLT units were only 16 and 2. Neither the results of the pre-reaction DAT nor the results of the visual inspection of the post-reaction plasma were reported. Although the recipient's post-PLT transfusion hemoglobin (Hb) decreased by 2.2 g/dl from the pre-transfusion value, no additional biochemical markers of hemolysis were reported. In the second case, 2 group O PLT units were transfused to a group AB recipient. The post-PLT transfusion DAT was positive and anti-A was detected in the eluate. The 2 group O apheresis PLT units had anti-A titers of 128 and 2,048. As in the first case, neither the results of the pre-reaction DAT nor the results of a visual inspection of the post-reaction plasma were provided. The recipient's post-PLT transfusion Hb decreased by 1.7 g/dl from the pre-transfusion value, and, again, no additional biochemical markers of hemolysis were reported. Thus in both of these patients, it is unclear if immune mediated hemolysis actually occurred following the ABO-minor mismatched PLT transfusions, and the authors concluded that the titers of anti-A and/or anti-B in the donor units were not predictive of which recipients would develop a HTR [5].

While most reported cases of HTRs due to ABO incompatible plasma involve group O donors, a recent case report identified a group A apheresis PLT donor with a 'high-titer' anti-B which caused a HTR in 2 different group B recipients [12]. The PLT product was collected from a donor who had previously donated >100 apheresis PLTs over many years. Approximately 3 weeks prior to the index PLT donation that caused the hemolysis, the donor had increased the amount of oral probiotic supplements that he had been taking compared to the dose that he had been taking during the period in which he had made his previous donations. The index apheresis product was split and transfused to 2 different group B recipients. The first patient experienced severe back and flank pain with hypertension and chest pain within the first 15 min of the PLT transfusion while the second patient had a syncopal episode after completing the PLT transfusion. The blood bank was consulted for a suspected transfusion reaction in both cases. The post-transfusion DATs were positive in both patients, and eluates from both recipients' RBCs contained anti-B. Further investigation of the donor demonstrated an anti-B titer of 16,384 at both room temperature and anti-human globulin (AHG) phases of testing. Samples from 4 of the donor's prior donations (over a period of 3 years) were available for testing and revealed that 2 of his PLT products had anti-B titers of 4,096 and the other 2 had titers of 8,192. There had been 2 group B recipients of this donor's prior donations where post-transfusion hemolysis was not reported to the blood bank. In retrospect, laboratory data from both of these earlier group B recipients demonstrated evidence of hemolysis in the days after the PLT transfusion, yet at the time the PLT transfusions were not suspected to have

been the etiological agent of the hemolysis. In vitro, the solubilized probiotic reduced the anti-B titer in a randomly selected group A PLT product by threefold, suggesting that the bacteria (or some other ingredient) in these probiotics may contain a B-like antigen capable of neutralizing the anti-B antibody in the group A donor plasma. This B-like antigen in the probiotic might also have been the stimulus for the donor's 'high titer' anti-B. Further investigation of the effect of oral probiotic supplements on the immune system is clearly warranted, and this case suggests that some form of antibody titer screening before mismatched transfusion might be needed if these widely taken probiotic supplements actually cause the production of high titers of antibody.

Guidance on the Transfusion of ABO-Minor Mismatched PLTs

Although the report from the 1996 National Institutes of Health (NIH) consensus conference on PLT therapy stated 'Administration of ABO-incompatible platelets is an acceptable transfusion practice' [13], there is a small but real risk of hemolytic complications following the transfusion of ABO-minor mismatched PLTs. There is neither a widely accepted incidence of hemolysis following ABO-minor mismatched PLT transfusions nor a definition of 'high titer' anti-A or anti-B antibodies. Currently, the AABB Standards require only 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 [14]. The British Committee for Standards in Haematology Guidelines recommends that ABO-identical PLTs be the first choice as much as possible and that group O PLTs be used for group A, B, and AB recipients as long as the PLTs have been tested and are negative for 'high titer' anti-A and anti-B antibodies. A definition for 'high titer' antibodies is not provided [15].

A recent survey of laboratories participating in 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 [10]. Of the transfusion services which have policies in place, there was wide variety in these practices ranging from the transfusion of exclusively ABO compatible plasma and platelet products, volume reduction of ABO-minor mismatched PLTs, and notification of the blood bank medical director and/or clinical team of the lack of ABO identical PLT products. Only 1–2% of transfusion services have both defined a threshold of, and screen for, 'high titer' anti-A or anti-B antibodies in the donor PLT units. An international survey performed by the Biomedical Excellence for Safer Transfusions (BEST) Collaborative collected data on PLT transfusion practices among predominantly European transfusion services with respect to ABO compatibility practices for PLT transfusion [16]. Approximately 80% of the respondents had a policy for issuing out of group PLTs, ranging from issuing any PLT

