Is it hot in here? The impact of febrile nonhemolytic transfusion reactions

Chills, increases in body temperature, and discomfort are all symptoms that are associated with hemolytic transfusion reactions as well as febrile nonhemolytic transfusion reactions (FNHTR). The former is a rare but very serious complication while the latter is fairly common, associated with approximately 1 in 8 transfusions, and is typically considered as a non-serious adverse reaction to transfusion.

Most often caused by inflammatory cytokines produced during blood product storage or from interaction of residual donor white blood cells with recipient anti-leukocyte antibodies, FNHTRs are commonly diagnosed after excluding other causes of fever in the transfusion recipient. These investigations involve laboratory tests to exclude errors in pretransfusion testing and immune hemolytic incompatibility, which result in substantial additional costs for hospitals. While many papers have focused on determining incidence, causal factors, and mitigating strategies, this report from Canada also tries to assess the care-related activities triggered by FNHTR and the resource consumption. Among their observations were that the overall per-product rate across all sites was 0.24% and blood cultures were collected in 79% of FNHTR events. Especially alarming to the authors, 15% of outpatient FNHTR events resulted in hospital admissions across the study sites!

FNHTRs result in significant distress for the patient and place a burden on hospital staff by way of (possibly unnecessary) added medications, laboratory and imaging studies, and interruption of transfusions and other patient care. Moreover, quality audits indicated that approximately one in five RBC transfusions at sites included in this study were not needed. While making these observations, the authors have suggested that mitigation steps such as premedication, pathogen inactivation, and implementation of a more restrictive transfusion policy could not only decrease the occurrence of FNHTR but avoid the need for many hospital resources.

Understanding complement activation during platelet storage and potential risks

In this era of blood management initiatives and decreased red cell transfusions, the demand for platelet components has increased, largely due to the central role these products play in management of treatment-related thrombocytopenia in oncology patients. This increased demand, coupled with the logistical and storage related challenges of platelets, has placed tremendous strain on an already limited resource.

Platelet safety, despite widely used leukoreduction, decreases with storage time and this has been a limitation to supply. With the introduction of pathogen reduction technology and improved testing to reduce the risk of bacterial contamination, the storage lesion remains a roadblock to increasing the shelf life of platelet concentrates. A change in shelf life from five to seven days that may be allowed based on “pathogen safe” criteria may improve platelet availability, but, as a recent study shows, there are time-dependent alterations occurring within the platelet bag with potential effects on recipients that are not understood yet should be considered.

This study of complement factor marker levels from various complement pathways demonstrates the potential for continuous activation during storage, the byproducts of which are capable of inducing an inflammatory response. The authors speculate that this could be particularly important for recipients with conditions, such as malignancy or sepsis, associated with complement activation. Further, their increase in components is greatest if stored more than 5 days and presents a potentially significant risk of inflammatory response in the transfused recipients. Additional study is warranted to understand this phenomenon as a potential cause of transfusion-related adverse events.


Platelet sterility testing: We’ve come a long way but still have a way to go

Room temperature storage of platelets in gas-permeable bags with constant agitation creates a welcoming environment for bacterial growth. As a result, bacterial contamination and possible sepsis pose a primary risk. For several years, AABB, FDA and blood centers around the globe have studied strategies to reduce this risk employing such measures as improved donor arm disinfection, use of a diversion pouch, routine culture-based bacterial screening, rapid bacterial detection, and most recently pathogen reduction technology.

AABB adopted a standard in 2004 requiring all member facilities to “implement measures to limit and detect bacterial contamination in all platelet components.” Although no single test method is 100% effective, culture-based bacterial screening of all platelet products was widely adopted as the “gold standard” for sterility testing of platelet concentrates. With the FDA in 2016 releasing further draft guidance for consideration, the authors of this Canadian Blood Services study offer a timely summary of their recent experience.

