An evaluation of methods for producing low-titer group O whole blood to support military trauma resuscitation

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The US Armed Services Blood Program (ASBP) focuses on establishing a robust distribution system that ensures a viable supply of blood components in remote combat environments in order to enhance the survival of soldiers injured on the battlefield. However, it is difficult to deliver and subsequently maintain blood components at their appropriate temperatures in austere conditions. Since World War I, great strides have been made to safely transport packed red blood cells (PRBCs), fresh frozen plasma (FFP), and even cryoprecipitate (CRYO) as far forward as possible, but platelets (PLTs) require special handling—room temperature storage limits their use to 5 days because of the risk of causing a septic reaction. As a result, PLTs are generally not available at remote locations.[1] Therefore, having the capability to collect and transfuse whole blood (WB) has supplemented the lack of PLT availability in austere locations.[2]

The concept of damage control resuscitation, transfusing PRBCs, FFP, and PLTs in balanced (1:1:1) ratios early in resuscitation, essentially attempts to recapitulate WB.[3] The use of damage control resuscitation use has been proposed in recent publications dealing with military and civilian trauma patients.[4–6] However, a 1:1:1 unit of reconstituted WB results in a product that has lower concentrations of red blood cells (RBCs), PLTs, and clotting factors compared with a WB unit that has not been fractionated. By contrast, a unit of WB contains approximately 500 mL and is only diluted by 70 mL of anticoagulant and preservative solution. As WB is typically not stored for as long as conventional RBC units before transfusion, an expectation is that any adverse effects of the RBC storage lesion on the recipient would be reduced by using a WB unit, although several recent randomized controlled trials have not found adverse effects from transfusing older RBC units in critically ill patients.[5,7–11] Nevertheless, in casualties requiring massive transfusions (commonly defined as ≥10 units of RBCs within 24 hours), it is appropriate to consider a WB-based approach in the initial resuscitation effort in order to provide a balanced response to the massively bleeding patient; recent retrospective studies suggest that WB-based resuscitation is at least equivalent if not more effective than component-based therapy.[4,12] Furthermore, WB represents a more logistically supportable option than component therapy for the provision of balanced transfusions in the prehospital and far-forward settings, as demonstrated by its successful use in World War II, the Korean War, and the Vietnam War.[13]

Despite the advantages of using WB for battlefield casualties, its use has been limited, particularly in the prehospital setting, by AABB Standard 5.14.1 that states that recipients shall receive ABO group–specific WB to eliminate the risk of acute hemolytic transfusion reactions (HTRs) caused by both the recipient's and the donor's naturally occurring anti-A and/or anti-B isohemagglutinins.[14] Whole blood has the potential to cause a major, minor, or bidirectional incompatibility. A major incompatibility results from infusing an RBC unit that is incompatible with the recipient's naturally occurring isohemagglutinins (e.g., a group A WB unit transfused to an O recipient). A minor incompatibility results from infusing plasma containing isohemagglutinins that are incompatible with the recipient's RBCs or PLTs (e.g., a group O WB unit transfused to an A recipient). A bidirectional incompatibility occurs when both a major mismatch and a minor mismatch are present (e.g., a group A WB unit transfused to a group B recipient). Because WB contains both RBCs and plasma, the compatibility of both components with the recipient must be considered. The risk of causing hemolysis due to a minor mismatched WB transfusion can be mitigated by the dilution of the donor's isohemagglutinins in the recipient's plasma volume; often, the presence of circulating A and B antigens in the recipient's plasma can also adsorb (neutralize) the donor's incompatible isohemagglutinins before they cause hemolysis. In light of these factors, group O WB, in which the RBCs are compatible with all ABO groups, represents the most practical option for transfusion in austere settings.

The use of "low titer" group O WB (containing low and presumably safe titers, or concentrations, of anti-A and anti-B antibodies) as a universal blood product in trauma scenarios is a concept that is regaining acceptance in the military and civilian literature.[5,13,15,16] Low-titer group O WB is a particularly attractive option for transfusion in the prehospital setting, close to the

