Natural born killers!

First described in 1975, natural killer (NK) cells have demonstrated the capacity to kill malignant cells in the human body spontaneously, without requiring prior sensitization. A decade-long study in Japan, published in the Lancet in 2000, showed that men and women who demonstrated higher levels than average of natural cytotoxicity by their peripheral blood NK cells had a significantly decreased risk of developing cancer. Since then, much activity has been devoted to the identification, kinetics, function and clinical utility of these cells, including at the University of Minnesota, from whence came several articles in a recent edition of Transfusion.

NK cells are a mixed group of immune cells that share several properties. They appear as large lymphocytes with abundant granular cytoplasm containing medium-sized azurophilic (bluish) granules. They are identified more specifically by cell surface markers, detected by flow cytometry and immunohistochemistry. They are commonly present in blood, as high as 30% of circulating lymphocytes and up to 15% of the cells in hematopoietic and lymphoid organs, such as the spleen and bone marrow. There are different subsets with different distributions, based on the cell surface markers they contain. Those with low “resting” cytotoxic capacity are generally found in more secondary lymphoid tissues and may be precursors to the more cytotoxic NK cells which are in circulation. Some NK cell subsets can express an Fc immunoglobulin G receptor that allows them to engage in antibody-dependent cellular toxicity. Other surface markers and other subsets of NK cells are being discovered. Just about all of the methods for preparing NK cells for treatment or research involve collections by peripheral blood apheresis. CD-19 positive B cells and T cells in the product need to be depleted, and methods to promote NK cell growth in the collected product are being examined. A number of methods designed to “enrich” the number of NK cells in the infusion product are being used, including the use of IL-2 to stimulate NK cell expansion in culture bags.

One of the first functional discoveries was that NK cells can recognize and lyse cells which lack a major histocompatibility complex (MHC) without any prior sensitization. NK cells themselves lack antigen-specific cell surface receptors. As they are maturing, though, they acquire killing functions based on inhibitory signaling (or a lack of it) of their naturally occurring killer functions. They can attack tumor cells, but they don’t cause graft vs. host disease, and protect against it to some degree. Although NK cells can be educated, or programmed, to kill certain cells under certain conditions, the process through which this occurs is not at all clear, and is under intensive investigation. Nonetheless, it is known that, for example, under certain conditions of hematopoietic stem cell transplantation, NK cells can have a major graft vs. leukemia effect; however, they do not exhibit a graft vs. host effect.

In the news

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Cover photo: Adenovirus, a cause of the common cold.
A number of trials have occurred and have been published using NK therapeutic products for a range of malignancies; various leukemias, melanoma, and carcinomas have been looked at in this way, using both autologous and allogeneic-derived NK cells. Almost all of the studies have occurred in patients who have had autologous hematopoietic stem cell transplantation. But, very importantly, NK cell therapy without prior stem cell transplantation is also being looked at, and the ability to enrich healthy donor cell products up to a level of 40% NK cells in a non-transplant setting has been shown to be effective. More than half the patients (with AML) had detectable donor cell engraftment and several entered complete remission following chemotherapy and such an NK cell infusion.

NK cell infusions are generally well tolerated, with febrile reactions being the most common side effect. Although some severe episodes of nausea, dizziness, rash, hypotension and other effects have been noted, no occurrences of anaphylaxis have yet been reported. In this same issue of Transfusion, there is a report of two cases of severe hemolysis that occurred after infusion of NK cell-enriched products that had a minor ABO mismatch. These two patients received significant numbers of B cells from Group O donors, leading to the development of red cell antibodies.

There are not a lot of centers that have the capacity to collect, store, enrich, activate and transplant these cells, and there are a variety of methods that have been employed for each of those steps. Resources and expertise in such advanced techniques will continue to be developed, certainly.


Killer potential

Just think, two articles in the same issue about killers, and both are examples of useful tools for blood bankers, and of particular benefit to patients. This article is presented as a way to introduce you to a much larger article in Vox Sanguinis, a critical review of pathogen inactivation (PI) in blood components. PI was developed by scientists at the New York Blood Center in 1991 and the material originally published in Blood in 1992 in an article titled: “Solvent-detergent plasma: a virus-inactivated substitute for fresh frozen plasma.”