Continues on next page
Have you ever wondered why some of the most chronically transfused patients, those who receive transfusions every few weeks for years, never seem to develop an antibody while others respond with antibodies after a single transfusion? Or why a mother with multiple children has not formed an antibody but other OB patients to this study, the culture system used appears particularly effective in identifying platelets contaminated with Gram-negative bacteria; not a single Gram-negative organism was isolated from expired platelets. In contrast, quality control testing of expired products indicated that slower-growing Gram-positive skin and mucosal contaminants including 10 coagulase negative streptococcus, 1 S. aureus, and 4 P. acnes had been missed during screening. During this time period, there were also six septic transfusion reactions reported to Canadian Blood Services, all involving platelets contaminated with Gram-positive organisms not detected by bacterial screening.

Several different strategies are being tested that appear to further reduce risk from bacterial contamination—delayed sampling has been tried by Hema-Quebec, methods to test closer to time of issue have been tried in Germany, pathogen reduction has been studied and deployed widely in Europe. The latter two strategies are now being proposed by the FDA. Time will tell if we have a panacea though there are already some promising results.

[Image of a pregnant woman]

**Triggered: Understanding blood group alloimmunization**

Since 2004, Canadian Blood Services has screened 100% of platelet products using an automated culture system and in 2009 implemented standardized monthly quality control sterility testing of expired platelet products. This study details the results of this testing from 2010 through 2016 of almost 600,000 platelet products. Data of routine bacterial screening, quality control testing, and reports of septic transfusion reactions, all come from this 7-year period during which current collection practices to reduce contamination and testing protocols were fully implemented. According to this study, the culture system used appears particularly effective in identifying platelets contaminated with Gram-negative bacteria; not a single Gram-negative organism was isolated from expired platelets. In contrast, quality control testing of expired products indicated that slower-growing Gram-positive skin and mucosal contaminants including 10 coagulase negative streptococcus, 1 S. aureus, and 4 P. acnes had been missed during screening. During this time period, there were also six septic transfusion reactions reported to Canadian Blood Services, all involving platelets contaminated with Gram-positive organisms not detected by bacterial screening.

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As expected, patient populations that require more transfusions (hemoglobin disorders such as sickle cell disease and thalassemia as well as myelodysplastic disorders) have significantly higher reported alloimmunization rates (3-10% in the chronically transfused) compared to the general patient population. This stands to reason since increased exposures to foreign RBC antigens is expected to result in increased rates of response. As a result, strategies such as providing "phenotypically matched" RBCs for these frequently transfused patients, have been implemented to limit the exposure of foreign RBC antigens and the potential for alloimmunization.

Even in patient populations where transfusion rates are highest, there is extreme variability in the patients who form antibodies ("responders") and those who do not ("nonresponders.") This suggests that a key to optimizing strategies to avoid alloimmunization triggers, and thus reduce or prevent alloantibody formation, lies in understanding what defines a "responder.".


Potential Triggers for Blood Group Alloimmunization

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<thead>
<tr>
<th>Donor/Antigen Related</th>
<th>RBC Unit Related</th>
<th>Recipient Related</th>
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<tbody>
<tr>
<td>Ethnicty</td>
<td>Storage age</td>
<td>Degree to which the recipient views the RBC antigen as foreign</td>
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<tr>
<td>Number of copies of antigen present on RBCs</td>
<td>Anticoagulant and/or preservative solution</td>
<td>Disease state</td>
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<tr>
<td>Immunogenicity of antigen</td>
<td>Number of residual platelets or leukocytes after processing</td>
<td>Immune state</td>
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<td>Donor health (inflammation)</td>
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Demand for O negative red blood cell units—The view from down under

Over the last five years (2010-2015) where data is available, the overall international demand for red blood cell (RBC) units for transfusion has been declining. During that same time period the demand for group O negative RBC units has increased significantly. The Australian Red Cross Blood Services studied this phenomenon among their hospital users and researched possible approaches to reduce demand for O negative RBC units.