Table 2. Definitions of 'high titer' anti-A or anti-B in various studies arranged by the isotype of the antibody, and the method employed to perform the titer

Reference	High titer IgM	Method employed for performing titer	High titer IgG	Method employed for performing titer
Josephson et al. [3]	≥64	buffered gel card	≥256	anti-IgG gel card
Cooling et al. [4]	NT	NT	≥128	anti-IgG gel card
Quillen et al. [6]	≥250	buffered gel card	NT	NT
Karafin et al. [5]	NT	NT	≥512	anti-IgG gel card
UK national guidance document [25]	≥128	tube	NT	NT
	≥100	automated instrument*		
Scottish National Blood Transfusion Service [24]	≥50	automated instrument*	NT	NT
Pittsburgh, USA [†]	≥100	tube	NT	NT
NT = Not tested.				
*Olympus PK7200.				
[†] Local practice at the Centralized Transfusion Service (Pittsburgh) of authors SKH and MHY.				

unit irrespective of its ABO group to providing only ABO-major mismatched PLT units. Approximately half of the transfusion services would issue an ABO-minor mismatched PLT without taking additional measures such as selecting PLTs with low levels of anti-A or anti-B or volume reducing the PLT unit. Of those transfusion services that use PLTs suspended in additive solutions, >60% would issue an ABO-minor mismatched PLT without modifying the unit.

Strategies to Reduce the Risk of Hemolysis

There are several strategies that have been used to reduce the risk of hemolysis from an ABO-minor mismatched PLT transfusion. Many of these strategies are reviewed by Josephson et al. [9]. Some hospitals elect to wash or volume reduce the incompatible plasma from the platelet PLT unit. Washing the PLT unit is effective in removing plasma proteins; however, it can potentially lead to decreased PLT function manifested by increased CD62 surface expression, a marker of PLT activation, and decreased PLT aggregation *in vitro* [17–19]. On the other hand, volume reduction of PLTs results in less PLT activation than washing but is not as effective in removing plasma proteins [19]. Volume reduction is a procedure often used in neonatal and pediatric populations as these patients may be more sensitive to a large volume of incompatible donor plasma due to their relatively smaller blood volume [20–22]. Thus, a strategy to reduce the risk of hemolysis due to passive anti-A or anti-B in the neonatal and pediatric transfusion setting might be to first provide ABO identical PLTs, and if they are not available then to provide either volume reduced ABO-minor mismatched PLTs or washed PLTs if none of the other strategies are suitable. Transfusion services must weigh the pros and cons of each method and determine the best strategy for their given patient population.

Another strategy to reduce the passive transfusion of anti-A and/or anti-B in an ABO incompatible recipient is simply to place a limit on the volume of incompatible plasma that can be transfused to an individual recipient over a certain period of time. For example, a transfusion service may decide on a maxi-

mum volume of ABO incompatible plasma that can be transfused to a patient over a certain period of time [5, 23]. If the recipient reaches the designated upper limit, then only ABO identical PLTs or ABO-major mismatched PLTs would be issued. Washing or volume reducing minor mismatched PLTs might also be considered in this situation. Given the variable definitions and incidences of 'high titer' antibodies (table 2) and the variable volume of incompatible plasma that has been reported to cause hemolysis, the determination of a threshold amount of incompatible plasma that can be safely administered seems arbitrary.

Some transfusion services designate a certain titer of anti-A and/or anti-B as a 'high titer' and screen their inventory of PLTs for units containing antibodies that exceed this limit. Those units with antibody titers above the designated limit are labeled as 'high titer' and used preferably for ABO identical or ABO-major mismatched recipients. Conversely, PLT products with antibody titers below the designated limit may be transfused to any recipient, regardless of ABO type, although as the discussion above has highlighted selecting a 'safe' antibody threshold is not straightforward given the titers at which anti-A and anti-B have been reported to cause HTRs (table 2). For example, in Scotland and England ABO-minor mismatched PLTs are only transfused to patients if the anti-A and/or anti-B titer is <50 and <100, respectively [24, 25]. The local practice at the authors' (SKH and MHY) institute in Pittsburgh is to measure the titer of the anti-A and anti-B antibodies in group O apheresis PLTs and to label those units that have titers of ≥100 as 'high titer'; these 'high titer' group O apheresis units are transfused only to group O recipients while PLT units with anti-A and anti-B titers <100 can be transfused to any recipient without any limitation on the volume of incompatible plasma. Neither the group A and B apheresis PLTs nor the whole blood-derived PLT units (of any group) are screened for 'high titer' ABO antibody, and they are transfused to any recipient.

