FOCUS ON PLATELETS

Apheresis Platelet Storage in Syringes for Newborns

Up to 9.4% of all infants in neonatal intensive care units need platelet transfusions. Hospital staff can volume-reduce units to get the small-volume platelet transfusions that newborn babies, especially premature infants, usually need. But this method is time consuming and often wastes product. Volume reductions can also damage platelet function and increase platelet activation. A potentially better alternative is aliquoting platelet concentrates into syringes. Putting prepared syringes directly into infusion pumps can prevent errors and contamination during the transfer of platelets from a transfer pack to a syringe.

Researchers from George Washington University and the American Red Cross studied how short-term storage of small apheresis platelet aliquots with and without agitation affects platelet quality. They collected double apheresis platelets in 100% plasma and split them into two identical products. They transferred aliquots from one bag of each pair to two syringes and stored them for six hours on a flatbed agitator or left the bag without agitation at room temperature. The other bag from each pair served as a control.

The results showed no differences between the quality of agitated and non-agitated syringes. Control bags and aliquots stored in syringes had different pH levels, 7.42 ± 0.1 in control apheresis bags, 7.19 ± 0.1 in agitated bags, and 7.19 ± 0.1 in non-agitated bags, after four days of storage. But pH at room temperature was higher than the critical level of 6.8 in all samples, regardless of whether they had been stored with or without agitation.

Storing small-volume apheresis platelet aliquots in syringes for up to six hours is therefore feasible and the quality of platelets stored with and without agitation is similar. Based on these results, storing syringes on reciprocal agitators is probably not necessary. Although the results showed some differences in quality between aliquoted and control samples, these differences were small and not likely to have any clinical effects.

According to earlier research, a 1:1 ratio of RBCs to plasma improves survival in trauma patients with a massive hemorrhage. Confirming these findings is challenging, however, because these patients often die quickly, before the right blood products can be thawed and delivered.

Researchers from the University of Maryland School of Medicine recently studied the outcomes of 844 adults who had been admitted to their hospital for trauma over five years. The analysis focused on 438 patients who had gotten at least five RBC units in the first 24 hours after hospital admission. All patients had aggressive RBC and plasma resuscitation within minutes of admission.

In patients who got 10 or more RBC units, plasma deficit status (units of RBC minus units of plasma), but not plasma ratio (units of plasma/units of RBC), was statistically associated with risk of death in the first 24 hours of resuscitation. But in patients who received five to nine RBC units, neither plasma deficit status nor plasma ratio predicted death in the first 24 hours. After three hours, a plasma deficit that was getting worse increased the risk of death in both patients who got five to nine RBC units and those who got 10 or more units. But plasma ratio still had no effect on risk of death in either group.

The results show that plasma deficit status might be more important than the ratio of RBCs to plasma in decreasing a trauma patient’s risk of death due to massive bleeding. Also, the impact of plasma repletion occurs within the first three hours of resuscitation in these patients. Finally, although repletion of plasma can improve outcomes, the effects in this study were less dramatic than earlier reports had suggested.


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Mitochondrial Function and Stored Platelet Concentrate Quality

The term “platelet storage lesions” (PSLs) refers to the different structural, functional and biochemical changes that happen to platelets during storage. PSLs can make platelets stored in platelet concentrates unsuitable for transfusion. Now that technologies can reduce the risk of transferring blood-borne pathogens through transfusion, PSLs have become the main barriers to lengthening the storage life of platelet concentrates.

A group of researchers from Osaka, Japan, theorized that one way to prevent PSLs is to maintain the function of mitochondria in platelets. Mitochondria are specialized structures that produce energy and help control the activities and even the survival of cells. Preserving the function of mitochondria in platelets could be important for maintaining the quality of platelets in stored platelet concentrates.

The researchers studied the effects of different compounds that cause mitochondria to malfunction during four days of storage that could affect the platelet quality.

Two of the compounds studied—2,4-Dinitrophenol and antimycin A—had no effect on platelet count, but they did increase the severity of the storage lesion. These compounds seemed to inhibit mitochondrial function almost completely and led to major deterioration in platelet quality. Both of these compounds inhibit an electron transport chain that helps mitochondria produce energy.