Since then, additional PI techniques for the treatment of platelets, plasma, red cells and even whole blood have been described; however, not all products are licensed for use, and especially not in the United States. Most of the technology is designed to target nucleic acids, thus killing pathogens and inactivating white blood cells, the latter generally making the product incapable of causing graft vs. host disease and febrile reactions, and perhaps reducing patient alloimmunization. Various combinations of treatment with chemicals and with photo-inactivation are used in various products, and some of these also have harmful effects on the function of various cells or proteins, somewhat limiting their effectiveness. A number of products are in use for platelets (in Europe) and plasma, whereas trials with whole blood or red cells are currently in

Continues on next page
the planning stages. The Council of Europe has “CE marked,” or approved, seven systems for use in plasma and platelets, including small “minipools” of FFP. Licensed products have been shown to effectively reduce significant numbers of bacteria and parasites (such as malaria, *T. cruzi*, *Babesia* spp.) as well.

Studies on the efficacy of these methods, chemicals with photo inactivation, the generation of reactive oxygen radicals and solvent-detergent treatment, have been shown to be effective on viruses such as HIV, HCV and HBV, but do not adequately reduce non-enveloped viruses—HAV, parvovirus B19 and others—when they are present in large amounts. Solvent-detergent methods, of course, can’t be used for cellular products. The author, a scientist at Edinburgh University, reviews the literature dealing with all of these methods, summarizes their efficacy and outlines some future directions. He concludes that, for the available technologies that inactivate plasma and platelets, evidence for PI is quite good, except for the non-enveloped variety noted above. The European and North American hemovigilance data suggest that loss of potency of these components is not a major problem, nor have neo-antigen formation or other adverse effects been noted for currently licensed products. After all, killing “bad guys” is one thing, but if the products kill the “good guys” as well—platelets, AHF, vWF and so on—they aren’t of much value.

One startling conclusion that he draws, which had never occurred to some of us before, is that perhaps the availability of PI methods could actually replace current testing for blood-borne pathogens, those we know of and those of which we don’t yet know. Given the licensing considerations for such use, especially here in the United States, this seems not very likely in the near future, and perhaps it is simply a futuristic goal. A major determinant of the extent of use of these products will also depend on cost-effectiveness, especially when evaluating their use in terms of QALYs—quality-adjusted life years—and the expenses required to achieve them.

**Bugs in your platelets: the more the merrier?**

Well, it turns out it’s the platelets we’re talking about, not the bugs. Estimates are that platelets collected by apheresis have a predictable rate of bacterial contamination, roughly 1 per 1,000. Bacterial testing at 3, 4 and 5 days of apheresis platelets that are negative at 24 hours has demonstrated residual rates of contamination of about 500 per million, or roughly 0.5 per 1,000. This, in spite of efforts to reduce such contamination, such as improving the skin prep, diverting the first 30–50 ml and performing culture-based quality control on all apheresis platelets, holding them for 24 hours. Thus, it seems we are only interdicting half of the potential danger from platelet contamination with bacteria.

Most centers inoculate the bacterial detection system with a fixed volume, from 4–8 ml. At the institution of one of the authors, more than half of the apheresis platelets are collected as double or triple procedures, which is more or less true around the country.

The article reasons that the greater the volume sampled from the collection, the greater the likelihood of detecting low levels of bacteria. It is generally believed that the presence of 5 colony-forming units (CFUs) per bag is the lower limit of bacterial detection at 24 hours, and that 24 hour false negatives are the cause of residual contamination and subsequent reactions in patients. The authors used a Poisson distribution model to determine if larger samples would increase detection at levels of contamination that seemed realistic (5–30 CFUs per bag). They compared (Model 1) fixed inoculations of 8 ml of the collection, whether a single, double or triple, equaling 3.2, 1.6 or 1.2 % of the collection volume, respectively, with (Model 2) 3.8% collections of the total volume for single, double and triple collections; these total volumes are, on average, 250 (9.5 ml sample), 500 (19 ml sample) and 750 ml (28.5 ml sample), respectively. This model would require the

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**Prowse, CV.** Component pathogen inactivation: a critical review. *Vox Sanguinis* 2013;104:183–199.
use of multiple culture bottles, since most of the currently used incubation systems have a maximum volume of 10 ml. The model also assumed that the system could detect the presence of 1 viable micro-organism in the sample.