Rather than screening for antibody titers in PLT products, some investigators have examined other ways of reducing or neutralizing the anti-A or anti-B antibodies. In one study, it was

demonstrated that including 1 non-group O whole blood-derived PLT unit in a pool of 3–4 group O PLTs decreased the titer of, or outright neutralized, the anti-A and anti-B antibodies in the pool [4]. This practice of adding a non-group O whole blood PLT unit in order to decrease the anti-A or anti-B titer should be used with caution as there is a potential for the formation of immune complexes, PLT activation, and an increase in PLT microaggregates which, in small studies, have been implicated in causing adverse clinical outcomes (discussed in more detail below) [26, 27]. Another study demonstrated that volume reducing group O apheresis PLTs followed by resuspension in group AB plasma reduced the anti-A and anti-B titers to ≤ 8 thereby creating a ‘universal donor PLT’ [28]. However, as discussed previously, PLTs are activated after centrifugation, and the authors did not report on the hemostatic potential of the PLTs after resuspension and storage in AB plasma. In addition, adding an AB plasma unit increases the number of donor exposures with each dose. An alternative resuspension media is a platelet additive solution (PAS) [29–32]. The use of PAS reduces the plasma volume and as a result may reduce the risk of hemolysis caused by passively transfused isohemagglutinins. While the use of PAS is becoming standard practice throughout Europe, they are not widely used in the USA at this time.

ABO Major-Mismatched PLT Products: Post-Transfusion Platelet Increments and Bleeding Outcomes

The ABH antigens on the PLTs can be a target for recipient anti-A and/or anti-B. Once coated with antibody, the PLTs can be cleared from the circulation. There is variability in the amount of ABH antigen expressed on the PLT surface, and PLT donors can usually be placed in to 1 of 2 categories: high-expressers and low-expressers. In 1993 the first report to associate PLTs that expressed high levels of B antigen with low post-transfusion PLT increments was published [33]. The authors described a case of apparent PLT refractoriness in a group O recipient of HLA-matched, group B donor PLT transfusions from 2 different donors. Enzyme-linked immunosorbent assay (ELISA), immunoblotting, and flow cytometry studies demonstrated that both of those PLT donors expressed more B antigen on their PLT surface compared to the donors of the other group B PLT units to which the recipient had demonstrated an appropriate post-transfusion PLT count increment. The finding of these 2 group B high-expressor donors led the authors to investigate the amount of A and B antigens on the PLTs of over 300 randomly selected Japanese donors. They found that 7% of Japanese donors demonstrated what they termed a ‘high-expressor’ phenotype, defined as an ELISA absorbance of >2 standard deviations above the mean population expression of A and B antigen. The Japanese group O recipient in this case report had anti-A and anti-B IgG titers of 512 and IgM titers of 128. Had the PLT refractoriness been solely due to the ‘high

titer’ anti-A and anti-B in his serum, refractoriness to all ABO-major mismatched PLTs would have been expected. However, the recipient received multiple ABO-major mismatched PLT units from ‘low expressers’ and had acceptable post-transfusion PLT increments following these transfusions. The recipient’s apparent refractoriness was only associated with the transfusions from the donors with the high expression phenotype; therefore, the authors concluded that donor PLTs with high levels of B antigen, in combination with recipient’s ‘high titer’ anti-B, were associated with lower than expected post-transfusion PLT increments. Subsequent studies have demonstrated that there are different patterns of A and B antigen high expression on PLTs (that PLTs from group A₂ donors tend to express group O levels of A antigen) and that the frequency of PLT A and B high expression is fairly constant between different populations (2–7%) [33–38].

In the bone marrow transplant setting, ABO-major mismatched PLT transfusions in addition to multiple other factors such as the presence of recipient HLA antibodies, large body surface area, steroid administration, and transfusion of RBCs on the same day of the PLT transfusion can be associated with lower than expected post-transfusion PLT increments [39]. Some retrospective studies in hematology-oncology patients have reported significantly lower post-transfusion PLT increments after ABO-major mismatched PLT transfusions compared to ABO identical and ABO-minor mismatched PLT transfusions [39–41]. While statistically significant, the absolute difference in PLT increments after ABO-major mismatched PLT transfusions compared to ABO identical and ABO-minor mismatched PLT transfusions was small in each study, ranging from approximately 2,000 to 4,000/ μ l, and bleeding was not evaluated as an outcome. In contrast, one study demonstrated post-transfusion PLT increments after ABO-major mismatched PLT transfusions that were not significantly different from the post-transfusion increments following ABO identical and ABO-minor mismatched PLT transfusions [42]; another study surprisingly demonstrated a lower PLT increment after ABO identical PLT transfusions compared to ABO-major and ABO-minor mismatched transfusions [43]. A recent prospective observational study analyzed 1,030 PLT transfusions in adult patients with trauma, medical, and surgical diagnoses. The post-transfusion PLT increments amongst ABO-major mismatched recipients was lower ($p = 0.0499$) than amongst ABO compatible (defined as ABO identical and ABO-minor mismatched) PLT recipients [44]. In another prospective study, the authors analyzed the post-transfusion PLT increments after 400 PLT transfusions in 50 pediatric patients with a variety of hematology-oncology malignancies. ABO-major mismatched PLT transfusions resulted in lower post-transfusion PLT increments compared to ABO identical PLT transfusions ($p = 0.034$) [45]. However, none of these studies reported any clinical outcomes in the recipients of matched or mismatched PLTs. Thus it is unclear if the smaller post-transfusion increments have any adverse clinical consequences on the recipients of ABO major mismatched PLTs.

