Immunologic risks of whole blood: ABO compatibility, D alloimmunization, and transfusion-related acute lung injury

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When it comes to deciding whether or not to use whole blood in the initial resuscitation of traumatically injured patients, one must weigh potential risks against potential benefits. Potential benefits of whole blood include increased in vitro and in vivo hemostatic properties of cold-stored platelets,[1–3] simplified logistics of resuscitation, and less dilutional coagulopathy resulting from both introducing plasma earlier in the resuscitation and transfusing a less dilute product compared to traditional component therapy (processing related dilution includes 63 mL of anticoagulant used for whole blood collection and 100 mL of additive solution to prolong red blood cell [RBC] shelf life).[4] Whole-blood transfusion, however, poses a risk for hemolysis when the product is non-ABO identical to the transfusion recipient, as well as other immunologic risks, including possible D alloimmunization, if D+ whole blood is used, and transfusion-related acute lung injury (TRALI).

This article provides an overview of the immunologic risks associated with whole blood transfusion, including ABO incompatibility (in terms of both red cell and plasma incompatibility), D alloimmunization, and TRALI, and describes how centers that currently use whole blood are mitigating these risks.

ISSUE #1: ABO COMPATIBILITY

ABO compatibility of RBCs is of paramount importance in transfusion medicine. When medical students are introduced to the concept of ABO compatibility, they are taught that ABO-identical blood components are the preferred choice for transfusion; however, ABO-compatible blood components are acceptable choices when patient's ABO is unknown. ABO-incompatible RBC transfusions should always be avoided due to the risk of hemolysis, which can be fatal.

ABO compatibility considers both the ABO antigens present on the RBC and the anti-A and anti-B antibodies present in the plasma. For RBC transfusions, all patients can receive group O RBCs since these lack A and B antigens and should therefore be compatible with any patient plasma. For plasma transfusion, all patients can receive group AB since this plasma lacks anti-A and anti-B antibodies and should therefore be compatible with any patient RBCs. These products are both considered "universal donors." Additionally, group O patients can receive plasma of any ABO group since their RBCs lack A and B antigens, and group AB patients can receive RBCs of any ABO group since their plasma lacks anti-A and anti-B antibodies. These patients are both considered "universal recipients." According to this construct, whole blood should be ABO identical as this product contains both RBCs and plasma (Table 1).

While this approach to ABO compatibility is a useful framework for understanding ABO antigens and antibodies, those of us who practice transfusion medicine recognize that ABO compatibility is a bit more nuanced than what we were taught in medical school. Although the rules of ABO compatibility are never broken for RBC transfusions, due to risk for potentially fatal acute hemolytic transfusion reactions, ABO-incompatible transfusions, in the form of plasma incompatibility, are common. ABO-incompatible platelet transfusions are standard practice for many transfusion services given the short shelf life of the product and frequent inventory shortages.[5] Due to the limited supply of group AB plasma, group A plasma units are increasingly being used during the emergent resuscitation of traumatically injured patients of unknown ABO group, without adverse outcomes.[6,7]

Although ABO-incompatible platelet and plasma transfusions both result in the transfusion of anti-A and/or anti-B antibodies, concern for clinically significant hemolysis in the transfusion recipient is low. This is due to several protective factors, including the presence of A and B antigen on endothelial cells, dilution of transfused plasma into the patient blood volume, and soluble A and B antigen in the

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TABLE 1. Traditional ABO compatibility teaching

Patient ABO Group	Compatible Red Cells	Compatible Plasma	Compatible Whole Blood
O	O	AB, A, B, O	O
A	A, O	AB, A	A
B	B, O	AB, B	B
AB	AB, A, B, O	AB	AB

plasma of secretors.[8] All of these factors serve to decrease risk for hemolysis of recipient red cells.

Transfusion services that permit ABO-incompatible platelet transfusion or use group A plasma for resuscitation of traumatically injured patients of unknown ABO group can utilize strategies to reduce the risk of hemolysis from the ABO-incompatible plasma. Options for platelet transfusion include limiting the volume of incompatible plasma, typically by placing limits on the volume or number of incompatible units that can be dispensed to a patient within a certain time period; screening units to identify those with high-titer anti-A and/or anti-B; or using volume reduction, washing, or platelet additive solution to reduce or remove the incompatible plasma.[5,9] For group A plasma transfusions, options for risk reduction are limited to screening units for high-titer anti-B and placing limits on volume or number of incompatible units that can be dispensed to a patient. Although these risk-reduction methods are available, they are not currently required for ABO-incompatible platelet or plasma transfusions.