Since O negative is the “universal” red cell blood type, i.e. compatible with recipients of any ABO blood group for transfusion, there has been a philosophy to overstock O negative RBC units to ensure an adequate supply for O negative patients and for emergencies when transfusion is required before ABO type can be determined. O negative units not used for these patients can be crossmatched and transfused to recipients of any ABO type before they expire. Although this approach has worked for many years, the report presented here suggests that it is time to

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reassess this approach and instead focus on decreasing demand for O negative RBC units and better aligning ABO type of transfused blood to that of the recipients. The authors propose numerous possibilities for accomplishing this goal including utilization of O positive RBC units in emergency situations for males and females outside of childbearing age, reevaluation of optimal inventory levels at smaller facilities and differential pricing (less expensive B and AB; more expensive A and O units for example) to encourage facilities to stock minor ABO groups. Another particularly interesting strategy presented was inventory data sharing among hospitals and potential for stock rotation among sites which could allow for the more efficient use of RBC units of all blood types not just O negative.

There is certainly no “one size fits all” solution but this review is intended to start discussions around the possibilities for reducing demand for these “universal” RBC units while providing the right product at the right time for the patient.


Rules are made to be broken: Use of group A plasma in trauma situations

Recent shifts toward a 1:1:1 ratio of plasma, platelets, and RBCs in massive trauma transfusion practice has resulted in increased plasma use, particularly group AB plasma which is the “universal blood type” of plasma components. The use of thawed plasma, with a shelf life of five days has become a useful tool for trauma centers and allows for provision of plasma as early as possible in resuscitation. However, if group AB plasma is always transfused in these situations, there is the risk of waste. How can we provide our trauma teams the best chance for success while conserving the limited supply of group AB plasma? Facilities have begun to use group A plasma in trauma situations at an increasing rate. As recently as 2016, a survey indicated that most level 1 blood services and trauma centers maintain thawed plasma inventories and that group A plasma was widely used initially for instances of trauma when the ABO group of the recipient was unknown. But what about the risks associated with incompatible plasma transfusions in group O or AB trauma patients? Although the data to support this practice is limited, it has become widely adopted and assumed to be safe based on the following:

1. Group A plasma is compatible for 8 of 10 trauma patients since 85% of the population is group O or A.

2. Initially in resuscitation, blood loss is replaced with group O RBCs resulting in a reduced volume of possibly incompatible patient RBCs that may be susceptible to hemolysis from group A plasma.

3. Historically, transfusion of ABO incompatible platelets has been necessary due to limited availability. Hemolysis from group A platelets is uncommon and reports of hemolytic transfusion reactions only involve group O platelets, which contain plasma with higher anti-A and anti-B titers.

4. Among group B and AB recipients, 80% secrete B-substance that might neutralize much of the anti-B in the transfused group A plasma.

This study, which the authors say is the largest to date, combined retrospective data from 17 trauma centers to...
Cold agglutinin disease (CAD) is a rare (1 case per million people per year) type of autoimmune hemolytic anemia (AIHA) and is commonly associated with infectious mononucleosis or Mycoplasma pneumoniae infections. Diagnosis of CAD is typically based on clinical indications of hemolysis and laboratory evidence of cold autoantibodies; i.e. splenomegaly, hemoglobinuria, increased LDH, positive anti-C3 DAT and an elevated cold agglutinin (CA) titer with a high thermal amplitude (reactive at near body temperature).

The CA titer is a time consuming manual procedure that is labor intensive, is not always ordered or interpreted correctly, and may require complicated sample handling. Results typically show the titer to be not elevated, particularly in patients with a negative anti-C3 DAT. To evaluate the negative anti-C3 DAT as a screening test to reduce the need for CA titer testing, a retrospective analysis was performed at three large academic institutions: Barnes-Jewish Hospital, Vanderbilt University Medical Center and Massachusetts General Hospital. Testing results were reviewed for a total of 401 different patients from the three hospitals who had anti-C3 DAT and CA titers performed; of these, 279 (68%) had a negative anti-C3 DAT. 98% of patients with a negative anti-C3 DAT also had negative (<64) CA titer results. Of the 131 (32%) of patients with a positive anti-C3 CAT, 46 (35%) also had elevated (>64) CA titer results.