Some studies have included other patient populations in their analysis of post-transfusion PLT increments and outcomes in addition to hematology-oncology patients. A large retrospective study of approximately 1,700 cardiac surgery patients over a 10-year period demonstrated that there was no statistically significant difference between the post-transfusion PLT increments amongst the patients whose first perioperative PLT transfusion was ABO identical and those whose first PLT transfusion was ABO mismatched [46]. In addition, there was no statistically significant difference in the number of patients who had to return to the operating room due to bleeding between the recipients of ABO identical PLTs and the recipients of ABO mismatched PLTs.

A recent systematic review and meta-analysis summarized the findings of 3 randomized controlled trials and 16 observational studies, some of which have been discussed in this review [47]. Overall this analysis found that ABO identical PLT transfusions were associated with higher post-transfusion PLT increments. However, survival, bleeding events, or transfusion reactions were not primary outcomes in any of the studies. A secondary analysis of the Platelet Dose Study (PLADO) [48], which was the largest randomized study to date that addressed the question of post-transfusion PLT increments and clinical outcomes in 1,272 hematology-oncology patients, evaluated the 4-hour post-transfusion PLT increments following 3,993 transfusions and the 24-hour post-transfusion PLT increment following 2,309 transfusions [49]. Recipients of ABO-major mismatched PLT units had lower absolute post-transfusion PLT increments as well as lower corrected count increments (CCI) at both 4 and 24 h after transfusion compared to recipients of ABO-identical PLT transfusions. This study also correlated the post-transfusion PLT increments amongst ABO identical and ABO mismatched recipients and the time (starting from the PLT transfusion) to their first episode of World Health Organization (WHO) $\geq$ grade 2 bleeding. In a multiparameter model, the authors adjusted for PLT characteristics including ABO matching status, PLT dose, and storage duration as well as recipient characteristics, and demonstrated that the nature of the ABO match between the donor and recipient did not predict the time to $\geq$ grade 2 bleeding ($p = 0.33$) [49]. Thus, while ABO-major mismatched PLT transfusions resulted in decreased post-transfusion PLT increments, the decreased PLT increment did not increase the risk of major bleeding events in hospitalized hematology-oncology patients.

ABO-Mismatched PLTs and Non-Bleeding Clinical Outcomes

Retrospective analyses of ABO mismatched PLT transfusions in the hematology-oncology patient population have focused mainly on post-transfusion PLT counts. However, a number of studies have addressed some other-than-bleeding clinical outcomes following the transfusion of mismatched PLTs. In one such study of lymphoma patients, ABO identical

PLT transfusions, in combination with leukoreduction of all blood products, was associated with a reduced mean number of days with fever ($\geq 38.5^\circ\text{C}$), fewer days of antibiotic administration, fewer units of PLTs and RBCs transfused, and faster neutrophil recovery compared to recipients of ABO mismatched PLTs [50]. In contrast, other studies have demonstrated that there was no difference in the number of PLTs and RBCs transfused to patients who received ABO-major mismatched PLTs compared to those who received ABO identical PLT units [51] as well as no difference in hospital length of stay, fever, or antibiotic use [52]. The TRAP study found no difference in the time to the next PLT transfusion between recipients of ABO identical PLT units and recipients of ABO-major mismatched PLTs [40]. A different study found an increased risk of hepatic veno-occlusive disease (VOD) in pediatric recipients of hematopoietic stem cell transplants who received ABO-minor mismatched PLTs [53]. However, the use of busulfan containing conditioning regimens in this patient population confounded the association between minor mismatched PLTs and VOD because hepatic VOD is a complication of busulfan containing treatments.

In the hematology-oncology patient population, a small randomized trial demonstrated that patients who received ABO mismatched PLT transfusions had a statistically significant shortening in the duration of their remission as well as an increase in mortality compared to the group who received ABO identical PLTs [54]. A separate prospective trial demonstrated that in a small group of patients with newly diagnosed hematologic diseases receipt of ABO identical PLTs resulted in fewer PLT transfusions in the first 30 days compared to those patients who received ABO mismatched PLTs [55]. Another small randomized trial consisting of 26 evaluable hematology-oncology patients found that the incidence of developing PLT refractoriness was higher amongst those who received ABO mismatched PLTs compared to those who received ABO identical PLTs [56].