But the antioxidants that the researchers studied did not decrease the quality of stored platelet concentrates. N-acetylcysteine, for example, had no effect on platelets. Acetyl-L-carnitine prevented decreases in platelet pH and slightly reduced structural changes. This compound also slightly decreased glucose consumption rates and lactate production. Ascorbic acid had no major effects on glucose consumption, lactate production, pH declines, or changes in platelet structure. But platelets stored in medium containing ascorbic acid had better aggregation after seven days of storage, although these effects disappeared after another four days.

None of the compounds that the researchers studied had an effect on the expression of CD62P, an antigen, on the surface of platelets during storage. CD62P is a marker of platelet activation.

These results show that mitochondria play an important role in maintaining platelet concentrate quality during storage. Preserving mitochondrial functioning seems to have a positive effect on maintaining platelet quality in platelet concentrates, but this benefit is limited. New ways to inactivate blood-borne pathogens are needed that don’t lead to mitochondrial dysfunction and can therefore prevent PSLs.


Platelets and Blood Vessel Formation

Although the best-known functions of platelets are to stop bleeding or hemorrhage and control thrombosis, they also play roles in inflammation, blood vessel formation and wound healing. Researchers from Harvard Medical School recently explored one of these roles—how platelets regulate the growth of new blood vessels—by studying their release of angiogenesis stimulators and inhibitors. The researchers treated platelets with several platelet agonists and used assays to measure the platelets’ release of pro-angiogenic vascular endothelial growth factor (VEGF) and anti-angiogenic endostatin.

The results show that platelets can promote or inhibit blood vessel formation. Platelets can also release substances into the tumor microenvironment that can support blood vessel formation and tumor growth.

Activation of human platelets with adenosine diphosphate (ADP), a platelet agonist, released VEGF but not endostatin. In contrast, thromboxane A2 (TXA2), another platelet agonist, released endostatin but not VEGF. The releasate from ADP activation encouraged the migration and formation of capillary structures by human umbilical vein endothelial cells, whereas the TXA2 releasate had the opposite effect. When platelets were exposed to a breast cancer cell line, they secreted VEGF. Aspirin, a platelet inhibitor, prevented platelets from releasing VEGF or forming new blood vessels after they were exposed to ADP or breast cancer cells.

The effects of aspirin on platelets’ release of proteins that support blood vessel formation could help explain why aspirin and other platelet inhibitors can interfere with tumor growth and metastasis.

Platelet Additive Solution

In 2009, the U.S. Food and Drug Administration recently approved InterSol®, the first platelet additive solution (PAS) approved for replacing some of the plasma used to store platelets. Storing platelets in PAS can reduce the volume of plasma transfused with platelets and decrease the frequency and seriousness of plasma-associated transfusion reactions, such as TRALI. But platelets lose some of their in vitro properties when they are stored for five days in PAS that lacks bicarbonate or glucose and has less than 20% of residual plasma.

Researchers from the Holland Laboratory of the American Red Cross and Fenwal, Inc., studied platelets suspended in 5% plasma and a solution (PAS-5) that contains potassium, magnesium, calcium, bicarbonate, glucose, and other components of InterSol®. In the first study, the researchers prepared 5% plasma/95% PAS-5 platelets directly on a separator, without any other offline washing steps. Platelet concentration, mean platelet volume, pH, HCO3-, pCO2, pO2, lactate dehydrogenase and hypotonic shock response did not change during the seven days of storage. Glucose levels and morphology scores decreased and extracellular lactate dehydrogenase levels increased slightly.

For the second study, the investigators collected hyperconcentrated platelets and used an extra centrifugal step to generate paired platelet products in 100% plasma and 5% plasma/95% PAS-5. Mean platelet volume, pH, glucose, pO2, hypotonic shock response and morphology were similar in control and test platelets during seven days of storage. Glucose consumption and lactate production were lower in test than in control platelets. The PAS-5 platelets had less shape change and fewer test platelets with CD62P and CD63 activation markers than the platelets stored in 100% plasma.

The platelets in 5% plasma and PAS-5 produced from an apheresis device or manually had similar in-vitro storage features to platelets collected through apheresis or manually using standard techniques, but with much less plasma. This lower plasma level could decrease the number of plasma-associated transfusion reactions and the need for ABO typing of platelet concentrates for some recipients.

