Prehospital low-titer cold-stored whole blood: Philosophy for ubiquitous utilization of O-positive product for emergency use in hemorrhage due to injury

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ABSTRACT: The mortality from hemorrhage in trauma patients remains high. Early balanced resuscitation improves survival. These truths, balanced with the availability of local resources and our goals for positive regional impact, were the foundation for the development of our prehospital whole blood initiative—using low-titer cold-stored O RhD-positive whole blood. The main concern with use of RhD-positive blood is the potential development of isoimmunization in RhD-negative patients. We used our retrospective massive transfusion protocol (MTP) data to analyze the anticipated risk of this change in practice. In 30 months, of 124 total MTP patients, only one female of childbearing age that received an MTP was RhD-negative. With the risk of isoimmunization very low and the benefit of increased resources for the early administration of balanced resuscitation high, we determined that the utilization of low-titer cold-stored O RhD-positive whole blood would be safe and best serve our community. (*J Trauma Acute Care Surg.* 2018;84: S115–S119. Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Whole blood; isoimmunization; resuscitation; hemorrhage; blood transfusion.

Trauma is a leading cause of death both globally and in the United States.[1,2] Approximately half of all trauma mortalities are a consequence of hemorrhage, and most of these patients die within 6 hours of injury.[2,3] Trauma patients in hemorrhagic shock have shown improved survival with the implementation of the massive transfusion protocol (MTP).[3] We sought to characterize MTP patients in a retrospective study to better understand how Rh status of whole blood in our prehospital transfusion program might affect our trauma patient population.

Our preliminary data, collected over the span of 1 and half years (presented at the Military Health System Research Symposium in 2017), consisted of 63 patients who received massive transfusion on arrival at our Level I trauma center. This group of patients had the following characteristics: average age of 40 years, 78% males, 75% blunt trauma, and average injury severity score of 29. All-cause mortality was 76% (n = 48). This high mortality rate raised a concern that perhaps there was more we could do to improve survival in severely injured patients. Realizing that in our data set, most hemorrhage-related deaths after arrival had less than a 30-minute prehospital time, prehospital transfusion appeared to be a potential strategy to decrease mortality in the MTP patient.

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As we prepared to initiate a prehospital transfusion program, we decided on the use of whole blood for a several reasons. Whole blood has been well established as a superior resuscitation therapy compared with crystalloid and colloid fluids.[1,4] Whole-blood administration is also a simpler procedure than transfusion of multiple components, without loss in safety.[1] With our decision to use low titer cold-stored O RhD-positive whole blood ($LTO^{+}WB$), we had to consider the potential risk of isoimmunization, especially in women of childbearing age.

Rh is a blood group system composed of many blood group antigens. The most immunogenic Rh antigen is RhD. An individual may possess or lack the RhD antigen and is referred to informally in clinical situations as Rh-positive or Rh-negative, respectively. The remainder of this article will refer the status of Rh as Rh− or Rh+ to infer the status of the RhD antigen.

PREPARATION OF RESOURCES

The herein described regional blood resuscitation program was developed, founded on the conceptual framework of the Trauma, Hemostasis and Oxygenation Research (THOR) network: an international community of civilian and military stakeholders specializing in emergency medical services, trauma, anesthesia, blood banking, and basic/translational science coalesced around the vision to improve outcomes from traumatic hemorrhagic shock. The THOR network is dedicated to the development and implementation of best practices in resuscitation from hemorrhagic shock through a multidisciplinary collaborative approach to research, education, training, and advocacy.[5,6] The longstanding relationship between South Texas Blood and Tissue Center (STBTC), UT Health San Antonio, University Hospital, San Antonio Military Medical Center, Institute for Surgical Research, and Southwest Texas Regional Advisory Council for Trauma (STRAC) provided the additional platform for discussions

about developing a program to transfuse LTO^{+}WB for prehospital trauma resuscitation by first responders. South Texas Blood and Tissue Center has been providing leukoreduced red blood cells and "never-frozen" liquid plasma to multiple helicopter bases in Texas for several years; therefore, providing whole blood on helicopters is a logical next step.