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point of injury, in order to ensure that a balanced resuscitation strategy that treats both shock and coagulopathy is initiated as early as possible. This was a practice successfully implemented in past military conflicts as early as World War II.[17,18] In those conflicts, group O WB was screened by serial dilution to determine the titers of anti-A and anti-B antibodies. Units that contained anti-A or anti-B titers greater than 256 were labeled as "dangerous universal donors" and could only be transfused to group O patients. Donor safety reflected only titer and did not distinguish between immunoglobulin M (IgM) and IgG titers, although the method used (saline dilution titer, immediate spin performed at room temperature) detects IgM antibodies. All units with titers less than 256 (IgM) were considered as "universal" or "low titer" blood products acceptable for transfusion to non–group O casualties. In one early study, this method of screening identified 66.4% and 22.8% as having titers of 256 or greater for anti-A or anti-B, respectively, from a sample of 250 group O donors.[19] Low-titer group O blood was nevertheless widely available and was extensively used in combat scenarios. Notably, in more than 750,000 documented transfusions, there have been very few reports of serious adverse reactions.[17,18] Transfusion practices later moved away from WB transfusions when component therapy was introduced in the 1970s, and the concern for "dangerous donors" dissipated because transfusion products were specifically chosen to be ABO compatible.

Current AABB standards (5.14.4) require transfusion medicine services to develop specific policies and procedures concerning the transfusion of components containing significant amounts of ABO-incompatible antibodies.[14] A College of American Pathologists survey compared the practices that laboratories in the United States have implemented to minimize patient exposure to anti-A or anti-B isohemagglutinins in minor ABO-incompatible PLT products. A PLT dose contains 200 to 300 mL of plasma, which is comparable to the quantity of plasma in a WB unit. This survey revealed that, overall, most laboratories have a policy that entails manipulation of the PLT product to reduce the plasma content, but only ~2% perform some method of prospective screening to detect "high titer" anti-A or anti-B in group O products.[20] It is not possible to reduce the plasma content in WB as this would result in a self-defeating, less potent product. To ensure the safety of group O WB in combat, a screening method and policy must be implemented to identify low-titer donors (if fresh warm WB is to be used) and collected units.

In concordance with the AABB and US Food and Drug Administration guidance, US military doctrine has until recently restricted the use of WB to ABO-identical recipients.[21] Combat environments are often chaotic and disorganized. The risk of clerical errors during compatibility testing of WB is dramatically increased and could potentially result in the inadvertent transfusion of ABO-incompatible RBCs. This could have catastrophic results, possibly leading to the recipient's death. In practice, therefore, military WB transfusion has occurred largely in combat support hospitals rather than in the prehospital setting, where it has the greatest potential to improve outcomes. Several studies have evaluated current techniques used to mitigate the hemolysis potentially caused by minor incompatibilities. The aim of this review is to inform policy making and identify safe and effective methods of screening group O WB that minimize the hemolysis risk in order to support WB transfusions in battlefield conditions and in civilian un–cross-matched transfusion settings, such as in the prehospital environment or early in the hospital-based trauma resuscitation process.

Current Serology Techniques

ABO blood groups result from differential glycosylation patterns on the H antigen, an abundant RBC membrane protein. A single gene (*ABO*) with multiple alleles facilitates the differentiation of blood groups. The A allele encodes for the α3-*N*-acetylgalactosaminyltransferase, which is responsible for the attachment onto the H antigen of the *N*-acetyl-D-galactosamine sugar that represents the A antigen specificity. The A allele is generally expressed at higher levels than the B allele. This leads to the conversion of practically all H antigen sites on the RBC to A antigen. Some 810,000 to 1,170,000 antigen sites exist on a type A_1 adult RBC.[22] The B allele encodes for the α3-D-galactosyltransferase, which is responsible for the expression of the D-galactose sugar that represents the B antigen specificity. Anywhere from 610,000 to 830,000 antigen sites exist on a type B adult RBC.[23] However, when both A and B alleles are inherited, the B enzyme appears to compete more efficiently for the H antigen than the A enzyme; therefore, the average number of A antigens on a type AB adult cell is approximately 600,000 compared with an average of 720,000 B antigen sites.[22] Type O individuals produce neither of these glycosyltransferase enzymes, and their RBCs lack both the A and B antigens.

The plasma component in group O WB always contains both anti-A and anti-B, generally of both IgM and IgG classes, which can potentially cause significant hemolysis when transfused to a group A, B, or AB patient. The risk of hemolysis is thought to be predicted by the titer of the antibody, with higher titers more likely to cause hemolysis in non–group O recipients, but titers as low as 16 have been implicated in acute hemolytic reactions.[24] The incidence of HTR associated with minor incompatibility PLT transfusions ranges from 1:9,000 to 1:100.[11,25] Immune-mediated HTRs caused by IgM anti-A or anti-B typically result in severe complement-mediated intravascular hemolysis. The liberation of free hemoglobin and RBC stroma in blood vessels can lead to end organ failure and be life-threatening. On the other hand, immune-mediated HTRs caused by IgG typically result in extravascular hemolysis, with its relatively mild and rarely life-threatening clinical signs and symptoms.[14] Antibodies to A and B antigens develop within the first year of life and are thought to result from exposure to similar bacterial antigens in the diet.

