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An approach to prevent the severe adverse events associated with transfusion of FDA-approved blood products

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ABSTRACT

There have been several retrospective studies reporting severe adverse events of mortality and morbidity associated with blood transfusions. Mortality and morbidity associated with posttransfusion infection, transfusion related acute lung injury (TRALI), and systemic inflammatory response syndrome (SIRS) have been reported in patients undergoing cardiac surgery, after massive transfusions for severe traumatic injuries, and after transfusions for elective and emergency indications. After 35 days of storage at 4 °C in additive solutions, RBC have 24-h posttransfusion survival values of 75% but do not function satisfactorily. For RBC to function satisfactorily shortly after transfusion, they should be stored at 4 °C for no more than 2 weeks. Yet while the FDA requires a 24-h posttransfusion survival value of 75%, there is no requirement for the function of the transfused RBC. It has been shown that red blood cells that circulate and function immediately or shortly after transfusion exert a very important hemostatic effect to reduce the bleeding time and nonsurgical blood loss in anemic and thrombocytopenic patients. Greater restoration of hemostasis is seen with viable and functional RBC transfusions than with platelets or plasma even though the platelets and plasma proteins may have satisfactory viability and function.

The length of storage of the blood products affects their survival and function and the transfusion of nonviable compatible RBC, antibodies to granulocytes and WBC HLA antigens and biologically active substances affects the patient's clinical outcome. One of the easiest ways to prevent the severe adverse events that have been observed is to ensure that the transfused blood products survive and function at an optimum level and that the levels of antibodies to granulocytes and WBC HLA antigens and biologically active substances are eliminated or reduced. The best way to ensure this is to store liquid-preserved leukoreduced human red blood cells at 4 °C in additive solutions for no more than 2 weeks and leukoreduced platelets at room temperature for no more than 2 days. These liquid-preserved blood products can be used in conjunction with frozen RBC, platelets, and plasma stored in −80 °C mechanical freezers and will avoid the need for fresh whole blood and prevent the severe adverse events associated with the transfusion of blood products.

1. Introduction

Recent publications have reported severe adverse events of mortality and morbidity associated with the transfusion of FDA-approved blood products. Transfusions of RBC, platelets and plasma have been associated with mortality and posttransfusion infections, transfusion related acute lung injury (TRALI) and systemic inflammatory response syndrome (SIRS), leading to increased hospitalization of these patients and increased healthcare costs. Even while reports continue to surface of severe adverse events suffered by recipients of these blood products and even though studies have shown that no such adverse effects have been

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seen with transfusions of previously frozen RBC and platelets, the reluctance to use freeze-preservation procedures continues. Combining freeze-preservation and liquid preservation procedures would prevent the severe adverse events now observed with blood products.

Current methods by which leukoreduced RBC are preserved in the liquid state at 4 °C maintain the viability and function of the RBC at 4 °C for only 2 weeks. If the leukoreduced liquid-preserved RBC are washed after 4 °C storage for 2 weeks it would reduce the antibodies to granulocytes and WBC HLA antigens which activate granulocytes in the recipient's lung and cause transfusion related acute lung injury (TRALI). Washing would also reduce cytokines and other biologically active substances in blood products that activate the recipient's granulocytes to produce oxygen free radicals and the systemic inflammatory response syndrome (SIRS).

After room temperature storage at 22 ± 2 °C for 2 days, leukoreduced platelets have acceptable in vivo recovery, lifespan and function immediately or shortly following infusion and can reduce the nonsurgical bleeding diathesis in anemic and thrombocytopenic patients.