They applied this model to 12 months worth of platelet collections at Blood Systems, Inc. (BSI), during which there were nearly 30,000 single apheresis procedures (35%), 45,000 doubles (53%) and 10,000 triple collections (12%). Perhaps more importantly, of the platelets issued, 26% were from single procedures, 59% from double procedures and 21% from triples, meaning that more large volume collections were utilized for transfusion.

As one might expect, the modeling shows that if there are 30 CFUs per bag, Model 2 increases the detection rate from 41% to 68%, and increases it from 9% to 17% in Model 1 at CFU levels of 5 per bag. These numbers fit well with actual contamination experience at BSI. Since it is the lower levels of contamination that currently evade detection using the standard inoculum of 8 ml, no matter what the collection volume, this increased sampling level might double the number of contaminated units detected. Given the wide use of apheresis platelets, especially for immuno-compromised and critically ill people, this would seem to be a potentially useful way to reduce serious problems at very little increase in costs. Look for further reports from other centers that are using larger inoculae.


Did anyone tell the cows?

Before and during the Great Depression, which followed the stock market crash of October 1929, farmers in the northern United States and Canada had seen outbreaks of fatal bleeding in their cattle herds. The problem was eventually linked to moldy silage, and specifically moldy clover in that silage. That there was a hemorrhagic factor in the silage had been noted by scientists in North Dakota. But it wasn’t until 1940 that a farmer in southern Wisconsin took a load of moldy silage into the laboratory of Dr. Karl Link, a biochemist at the University of Wisconsin’s College of Agriculture, that the causative agent was discovered. Dr. Link successfully synthesized the compound in 1948 and called it “warfarin”—WARF standing for the Wisconsin Alumni Research Foundation. It was first licensed (and is still used) as a rodenticide and then became licensed for human use as an anticoagulant in 1954. As such, it has contributed hugely to the funding of basic research at that large Midwestern university. It is the most widely used anticoagulant in the world.

It is used as prophylaxis to prevent thromboembolism in patients with artificial heart valves, atrial fibrillation and those with deep venous thrombosis. It works by inhibition of the coagulation factors that depend on Vitamin K for their synthesis: Factors II, VII, IX and X. Thus, Vitamin K is its antagonist, and is used to reverse the effects of warfarin (licensed as coumadin). About 31 million prescriptions for it were issued in 2004; the number has likely not changed much recently, although direct inhibitors of thrombin are now seeing increased use in some circumstances.

The problem with warfarin has always been its narrow therapeutic index; its effects are inhibited by certain foods and drugs and increased by others. Using the INR, we like to keep the range between 2.0 and 3.0, recognizing that over a week’s time the actual measurement likely varies both above and
Treating patients with hemophilia, and teaching them how to treat themselves, was very difficult (and still is, in some circumstances) until the mid-1960s when Dr. Judith Pool and colleagues developed cryoprecipitate, a cold-insoluble protein fraction removed from fresh frozen plasma (FFP) that contained a concentration of Factor VIII, fibrinogen and von Willebrand factor. This was followed, years later, by the development of Factor VIII concentrates. But significant numbers of hemophilia patients developed antibodies to Factor VIII, some of them “strong” enough, or in high titer, such that the concentrates were no longer effective. Antibodies to Factor IX, Christmas disease or hemophilia B were also known, with similar resistance to therapy. In more recent years, recombinant DNA-produced activated Factor VIIa was developed. As you will recall, this activated form of Factor VII acts directly by converting prothrombin to thrombin, in the presence of tissue thromboplastin, thus bypassing the so-called “intrinsic pathway” of coagulation in which normal levels of Factors VIII and IX are required to achieve the same result. As you can imagine, this was a great breakthrough for such patients.

Dr. Joe Sweeney and colleagues (see reference) looked at actual clinical practice in their tertiary care university hospital in Rhode Island, examining 135 patients meeting criteria for analysis who received Vitamin K, 81 by mouth and 54 by IV. These were selected from a total of 1,276 Vitamin K dose-episodes. All occurred within a 14-month period following a significant decline in FFP use for coumadin reversal resulting from an educational effort. Pre-K administration INRs were similar in the two groups. Post-administration INRs were significantly shorter in the IV group, as were the times to improvement. There were no anaphylactic reactions.