In a general surgical and trauma patient population, a retrospective analysis found that when ABO identical PLTs and cryoprecipitate were not exclusively provided by policy, group B and group AB recipients received significantly more ABO mismatched PLT transfusions ($p = 0.0004$) and more RBC transfusions ($p = 0.04$) and their average post-operative hospital length of stay was approximately 11.5 days longer ($p = 0.039$) compared to group O and group A recipients [57]. In-hospital mortality was not different ($p = 0.4$) between the group B and AB recipients compared to group O and A recipients. After a policy of providing ABO identical PLTs and cryoprecipitate to all patients was implemented, there was no significant difference in the hospital length of stay ($p = 0.81$) between the group B and AB recipients versus the group O and A recipients without a change in the in-hospital mortality trend. The authors cited the formation of immune complexes as a possible explanation for the increased RBC transfusion needs among recipients of ABO-minor mismatched PLTs and/or cryoprecipitate (see below) [57].

A retrospective cohort study looked at a variety of clinical outcomes amongst approximately 150 patients who underwent primary coronary artery bypass graft or coronary valve replacement surgery and who received ≥ 1 PLT transfusion during their hospital stay [58]. Patients who received ≥ 1 ABO mismatched PLT transfusions had significantly longer hospital stays ($p = 0.048$), had a greater number of days with fever ($p = 0.026$), accrued greater hospital charges ($p = 0.042$), received more RBC transfusions ($p = 0.0049$), had trends, while not statistically significant, towards longer stays in the intensive care unit ($p = 0.099$), and had a higher percentage of in-hospital deaths ($p = 0.07$) compared to patients who received exclusively ABO-identical PLT transfusions. However, this study included patients at only 1 institution under the care of 2 cardiothoracic surgeons. In contrast, another study of approximately 1,700 cardiovascular surgery patients demonstrated no association between the ABO matching of perioperative PLT transfusions and 30-day mortality or postoperative length of stay [46]. While retrospective, the larger study included 6 cardiothoracic surgeons and was powered to detect a statistically significant difference in mortality and postoperative length of stay.

There are 2 studies that demonstrated that ABO mismatched PLTs are not associated with an increased incidence of transfusion reactions. A study of the PLT units that were involved in 162 FNHTRs found that ABO mismatched PLTs were not over-represented amongst the units that caused the reaction compared to other PLT units that did not cause a FNHTR [59]. In addition, ABO mismatched PLTs were not over-represented amongst the PLT units that were issued in the 30 days leading up to the FNHTR, suggesting that a critical quantity of ABO immune complexes is not required for a FNHTR to occur. Similarly, another study analyzed the incidence of FNHTRs in recipients of ABO-minor mismatched apheresis PLTs and demonstrated there was no difference in the incidence of FNHTRs compared to recipients of ABO identical and ABO-major mismatched PLTs [5].

In contrast, a recent study reviewed the incidence of transfusion reactions prior to and after implementing a policy of providing only ABO identical or washed PLTs [60]. The authors demonstrated that in the period after implementation of the ABO identical or washed PLT transfusion policy there were 46% fewer FNHTRs to PLT transfusions compared to the period when ABO mismatched PLTs were routinely issued. In addition, a 23% decrease in allergic transfusion reactions was observed as well as a 50% decreased incidence of alloimmunization to RBC antigens in the 4 years after the ABO identical PLT policy was implemented. However, the authors included in their analysis FNHTRs and allergic reactions to all blood components transfused at their institution, and the rates associated with PLT transfusions were not reported separately. Thus, the incidence of FNHTRs and allergic reactions caused by ABO mismatched PLT transfusions remains unknown.

A commonly invoked explanation for the reported adverse events following mismatched PLT transfusion is the formation

of ABO immune complexes. These complexes form between the soluble A and B antigens and the corresponding antibodies in the recipient's plasma or that which accompanied the PLTs. ABO immune complexes have been reported to alter PLT function [61] and trigger complement activation and inflammation which has been reported to lead to transfusion reactions and RBC alloimmunization [26, 62, 63]. ABO immune complex formation was also implicated in higher mortality rates amongst recipients of ABO compatible but not identical plasma recipients [64] and was associated with the development of acute respiratory distress syndrome and sepsis in trauma patients who received ABO compatible but not identical plasma transfusions [65]. However, these studies are limited by their retrospective nature and are confounded by the fact that recipients of ABO mismatched plasma were more severely injured and received more blood products compared to recipients of ABO identical plasma.

Part 2: PLT Transfusion and the D Antigen

The terms 'Rh positive' and 'Rh negative' refer to the presence or absence of the D antigen on the RBCs. Rh antigens are on the Rhesus polypeptides and are not expressed on the platelet membrane (neither Duffy, Kidd, Kell, and Lutheran antigens) [66]. However, PLT concentrates should be labeled with their D blood group because they contain RBCs. D matching is currently recommended to prevent anti-D alloimmunization, especially for immunocompetent D- women of childbearing potential [67]. However, logistic constraints often dictate that D- patients are given platelet transfusions from D+ donors. If D compatibility is not present, Rh immune globulin (RhIG) should be administered.