Low-titer cold-stored O RhD-positive whole blood is a licensed blood product, so there were no issues related to Food and Drug Administration approval. The selection and validation of non-leukoreduced whole blood bag with citrate phosphate dextrose adenine (CPDA-1) anticoagulant with a 35-day expiration was done in consultation with military partners including the Institute for Surgical Research, the Army Blood Program and the Mayo Clinic Low Titer O Whole Blood (LTOWB) team. The overall donor base was evaluated and donor testing was conducted with anti-A/anti-B agglutinin titer of 1:256. All processes and supplies used in collection, labeling, and transporting of LTO^{+}WB were validated by the STBTC. Further, a collection, delivery and rotation schedule was created to achieve best practice utilization of product resources.

South Texas Blood and Tissue Center standardizes to the same low-titer value as our military partners and QualTex Laboratories conducted low-titer testing for both STBTC and military donors. Only male donors are used in the program because of the risk of transfusion-related acute lung injury from female donors. STBTC has tested more than 2,000 individual donors, 85% of whom met the low-titer qualification for inclusion in the program.

Community engagement was a key element in initiating this program. It required collaboration of community leaders, the medical community, as well as improved outreach to potential donors. A grant was awarded from the San Antonio Medical Foundation to decrease the cost of development of this new product line. To recruit the necessary additional donors, STBTC developed the "Brothers in Arms" branded program with specific "Brothers in Arms" messaging to enlist and prepare potential donors for inclusion in the program.

Figure 1. Logo for STBTC branded donor recruitment logo.

PREHOSPITAL TRANSFUSION PROTOCOL

The STRAC serves 22 counties, over 26,000 square miles. There are 18 helicopters within this region that provide whole blood to trauma patients prior to arrival to a Level I trauma facility. All personnel administering products have received whole blood training by STRAC using physiologic inclusion criteria-based transfusion protocol. Penetrating trauma inclusion will require one abnormal physiologic parameter and blunt trauma will require two abnormal physiologic parameters. Examples of inclusion criteria include the following: physical signs of hemorrhage, systolic blood pressure less than 90 mm Hg, heart rate greater than 120 bpm, point of care lactate greater than 5, and so on. Patients that meet the criteria will be transfused up to two units of LTO^{+}WB in the prehospital setting. The importance of clear communication with the receiving facilities and documentation of products transfused is strongly emphasized. Careful monitoring for transfusion reactions and hypothermia is key component of education. After 14 days, the whole blood products that have not been utilized in the prehospital setting are exchanged from the helicopter and brought to the hospital to be used in trauma patients before expiration at 35 days.

EMERGENCY DEPARTMENT TRANSFUSION PROTOCOL

Our University Hospital emergency release blood protocol is initiated by an automated page to the blood bank with all of our highest level trauma activations (Level I alpha) or by phone call for lower level activations. A member of the blood bank promptly responds to the page with two coolers of blood each containing four units of packed red blood cells (pRBCs) and four units of type AB or A plasma. Rh− prRBCs are preferentially administered to females younger than 50 years or males younger than 10 years (AB plasma). Rh+ pRBCs are preferentially administered to males older than 10 years and females older than 50 years (type A plasma). Alternatively, when LTO^{+}WB is available in inventory, four units of LTO^{+}WB replaces the Rh+ pRBCs and plasma. In situations where prehospital blood was not used, and there is a lack of additional available Rh− components in a male younger than 10 years or a female younger than 50 years, the Rh+ products are used as a secondary option. As opposed to an arbitrary limit of 2-4 units of WB, following the long established historical precedent (before component therapy was available), there is no established limit to the number of LTO+WB units that could be transfused.