There have been a number of previous studies along these lines, which the authors review. They have added much information, however. They noted considerable delay in the reporting of abnormal INRs from the lab to the floor and lowered the threshold and shortened the time lag. Their patients received a large variety of doses and they provide data which conclude that large doses of Vitamin K, in the 10 mg range, are no better than smaller ones. They can confidently predict that an IV dose of 1–5 mg can correct an INR to 2 in as early as 6–12 hours in a majority of patients, and close to 1.5 in 24 hours, and that it does so more reliably and in roughly half the time compared to an oral dose. They point out that anaphylaxis to IV Vitamin K as well as to plasma transfusion is rare. Both occur at roughly similar rates, and in life- or limb-threatening situations, both should be administered for sustained warfarin reversal.


Recombinant factor VIIa off-label: more uses?

Treating patients with hemophilia, and teaching them how to treat themselves, was very difficult (and still is, in some circumstances) until the mid-1960s when Dr. Judith Pool and colleagues developed cryoprecipitate, a cold-insoluble protein fraction removed from fresh frozen plasma (FFP) that contained a concentration of Factor VIII, fibrinogen and von Willebrand factor. This was followed, years later, by the development of Factor VIII concentrates. But significant numbers of hemophilia patients developed antibodies to Factor VIII, some of them “strong” enough, or in high titer, such that the concentrates were no longer effective. Antibodies to Factor IX, Christmas disease or hemophilia B were also known, with similar resistance to therapy. In more recent years, recombinant DNA-produced activated Factor VIIa was developed. As you will recall, this activated form of Factor VII acts directly by converting prothrombin to thrombin, in the presence of tissue thromboplastin, thus bypassing the so-called “intrinsic pathway” of coagulation in which normal levels of Factors VIII and IX are required to achieve the same result. As you can imagine, this was a great breakthrough for such patients.
Inevitably, and despite the initial enormous cost of the drug, it was used for the treatment of severe, refractory bleeding in numerous situations, and found some utility in patients with severe trauma, neurosurgery and cardiac surgery. Certainly it has been greatly overused as a “last resort” in some patients, and it’s not hard to understand why.

Lung transplantation is considered the gold standard for treatment of patients with end-stage lung disease, say the authors of this paper from Brisbane, Australia (see reference). More than 25,000 have been performed around the world. Routinely, these patients have significant perioperative bleeding; bleeding in the operating room, particularly in the more extreme instances of intervention, is generally identified as surgical bleeding (reparable with sutures or cautery) or coagulopathy (lack of adequate coagulation factors). Excessive intraoperative bleeding leads to transfusion of large volumes of blood, which contributes to development of increased morbidity, multi-organ failure, acute respiratory distress syndrome (ARDS), transfusion-related acute lung injury (TRALI), immune modulation, fluid overload and more.

This retrospective observational study looked at all patients having a single or double lung transplant over a 5-year period between 2005–2011. Of the 95 patients, 15 received Recombinant Factor VIIa (rVIIa). Examination of the records of these patients showed that—unlike patients not receiving rVIIa—they had no increases in mechanical ventilation time, time in the ICU or in the hospital stay. In addition, they documented a decrease in requirements for red cells, FFP and platelets compared to the norm. There were no clearly defined thromboembolic events, either, which have been noted in some other rVIIa studies.

The authors attempt to show that rVIIa was used only after surgical repair was completed and non-RBC support (FFP, cryo, platelets, DDAVP and antifibrinolytics) had failed. Since this study was retrospective, it is hard to know exactly what the criteria for treatment were at the time of intervention. Some rFVIIa was given after heparin reversal and before sternal closure (5 patients), some in the ICU and after re-exploration for bleeding (10 patients). The authors also point out their use of non-parametric statistical analysis, since most of the variables didn’t fit with normal distributions. The 95 patients had several types of lung transplant. Eight of the 15 given rFVIIa had previous pleurodesis (obliteration of the pleural space).

Thus, the study adds information regarding the use of rVIIa, but is not conclusive about its indications. Randomized studies are exceedingly difficult in such situations, but more data are needed regarding timing, dosage and patient selection for the use of this product off-label.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a serious red cell enzyme deficiency that affects approximately 400 million people worldwide, the authors tell us. Red cells (RBCs) so affected are very sensitive to oxidative stress, and hemolysis can result from exposure to such stress, in the form of infection, some drugs and ingesting fava beans. The process of delivering O2 to the tissues normally results in the oxidation of heme iron from the 2+ (ferrous) state to the 3+ (ferric) state. This is called methemoglobin (MHb), instead of oxyhemoglobin. In that condition, hemoglobin cannot bind O2, so our red cells contain another system of reactions centered around MHb reductase to return the molecule to its O2-carrying form. Normally, about 1% of our Hgb in circulation is in the form of MHb. G6PD is a critical enzyme in this activity. Continued oxidative stress on MHb leads to formation of insoluble Hgb products that we call sulfhemoglobin, so named since sulfa compounds can produce these in vitro (or in vivo). Red cells deficient in the enzyme G6PD are much more susceptible to oxidative stress and hemolysis, since the insoluble Hgb forms Heinz bodies (precipitated, oxidized Hgb) which leads to RBC destruction by a number of mechanisms, both intravascular and extravascular.