The concentration of an antibody in plasma can be determined (i.e., titrated or titered) using a semiquantitative test. The titer techniques used in blood banks are based on the principle of interaction between antigen and antibody and subsequent agglutination or hemolysis of reagent RBCs. These methods cannot predict with certainty whether, or to what degree, an antibody will cause RBC destruction; they only quantitate the amount of antibody that is present. Antibody titration can be performed using the conventional serial tube dilution method, the microcolumn gel agglutination technique, enzyme immunoassay, flow cytometry, and a variety of other methods. Most laboratories use a conventional tube method primarily because

of its low material cost and their extensive experience with this platform, but this technique is limited by difficulties in automation, standardization, and reproducibility.[26]

For most blood banks, screening group O WB for high titers of anti-A or anti-B can most practically be accomplished by performing a doubling dilution of the donor's plasma and then exposing it to reagent RBCs in either a saline tube or gel. A study conducted by Josephson and colleagues[27] suggested that the use of gel technology offered a more standardized approach with comparable sensitivity to tube testing. Alternatively, the study performed by Cooling et al.[28] determined that the gel method resulted in titers one to two dilutions higher when compared with tube testing; the increased number of higher titers would result in larger percentages of falsely labeled "dangerous" units and thus limit the donor pool.

The gel microcolumn assay is in widespread use throughout the transfusion community primarily for ABO/Rh typing and antibody screens. The technology is based on a principle of controlled centrifugation of RBCs through a dextran-acrylamide gel. This method can be used for detection of antibody-antigen reactions, either directly during RBC phenotyping or indirectly during an antibody detection test. Like the tube technique, RBCs and plasma or serum (or antiserum) are combined to produce a visual readout of a positive antibody-antigen reaction. However, instead of combining the mixture in a tube, the mixture is pipetted to the top of the column; the column is then placed in a specialized centrifuge to separate agglutinated RBCs from nonagglutinated RBCs. Like conventional tube testing, there are varying degrees of positive reactions ranging from 1+ (weakest) to 4+ (strongest). Negative reactions are denoted as a pellet of RBCs formed at the bottom of the microtube without any agglutinated RBCs above the pellet.

The methods used to prepare a plasma or serum sample for titer testing can vary depending on the targeted immunoglobulin class (i.e., IgM or IgG). The IgM isotype is best detected at either cold temperatures or at room temperature using a saline dilution, which can be read immediately or after a predetermined brief incubation time. The IgG type is detected using an indirect antiglobulin test. The indirect antiglobulin test is performed to detect IgG antibodies coating the RBCs. This process requires a 37°C incubation of the plasma or serum and RBC mixture, which allows the antibody to bind with its corresponding antigen, if present. The incubation is followed by a washing step that removes any unbound antibody. Antihuman globulin (AHG) antiserum is then added to the mixture to agglutinate RBCs that are coated with IgG antibody, as IgG antibodies cannot cause direct RBC agglutination because of their small size, unlike larger IgM antibodies .[14]

Antibodies of the IgM type are large pentameric structures found in blood and lymphatic fluid, the first line of the intravascular humoral defense to foreign antigens. They react best with carbohydrate antigens and are the most efficient complement-fixing immunoglobulin. Approximately 10% of normal plasma antibodies are IgM type. Immunoglobulin G antibodies, produced as part of the secondary immune response, are much smaller monomer structures yet are the most common of all antibody types in circulation, comprising ~75% of the total. Unlike IgM, IgG antibodies are capable of crossing the placenta and binding to fetal RBCs. Most IgG antibodies cannot fix complement, and thus RBCs coated with these antibodies tend not to lyse in the vessel. Instead, they are removed by macrophages with IgG receptors in the reticuloendothelial system. Dithiothreitol treatment can be used in either the conventional tube test (CTT) or gel to distinguish IgG from IgM antibodies. Treating IgM antibodies with the sulfhydryl reagent dithiothreitol disrupts the disulfide bonds between cysteine amino acid residues, destroying the pentameric nature of the antibody and preventing both direct RBC agglutination and complement-binding activity. This method is used to detect any coexisting IgG antibodies.[14] Currently, there is no consensus as to which antibody type (IgM or IgG) is more significant when attempting to predict hemolytic potential caused by isohemagglutinin antibodies.