2. Freeze preservation of RBC, platelets, and plasma

Frozen blood products have been shown to be safe and therapeutically effective as a supplement to liquid-preserved products. Group O Rh positive and group O Rh negative RBC, single donor leukoreduced platelets collected by plateletpheresis and AB plasma can all be frozen and stored in a −80 °C mechanical freezer [1]. The Naval Blood Research Laboratory for 45 years conducted studies to preserve cryopreserved blood products for military and civilian use. These studies were supported by the US Navy's Bureau of Medicine and Surgery; the Office of Naval Research (ONR); and the Congress of the United States. Frozen blood products stored in −80 °C mechanical freezers were deployed to supplement the supply of liquid-preserved blood products to treat wounded casualties. During the Vietnam War, the Huggins cytoglomerator was used to deglycerolize RBC frozen with 40% W/V glycerol and stored at −80 °C. The Haemonetics Blood Processor 115 was used in the Persian Gulf War and the Haemonetics ACP215 is being used in Iraq, Afghanistan and Bosnia [1,2]. With the functionally closed Haemonetics ACP215 instrument, group O Rh positive and group O Rh negative RBC are treated with 40% W/V glycerol, the supernatant glycerol is removed, and the RBC containing 40% W/V glycerol are frozen in a −80 °C mechanical freezer. These RBC have been stored at −80 °C for at least 10 years, and following deglycerolization, leukoreduction and washing, the RBC have been stored at 4 °C in the additive solution AS-3 (Nutricel) for 2 weeks.

Single donor leukoreduced platelets obtained by plateletpheresis have been treated with 6% DMSO, and after the supernatant DMSO is removed, the platelets are frozen in a −80 °C freezer and stored at −80 °C for 2 years. After dilution of the thawed platelets with 10 ml of 0.90 NaCl the platelets can be stored at room temperature without agitation for 6 h. Type AB plasma has been frozen in a −80 °C mechanical freezer and stored at −80 °C for 7 years, and after thawing can be stored at room temperature for 8 h or at 4 °C for 24 h. All these blood products have been shown to be safe and therapeutically effective [1].

A −80 °C dual cascade air-cooled mechanical freezer maintained at a mean temperature of −80 °C with a range from −65 °C to −90 °C is used for frozen storage of these blood products. There is no need to control the rate of freezing using a special programmed rate freezer and liquid nitrogen. The blood product is frozen in a polyvinyl chloride (PVC) plastic bag inside a polyester plastic bag placed in a rigid cardboard box in a −80 °C mechanical freezer. The frozen blood products can be transported on dry ice in polystyrene foam shipping containers which maintain the temperature at −80 °C. In our experience, frozen RBC in PVC plastic bags showed an incidence of breakage of about 6% following transportation compared to an incidence of about 3% in PVC plastic bags that were not subjected to transportation [1].

3. Severe adverse events related to liquid-preserved blood products

Since 1985 the effects of the length of storage of liquid-preserved RBC in the additive solutions CPD AS-1 (ADSOL), CP2D AS-3 (Nutricel) and CPD AS-5 (Optisol) on mortality and morbidity in patients have been debated. Numerous publications have discussed whether or not the length of storage of liquid-preserved RBC at 4 °C in the current FDA-approved additive solutions is responsible for the severe adverse events that have been reported in patients. The articles by Zimrin and Hess [3], Lelubre and associates [4], Bennett-Guerrero and associates [5] and the editorial by Steiner and Stowell [6] report on this controversy. Bennett-Guerrero and associates [5] report a prospective, double-blind, randomized clinical feasibility trial of controlling the storage age of red blood cells for transfusion in cardiac surgical patients. The report by Bennett-Guerrero and associates [5] is a limited prospective randomized study in 23 patients who received liquid-preserved red blood cells, 12 patients received RBC stored at 4 °C for 7 ± 4 days and 11 patients received RBC stored at 4 °C for 21 ± 4 days. The authors report that most (94.4%) of the RBC units were AS-1 or AS-3 units which have a shelf life of 42 days; however 12 of the units (5.6%) were CPDA-1 units which have a shelf life of 35 days. The authors do not report the clinical outcome in these 23 patients. This limited prospective randomized study failed to assess liquid-preserved RBC stored in additive solutions (AS-1 or AS-3) at 4 °C for 35–42 days.