G6PD deficiency is most commonly found in people from sub-Saharan Africa, the Mediterranean and southeast Asia, parts of the world in which we also see more prevalence of sickle cell anemia, and beta and alpha thalassemia. Unlike those disorders, G6PD deficiency is X-linked, making it much more common in men than in women. It is known that storage of G6PD-deficient RBCs further depletes that enzyme, making them very susceptible to oxidative hemolysis, particularly if the patient is taking anti-malarials, sulfas or has other oxidative stresses, such as infection. Blood donors are not routinely screened for G6PD deficiency. The authors looked at blood in their large, hospital blood bank in New York City.

First, they determined that screening red cells from an attached segment was a reliable predictor of the G6PD status of the RBCs in the unit itself. They looked at 301 randomly selected units and defined G6PD deficient RBCs as enzyme activity less than 60% of the normal established mean. G6PD levels in the normal units did not decrease over the storage time of 42 days. The frequency of negative units in their sample was 0.3% (roughly 1 in 300 units), with a wide and low confidence interval, given the low frequency and small sample size. Given that about 15 million units of RBCs are transfused each year in the United States, this suggests that about 45,000 units of deficient RBCs are administered.

However, when the authors examined 73 units of RBCs that had been screened and found to be D+C-E-, 9 of the units, or 12.3%, were found to be deficient in G6PD, with a high confidence interval. Since these units are preferentially allocated to be given to patients with sickle cell disease, so as to decrease or slow rates of alloimmunization, these deficient units could conceivably be given more frequently to patients already at risk for hemolysis, infection and shortened RBC survival. Interestingly, the authors cite a reference from the military in which G6PD deficiency was found in 0.3% of Caucasians and 12.2% of African-American personnel.

Since no studies have been performed, or the effects of transfusing these deficient units into patients with sickle cell disease evaluated, the authors are pursuing this line of inquiry. They suggest that since normal G6PD levels are well preserved in RBC unit segments, units specially screened for patients with sickle cell disease could easily be tested prior to transfusion.

Neonatal alloimmune thrombocytopenia

Dr. William Harrington, in 1953, described two cases of newborns with severe thrombocytopenia whose mothers both had normal platelet counts. In 1951, two years prior to this, Dr. Harrington and several colleagues achieved some notoriety for demonstrating that some cases of severe thrombocytopenia (in adults) were due to the presence of a platelet antibody in the plasma. They did so by injecting themselves with the plasma of patients with acquired thrombocytopenia. The results were dramatic, and quite severe! These cases became known as ITP—immune thrombocytopenia. When the antibodies arose de novo in previously normal people, it was called AITP—autoimmune thrombocytopenia. In these cases in newborn infants, it was shown later by Shulman and others that the cause was an antibody formed by the mother in response to a platelet-specific antigen present in the fetus (and father) but not the mother. Since this was an alloantibody, not an autoantibody, the condition was called neonatal alloimmune thrombocytopenia, or NAIT, for our purposes. It is generally due to an IgG antibody directed against a paternal platelet antigen lacking in the mother.

Dr. Shulman’s work demonstrated that antibodies directed against a platelet antigen called PlA1 were the cause of platelet destruction, and the antigen was later found to be identical to one called Zw4 by Dutch workers. This is now called “HPA-1a” for human platelet antigen 1a, and other human platelet antigens have been found that cause NAIT. Many cases of NAIT are relatively mild, but it is still a very significant cause of neonatal morbidity, and mortality, and is the most common cause of intracranial bleeding in newborns who are fully mature. Although numerous antigens have been identified in the system, anti-PlA1 (HPA-1a) remains the most common antibody associated with NAIT. Surveys have shown that in women who lack HPA-1a, as many as 1 in 2,000 infants had neonatal thrombocytopenia caused by maternal antibodies. Most cases of NAIT occur not with the first pregnancy, but later, leading one to assume immunization occurs most frequently at the time of delivery.