Focused Review of Methodological Studies

A review of the literature identified six prior studies that compared the performance of gel versus CTT for detection of IgM and IgG antibodies in plasma by titration.

Duez et al.[29] performed antibody titrations on 136 antibodies found in alloimmunized pregnant women using both CTT and gel cards (Scan gel anti-IgG; Bio-Rad, Marnes La Coquett, France). The titrations were performed using serial twofold dilutions of the plasma in normal saline. The reagent RBCs were washed three times in normal saline and resuspended to a final concentration of 4% for tube and 0.8% for gel test methods. Then the plasma dilutions were dispensed into each tube or gel card. The CTT tubes were incubated at 37°C for 30 minutes and the gel card at 37°C for 15 minutes. After the 30-minute incubation, the CTT RBCs were washed three times, and AHG was added. Gel cards do not require washing or addition of AHG. Both methods used a final centrifugation, and agglutination was read.

Quillen et al.[30] tested 11 samples using buffered gel cards (Ortho Clinical Diagnostics, Raritan, NJ), as well as CTT. This study described a slightly different technique for diluting the plasma sample compared with the technique of Duez et al.,[29] where doubling dilutions were used. A 1:150 dilution of the test plasma was prepared by adding 5 μL of plasma to 745 μL of normal saline. This mixture was then added to pooled and separated 0.8% A_1 and 0.8% B reagent RBCs in buffered gel cards that were incubated at room temperature for 15 minutes before centrifugation. Testing was also performed in CTT tube using 3% suspensions of pooled and separate A_1 and B RBCs.

Novaretti et al.[31] performed a comparison between testing anti-D titers in CTT and the gel microcolumn assay (DiaMed Latino America, Lagoa Santa, Minas Gerais, Brazil) using 79 serum samples. They described using serial twofold dilutions (2–2,048) to prepare the serum samples. The reagent RBCs were washed three times in isotonic saline before adjusting the final concentration to 3% for the tube and 0.8% for the gel assay. For CTT, 100 μL of each serum dilution was dispensed into tubes alongside 50 μL of 3% RBC suspension. The tubes were incubated for 60 minutes at 37°C. After washing three times, 100 μL of AHG (monoclonal rabbit antihuman IgG and anti-C3d; DiaMed Latino America) was added. The tubes were centrifuged for 15 seconds at 3,400 G and examined for agglutination. The sample preparation for the gel microcolumn assay was as follows: 25 μL of each serum dilution and 50 μL of 0.8% reagent RBCs were dispensed into each microtube. The cards were

incubated for 15 minutes at 37°C, centrifuged for 10 minutes at 895 ± 25 G, and then examined for agglutination.

Finck et al.[32] tested 48 samples from alloimmunized patients to compare the performance between CTT and gel card (Micro Typing Systems Inc., Pompano Beach, FL). This group used reagent RBCs (Immucor, Inc., Peachtree Corners, GA) in a 4% suspension for CTT. For the gel assay, a 0.8% suspension was prepared from the same 4% RBC suspension using a commercially available dilution solution (Ortho MTS Diluent 2; Ortho Clinical Diagnostics). To prepare the sample dilutions for both CTT and gel assay, 10 serial twofold dilutions were prepared using phosphate-buffered saline. For the CTT, 100 μL of each serum dilution and 50 μL of 4% RBC suspension were dispensed into 10 test tubes. The tubes were incubated at 37°C for 30 minutes. The tubes were washed three times, and two drops of AHG (Gamma Clone; Immucor, Inc.) were added to each tube, mixed, centrifuged, and read for agglutination. The gel assay titration was prepared by combining 25 μL of each serum dilution along with 50 μL of the 0.8% RBC suspension into separate gel microcolumns. The cards were incubated at 37°C for 15 minutes, centrifuged, and read for agglutination.

Josephson et al.[33] studied the anti-A/A,B IgM and IgG titers in 100 group O single-donor PLT (SDP) units. Segments containing 300 to 1,000 μL of plasma were removed from each unit to be tested. A serial twofold dilution (32–1,024) of plasma from each group O SDP unit was prepared in 0.9% saline, and 25 μL of titrated plasma was combined with 50 μL of 0.8% suspension A_1 cells (Ortho Clinical Diagnostics). After 15-minute room-temperature (20–24°C) incubation in buffered gel cards and 15-minute 37°C incubation with anti-IgG gel cards (Micro Typing Systems Inc.), the cards were centrifuged and read for agglutination.