The study showed that apheresis platelets can be safely stored in 100% plasma or PAS-5 for at least seven days, probably because of their buffering capacity and low glycolysis rate. If issues with bacterial contamination could be resolved, the shelf life of platelets might be extended.

Radwanski K, Wagner SJ, Skripchenko A, Min K. In vitro variables of apheresis platelets are stably maintained for 7 days with 5% residual plasma in a glucose and bicarbonate salt solution, PAS-5. Transfusion. 2011 [Ahead of print].

High-Concentration Platelet Collection

The newest cell separators can yield platelet concentrations of up to 5 x 10⁶/μL platelets. Collecting high concentrations of platelets using apheresis devices and then re-suspending them in additive solution can dilute naturally occurring ABO-related hemolytic antibodies right away. The final titer of less than 1:64 could be particularly useful when health centers can’t test naturally occurring antibodies.

Hyperconcentrated group O apheresis platelet units resuspended in additive solution can be safely transfused into non-group O recipients, reducing discard rates and increasing the number of “universal” platelet units available for transfusion. High-concentration apheresis platelet units from donors who have been pregnant several times can be transfused with minimal TRALI risk.

Finally, autologous high-concentration platelet apheresis units collected and frozen during remission could decrease the number of platelet transfusions that patients with cancer need, avoid future volume decreases and reduce the total amount of dimethyl sulfoxide used.

Human Herpesvirus-8 in Transfused Blood

About 3–5% of U.S. blood donors test positive for human herpesvirus-8 (HHV-8), which can cause Kaposi’s sarcoma and certain rare kinds of B-cell cancers. Some patients have detectable levels of HHV-8 antibodies after blood transfusions during heart surgery and solid-organ transplantation. Recipients of blood products that are seropositive for HHV-8 have a much higher seroconversion rate than recipients of seronegative blood.

Researchers from the University of Pittsburgh treated peripheral blood mononuclear cells (PBMCs) from blood donors with reagents that stimulate lytic viral replication from cells latently infected with HHV-8 in an attempt to make the HHV-8 detectable. They then used highly sensitive PCR to search for HHV-8 genomes in the PBMCs. The goal was to find out whether just a few cells that had been latently infected with HHV-8 might be in circulating blood at a level that is too low for detection by PCR amplification.

The researchers found antibodies to HHV-8 in PBMCS from seven of 164 donors. But they did not detect HHV-8 genomes in any of their samples, including the samples from donors with HHV-8 antibodies. These results, along with data from earlier studies, show that HHV-8 cannot be induced from PBMCs. Also, HHV-8 genomes are extremely rare in the circulatory blood of U.S. donors and are basically undetectable. The available evidence does not support the theory that HHV-8 can be transmitted through blood transfusion.


Storage Age of Transfused Red Blood Cells

According to some studies, storing RBCs for too long before transfusing them into cardiac surgery patients can cause infections and other problems. Other studies suggest that these negative effects are associated with the volume of blood transfused, as an indicator of the severity of the patient’s underlying disease.

Researchers from St. James’s Hospital in Dublin, Ireland, studied whether the storage age of transfused RBCs or the number of RBC transfusions was responsible for complications and deaths in 1,153 patients who had undergone cardiac surgery at their institution.

Patients who received transfusions of RBCs that had been stored for more than 14 days (about the length of time it takes for storage lesions in blood to become noticeable) had higher rates of death, lengthy postoperative ventilation, new cases of renal failure needing dialysis, and infections than patients transfused with newer RBCs. Patients who received the older blood products also stayed in the intensive care unit (ICU) longer and spent more time on ventilation, on average, than those who received the newer blood products. But all of these differences disappeared when the researchers took potential confounding factors—especially the number of transfused RBCs—into account.

Patients who received transfusions of more RBC units spent longer, on average, in the ICU or on ventilation. Also, the more transfused RBC units a patient received, the more complications he or she was likely to experience.

These results show that transfusions with RBCs stored up to 35 days do not increase a patient’s chances of major complications or death after heart surgery. But getting more RBC transfusions, especially at least five RBC units, increases the chance of major complications and death after heart surgery.

Blood Transfusions in Older Adults

No national data are available on the characteristics of Americans who get blood transfusions. This information could be useful in determining the reasons for the major variation in the types and amount of blood components transfused into patients going through the same surgical procedures.