Institutional practices regarding ABO-incompatible platelet transfusions vary in the United States.[5] *AABB Standards* require that hospitals have a policy for transfusion of components containing significant amounts of ABO-incompatible plasma.[10] Compliance requires only the existence of a written policy; specific procedures to mitigate risk for hemolysis from ABO-incompatible plasma are not specified.[11]

A recent survey of level 1 trauma centers in the United States revealed that the majority of centers that maintain thawed group A plasma use it for trauma patients of unknown ABO group. Among these centers, the majority do not limit the amount of plasma that can be transfused to patients of unknown ABO group nor titer for anti-B.[6]

Whole blood contains both RBCs and plasma, the compatibility of both requiring consideration. Group O whole blood provides "universal donor" RBCs when the ABO group of the recipient is unknown. The unwanted consequence of group O whole blood is that it also provides both anti-A and anti-B to non-O recipients. One unit of whole blood contains about the same volume of plasma as contained in an apheresis platelet unit. Thus, extrapolating from data published on ABO-incompatible platelet transfusions, hemolysis from anti-A or anti-B in one unit of group O whole blood is a very rare complication. A review of 25 published case reports described 30 patients with severe hemolytic transfusion reactions following platelet transfusion, with the vast majority of hemolytic cases from group O platelet units transfused to group A or group AB recipients.[12]

Risk-mitigation strategies that are commonly used to help prevent hemolytic reactions from ABO-incompatible platelet and/or plasma transfusions can be adopted for group O whole blood: limit the volume of incompatible plasma given (i.e., place a limit on the number of whole blood units a recipient can receive over a given period of time) and screen for high-titer anti-A and/or anti-B (i.e., give a "low-titer" group O whole blood unit). Factors that may influence the likelihood of hemolysis in the non-group-O recipient of group O whole blood are the same as those in a recipient of ABO-incompatible platelet and/or plasma transfusion and include the titer of the component, the volume of incompatible plasma transfused, the blood volume of the patient, the fraction of recipient red cells susceptible to hemolysis in circulation, and the secretor status of the recipient.

AABB Standards allow for use of low-titer group O whole blood for non-group-O recipients or for recipients whose ABO group is unknown. The definition of what constitutes a "low-titer" unit must be made by the transfusion service. In addition, the transfusion service must have a policy specifying 1) which patients are eligible to receive whole blood, 2) the maximum quantity of whole blood allowable per patient, and 3) how to monitor patients for potential adverse events post-transfusion.[10]

Although these are reasonable strategies to help mitigate risk for hemolysis, there is limited evidence-based guidance for transfusion services trying to meet these requirements. For example, there is no definition of a safe volume of incompatible plasma that can be transfused without the consequence of hemolysis, nor is there a definition of a "low titer" of anti-A and/or anti-B. Since titer measurement utilizes agglutination, a subjective endpoint, titers are poorly reproducible. Even when using a uniform titer method, the same sample may vary in titer result by $\pm$ one dilution.[13] Published data support anti-A and anti-B titers below 100–200 (IgM saline immediate spin method) and 250–400 (IgG techniques) as reasonably safe for transfusion of ABO-incompatible platelet units, and may be extrapolated to whole blood.[12,14–17]

However, higher titers do not guarantee hemolysis in the incompatible recipient as reported from one center where 26% of group O apheresis platelets had anti-A and/or anti-B IgG titers > 256 and almost 10% had titers > 512 without evidence of hemolysis.[18] Variables such as antibody subclass and soluble ABH antigens that exist in approximately 80% of the population may help explain why hemolysis is a rare complication of incompatible plasma transfusion.[8] The absolute amount of incompatible anti-A and anti-B that can be safely transfused to recipients has yet to be defined.

In summary, high-titer anti-A and anti-B do not guarantee hemolysis, and low titers do not absolutely protect transfusion recipients from hemolysis. To date, published experience from centers currently providing low-titer group

O whole blood to civilian trauma patients report no adverse outcomes when using a low-titer definition of < 50 (immediate spin, saline) and limiting the number of units provided to a maximum of four.[19]

However, "low-titer" definitions are far from standardized and vary in both method used to measure the titer and the absolute number used for the titer cut-off.[20,21] At one system that supplies whole blood for four trauma hospitals, whole blood units are collected in citrate–phosphate-dextrose (CPD) anticoagulant and stored at 1–6°C for up to 21 days. An immediate-spin saline tube method is used to perform titers with a critical titer cut-off of < 50. High-titer units are processed into RBC units with a 21-day shelf life.[19] Other published whole blood titer thresholds include < 200, and < 256.[21]

Transfusion services must determine their own definitions and policies to help prevent hemolysis from incompatible anti-A and anti-B in the non-O recipient, with limited evidence on the safest thresholds for both titer and volume. Additional studies are needed to guide the development of evidence-based protocols for the use of non-ABO identical whole blood.