Zimrin and Hess discussed the quality of liquid-preserved RBC stored in the three additive solutions (CPD AS-1, CP2D AS-3, and CPD AS-5) approved by FDA for 42 days [3]. They tabulated the adverse events reported in 19 retrospective studies and they concluded that there is no need for an immediate change in the current transfusion practice [3].

There have been two retrospective studies showing that the mortality and morbidity of recipients are related to the storage of the RBC in FDA-approved additive solutions for 42 days [7,8].

Basran and associates have reported that morbidity and mortality were related to the length of storage at 4 °C of allogeneic RBC transfused to patients subjected to reoperative cardiac surgery [7]. Their retrospective study included 434 patients who had undergone repeat median sternotomy for coronary artery bypass surgery or valve surgery. The authors reported that 321 (74%) of the 434 patients met the criteria for eligibility. After adjusting for the effects of confounding variables and total number of RBC transfusions, the investigators determined that the longer the RBC storage before transfusion, the greater the incidence of in-hospital and out-of-hospital mortality. Moreover, they determined that the length of storage before transfusion was related to the incidence of acute renal dysfunction and length of stay in the intensive care unit and hospital. They also reported a relationship between in-hospital mortality and the mean duration of storage of the RBC in the additive solution AS-1/ADSOL.

The increased incidence of acute renal dysfunction observed in these patients suggests that the allogeneic red blood cells that did circulate did not release oxygen immediately or shortly after infusion sufficient to maintain the critical tissue p_{O_2} tension in the kidney [7].

This retrospective study underscores the importance of analyzing both the survival and function of RBC after storage in the additive solution AS-1 (ADSOL) at 4 °C for as long as 42 days [7]. Our data have shown that in order to maintain satisfactory 24-h posttransfusion survival RBC should be stored for no more than 35 days in the ADSOL (AS-1) solution at 4 °C [9–11]. It is important that preserved RBC have normal function immediately after transfusion so that they can deliver oxygen at high tissue p_{O_2} tension to the brain, heart and kidneys and restore hemostasis in patients with a nonsurgical bleeding diathesis. Even though a mean 24-h posttransfusion survival of 75% can be maintained during storage at 4 °C for 35 days, the function of the RBC decreases significantly after only 2 weeks of 4 °C storage in AS-1. Nevertheless, the FDA has approved 4 °C storage of RBC in AS-1 for 42 days [10,11].

Because red blood cells stored at 4 °C in CPD, CPDA-1, CPD AS-1, CP2D AS-3, or CPD AS-5 for longer than 2 weeks have significantly impaired oxygen transport function [11], they require time in the recipient's circulation for the functional defect to be repaired so that they will release oxygen at high tissue p_{O_2} tensions. For optimum survival and function, liquid-preserved red blood cells should be stored for no more than 2 weeks at 4 °C in any of the FDA-approved preservative solutions [11]. Any patient who is sick enough to require a transfusion should receive red blood cells that have not only maximum viability but maximum function as well.

The FDA has never provided any standard for the ability of preserved red blood cells to adequately deliver oxygen immediately after infusion. The basic assumption is that even though the transfused red blood cells have reduced 2,3 DPG levels, the respiratory defect of an increased affinity for oxygen will be restored following transfusion. However, when patients are hypovolemic and hypotensive and are acidotic and have reduced levels of inorganic phosphorus, the in vivo restoration of the respiratory defect in vivo is impaired [10].

Further compromising the well being of the patient who receives liquid-preserved RBC that have been stored in AS-1 for 42 days is the fact that there are compatible nonviable RBC that are infused which must be removed by the reticuloendothelial system. Removal of these nonviable RBC may affect the ability of the reticuloendothelial system to clear infectious agents, tumor cells and particulate matter from the circulation [10,11]. The number of nonviable compatible RBC that can be administered to sick patients without causing harm has never been determined.