Researchers from the University of Michigan and University of Rochester analyzed data collected between 1992 and 2006 by the nationally representative Health and Retirement Study and Medicare. They wanted to find out how often patients aged 65 and older in the United States use blood and what types of older patients get a blood transfusion.

Slightly less than a third, or 31%, of older Americans had had at least one blood transfusion in the previous 10 years. Transfusion rates were highest (34%) in the South and lowest in the West (26%). Adults 85 years of age or older, men, African Americans, Mexican Americans, underweight adults, smokers, nondrinkers and those without a high school diploma were more likely to have had a transfusion during this time. Adults with a history of cancer, diabetes, end-stage renal disease, heart disease or lung disease also had a higher chance of receiving a transfusion. For 81% of older Americans, the transfusions happened in a hospital, and 46% of blood recipients received a transfusion during more than one visit.

In every region of the country, people with a low body-mass index (BMI) had the highest chance of being transfused. Transfusion use was lower in people with a normal BMI and slightly higher in those with a high BMI in every region, except the West.

Although African Americans had a higher transfusion rate than other racial and ethnic groups, their usage rates of medical services—such as critical care unit or emergency department services or major surgery—that tend to require transfusion were similar to the usage rates of other populations. Also, 70% of Mexican Americans who had major orthopedic surgery had a blood transfusion, compared to just 44% of other Hispanics and 53% of people who were not Hispanic.

Different personal characteristics and conditions did not explain the wide variation in transfusion use in different areas of the country. But one possible explanation for the higher transfusion rates in older African Americans and underweight adults is that these groups have higher rates of anemia.


In Memoriam:
Douglas Surgenor, Ph.D., 1918-2011

Dr. Douglas MacNevin Surgenor, former president of the American Red Cross Blood Services, Northeast Region, passed away in August after a life dedicated to learning and community service. Over his long career, Surgenor mentored young scientists and physicians in transfusion medicine, biochemistry, epidemiology and leadership.

He earned a master’s degree from the Massachusetts State College at Amherst and in 1946 a Ph.D. in chemistry from the Massachusetts Institute of Technology. Surgenor was part of a stellar research team that made ground-breaking discoveries related to protein chemistry, plasma fractionation and human serum albumin.

After 15 years on the faculty of Harvard Medical School he joined the faculty of the University of Buffalo, School of Medicine, which in 1962 became the SUNY Buffalo School of Medicine. Surgenor served on several hospital boards and executive committees including Millard Fillmore Hospital and Buffalo General Hospital.

Surgenor’s career brought him to the American Red Cross when he was named President of the American Red Cross Blood Services, Northeast Region. He also became a Visiting Professor in Pediatrics at the Harvard Medical School.

A lifetime of research and collaboration led Surgenor to author more than 150 scientific and medical papers on the chemistry of proteins and the formed elements of blood and related topics. He is survived by his wife, Lois, five children, fifteen grandchildren and two great grandchildren.
Making Blood Donations Safer for Teens

The number of Red Cross blood donations from high school-aged donors is growing quickly. Between 2006 and 2010, the proportion of donations from teens aged 16-18 increased, doubling from about 8% to 16%. A major reason for this increase is the recent growth in the number of states that permit teens as young as 16 to donate blood. Unfortunately, these younger blood donors face certain adverse side effects more than other donors. Specifically, they are much more likely to faint or feel lightheaded or dizzy post-donation. Although most young people recover quickly, in very rare instances these symptoms can have long-lasting, negative effects on their health. Even when donors recover completely from these unpleasant experiences, they might feel deterred from future blood donations.

In 2008 and 2009, the American Red Cross experimented with several new measures at its blood drives, including high school drives, to make donating blood safer for whole-blood donors younger than 19. For example, educational materials were distributed and dedicated supervisors were assigned to high school blood drives with more than 25 donors. American Red Cross regional blood centers also started requiring donors aged 16-18 years to have an estimated blood volume of at least 3.5 L based on height, weight and sex.

These strategies were found to improve young donor safety. For example, in blood drives that followed the new requirements, donors aged 16-19 had 18-33% fewer fainting or dizzy spells. Moreover, their complication rates were similar to rates in 19-year-old donors. The number of whole-blood collections from 16-year-olds increased between 2005 and 2009, showing that the new requirements didn’t have a negative impact on donations from teens.