This section of the review will focus on the three main factors present in the clinical scenario when PLTs obtained from D+ donors are transfused to D- recipients: first, the volume of D+ RBCs contained in the PLTs; second, the anti-D alloantibody detected in D- recipient's sera after receiving PLTs obtained from D+ donors; third, the immune status of the D- recipient.

Quantity of RBCs in PLTs

The content of RBCs per PLT transfusion varies considerably and is higher in whole blood derived PLTs than in those collected by apheresis (table 3) [68]. PLTs derived from whole blood, either by the platelet rich plasma or the buffy coat method, contain similar amounts of RBCs [69]. Moreover, today's PLTs contain similar amounts of RBCs as PLTs produced decades ago [68].

By contrast, PLTs prepared by apheresis methods contain only traces to small numbers of RBCs, and these have decreased over time [68].

These results are concordant with recent data published by

Table 3. Summary of studies analyzing anti-D formation after D-incompatible PLT transfusions

Reference	RBC in WB-PLT ⁽ⁱ⁾	RBC in A-PLT ⁽ⁱⁱ⁾	Reported anti-D detection rate ⁽ⁱⁱⁱ⁾	Anti-D > 4 weeks ^(iv)	Follow-up length, days ^(v)	ABO compatible, % ^(vi)
Goldfinger and McGinniss, 1971 [83]	0.42	NR	8/102	7/102	36(2-174)	NR
Lichtiger et al., 1983 [84]	0.53	3.00	0	0	14(2-97)	88.56
Baldwin et al., 1988 [88]	0.20	2.00	9/73	4/73	16(2-55)	90
McLeod et al., 1990 [89]	0.42	1.50	3/16	1/16	3(2-12)	NR
Heim et al., 1992 [108]	0.30	0.80	1/37	1/37	27(4-104)	NR
Zeiler et al., 1994 [94]	NR	0.75	0	0	22(12-52)	NR
Atoyebi et al., 2000 [86]	0.30	0.005	0	0	8(2-76)	NR
Molnar et al., 2002 [87]	NR	0.00017	0	0	27(2-223)	79
Cid et al., 2002 [91, 92]	0.59	NR	0	0	8(1-37)	89
Misso et al., 2006 [93]	0.42	NR	0	0	12 (range NR)	43
Bartley et al., 2009 [90]	NR	NR	0	0	NR	NR
Cid et al., 2011 [95]	0.036	0.00043	49/1014	12/315	29(4-718)	65-73

NR = Not reported.

⁽ⁱ⁾Red blood cell content (in ml) in PLT concentrates obtained from whole blood donations.

⁽ⁱⁱ⁾Red blood cell content (in ml) in PLT concentrates collected by apheresis.

⁽ⁱⁱⁱ⁾Reported frequencies of anti-D development.

^(iv)Frequencies of anti-D development after 4 weeks of the first Rh (D) incompatible PLT transfusion.

^(v)Median (range) of follow-up in weeks.

^(vi)Percentage of ABO compatibility in Rh (D) incompatible PLT transfusions.

Kitazawa et al. [70] who stained RBCs and PLTs in the PLT samples with anti-glycophorin A and anti-CD41 to count residual RBCs and residual RBC derived microparticles. Microparticles are cell derived membrane vesicles that range in size between 0.1 and 1 µm [71] and express D antigen on their surface, if they are obtained from a D+ donor [72]. These authors estimated that PLTs collected by apheresis contained a residual RBC volume and RBC derived microparticle volume in the range of 0.0004–0.0008 ml and 0.0001–0.001 ml, respectively [70].

Amount of RBC Necessary for Anti-D Alloimmunization

When a relatively large amount of D+ RBCs (200 ml or more) is transfused to healthy D– subjects, within 2–5 months, anti-D can be detected in the plasma of some 85% of the recipients [73–75]. The remaining 15% of D– subjects fail to make anti-D within the following months. About half of these subjects who did not become alloimmunized after the first injection of D+ RBCs also fail to make anti-D after further injections of D+ RBCs and are termed non-responders [76]. Although small doses of D+ RBCs, such as the quantity that is present in PLTs, can provoke primary immunization, the number of patients who respond is fewer and the titers of anti-D that patients produce are lower than is observed after exposure to larger doses of D+ RBCs.