The release of cytokines and biologically active substances that occur after 2 weeks storage of RBC at 4 °C may affect the patient's immune system and render the patient susceptible to the adverse events [12,13]. Washing of the liquid-preserved leukoreduced RBC stored at 4 °C in the additive solutions would reduce the cytokines and biologically active substances that may be responsible for the occurrence of adverse events in the recipients [13].

In a recent paper published in the New England Journal of Medicine together with an editorial, the authors reported that when patients undergoing cardiac surgery were transfused red cells that had been stored for more than 2 weeks, they exhibited a significantly increased risk of postoperative complications as well as reduced short-term and long-term survival rates [14]. These patients had received red cell transfusions during coronary artery bypass grafting, heart valve surgery, or both, between June 30, 1998 and January 30, 2006. A total of 2872 patients received 8802 units of blood that had been stored for 14 days or less ("newer blood"), and 3130 patients received 10,782 units of blood that had been stored for more than 14 days ("older blood"). The median age was 11 days for the newer blood and 20 days for older blood. Patients who were given older units had higher rates of in-hospital mortality (2.8% vs. 1.7%, $p = 0.004$), intubation beyond 72 h (9.7% vs. 5.6%, $p < 0.001$), renal failure (2.7% vs. 1.6%, $p = 0.003$) and sepsis or septicemia (4.0% vs. 2.8%, $p = 0.01$). A composite of complications was more common in patients who were given older blood (25.5% vs. 22.4%, $p = 0.001$) [8].

Dzik has written a commentary on the Koch and associates article "Fresh blood for everyone? Balancing availability and quality of stored RBCs." [8,15]. The effects of the length of storage of RBC at 4 °C in AS-1 (ADSOL) for 49 days on red blood cells survival and function were discussed at the Massachusetts Committee on Blood Banks meetings between 1980 and 1985. At that time, the NBRL was requested by the American Red Cross Northeast to study whether or not RBC stored at 4 °C in AS-1 for 49 days could be biochemically modified and frozen. The data reported by the NBRL demonstrated that RBC stored at 4 °C in AS-1 could be stored for only 35 days to provide RBC with 24-h posttransfusion survival of 75% and RBC stored at 4 °C in AS-1 could be stored at 4 °C for only 2 weeks to provide RBC that circulated and functioned shortly following infusion [9,10]. The discrepancy between the NBRL data and the data reported by several other investigators was presented at an FDA meeting conducted in 1985. The result of the Food and Drug Administration meeting was to reduce the length of storage of RBC stored at 4 °C in AS-1 from 49 days to 42 days. At that time, the NBRL reviewed the length of storage of RBC transfused at two hospitals

in the Boston area. Hospital 1 was University Hospital at Boston University Medical Center (BUMC) and Hospital 2 was the Boston City Hospital. The results of the study of the patients and the total number of units transfused and the length of storage of the RBC at 4 °C in AS-1 were reported in the chapter on the Physiology of Blood Transfusion in the book Surgical Intensive Care edited by P.S. Barie and G.T. Shires published in 1993 [16].

Table 22-5 in the article reports the length of storage for RBC in AS-1 at 4 °C in 26 patients who received 207 units of RBC in Hospital 1 (University Hospital) and 97 patients who received 321 units of RBC in Hospital 2 (Boston City Hospital). Twenty-two percent of the patients at Hospital 1 and 21% of the patients at Hospital 2 received RBC stored at 4 °C for greater than 42 days. At Hospital 1, 61% of the patients received 2 units of RBC stored at 4 °C for greater than 35 days and at Hospital 2, 81% of the patients received 2 units stored at 4 °C for greater than 35 days. The clinical outcomes in these 123 patients were not investigated. At the Massachusetts Committee of Blood Banks meetings, discussions with Dr. Dzik and other members of this committee recommended the need to study the clinical outcomes in patients who received multiple units of RBC stored in the additive solution (AS-1) at 4 °C for as long as 49 days. During the past 25 years, numerous clinical studies have reported severe adverse events associated with the transfusion of blood products [3–6]. The recent retrospective studies reported by Basran and associates [7] and Koch and associates [8] have reported mortality and morbidity associated with older stored RBC compared to those observed with newer stored RBC.