Leukopheresis Treatment for Patients with Acute Myelogenous Leukemia

Some patients with acute myelogenous leukemia (AML) develop leukostasis, or an extremely high number of leukocytes clogging the vasculature. Leukostasis can damage the respiratory system, central nervous system, and renal function.

In a center in Sao Paulo, Brazil, two leukapheresis procedures decreased white blood cell counts in patients with leukostasis from 279.2 x 10^9/L to 105.9 x 10^9/L. Continuing the leukapheresis after a certain point did not seem to further decrease the number of circulating white blood cells. The study confirmed that leukostasis is almost never fully reversible, even with efficient leukoreduction.

**Role of Periodontitis in Heparin-Induced Thrombocytopenia**

Platelet factor 4 (PF4), a cytokine that helps control the immune system, can bind to heparin. PF4/heparin complexes induce antibodies that can cause heparin-induced thrombocytopenia and thrombosis. Patients who are treated with heparin for the first time can develop anti-PF4/heparin IgG antibodies within just four days of starting the heparin. This suggests that patients are pre-immunized to PF4/heparin complexes.

A German research group studied the role of periodontitis in causing this pre-immunization. Most adults have at least some periodontitis, and up to 20% have severe levels of this infection. Periodontitis can activate platelets, so it could trigger the release of PF4 that binds to bacteria. The data came from in vitro studies, a case-control study of 40 people with natural PF4/heparin antibodies, and a prospective, population-based study with 3,500 participants.

**Blood-borne Viruses in Health-Care Workers and Patients**

Hepatitis B virus, hepatitis C virus, and HIV account for most transmissions of blood-borne viruses in health-care workers. These viruses are common and the infections they cause are severe. But health-care workers can lower their risk of getting a blood-borne infection by washing their hands after they touch patients, using gloves and touching sharp instruments and devices as little as possible, as well as disposing of sharp instruments and devices in safe containers. All health-care workers who ever come into contact with patients, blood or other body fluids should be trained in safety precautions and have a hepatitis B virus vaccination.

If a health-care worker or a patient does have a percutaneous injury, the exposed skin should be washed with soap and water immediately. Source patients (patients who are potential sources of infection) who have been recently exposed to HIV or hepatitis C virus infection should be tested for acute infection. Periodontitis was strongly associated with anti-PF4/heparin antibodies in the case-control and population-based studies, regardless of whether participants had markers of inflammation.

Pre-immunization to complexes of PF4 and bacteria by periodontitis and other infections probably explains why many people have natural anti-PF4/heparin antibodies. These antibodies might also be the reason why many people develop heparin-induced thrombocytopenia so quickly after starting heparin treatment for the first time.


The first symptoms of transfusion-related acute lung injury (TRALI)—the most common cause of serious transfusion side effects and deaths—usually start within just a few minutes of a transfusion with blood products containing plasma. Typical symptoms include severe breathing problems, fever, cough, chills and low blood pressure. TRALI happens more often after transfusions with plasma and, to a lesser extent, platelet concentrates than after packed red blood cell (RBC) transfusions.

Immune, or antibody-induced, TRALI occurs when transfused antibodies from the donor activate the recipient’s neutrophil granulocytes. The recipient’s neutrophils release substances (proteolytic enzymes and reactive oxygen species) that increase the permeability of the endothelium that lines the blood vessels of lungs. As a result, fluid enters the lungs.

The leukocyte-reactive antibodies against human leukocyte antigens (HLAs) and human neutrophil antigens (HNAs) that can cause immune TRALI are found most frequently in plasma of women who have been pregnant. Antibodies to HLA and HNA-3a antigens in fresh frozen plasma and platelet concentrates, in particular, can cause severe or even fatal TRALI in critically ill patients because of the high levels of these antibodies react with the patient’s leukocyte antigens. Fortunately, leukocyte-reduced blood components have made TRALI from leukocyte-reactive antibodies in patients’ blood very rare.