Cooling et al.[28] performed CTT and gel titration testing on 124 WB-derived PLT concentrates for anti-A and anti-B using the MTS system.[28] In their study, a serial twofold dilution of plasma was prepared in 0.9% saline. Conventional tube test was tested by adding two drops of diluted plasma and one drop of commercially prepared 3% pooled A_1 and B RBCs (Ortho Clinical Diagnostics). The tubes were centrifuged without incubation for 15 seconds at 1,290 G. The results were read and recorded immediately after centrifugation. The gel assay was tested by combining 50 μL of diluted plasma and 50 μL of commercially prepared A_1 and B RBCs in buffered gel cards and centrifuging for 10 minutes at 90 rcf (relative centrifugal force). The results were read and recorded immediately after centrifugation.

The six studies compared different titer methods using either plasma or serum from a variety of sources, and none used identical procedures or reagents when preparing or processing their titration samples (Table 1). Although the methods varied, each laboratory took measures to validate the process using known positive and negative samples. The study by Josephson and colleagues[33] demonstrated a 60% single-tube discrepancy between the two titer methods, with the CTT titer demonstrating the higher titer than that found with the gel assay. Conversely, in the investigation conducted by Cooling et al.,[28] it was determined that the titers were consistently one to two dilutions higher by the gel assay than by CTT, and Duez et al.,[29] Quillen et al.,[30] and Novaretti et al.[31] also found that the gel assay resulted in higher titer levels compared with CTT. Finck et al.[32] detected a relatively even distribution of differences in their 48 samples: 21 samples had identical titers between the two methods, 11 CTT titers were higher than gel, and 16 gel samples were higher than CTT.

TABLE 1. Differences in Titration Methods in Studies Comparing Tube and Gel Determinations of Antibody Reactivity

Source	Dilution	Tube Incubation	Gel Incubation	Type
Duez et al.[29]	Serial twofold	37°C, 30 min	37°C, 15 min	IgG
Quillen et al.[30]	1 in 150	RT, 15 min	RT, 15 min	IgM
Novaretti et al.[31]	Serial twofold	37°C, 60 min	37°C, 15 min	IgG
Finck et al.[32]	Serial twofold	37°C, 30 min	37°C, 15 min	IgG
Josephson et al.[33]	Serial twofold	RT, 15 min	RT, 15 min	IgM
Cooling et al.[28]	Serial twofold	None	None	IgM

RT, room temperature.

Quantifying the sources of variance in the studies described previously presents an array of analytic variables for consideration. These include pipetting and dilution of plasma; incubation time and temperature; gel versus tube method; source, concentration, and washing of RBCs; centrifugation and agglutination scoring; immunoglobulin class detection; and underlying source of test plasma or serum. The degree to which any one of these variable contributed to the reported discrepancies in results is difficult to estimate with any confidence. It is clear that no indisputable standard technique for titering agglutinating antibodies emerges from the literature. Furthermore, these studies did not explicitly determine how agglutination translates into risk of hemolysis. These studies used EDTA-chelated plasma or serum of undetermined complement activity. Use of fresh (<3 hours since collection) serum containing active complement may more accurately predict hemolytic potential of antibodies bound to RBCs. The current state of the art in assessing risk of hemolysis from transfusion of out-of-group plasma-containing blood products thus relies on a collection of semiquantitative, imprecise methods of varying complexity that do not directly predict hemolysis. Opportunities exist for innovation in this field through rigorous testing of analytic variables and optimization of existing methods or development of new approaches.

Review of ABO-Mismatched Platelet Transfusions

We reviewed 23 publications of HTRs following transfusions of group O blood products to non–group O recipients between 1977 and 2012. Of these case reports, three involved PRBC transfusions that contained residual amounts of plasma, whereas the remaining 20 cases involved SDP or pooled PLT concentrate units. In one period of the Vietnam War (September 1967 to February 1969), more than 230,000 WB units were transfused with 24 reported HTRs—only one involved group O blood, and this was a high-titer unit incorrectly transfused to a group A patient.[34] Since the introduction of component therapy, the concern for hemolysis caused by ABO-mismatched plasma-containing components has been reduced by virtue of the large numbers of mismatched transfusions that have been administered without incident. However; ABO mismatched PLT transfusions still pose a risk that in theory is comparable to the risk

of a minor mismatched WB transfusion. There are approximately 2 million PLT transfusions administered in the United States per year, but only a few severe HTRs have been reported in the literature.[25,35] The University of South Florida College of Medicine reported their incidence of HTRs secondary to the presence of ABO-incompatible plasma in PLTs as 1 in 46,176 (between 1986 and 1996).[36] Mild HTRs are likely unrecognized or underreported; thus, it is difficult to quantitate the residual risk to patients.[35]