The retrospective study by Koch and associates [8] has raised several issues by Dr. Dzik that need to be resolved by prospective randomized studies [15]. Dr. Dzik has suggested that group O recipients received newer red blood cells, patients with left ventricular failure, peripheral vascular disease, and mitral regurgitation received older red blood cells, and patients who received leukoreduced older red blood cells may have represented higher risk patients. Dr. Dzik reported that group O patients have a reduced incidence of the coronary syndrome because group O recipients have reduced level of von Willebrand factor compared to group A recipients [15]. The major issue which is not emphasized by Dr. Dzik is that the median age of the newer red blood cells was 11 days compared to that of the older red blood cells which was 20 days. The current FDA dating period of RBC in additive solutions at 4 °C for AS-1 (ADSOL), AS-3 (Nutricel) and AS-5 (Optisol) is 42 days. The quality of the old red blood cells reported in the Koch and associates' article [8] was significantly better than the quality of red blood cells stored at 4 °C for 35–42 days.

Table 22-5
Length of storage of red blood cells in CPD–ADSOL solution at 4 °C transfused to patients at Hospital 1 from December 1983 to July 1987 and at Hospital 2 from December 1983 to February 1985. From Valeri CR: Surgical Intensive Care, edited by P.S. Barie and G.T. Shires, 1993, p. 695 (with permission).

	Hospital 1	Hospital 2
Number of patients	26	97
Total number of units	207	321
<14 days old (%)	7.7	8.7
>30 days old (%)	58.9	81.6
>35 days old (%)	45.9	48.6
>42 days old (%)	22.2	20.6
Percent of patients receiving 2 units >35 days old	60.9	81.4

Key: CPD = citrate–phosphate–dextrose; ADSOL = adenine, sodium chloride, glucose, mannitol.

Dr. Dzik suggests that prospective randomized studies are needed to assess clinical outcomes in patients randomized to receive exclusively red blood cells stored at 4 °C for 10 days or less with those assigned to receive exclusively red blood cells stored at 4 °C for 35 days and longer. Dr. Dzik suggests that RBC exchange transfusion administered to patients with sickle cell syndrome should evaluate newer red blood cells stored for less than 2 weeks to older RBC stored at 4 °C for 35 days and longer in a randomized prospective study in the same patient.

The experiences of the NBRL and that of the Netherlands military have demonstrated that fresh whole blood is no longer needed when frozen RBC, frozen platelets and frozen plasma stored at −80 °C in mechanical freezers are available [1,2]. Safe and therapeutically effective group O Rh positive and group O Rh negative glycerolized RBC frozen for 10 years, single donor leukoreduced DMSO platelets frozen for 2 years, and AB plasma obtained from only male donors frozen for 7 years are now available for use by both the military and civilian communities [1,2]. Studies are now needed to compare the use of these frozen blood products to liquid-preserved blood products stored at 4 °C in additive solution AS-1, AS-3 and AS-5 for 42 days, platelets stored at room temperature for 5 days, and fresh frozen plasma stored at −20 °C for 1 year. Morbidity and mortality associated with posttransfusion infection, TRALI and SIRS need to be assessed to compare the frozen blood products to the liquid-preserved blood products with regard to safety, therapeutic effectiveness, and cost to the military and civilian communities responsible to provide blood products to treat patients.