If MTP is initiated, only Rh+ units are utilized. Our MTP is verbally initiated based on the predicted need for additional

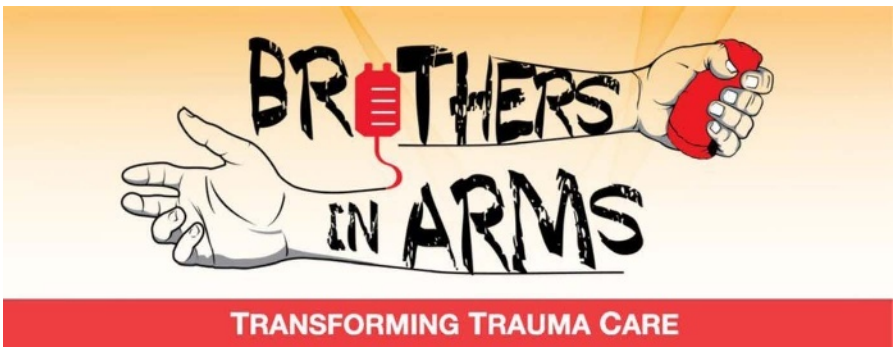

Figure 1. Logo for STBTC branded donor recruitment logo.

products (beyond the first one or two coolers) and is automatically continued in a balanced manner until verbal cessation.

JUSTIFICATION FOR RH-POSITIVE WHOLE BLOOD

The logistics of supplying and maintaining 18 helicopters with two units of LTO$^+$WB led to a lengthy discussion of feasibility and analysis of the South Texas donor population. At minimum, this effort would use 36 units of LTO$^+$WB every 14 days. All LTO$^+$WB are supplied by the STBTC. Type O Rh− individuals generally represent approximately 6% of the population compared with individuals that are Type O Rh+ which represent approximately 38% of the population. Our population is 63% Hispanic and 7% African American, and there are marked differences in Rh prevalence based upon ethnicity. Rh− prevalence in the Hispanic and African American populations, across all blood types, is reported at 7%, while in the white population it is 18%.[7,8] Thus, over two thirds of our possible donors and potential recipients have Rh+ blood which both not only limit the supply of Rh− products but also decrease the likelihood of exposure of Rh− recipients to Rh+ products. Our analysis of these factors lead us to believe that O− donors would not be able to maintain the inventory of LTO$^+$WB that we predict is necessary to impact our region.

Approximately 80% of the patients that receive emergency blood products are Rh+.[9,10] To avoid shortages of O− blood products, several facilities have instituted the selected use of O+ products for emergency release blood and especially for MTP.[9,11] However, in these circumstances, the risk of developing isoimmunization following transfusion of Rh+ products is a concern. However, the exact risk of isoimmunization after transfusion of LTO$^+$WB in the emergent trauma population is unknown.

Our protocol also continues inpatient LTO$^+$WB in all patients who receive prehospital LTO$^+$WB. It has been suggested that patients that do not develop anti-D antibodies after the first four units of blood products have a very low risk of forming antibodies from subsequent transfusions.[12]

RH SYSTEM

Rh is a blood group system composed of many blood group antigens. The most immunogenic Rh antigen is RhD. An individual may possess or lack the RhD antigen and is referred to informally in clinical situations as Rh-positive (+) or Rh-negative (−), respectively. Rh+ cells transfused to an Rh− recipient may cause induce alloimmunization (IgG response) that may cause hemolytic reactions to subsequent transfusions or induce hemolytic disease of the fetus and newborn in subsequent pregnancies in a female of childbearing age.[13]

The probability of development of anti-D antibodies in Rh − patients has been evaluated in multiple populations. The risk has been stated as greater than 80% in healthy volunteers and demonstrated as low as 0% in AIDs patients.[14,15] In a recent review of their institution, Selleng and colleagues[9] found an overall 3% to 6% risk of inducing anti-D antibodies with O+ emergency transfusions. This review included all emergent transfusions, with 70% in surgical or trauma patients. The immunosuppression of trauma patients has been well described, with impaired function of both adaptive and innate immunity.[16] This fact suggests that trauma patients may be at a decreased risk for isoimmunization.