A PLT concentrate can be extracted from a WB donation or collected by apheresis. A therapeutic dose of WB PLTs for an adult is usually obtained by pooling 4 to 6 WB PLT units together. As each individual unit in the pool contains approximately 60 to 70 mL of plasma, the volume of plasma in a pool is roughly equivalent to that of a single-donor apheresis unit (typically 200–300 mL). As PLT transfusions are mostly administered to hospitalized patients, hemolytic reactions can be quickly detected and linked to the transfusion if they occur in temporal association with it. Each of the 23 HTR patients reported had a malignancy diagnosis and had likely been receiving many antecedent PLT and RBC transfusions before the hemolysis event. Furthermore, disease and treatment effects, such as surgery, chemotherapy, and radiation, may have increased the susceptibility of native and transfused RBCs to lysis. Of these patients, three deaths were attributed to HTRs. Table 2 summarizes these case reports. While the nature of these HTRs is serious, these 23 cases are but a miniscule fraction of the millions of transfusions that have been conducted without incident over the timeframe.

TABLE 2. Summary of 23 HTR Case Reports Resulting From Transfusion of Group O Blood Products

Source	Recipient ABO	Component	IgM Titer	IgG Titer
Fontaine et al.[37]	A	SDP	512	2,048
Sadani et al.[38]	A	SDP × 3	NR	640
Sapatnekar et al.[39]	A	SDP	2,048	16,384
Angiolillo and Luban[40]	A	SDP	128	NR
Barjas-Castro et al.[41]	A	PRBC**	1,024	NR
SHOT (Serious Hazards of Transfusion)[42]	A	SDP	>1,024	>8,192
Larsson et al.[43]	A	SDP	16,384	NR
Valbonesi et al.[44]	A	SDP	128	NR
McManigal and Sims[45]	AB	SDP	NR	NR
Mair and Benson[36]	A	SDP	128	NR
Boothe et al.[46]	B	PRBC*	16,384	>64,000
Chow et al.[47]	AB	SDP, PC	1,024	NR
Murphy et al.[35]	A	SDP	512	2,048
Reis and Coovadia[48]	B	SDP	NR	4,096
Ferguson[49]	A	PC	256	>4,000
Pierce et al.[50]	A	SDP	512	32,000
Pierce et al.[50]	B	PC	512	2,048
Conway and Scott[51]	A	SDP	8,192	NR
Inwood and Zuliani[52]	A	PRBC†	8,192	NR
Lundberg and McGinniss[53]	A_2B	PC	64	128
Keidan et al.[54]	A	WB	128	256
McLeod et al.[55]	A	PC × 2	32/64	10,240
Zoes et al.[56]	A_1B	SDP	NR	8,192

*Group O RBCs suspended in AB plasma.
**RBCs with approximately 55mL plasma.
†RBCs with 95 mL plasma.
NR, not reported; PC, PLT concentrate.

There are several clinical features of the recipient that are associated with the HTRs reported besides the isohemagglutinin titer of the donor PLT, including age, transfusion volume, and transfusion history. Angiolillo and Luban,[40] Pierce et al.,[50] and Sapatnekar et al.[39] each describe pediatric cases of severe HTR subsequent to out-of-group PLT transfusions; they suggest that pediatric patients with small blood volumes are at high risk of such a complication, which can be compounded if the donor unit contains a high-titer isohemagglutinin.[39,40,50] Adults with larger total blood volumes can dilute or neutralize the incompatible antibodies.[14] Sadani et al.[38] described a 65-year-old group A woman who received five SDP units, of which three were ABO-incompatible, within an 11-day period after her first cycle of chemotherapy. Following the fifth unit, she was noted to be anemic. Laboratory testing revealed a positive direct antiglobulin test with IgG and C3d, and anti-A was eluted from her RBCs. Only the final PLT unit transfused was available for titer testing, which revealed an anti-A titer of 640; this is a relatively high-titer antibody that, perhaps with the accumulation of anti-A from the two previous major mismatched PLT transfusions, caused her to hemolyze. However, the finding of anti-A or anti-B in an eluate prepared from a recipient's RBCs following an incompatible PLT transfusion is relatively common and not usually associated with hemolysis.