There is some evidence that the minimum dose of RBCs necessary for primary immunization is only 0.03 ml [77]. This arises from a few observations following transfusions of less than 0.05 ml of D+ RBCs. In one series, 0.05 ml of unstated D

genotype (DD or Dd) RBCs were given at 6-weekly intervals to 15 D– subjects, four of whom formed anti-D. In another series, one male formed anti-D after six injections of 0.005 ml of D+ RBCs of predicted phenotype R2r, i.e., after a total of about 0.03 ml of RBCs. Finally, another male formed anti-D after ten injections of about 0.05 ml of D+ RBCs. In conclusion, these findings suggest that a cumulative dose of not more than about 0.03 ml of RBCs is capable of inducing primary D immunization, but they do not define the frequency with which such a dose is effective in causing alloimmunization [77, 78]. From these data, it can be concluded that PLTs derived from whole blood donations contain a sufficient number of RBCs to produce an antibody response. PLTs obtained by modern apheresis methods, however, contain only traces of RBCs, a quantity perhaps insufficient to provoke an immune response, particularly in immunosuppressed patients.

However, as stated before, recent data from Kitazawa et al. [70] showed that PLTs collected by apheresis contained a residual RBC volume and RBC-derived microparticle volume in the range of 0.4–0.8 µl and 0.1–1 µl, respectively. On a per-volume basis, these potential antigenic stimuli matched to within an order of magnitude, but the authors hypothesized that the smaller, more numerous RBC derived microparticles may be more immunogenic than RBCs themselves, because they could easily be phagocytosed by recipient antigen presenting cells. However, the authors concluded that the alloimmunization potential of such a small volume of RBC derived microparticles was theoretical, but their existence would require further research aimed at minimizing the immunological side effects associated with their transfusion [70].

The earliest time at which anti-D can be detected in primary immunization in immunocompetent individuals ranges from 4 to 10 weeks [79–81]. Production of anti-D within a few weeks of a first stimulus has been observed after the injection of specially treated D+ RBCs. The cells were incubated in low-ionic-strength medium at 37 °C and at the time of injection reacted strongly with anti-C4, anti-C3 and anti-C5. One out of 7 subjects first developed anti-D 15 days post-transfusion, and 5 others developed anti-D between 41 and 71 days after injection. It was considered that the time before the appearance of antibody was not significantly shorter than that observed following the injection of untreated RBCs [82].

Taking into account the previous data, we can calculate the frequency of anti-D alloimmunization after excluding those patients who developed anti-D as a result of a secondary immune response, which generally appears within 4 weeks after the provoking stimulus. If we exclude those patients, the percentage of D alloimmunization varies as shown in table 3. Overall, the incidence of D alloimmunization in immunosuppressed patients after D incompatible platelet transfusions is reported to be between 0 and 19% [83–94], but keeping some recently published data in mind [95], we can conclude that the frequency of anti-D alloimmunization actually ranges between 0 and 6.8% [96].

The observation interval after transfusion may have played a role in the frequencies of anti-D development that had been reported in the literature. The response times ranged from approximately 1 to 5 months in immunocompetent individuals, with the majority of responding recipients producing anti-D within 3 months [79–82]. The reported length of serological follow-up in immunosuppressed patients is quite variable. There are two papers whose authors reported no anti-D alloimmunization after D incompatible platelet transfusions with a median follow-up of less than 3 months, an interval perhaps insufficient to detect the formation of an antibody in immunosuppressed patients. Atoyebi et al. [86] found no anti-D alloimmunization in a group of 24 patients with hematological diseases after a median follow-up of 8 weeks (range 2–76 weeks). These authors reported an anti-D alloimmunization frequency of 13.5% in a group of 59 patients with non-hematological diseases after a median follow-up of 38 weeks (range 2–133 weeks). Cid et al. [91, 92] reported no anti-D formation in 22 D– patients with hematological diseases who received D+ PLTs after a median follow-up of 8 weeks (range 1–37 weeks). Of note, 41% of the patients did not survive beyond 7 weeks. Moreover, the paper by McLeod et al. [89] reported the highest frequencies of anti-D alloimmunization after D incompatible platelet transfusions in immunosuppressed patients. These authors, as mentioned before, reported two patients who formed anti-D very quickly, and the median length of serological follow-up in this series was only 3 weeks (range 2–12 weeks).

A possible protective effect of ABO incompatibility against D alloimmunization was first reported after analyzing groups of parents of infants with hemolytic disease of the fetus and newborn caused by anti-D. This protective effect was first demonstrated experimentally by Stern and colleagues [97, 98] who injected a group of D– male volunteers with D+ RBCs which were either ABO compatible or incompatible.

In some of the reports on D alloimmunization after D incompatible PLT transfusion, the percentage of ABO incompatible transfusions is provided; it ranges between 43 and 90% (table 3) [84, 87, 88, 91, 93, 95]. Thus, the protective effect associated with the transfusion of ABO and D mismatched RBCs is unlikely to be seen in current clinical practice given the high incidence of ABO matched transfusions in the studies reported [99].

D-Alloimmunization and the Level of Immunosuppression in Patients

Obviously, the level of immunosuppression affects the response to D+ RBCs in D– patients. There are several reports concerning anti-D alloimmunization in D– immunosuppressed patients after transfusing D+ RBC units. In all of these reports, the authors did not use RhIG as a preventative measure after D incompatible RBC transfusions, and only 0–16% of the patients developed anti-D alloantibody [100–103].