Blood centers can reduce the risk of TRALI: approaches include testing plasma products for leukocyte antibodies and reducing the transfusion of blood products from female donors. However, centers that avoid blood products from female donors seriously restrict the number of products available for transfusion. One way to get around this problem is to test women who have been pregnant for HLA class I, class II and HNA antibodies and use products only from women with negative test results.

Demographic Changes and the Future Blood Supply

Birth rates in the eastern German state of Mecklenburg-Pomerania have dropped quickly and dramatically, by 50%, since 1989. The migration of young people from this region to western Germany has decreased the number of young adults in the area even further. These demographic changes, coupled with the growing number of older residents, will probably lead to major blood shortages in the coming decades.

A model developed by a group of German researchers predicts that demand for RBC units in the 34 Mecklenburg-Pomerania hospitals will increase by 25% by 2020 because the number of people 65 or older will increase by 26.4%. At the same time, the potential donor population (those aged 18–68 years) will drop by 16.1%. Because the number of young adults, who are a major source of blood donations, is decreasing so quickly, the number of donations will decrease by 27%. Mecklenburg-Pomerania hospitals will probably face a shortfall of 56,000 RBC units for hospital inpatients, or 47% of available units, in 2020.

In an editorial, Richard Benjamin of the American Red Cross and Barbie Whitaker of AABB point out that this study should serve as a warning to U.S. blood centers of the likely impact of current demographic changes in the United States. The probable U.S. donor population (those aged 16–64) will only grow by about 5.2% by 2020, whereas the number of people aged 65 and older (who could need 55–60% of transfused blood) will increase by 36.2%. Absolute RBC needs could rise by 24.3% in the next 10 years and transfusion demand will exceed collections in the United States by 2020. The authors warn that the United States is not well positioned to respond to these changes.


Impact of Blood Donor Age and Gender on Fresh Frozen Plasma Quality

With the aging of the European and North American populations and the corresponding increase in demand for blood transfusion, it is time to rethink the upper age limits for blood donors. A group of German researchers conducted a study to find out whether the changes in the hemostatic system that happen with age could affect the quality of therapeutic fresh frozen plasma from elderly donors. Their study included three cohorts of whole-blood donors aged 50–52, 66–68, and 69–71 years.

Partial thromboplastin time dropped slightly with age but differences between the cohorts were very small. Mean fibrinogen concentrations increased slightly with age. International normalized ratio values in the oldest male donors, but not the oldest female donors, were much higher than in the two younger cohorts. Factor VIII was much more active in the plasma of the two older cohorts, especially in plasma from male donors, than in the plasma of the youngest group. Coagulation factor V, antithrombin and plasminogen levels were similar in the three cohorts.

The study concludes that the normal ranges of older donors for all of the parameters that the authors examined were only marginally different from those of younger donors. Therefore, blood from older donors could be useful for making therapeutic or fractionated plasma.

The American Red Cross encourages blood bank staff and physicians at the hospitals it serves to take advantage of the SUCCESS™ continuing education program. The program features courses on a variety of topics including antibody detection and identification, transfusion practices and case studies. New courses are added regularly. Sample courses include:

**Transfusion Management of the IgA-Deficient Patient**
The laboratory evaluation and transfusion management of IgA-deficient individuals, who may or may not have a history of prior anaphylaxis, is reviewed. American Rare Donor Program blood product release criteria and blood product selection are also discussed.

**Type O Negative Red Blood Cell Utilization: Preserving this Rare and Lifesaving Resource**
Type O negative blood – every hospital wants a supply on its shelves. But with a prevalence of only seven percent within the population, this valuable resource must be managed with care. Ways to best use lifesaving type O negative red blood cells are discussed.

**Case Study: ABO Discrepancy & Positive Antibody Screen**
This course is an interactive case study wherein the user directs the work-up. Abnormal pre-transfusion test results, including an ABO discrepancy and positive antibody screen require investigation. A discussion of ABO discrepancy resolution and literature bearing upon the cause of this particular discrepancy is reviewed.

Visit [success.redcross.org](http://success.redcross.org) to learn more.

**Publications Corner**
Recent publications by American Red Cross scientists and physicians:


- **Moroff GM, Kurtz J, Seetharaman S and Wagner SJ.** Storing apheresis platelets without agitation with simulated shipping conditions during two separate periods: immediately after collection and subsequently between Day 2 and Day 3. *Transfusion* 51: 636–642.