CLINICAL SIGNIFICANCE OF ISOIMMUNIZATION

The incidence of Anti-D is decreased due to the long-term practice to transfuse Rh− individuals with Rh− blood preferentially. In addition, Rh− females are provided with antepartum and postpartum doses of Rh Immunoglobulin (RhIg) as indicated to reduce the rate of isoimmunization. Anti-D can induce hemolytic transfusion reactions. Although rare, hemolysis may be clinically significant with a drop in hemoglobin and bleeding complications. Characteristically, this process presents with extravascular hemolysis and is classified as a delayed hemolytic transfusion reaction. It can take months for the blood counts to normalize depending on the immune response. Reticulocytosis, elevated lactate dehydrogenase (LDH) and elevated free hemoglobin can aid in the diagnosis of immune hemolysis along with a positive direct antiglobulin test.[14]

Women of childbearing age are the main focus of isoimmunization, as one of the most well described and most concerning associated reactions is Hemolytic Disease of the Fetus and Newborn. Women who develop anti-D antibodies may adversely affect an RhD-positive fetus. The anti-Rh antibodies (IgG) can cross the placenta during pregnancy and induce hemolysis of the fetal red blood cells, resulting in severe morbidity and mortality. Decreasing the sensitization of the immune system can be reduced by the administration of anti-D immune globulin (i.e., RhIg).[17,18]

Rh− females that receive Rh+ pRBCs or LTO$^+$WB, require additional testing to calculate the amount of immune globulin needed to reduce the risk of isoimmunization. Additional vials of immune globulin can be administered until the desired dosage has been reached.

RETROSPECTIVE DATA

In developing this protocol, we wanted to more fully understand the risk and impact of this potentially "at-risk" population. The number of female patients of childbearing age that are Rh− was predicted to be low.

To substantiate this assertion, we performed a query of our retrospective MTP study data to formulate a better estimate. With updated data from our initial MTP review, we reviewed a total of 124 patients (with the review expanded 30 months): 26 (21%) of these patients were women, 18 of 26 women (15% of the total study population) were of childbearing age and 10 of those 18 female patients died (56%). Of the 18 women, 16 had blood typing and screening performed (89%). Only one of these 16 women was Rh− (6.3%), and this patient survived.

POSTTRANSFUSION PROTOCOL FOR AT RISK PATIENTS

With prehospital utilization of Rh+ blood products, there will be a potential increase in patients who are Rh− receiving Rh mismatch products, and thus at risk for isoimmunization. We have collaborated with our pathology and obstetric colleagues to implement appropriate screening and as needed RhIg

administration. Each patient who has an Rh mismatch will have a pathology consultation within 24 hours. Any woman of childbearing age with an Rh mismatch will also receive a consult by our obstetric colleagues.

Women of childbearing age, who receive Rh+ pRBCs or LTO$^+$WB will be evaluated for RhIg administration candidacy. Administration of RhIg is not without risk, including hemolysis of the transfused Rh−positive cells and immediate hypersensitivity-type reactions. The risks and benefits, the patient's desires of future pregnancy, clinical condition/fitness and prognosis must be taken into account when deciding to administer RhIg.

When larger volumes of Rh incompatible red cells are transfused, the RhIg administration to prevent isoimmunization is 10 μg/mL to 15 μg/mL red cells and doses are divided to prevent hemolysis. In addition, if the volume of transfused red blood cells exceeds 20% of the patient's total blood volume, RhIg administration is generally not favored due to the potential for marked red cell splenic sequestration or hemolysis which could compromise the hemodynamic stability of the patient. In these cases, red cell exchange with Rh− may be utilized to remove up to 65% of circulating Rh+ red cells.

After completion of RhIg dose administration, evaluation for clearance of Rh+ is done with evaluation by a Rosette Test. This test allows for visualization of Rh+ red cells within an Rh − individual. A direct antiglobulin test (DAT) can also be performed which can detect passive antibody (RhIg) adherent to the transfused Rh+ red cells circulating in the patient. When the DAT becomes negative, it may indicate that the Rh+ transfused cells have been successfully removed from circulation by RhIg.