In contrast, when immunocompetent patients receive D+ RBC units, current transfusion medicine text books indicate an anti-D alloimmunization frequency exceeding 80%; these data are based on data obtained in 1970s with healthy volunteers and not hospitalized patients [77]. However, one prospective and three retrospective observational studies published in the last decade showed us two intriguing sets of data [104–107]. First, the frequency of anti-D alloimmunization following D+ RBC transfusion ranged from 21 to 33% in D– patients who were not iatrogenically immunosuppressed. Second, patients who did not develop anti-D received more D+ RBC units when compared with patients who developed anti-D, although the difference was not statistically significant in one study [104], and the statistical analysis was not reported in the remaining two studies [105, 106].

Finally, the frequency of anti-D alloimmunization in D– immunosuppressed patients who had received PLTs from D+ donors is shown in table 3. Some authors reported no incidence of anti-D alloimmunization in this group of patients [84, 86, 87, 90–95]; other authors, however, reported an incidence of anti-D alloimmunization that ranged from 2.7 to 18.7% [83, 88, 89, 108].

Stern et al. [98] first reported that if D+ RBCs were coated in vitro with anti-D before being injected into D- subjects, there might be no antibody response. Pollack et al. [109] reported the most valuable experiment in defining the dose of anti-D required to suppress D immunization. A fixed amount of IgG anti-D, namely 267 µg, was given to groups of D- subjects who received doses of D+ RBCs varying from 11.6 to 37.5 ml. Control subjects received the same dose of RBCs without anti-D. All of the subjects were given a challenge dose of 0.2 ml of whole blood 6 months after the initial D+ RBC transfusion and were tested for the formation of anti-D 1 week later. Of the controls, 49 (57%) out of 86 formed anti-D. In the treated-RBC group, only 15 (16%) out of 92 formed anti-D. From the results in the treated-RBC group, the authors concluded that 267 µg of anti-D was completely effective against about 13 ml of RBCs and was partially effective against larger amounts. From this and other studies it was concluded that about 20 µg of anti-D per milliliter of D+ RBCs is sufficient to suppress primary D immunization [74, 78, 79, 81, 109].

The availability of IV RhIG provides a more convenient and less painful approach to Rh immunoprophylaxis for those uncommon events when a D- person has been transfused with D+ RBC. Anderson et al. [110] published the first report using FDA approved i.v. RhIG for this indication. The manufacturer's recommended dose for i.v. RhIG is 18 µg/ml of D+ RBC, administered as 600 µg (3,000 IU) every 8 h until the total dose is reached. According to this, an injection of the 120 µg (600 IU) vial of i.v. RhIG should be adequate for preventing D alloimmunization with a wide margin of safety after transfusion of a pool of random donor PLTs or apheresis PLTs. There are two reports that support this view. Heim et al. [108] reported successful prevention of D alloimmunization in 36 D- patients who received 200 µg (1,000 IU) of anti-D just before the transfusion of platelets from D+ donors. Only 1 patient developed detectable anti-D, and it was 5 weeks after the transfusion of 1 D incompatible PLT unit. In that case, anti-D had not been administered. In the other report, Zeiler et al. [94] used only 20

µg (100 IU) to prevent D alloimmunization. Anti-D was added directly to the concentrate or given i.v. in advance of the transfusion. None of the 20 patients developed anti-D within a median follow-up of 5.5 months (range 3–13 months).

The use of anti-D is not free of adverse effects although overall it is very safe. The first reports about the i.v. route of administration of anti-D showed pyrexia (fever) and hemoglobinuria as the only untoward effect [77, 111].

Conclusions

This review has analyzed some of the assumptions that are made when selecting the ABO and D group for PLTs that are to be transfused. Overall the risk of hemolysis following the transfusion of ABO-minor mismatched PLTs seems small but not negligible. Questions about the clinical consequences of ABO immune complex formation following minor mismatched PLT transfusions need to be assessed in large, multicenter trials. Although the post-transfusion PLT increments are smaller following a major mismatched transfusion compared to matched transfusions, this does not appear to translate into increased bleeding for the recipient. The rate of anti-D alloimmunization following the transfusion of D+ PLTs to a D- recipient is low and might depend on a variety of clinical circumstances including the type of PLT product transfused and the recipient's immune status at the time of the transfusion. It is reasonable to try and prevent the transfusion of D+ PLTs to females of child-bearing age, and to use RhIG if D+ PLTs are administered to this type of recipient due to inventory pressures or the emergent nature of the bleeding. Using the latest literature, and being wary of unanswered questions, each transfusion service must decide on its own priorities for providing safe PLT transfusions

Disclosure Statement

The authors have no conflicts to disclose.

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