The authors of this review article (see citation below) have provided some excellent tables and figures that illustrate and list the variety of antigens on platelets that have been associated with cases of NAIT. A visit to the article is well worth the time. Although HPA-1a is the most common, there are many other antigens that have been identified, all carried on 4 different platelet membrane glycoproteins (GPs), including GPIa-GPIIa, GPIIb-GPIIIa, GPIIb-IIIb, and CD109. Platelet typing is now carried out best by DNA methods using fluorescent-labeled probes, which will identify the most commonly involved antigens in NAIT: HPA-1 through HPA-6 groups, and HPA-9 and -15 systems. NAIT is not, however, identifiable as the cause of most cases of thrombocytopenia in infants. Some are due to sepsis, to drugs present in the maternal circulation near the time of birth, and to maternal auto-antibodies that are not detectable in laboratory assays. Some are believed to be due to Class I HLA antibodies.

Some larger centers have donors who are known to be negative for, say, HPA-1a, who can provide platelet support via apheresis. The infant’s mother may also be a source of compatible platelets, but the product must be thoroughly washed to remove antibody-containing maternal plasma. Milder cases are self-limited, and antibody levels fall in all cases over a few weeks. Random ABO-compatible platelets may also be used, which will provide at least a temporary boost, but all transfusions should be CMV-negative and irradiated, and generally volume-reduced. IVIG has also been of value.

Not everyone realizes, though women do much more than men, that our small children are not just tiny versions of ourselves. This is particularly true of infants. Their digestive systems, immune systems, neurological systems—all are far from being functional in a “mature” way. (Of course, being an adult doesn’t guarantee maturity, either!) Well, with regard to hematologic parameters, blood volume and transfusion requirements, they also differ markedly. Whereas in adult men and women, estimated total blood volume (TBV) averages about 6.5% (65 ml/kg) and 6.0% (60 ml/kg), in newborns it is 8–9% (80–90 ml/kg), more in preemies, and even older than a few months it is still about 10 ml/kg more than in adults.

In view of the many changes, hopefully advances, we have witnessed over the last few years in massive transfusion (MT) in adult patients following large-volume blood loss from trauma or surgery, Dr. Diab and colleagues, including Dr. Naomi Luban, from the Children’s National Medical Center in Washington, D.C., have recently published an excellent review of these issues (see reference). Data on the management of MT in children and neonates is pretty much unknown, and various institutions have developed their own protocols without the benefit of large-scale controlled trials now being seen for adults. A definition of MT in children isn’t established, and adult definitions are just not applicable. The authors go over the newer concepts of massive bleeding and MT, in an attempt to lay out basic principles supported by a review of available data. They suggest that pediatric MT in children applies for >50% TBV replacement in 3 hours, >100% TBV in 24 hours or less, or >10% TBV per minute. When an infant weighs but a few kilos, it’s easy to see how delicate this balance can get.

The authors review the unique parameters of MT and the pathophysiologic considerations of hemostasis in infants, particularly, since they lack the production reserves of more mature humans. This means that the hemostatic effects of MT are much more profound and the risks of bleeding greater. As with adults, there is an early-presenting coagulopathy associated with trauma in children manifest by systemic anticoagulation and hyperfibrinolysis which is present even before resuscitation and transfusion are begun. They continue with a discussion of the effects of hemodilution, volume replacement, lack of some clotting factors in transfused blood products, and the delicate balancing act to approach normal coagulation that is required. Deficiencies of fibrinogen and of Factor XIII, of antifibrinolytic proteins and of platelet-derived plasminogen activator inhibitor-1, are all reduced. The result, as in adults, is that fibrinolytic activity remains intact, even enhanced, aggravating an already difficult dilutional situation. Problems with citrate overload (acidosis and hypocalcemia) may develop, further diminishing coagulation capacity.