In an article in Vox Sang, Maegele and associates reported on a retrospective analysis of mortality in patients with severe traumatic injuries who received massive transfusions of packed RBC (pRBC) and fresh frozen plasma (FFP) [17]. They reported that patients who received pRBC:FFP > 1.1 showed a significant increase in mortality compared to patients who received pRBC:FFP 0.9–1.1 or pRBC:FFP < 0.9, but they left out important information about the blood products. For instance, there was no information about the quality of the packed RBC, the type of anticoagulant used to collect the blood, whether the RBC were leukoreduced, the additive solution used to preserve the pRBC, or the length of storage of the pRBC at 4 °C before transfusion. The study by Maegele and colleagues is yet another retrospective study suggesting a relationship between observed severe adverse effects and the quality of transfused RBC [9–11].

Another publication that also lacks information necessary to interpret the data is a commentary by Hess and associates, "Giving plasma at a 1:1 ratio with red cells in resuscitation: who might benefit?" [18]. They do not report

whether resuscitative fluids were used and, if they were, what was the volume and composition? What anticoagulant was used to collect the blood and what was the quality of the RBCs? Was whole blood infused or nonleukoreduced or leukoreduced red blood cell concentrates? What additive solution was used during storage of the RBC at 4 °C and how long was the whole blood or RBC stored in the additive solutions at 4 °C prior to transfusion [9–11]? All of these are very important questions that must be answered. To state that a coagulopathy in patients in hemorrhagic shock can be successfully treated with equal volumes of plasma and red blood cells is not only misleading but can be dangerous especially when the authors do not report or know what resuscitative solutions were utilized, the volume and composition of these solutions, and what the survival and function of the transfused RBC were. RBC that circulate and function immediately following infusion exert a hemostatic effect greater than that of platelets or plasma to reduce bleeding time and nonsurgical blood loss in anemic thrombocytopenic patients [19]. The survival and function of the RBC are more important than are platelets or fresh frozen plasma in the restoration of hemostasis and reduction of bleeding time and nonsurgical blood loss in anemic and thrombocytopenic patients [19].

A committee convened by the Health and Human Services Agency to review the incidence of severe adverse events associated with blood products has recommended that multi-center prospective randomized studies be conducted to compare clinical outcomes in patients transfused with leukoreduced red blood cells stored at 4 °C for less than 2 weeks with outcomes for patients receiving 35- to 42-day-old leukoreduced red blood cells.

As far back as 1985, our laboratory sent a letter to the editor of the New England Journal of Medicine regarding the length of storage of human red blood cells at 4 °C in the CPD/AS-1 additive solution (ADSOL). We recommended that these RBC be stored at 4 °C for no more than 35 days in order to have an acceptable 24-h posttransfusion survival value of 75% [9]. We also recommend that in order to maintain acceptable 24-h posttransfusion survival and satisfactory function immediately or shortly after transfusion, human RBC should be stored in AS-1 (ADSOL) at 4 °C for no more than 2 weeks. We also reported these data at an FDA meeting in August 1985 at a time when the FDA had approved the storage of RBC in AS-1 (ADSOL) at 4 °C for 49 days. Four of the other investigators at the FDA meeting, all of whom were being funded by commercial companies, stated that RBC could be stored at 4 °C for 49 days and have 24-h posttransfusion survival values of at least 70%. At that time, we reported on a method to measure the in vivo survival of autologous RBC stored at 4 °C in AS-1 (ADSOL) using a 51Cr/125I double label procedure and a method to measure the in vivo survival of allogeneic compatible but identifiable RBC assessed by the automated differential agglutination (ADA) procedure and the 51Cr double label procedure. Using these two methods, we determined that a 24-h posttransfusion survival of 75% can be maintained at 4 °C for only 35 days, and that CPD/AS-1 (ADSOL) RBC can be stored at 4 °C for only 2 weeks in order to function immediately or shortly after infusion [10]. When ADSOL preserved RBC were stored at 4 °C for 49 days they had 24-h posttransfusion survival of only 55% and showed no evidence of restoration of ATP and 2,3 DPG levels during the 24-h posttransfusion period. Forty-eight hours after transfusion, the ATP level did rise significantly higher than expected levels but the 2,3 DPG level did not [10]. The reduced 2,3 DPG levels were not restored at a rate comparable to that reported for normal volunteers by Heaton and associates [20]. The rate at which the RBC 2,3 DPG level is restored is influenced by the patient's pH and level of inorganic phosphorus. In normal volunteers, in vivo conditions are optimal for the synthesis of preserved RBC with reduced 2,3 DPG levels. But comparisons cannot be made of in vivo restoration of RBC 2,3 DPG levels in normal volunteers infused with compatible but identifiable preserved RBC with low 2,3 DPG levels and the in vivo restoration in patients who may receive multiple units of compatible but identifiable preserved RBC [10,21–24].