American Red Cross

D variant genotyping candidate identification: A three criteria screening strategy

Following the ABO system, the Rh system is the most recognized and clinically significant blood group system. The five primary Rh antigens (D, C, c, E, and e) represent the most important antibodies in the system, with the D antigen being the most immunogenic antigen. To date, more than 200 variants of RHD, the gene that encodes the D antigen, have been described. Based on these variant genotypes and the potential of the individual to form an allogeneic anti-D antibody, these variants are broadly categorized in subgroups as weak D, partial D and DEL.

There has been much discussion recently regarding the current practice for interpretation a patient’s D type when a serologically weak result is observed and deciding whether genotyping is needed. The conversations have primarily focused on the lack of a widely accepted standard algorithm for resolving these weak D results, which is somewhat troubling since some of these patients have the potential to make an allogeneic anti-D antibody if managed incorrectly and are then subsequently exposed to RhD positive red cells, either by transfusion or pregnancy. The current edition (30th) of the AABB Standards for Blood Banks and Transfusion Services only requires serologic weak D testing of blood donor RBCs and of RBCs from the fetus or newborn when needed to determine the RhIG candidacy of an RhD-negative mother.

The authors of this study have proposed using a 2 serologic method strategy that is more sensitive for observing weak reactivity to D than a single serologic method, and a three-criteria approach for identifying candidates for RHD genotyping; (1) gel D typing at least 2x stronger than tube typing, (2) serological weak reactivity of ≤2+ in gel or ≤1+ in tube and (3) the presence of suspected alloanti-D in D-positive patients. This study demonstrated the high positive predictive value (98% of this approach as a useful screening tool for identifying RHD genotyping candidates (49 of 50 confirmed as D-variants) . By integrating the two commonly utilized and available serologic methods (i.e. gel and tube typing) this approach also satisfies the current AABB and CAP requirement for two blood type tests/specimens, so that these criteria can be implemented with minimal increase in cost and workload.


Finding the right type: ABO discrepancies

It has been a long night in the blood bank; with emergency release of uncrossmatched blood, traumas and massive transfusion protocols but the end of shift is in sight! A routine sample accompanies the sunrise and is all that stands between completing a night’s work and heading home for some rest, except... the forward and reverse ABO groups do not match! Testing is repeated with the same results. A new sample is requested and again forward and reverse ABO do not match. No patient history is available to review. What’s next? Are the reagents performing appropriately? Is there a problem with the centrifuge? So much for leaving on time this morning! This brief review examines causes for the discrepancies, methods for resolution, and a case-example approach to explore transfusion options.

ABO discrepancies are typically divided into two groups: red cell and plasma discrepancies. These are then further categorized as exhibiting weak/missing expected reactivity or extra reactivity. In addition, discrepancies may be due to technical problems such as failure to follow procedure or manufacturer’s instructions and reagent or equipment malfunction. Most ABO discrepancies can be resolved simply by performing a few additional serologic tests; subgroup of A with anti-A1 for example, or obtaining additional patient information; a recent transfusion may explain a mixed-field result. Others are more troublesome, such as a wrong blood in tube (WBIT) error which may involve other samples received in the laboratory and subsequent mistakes involving the results from analysis of those samples. Molecular methods have proven to be very useful in resolving Rh system discrepancies but, for now, are not used routinely for ABO discrepancy resolution, except in cases of suspected chimerism, which would make for a very “interesting” workup!