DISCUSSION

A retrospective analysis of 488 patients treated by US Army forward surgical teams in Afghanistan between 2005 and 2010 indicated that WB usage was independently associated with improved survival when used in conjunction with RBCs and FFP as compared with component therapy alone,[2] a finding that warrants further study. However, it should be noted that another retrospective study of 369 patients who had massive transfusion treated in Iraq between 2004 and 2006 indicated that those receiving fresh WB did not have a significant improvement in survival versus patients receiving apheresis PLTs (both groups additionally received a combination of FFP and RBCs), suggesting that the addition of PLTs to component therapy results in efficacy comparable to WB.[12] Nevertheless, WB appears to be efficacious in the resuscitation of bleeding patients and would be useful in the far-forward environment. The ASBP reported that as of June 30, 2016, more than 340,000 blood product transfusions had been performed since the beginning of Operation Iraqi Freedom and Operation Enduring Freedom; of those, only 10,245 were WB transfusions (ASBP communication). An expansion of WB availability could benefit many patients. For austere environments such as in military or civilian prehospital settings, in locations with severely constrained blood product inventories, or in mass casualty events with elevated risks of clerical cross matching, the use of group O low-titer WB could be an important expansion of the transfusion armamentarium. As documented, a rare complication associated with transfusing group O WB into non–group O recipients is acute hemolysis due to the passive administration of anti-A or

anti-B. No international consensus exists on the optimal testing method or critical titer level that should be adopted to reduce the risk of this complication, and this review highlights the difficulties inherent in creating a standardized policy for titering group O WB. Furthermore, there is no consensus on whether IgM or IgG or IgG subclasses or both IgM and IgG should be tested. Indeed, none of the studies reviewed here presented data on IgG subtypes, and overall, very is little is known about the effects of IgG subtypes on the risk of anti-ABO hemolysis. The United Kingdom and other European countries have taken a proactive approach in preventing HTRs from ABO-incompatible products by choosing threshold titers in blood donors, such that if their titers of anti-A and/or anti-B are above that threshold, the product is labeled as such used only in ABO-compatible recipients.[57] However; there are significant differences in the approaches taken by these countries, and a standardized approach would be beneficial.

A comprehensive reassessment of WB standards should consider other challenges such as transfusion transmitted diseases (TTDs) and transfusion-associated graft-versus-host disease (TA-GVHD). In a retrospective review of 2,831 units of virally untested WB transfused in Iraq between May 2003 and February 2006, it was discovered that three units were hepatitis C virus positive (0.11%), and two were human T-cell lymphotropic virus type 1 positive (0.07%).[58] In addition, a group A combat casualty who received group O WB (among other blood products) developed clinical symptoms of TA-GVHD 11 days after transfusion and ultimately died.[59] It is not clear if the TA-GVHD was caused by the WB, as any component with viable lymphocytes could have caused this disease in a susceptible recipient. Nevertheless, over the course of the past 13 years of conflict in Southwest Asia, only one case each of hepatitis C virus and human T-cell lymphotropic virus transmission and one case of TA-GVHD have occurred. These risks must be considered in light of the much more immediate risk of exsanguination from hemorrhagic shock; untested WB transfusion has undoubtedly saved many lives when screened components were either unavailable or ineffective. Outside extreme circumstances such as combat operations, TTD risks of WB could clearly be mitigated by testing. In addition, pathogen reduction technologies such as the Mirasol platform (Terumo BCT, Lakewood, CO) reduce the risk of TTD and TA-GVHD by utilizing riboflavin and ultraviolet light to damage nucleic acids and inactivate both pathogens and white blood cells.[60] Therefore, to maximize the safety of group O WB, we must develop clear policies on donor screening methods and pathogen risk reduction techniques.