In our institution, before RhIg administration, an indirect antiglobulin test (IAT), also known as an antibody screen, is performed and should be negative for Anti-D. After administration of the RhIg, the IAT is repeated and will demonstrate RhIg (passive Anti-D) administration. Passive Anti-D (RhIg) and true isoimmunization by Anti-D is indistinguishable by laboratory methods. Therefore, clinical correlation with RhIg administration is required. The half-life of RhIg is approximately 21 days and should become undetectable within 3 months to 6 months after administration. A repeat IAT is performed 3 months to 6 months after administration. Persistence of passive RhIg after 6 months is uncommon, and persistence of anti-D may be more consistent with isoimmunization with anti-D.

If RhIg administration is not pursued to reduce the risk of isoimmunization, repeat IAT monthly for up to 1 year to evaluate for the formation of anti-D may be recommended. If the antibody screen is positive for an Anti-D within 120 days of the transfusion event, a DAT is performed. If the DAT demonstrates IgG coating the red cells, an eluate is performed to further characterize whether the coating IgG antibody is Anti-D. Further evaluation for clinical evidence of hemolysis may be recommended if laboratory findings are suggestive of a delayed hemolytic transfusion reaction.[13] Screening protocols and procedures are variable. The ideal duration to perform screening is unknown. Isoimmunization has been seen to occur even a year following transfusions.[19] In the previously discussed study by Selleng, only one patient developed anti-D within 4 weeks, all others were detected after 4 weeks.[9]

CONCLUSION

With one Rh− woman of childbearing age in 30 months potentially getting LTO$^+$WB and an anti-D conversion rate between 3% and 30% (per the literature), there would be a risk of isoimmunization of 0.012 to 0.12 patients/year. In other words, it would take 3000 months (250 years) at the current rate to have 100 Rh− women of childbearing age receive LTO$^+$WB and approximately somewhere between 3 and 30 of them would develop isoimmunization without the administration of RhIg. RhIg administration decreases the isoimmunization rate to 1%, thus 0.3 Rh− women of childbearing age could develop isoimmunization. Without transfusion of LTO$^+$WB in the prehospital setting over this period, it is estimated that nearly 500 women of childbearing age would die of hemorrhage. The risk:benefit ratio appears to strongly favor this approach.

To appropriately state the overall risk with emergency transfusion of LTO$^+$WB, we must consider the risk to the population as a whole and not just consider the Rh− population. Rh status is almost always unknown at the time of prehospital transfusion. The incidence of Rh− individuals is 15% of the overall population, but even lower in the catchment area we serve, indicating that more than 85% of the patients with unknown blood status in our community are likely to be Rh+ prehospital or on arrival to our trauma center, and have no risk of isoimmunization due to utilization of LTO$^+$WB. Further, the low incidence of Rh− blood type in our donor pool would prohibit us from having enough emergency release LTOWB if we used only Rh− donors. In addition, currently women of childbearing age are preferentially transfused RhD-negative blood unless an MTP is called or there is not otherwise timely provision of RhD-negative blood components. Mortality in patients with severe hemorrhage still remains critically high. Our philosophy is better to have an alive patient with isoimmunization than a dead patient as a result of hemorrhage.

AUTHORSHIP

A.MG. participated in data interpretation. C.Z. participated in data collection and analysis. L.G. participated in process development, blood bank/transfusion medicine expert/consultant. E.X. participated in obstetrics and gynecology expert/consultant. E.W. participated in process development. E.E. participated in process development. D.C. participated in data collection. R.J. participated in data collection. S.N. participated in data collection. B.E. participated in process development, critical revision. R.S. participated in process development. D.J. participated in the process development, data analysis and interpretation, critical revision.

DISCLOSURE

The authors declare no conflicts of interest

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