Weiskopf and associates studied six female and three male healthy volunteers, with mean age of 23 in a range of 21–25 years, who were made anemic and isovolemic [25]. They reported that autologous compatible RBC concentrates stored at room temperature for 3.5 h (fresh RBC) and autologous CPDA-1 RBC concentrates stored at 4 °C for 23 days (old RBC) had normal and equivalent oxygen transport function when assessed by measurements of neurocognitive function and heart rate. These findings are similar to those observed in two of our studies in baboons published in 1975 which reported on systemic and cerebral oxygen extraction. In one study, healthy male baboons were subjected to hyperventilation and phlebotomy [26]. In the other study of 11 passively hyperventilated anemic male baboons, six baboons were transfused type specific allogeneic red blood cells with reduced 2,3 DPG levels and increased affinity for oxygen and five baboons received RBC with increased RBC DPG levels and reduced affinity for oxygen [27]. Immediately after the transfusion of red blood cells with decreased 2,3 DPG levels and increased affinity for oxygen, we observed a decrease in p50 in vivo and a 50% increase in cerebral blood flow and within 2 h after the transfusion the cerebral blood flow, had returned to pre-transfusion level. The 2,3 DPG levels of these RBC were restored to 40% of normal within 4 h of transfusion and the rapid increase in 2,3 DPG level was associated with an increase in blood pH and inorganic phosphorus levels. We did not observe any significant change in systemic or cerebral oxygen extraction [27].

The similarities in neurocognitive function observed in the young healthy anemic female and male volunteers in the Weiskopf study [25] and the maintenance of systemic and cerebral oxygen extraction in the baboons in our study indicated that rapid synthesis of the RBC 2,3 DPG level occurred accompanied by a significant increase in cerebral blood flow immediately after transfusion [27]. The subjects in the Weiskopf studies were young healthy anemic volunteers; studies should be conducted in anemic patients with cerebrovascular disease to evaluate neurocognitive function in these patients comparing leukoreduced RBC that have been stored at 4 °C for less than 2 weeks and leukoreduced RBC stored at 4 °C in the FDA-approved additive solutions for 42 days. Studies in anemic patients with

cerebrovascular disease will give a more accurate picture of the effect of the length of storage on neurocognitive function. Such studies will provide data necessary to determine the accuracy of the statement by Weiskopf and associates "RBC that are fresh and those that are old deliver oxygen equivalently and normally in humans" [25].

4. The survival and function of preserved RBC and platelets

One should not assume that because the survival of preserved RBC and preserved platelets is satisfactory, their ability to function following transfusion will also be satisfactory [19]. Yet, while the FDA requires that preserved RBC have 24-h posttransfusion survivals of 75%, they make no regulations requiring that the RBC function satisfactorily immediately or shortly after transfusion. The FDA also assumes that when a sick patient receives 25% of nonviable compatible RBC with a transfusion, the recipient will suffer no adverse effects. Although there are no data to show how many nonviable compatible RBC can be safely administered to sick patients, it is known that if a patient receives 10 units of RBC with a 24-h posttransfusion survival value of 75%, then 2.5 units of the RBC will be nonviable. The removal of these compatible nonviable RBC by the reticuloendothelial (RE) system may interfere with the removal of infectious disease agents, tumor cells and particulate matter present in the stored RBC.