ISSUE #2: D ALLOIMMUNIZATION

The presence or absence of the D antigen on both the donor and the recipient RBCs is the second-most-important consideration, after ABO compatibility, when choosing to transfuse blood products in emergency situations. In an ideal world, D− RBCs are given to anyone whose D type is unknown in order to prevent the possible formation of anti-D. This is because the antibody is not naturally occurring, and exposure is required for alloimmunization. Among D− hospitalized recipients who receive at least one unit of D+ RBCs, 20%–22% of them will form anti-D antibodies.[22,23] D alloimmunization is of most concern in women of childbearing potential where the antibody could complicate a future pregnancy with hemolytic disease of the fetus/newborn (HDFN). D− RBCs are less common in the blood supply, due to a population frequency of only 15%.[24] Thus, many transfusion services have policies to provide D+ RBC units to D− males and women beyond the age of childbearing potential, especially in emergent situations and during times of D− inventory shortages.[25] Moreover, D− inventory can become so low that some transfusion services may supply only group O, D+ RBCs in the trauma bay, even for females of childbearing potential whose D type is unknown.[25,26]

In traumatically injured women of childbearing potential, the benefits of receiving low-titer group O D+ whole blood early in the resuscitation, potentially decreasing risk of death from exsanguination, must be weighed against the potential of making an anti-D that could affect future pregnancies. However, not all anti-D causes HDFN. Only 20% of pregnancies where maternal anti-D is detectable will have the potential for severe HDFN.[27] Combined with the fact that only one quarter of D− recipients will make an anti-D, the chance of a future pregnancy being affected by severe HDFN is less than 5%, if the child is D+. Finally, the odds are low (1%–1.5%) that a trauma victim needing low-titer group O whole blood will be an D− female of childbearing potential.[25,26] Given the logistical difficulty in keeping a dual inventory of D+ and D− low-titer group O whole blood and the frequent inventory shortages of D− blood products, some transfusion services may choose to supply only D+ whole blood to all trauma patients because the benefits outweigh the consequences of potential anti-D formation in traumatically injured women of childbearing potential.

Published experience from centers currently providing low-titer group O whole blood to civilian trauma patients varies in terms of risk mitigation strategies for D alloimmunization. One system with four trauma hospitals stocks only D+ whole blood as the majority of their trauma patients are males. In this system, females under the age of 50 are not eligible to receive whole blood.[21] Another hospital treating adult trauma patients stocks only D+ whole blood and uses it for all traumatically injured patients, including women of childbearing potential.[26] Finally, one pediatric trauma hospital stocks only D− whole blood so that traumatically injured boys and girls (all of whom are presumed females of childbearing potential) can both receive this product.[21]

ISSUE #3: TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI)

One final consideration for whole blood is TRALI risk mitigation.[28] TRALI prevention is the issue of least debate because *AABB Standards* specify that "Plasma and whole blood for allogeneic transfusion shall be from males, females who have not been pregnant, or females who have been tested since their most recent pregnancy and results interpreted as negative for human-leukocyte antigen (HLA) antibodies."[10] The current risk of TRALI from platelets or plasma with these mitigation factors in place is estimated at 0.4 to 1.0 per 100,000.[29]

Human neutrophil antigen (HNA) antibodies have also been associated with TRALI, but the incidence of HNA mediated TRALI is low, as is the frequency of antibodies in the donor population. Further TRALI risk mitigation could include screening donors for HNA antibodies, although this testing is not routinely performed by blood collection centers in the United States.[30]

Published experience from centers currently providing low-titer group O whole blood to civilian trauma patients indicates that centers are meeting this requirement by producing whole blood units either from male donors or from female donors without a pregnancy history.[21] Testing of female donors with a history of pregnancy remains an additional option if utilization of whole blood production

increases and blood suppliers need to increase the donor pool for this component.

Although leukoreduction does not mitigate TRALI risk, some centers currently using whole blood are performing leukoreduction using platelet sparing filters.[21,31,32]

SUMMARY

The risks associated with providing group O whole blood to civilian trauma patients are, to many, offset by the benefit of transfusing RBCs, plasma, and platelets to patients in a timely, logistically simplified, and less dilute manner. When transfusion services follow the requirements specified by the *AABB Standards* to mitigate risk for hemolysis due to incompatible anti-A and anti-B in group O whole blood, the risk of clinically significant, or even detectable, hemolysis appears to be low.[19] The decision to supply group O, D+ whole blood to all traumatically injured adult patients, including women of childbearing potential, is an institution-specific choice and may largely depend on the availability of group O, D− whole blood from the blood supplier and local demographics of the trauma population. Finally, whole blood must be TRALI-risk reduced, that is, collected only from males, never pregnant females, or previously pregnant females who are negative for HLA antibodies.

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