Anti-CD38 therapy: Practical and financial considerations for pretransfusion testing

Multiple myeloma (MM), a cancer of malignant plasma cells in the bone marrow, represents 1.8% of all newly diagnosed cancer cases. Although considered incurable, MM is treatable and in November of 2015 the United States Food and Drug Administration (FDA) granted accelerated approval for the use of daratumumab (DARA). DARA is the first monoclonal antibody approved for the treatment of MM in relapsed or refractory patients and specifically targets human CD38 which is strongly expressed on MM cells. CD38 is also weakly expressed on normal red blood cells (RBCs) and this was observed to cause interference with antibody screening tests during clinical trials. The DARA anti-CD38 in the patient’s plasma binds to the CD38 expressed on the reagent RBCs (RRCs) used in antibody screening, causing a weak false positive result in the indirect antiglobulin test (IAT) and may mask underlying clinically significant antibodies in the patient’s plasma. The goal of this research was to study the practicality and financial impact of different strategies for eliminating this interference and providing safe transfusions to those patients receiving DARA therapy. The strategies studied were:

1. Using thiol-treated (AET, DTT) RRCs for antibody screening and providing K negative RBCs for transfusion
2. Patient phenotyping and providing phenotype matched RBCs for transfusion
3. Patient genotyping and providing genotype matched RBCs for transfusion
4. Combination of interval (every 4 weeks) thiol-treated RRCs for antibody screening and providing genotype matched RBCs for transfusion

They did not attempt to look at neutralizing antibody strategy (i.e. either CD38 antigen or anti-DARA antibody) to remove DARA as the reagents are currently very expensive and generally not available. While there is not currently a “one size fits all” approach for mitigating DARA interference in serological testing, this study suggests that the benefits of genotyping should not be overlooked when considering the best fit for your institution. Genotyping holds a practical advantage over phenotyping in that it provides a more accurate and comprehensive RBC antigen profile by detecting and identifying variants (Rh) and mutations (Fyb silencing) which are more common in African Americans, who are twice as likely to be diagnosed with MM as Caucasian Americans. Also, unlike reliance on antibody screens using thiol-treated RRCs that expire and must be repeated at defined intervals with a newly collected specimen, the genotyping and matched RBC strategy eliminates delays, 2-4 hours in most cases, and allows for RBCs to be issued and transfused without collecting a new blood specimen from the patient. Using thiol-treated RRCs for antibody screening and providing K matched units for transfusion was found to be the least expensive method for a single testing event however, when the increased technologist time and reagent costs associated with these workups are considered, implementing a strategy to genotype all DARA patients and providing genotype matched RBCs for transfusion may ultimately be more cost effective. The authors further argue that with additional new treatments that will soon continue to test the limits of serologic methods, a single genotyping approach can avoid piecemeal solutions and become more financially and practically efficient.

Although sometimes necessary and potentially lifesaving, there are risks associated with blood product transfusions. The patient presented in this case study showed signs and symptoms of two rare but serious complications: transfusion-related acute lung injury (TRALI) and hemolytic transfusion reaction (HTR.) According to the FDA, between 2011 and 2015, TRALI caused the highest number of reported fatalities at 38% while 14% were the result of non-ABO HTRs.

The patient is a 53 year-old female with a medical history that includes cirrhosis and immune thrombocytopenia (ITP) who presented for laparoscopic repair of a ventral hernia. Her pretransfusion workup indicated the presence of anti-E and anti-K alloantibodies. Complications from the procedure resulted in activation of a massive transfusion protocol (MTP): as a result within 24 hours post-procedure the patient received three units of platelets, three units of fresh frozen plasma (FFP), four units of packed red blood cells (negative for E and K antigens) and ten units of cryoprecipitate. The patient’s hemoglobin and hematocrit increased briefly following the MTP but quickly dropped soon afterwards. Additional blood products were transfused and the patient’s hemoglobin remained steady at 6-7 gm/dL despite multiple red cell transfusions and treatment of thrombocytopenia with IVIG and platelet transfusion in the week after the MTP.