In a study of 19 healthy group O individuals, the range of blood group isohemagglutinin titers was low: between 4 and 512 for both IgM and IgG anti-A and between 2 and 256 for both IgM and IgG anti-B.[61] There was very little difference in antibody titers on repeated measurements of the same donor over the course of 1 year. These titers were all performed on an automated solid phase instrument (NEO; Immucor, Inc.), which as suggested by some studies may be very sensitive to the presence of agglutinating antibodies compared with CTT. High titers are believed to result from heavy previous antigen exposure, such as multiple vaccinations or pregnancies. Development of titer screening programs to identify truly dangerous high-titer donors is complicated by the fact that there are no clinical trial data to guide the determination of a critical anti-A or anti-B titers in addition to the lack of an established reference method. In addition, given the relative rarity of the adverse reaction, there is concern about whether this testing is cost-effective. Josephson et al.[27] calculated a rough cost-based analysis of implementing prospective testing using the gel technology and determined that the cost per test is dependent on the testing strategy. In their scenario, the units were tested for both IgM and IgG using the gel assay, with the gel card permitting screening of up to six units per card; of 9,000 units collected, more than 50% were group O. If the policy is to screen only group O units needed for non–group O transfusions, then only 20% to 25% of the units would require testing, and the cost would be approximately $3,000 per year; to screen all group O units would cost approximately $6,000. In this scenario, the cost per unit was $1.20, which appears modest compared with most other transfusion safety interventions and would likely mitigate unforeseen adverse transfusion reactions.

Careful consideration must be given to determining the critical antibody threshold. Quillen et al.[30] found that when they implemented a critical threshold of 150, half of their group O donors were excluded. When they raised the critical threshold to 200, 36% of their group O donors were excluded as universal donors. Finally, they raised the critical threshold to 250, and 25% of the group O donors were excluded from the universal donor group. In the 2 years since implementing their screening protocol, no associated HTRs were reported of 9,800 PLT transfusions. A commonly cited titer is 200 when testing in saline (without further distinguishing between IgM and IgG); this threshold has been documented to exclude between 5% and 30% of donors as unacceptable for universal use.[62] The recent experience of the Mayo Clinic Transfusion Laboratory using cold-stored WB transfusions supports this threshold as well. From November 2015 through September 2016, 93 WB units have been collected, of which nine were excluded for having titers of more than 200; of those not excluded, 30 were transfused without incident. With PLTs, however, their results have been less clear: Forty-seven of 595 donors had conflicting positive and negative results on different donation dates, with past experience having no predictive value on future screening outcomes (data under review for publication).

The experience of the University of Pittsburgh with using group O un–cross-matched WB in the initial resuscitation of adult trauma patients suggests that when recipients are administered a small dose of incompatible plasma, generally 2 WB units or fewer, no clinical or biological evidence for hemolysis is found in the ensuing 2 days.[15,T6] All group O units at this institution have an anti-A and anti-B titer less than 50 by gel card testing (room temperature immediate spin for IgM) before they are used in potentially non–group O trauma patients. Thus, while it is unclear what the definition of a low-titer unit and the method of performing the antibody titer should be, current practices appear to be safe.

Although comparisons of the CTT and the gel assay have been inconclusive, the gel assay can be automated and is thus amenable to high-throughput screening of large donor populations. The gel cards do not require refrigeration and are stable for up to 1 year. In addition, the results of gel testing are durable (i.e., the visual indication of agglutination can last for days),

which is not true for the end point of CTT testing, which therefore lends itself to obtaining a "second opinion" on an equivocal result. The gel sample volume requirements are much lower than CTT, and certain platforms offer a means of automation with a small footprint. Although CTT is limited by difficulties in reproducibility, automation, and standardization, most laboratories continue to use CTT primarily because of its low material cost and decades of experience with the technique. Implementation of CTT would be relatively simple requiring minimum equipment additions (calibrated pipettes and pipette tips), whereas implementing a gel system could mean having to introduce an entirely new methodology into the laboratory. Gel testing is also considerably more expensive than CTT testing for small batches of tests, although economies of scale could become apparent with reduced labor costs for automated large-volume testing.

Based on the data examined in this review, further studies are needed to establish confirmatory thresholds across testing platforms. At this time, choosing a maximum antibody threshold of 200 or 256 established by saline dilution, room-temperature immediate spin (IgM) as historically performed by the US military seems reasonable in light of the extensive wartime experience and the extremely low incidence of HTRs. Other methods in current civilian practice also appear very safe. There is currently an opportunity for innovation and standard-setting research to define universal WB for hemorrhage resuscitation in the 21st century.

AUTHORSHIP

T.R.B. performed the literature review and wrote the manuscript. M.H.Y., M.A.M., and A.P.C. reviewed the literature, gave critical reviews, and contributed to the writing of the manuscript. R.L.F. and W.W.M. provided critical reviews of the manuscript. J.R.S. contributed to the writing of the manuscript.

DISCLOSURE

The authors declare no conflicts of interest.

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