The review continues with critical discussions of current in-use MT practices and protocols, including some of their own versions at Children’s National Medical Center. They review transfusion approaches to extracorporeal membrane oxygenation (ECMO) and cardiac surgery in small infants and neonatal exchange transfusion, with discussion of anticoagulants and the age and sickle cell status of RBCs. The article then proceeds to outline specific emerging strategies for MT, especially for specific coagulation abnormalities based on sophisticated point-of-care coagulation testing, not available in many hospitals except for major medical centers, including diagnostic thromboelastography. A discussion of “novel adjuvant” therapy follows; agents such as desmopressin, antifibrinolytics and coagulation concentrates. They conclude this really excellent review pointing out how little we really know about optimal MT strategies in children and neonates and the need for MT protocols to be evaluated in large, prospective multicenter studies, as is just starting to happen in adult care in the past few years. Readers of this are urged to review the entire article.

In the western, and primarily northern, world, we are actively defining improved methods to decrease blood transfusion, to reduce unnecessary risk and to help control the ever-increasing costs of health care. We sometimes forget how fortunate we are, in a way, to have such problems with which to grapple. Early accounts of blood transfusion—some hypothetical, not actual—centered around restoring vigor or vitality by infusing blood from a bull or other animal into an ailing or aged man. It wasn’t until the studies of Vesalius, William Harvey and Marcello Marpighi in the 16th and early 17th century that blood circulation was actually understood. In the late 17th and 18th centuries, blood transfusion fell into disrepute, due to some major failures leading to hemolysis and death. Finally, in the 19th century, Dr. James Blundell, also credited as the father of modern obstetrics, successfully transfused human blood into women dying from severe post-partum hemorrhage. Many were saved; however, it wasn’t until the turn of the century and Karl Landsteiner’s description of the ABO blood group systems that the practice spread. World War I and subsequent events led us into the more modern day.

A recent article in Vox Sanguinis reminds us of the critical importance of transfusion in the less-developed world, where lack of adequate transfusion services makes difficult obstetrical cases even more so. The authors, from four institutions in the Netherlands, developed a model to quantify the clinical and human benefit of red cell transfusion in obstetric care. They took data from a nationwide study on severe maternal morbidity, including obstetrical hemorrhage that required 4 or more units of blood, and calculated the maternal morbidity rate (MMR). They then created a hypothetical group of similar women for whom transfusion was not available. They calculated the observed MMR and relative risk in the Dutch group, and then applied the same criteria to the hypothetical group of women. Part of the study involved a survey of experts in major obstetrical hemorrhage to choose a critical number of red cell units at which a woman with such a problem would likely die without blood transfusion.

Obstetrical hemorrhage is the leading cause of maternal mortality worldwide and accounts for 25% of all maternal deaths worldwide, an estimated 140,000. This number is much higher in less developed areas. This problem is also the most common cause of maternal morbidity outside of death. During the course of the study, there were 371,021 maternities in the Netherlands. All 98 obstetrical facilities in the country contributed data. A total of 1,567 women had major obstetrical hemorrhage requiring 4 or more units of blood. Eight women died from hemorrhage, 7 of them receiving 9 or more units of blood, the other, a Jehovah’s Witness, received none. There were 270 women who received 9 or more units of blood and lived because the blood was available, and transfused successfully. Thus, the observed MMR was 13 per 100,000 deliveries in the group. In the hypothetical group, by extrapolation of these figures, the death rate would have been 87 per 100,000, 6.5 times more common.

Given the cutoff of 9 units, and knowing something of the transfusion capacities in some parts of the world, it is clear that in some areas the rates would be very much higher. It is generally assumed that we all know how important such blood transfusions are for the health—and survival—of women in resource-poor settings, and how important the presence of these women is to the health and welfare of their families and community. But, all too often we forget, and there is still much to be done.

The American Red Cross is committed to working with its valued hospital partners on optimizing use of our blood supply so as to best serve patients in need. With that aim, a series of continuing education courses in patient blood management is currently being developed. The first of these courses are now available online at success.redcross.org.

**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 7 percent within the population, this valuable resource must be managed with care.

**Overuse of Blood Transfusions and Growing Evidence in Favor of Patient-Centered Blood Management**
This program describes the growth of, and drivers for, Patient-Centered Blood Management which promotes the transfusion of fewer blood products for more appropriate, evidence-based indications, promising better patient outcomes and more efficient use of health care resources.

**Red Cell Transfusion Triggers**
Hospital Transfusion Committees should establish consensus transfusion guidelines using evidence-based triggers and educate their physicians regarding implementation of these practices.

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


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**Remember these Websites**

- *Immunohematology Journal*
  redcross.org/immunohematology
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  success.redcross.org

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