Although a diagnostic test for TRALI does not exist, the US National Heart, Lung and Blood Institute Working Group has developed criteria for diagnosis which include the onset of respiratory distress within six hours of transfusion of blood products, newly developed bilateral pulmonary infiltrates on a chest X-ray, and the exclusion of other possible causes. Within four hours of the first transfusion, the patient exhibited respiratory distress and a presumptive diagnosis of TRALI was made after chest X-rays showed bilateral lung infiltrates.

All blood products that contain plasma have been associated with TRALI and even though they contain very small amounts of plasma, packed red blood cells have been implicated in the largest number of reported cases. TRALI is suspected to be triggered by the presence of anti-HLA or anti-granulocyte antibodies most commonly present in the plasma of female donors who have been pregnant or donors who have received transfusions.

On post-operative day 16, laboratory technologists noticed a change in the appearance of the patient’s serum from icteric to hemolytic. Subsequent laboratory studies were consistent with in vivo hemolysis and additional immunohematology studies were performed. The patient’s pre- and post-operative direct antiglobulin tests (DAT) had been negative but the DAT was positive when hemolysis became evident. The previously identified anti-E and anti-K antibodies were confirmed but an additional antibody was detected that could not be identified and repeat testing showed previously compatible donor units were now incompatible. Additional tests done by a reference laboratory also did not identify the 3rd antibody though they did exclude many possible uncommon antibodies. No additional blood products were transfused during the patient’s hospital stay. The patient soon began to improve significantly, laboratory results and vital signs normalized and she began to move about and tolerate a regular diet.

This case demonstrates the importance of considering the diagnosis of TRALI when post-transfusion respiratory signs and symptoms are observed, recognizing the risk for HTRs, especially in patient populations with known prior alloantibody formation, and understanding when transfusion is inappropriate.

Patient Services: Focus on Perioperative Autologous Cell Salvage

Among the multiple direct patient care services the Red Cross provides is perioperative autologous cell salvage (PACS). PACS services are recognized as a key component of blood management programs and are an effective option for surgical patients who need blood transfusions. The benefits of autologous transfusion include:

- Conservation of allogeneic donor blood inventories.
- No risk of disease transmission or a transfusion reaction.
- Prevention of alloimmunization to red blood cell, platelet and leukocyte antigens.

In addition to the clinical benefits of cell salvage, there are operational upsides:

- A Red Cross technician works independently, reducing the need for additional hospital personnel in the operating room.
- Red Cross can provide the equipment, reducing your hospital’s need for capital expenditures.
- Red Cross has in place strict training and certification protocols and extensive experience performing PACS procedures, ensuring technician excellence.
- Reduction in the expense of transfusion support through elimination of blood purchase and compatibility testing.

In addition to PACS Red Cross offers to most patients:

- Therapeutic phlebotomy
- Directed donor collections

Contact your Red Cross representative to learn more.

To view previous issues of PLUS, go to redcrossblood.org/hospitals/plus-quarterly
Publications Corner

Recent literature from Red Cross scientists and physicians

Discriminating complement-mediated acute transfusion reaction for type O+ red blood cells transfused into a B+ recipient with the complement hemolysis using human erythrocytes (CHUHE) assay. Cunnion KM, Hair PS, Krishna NK, Whitley PH, Goldberg CL, Fadeyi EA, Maes LY. 

The Growing Need for Diverse Blood Donors. MacIntyre LM. 


Remember these websites:

Immunohematology Journal
redcross.org/immunohematology

Reimbursement
redcrossblood.org/hospitals/
educational-resources/reimbursement

SUCCESS®
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The Mission of American Red Cross Biomedical Services

To fulfill the needs of the American people for the safest, most reliable and most cost-effective blood services through voluntary donations.

The American Red Cross empowers ordinary people to perform extraordinary acts of service. Our blood donors are ordinary people – high school students, factory and office workers, business executives, parents and grandparents, and people from every walk of life. But they share one thing – a generous spirit, a desire to give back to their community and help others. Blood donors play an integral role in the delivery of modern healthcare. Many life-saving medical treatments and procedures involve blood transfusions and would not be possible without a safe and reliable blood supply.