The FDA considers preserved platelets to be acceptable if the in vivo recovery is 66% that of fresh platelets and the lifespan is 50% that of fresh platelets but again offers no guidelines with regard to function [19]. Although in vitro levels of RBC ATP, DPG and p50 levels give an indication of the in vivo function of the preserved RBC, there are no in vitro tests that can predict the in vivo function of fresh and preserved platelets. Studies at the NBRL have shown that when fresh and preserved platelets function satisfactorily they will correct an aspirin-induced increased bleeding time in normal volunteers and in healthy baboons [19].

The survival and function of preserved red blood cells are critical especially for patients with cerebral, myocardial or renal insufficiency and for patients with a nonsurgical bleeding diathesis. Studies at the NBRL have shown that preserved red blood cells stored in CPDA-1 or in any one of the additive solutions CPD/AS-1, CP2D/AS-3, or CPD/AS-5 at 4 °C for 2 weeks have acceptable 24-h posttransfusion survival values of greater than 75% and only moderately impaired oxygen transport function at the time of infusion [11,19].

Liquid-preserved autologous platelets that were stored at room temperature (22 ± 2 °C) with agitation for 48 h were found to have acceptable in vivo recovery and survival and were able to function to reduce an aspirin-induced prolonged bleeding time in normal volunteers and healthy baboons [19]. On the other hand, when autologous platelets were stored at room temperature for 3 or 5 days, they did circulate but did not reduce the prolonged bleeding time in aspirin-treated healthy baboons [19,28].

Between 1988 and 1992, the NBRL collaborated with Dr. Shukri Khuri, Chief of Surgery and Chief of Cardiothoracic Surgery at the West Roxbury Veteran's Administration Hospital, to evaluate liquid-preserved and washed previously frozen platelets transfused to cardiopulmonary bypass (CPB) patients [29]. The previously frozen platelets had been frozen with 6% DMSO at 2–3 °C/min and stored at −80 °C for a mean of 289 ± 193 days (SD) and for as long as 2 years and were washed prior to transfusion. A prospective randomized study was conducted in 73 patients undergoing CPB surgery. The study was designed to measure nonsurgical blood loss, i.e. blood loss unrelated to the surgical procedure and not controlled by surgical intervention, after neutralization of the heparin with protamine sulfate. Nonsurgical blood loss was collected intraoperatively and during the 24-h postoperative period [29].

The allogeneic single donor washed previously frozen platelets transfused to these patients had been processed in the following manner. The platelets were frozen at the University of Massachusetts in Worcester, Massachusetts, and transported in the frozen state with dry ice to the NBRL where they were stored in −80 °C mechanical freezers for at least 3 months. They were then transported in the frozen state with dry ice to West Roxbury Veteran's Administration Hospital Blood Bank where they were stored at −80 °C in mechanical freezers. After thawing, the platelets were washed and stored in ACD plasma at room temperature without agitation for as long as 5 h. The platelets were transfused to the patients after the CPB surgery [29].

The prospective randomized study compared the need for allogeneic RBCs and FFP to treat the nonsurgical blood loss in two groups of patients. One group received previously frozen washed platelets and the other group received liquid-preserved platelets. Patients who received previously frozen washed platelets showed a reduction in the nonsurgical blood loss and required fewer units of allogeneic RBCs and FFP than the patients who received the liquid-preserved platelets stored at 22 °C for 3.4 days. The platelet survival 2 h after transfusion was 37% for patients who received the liquid-preserved platelets compared to 24% for the patients who received the previously frozen washed platelets [29].

The total number of liquid-preserved platelets infused was $6.9 \times 10^{11} \pm 3.9 \times 10^{11}$ per patient which was significantly greater than the $4.5 \times 10^{11} \pm 2.1 \times 10^{11}$ per patient for the previously frozen washed platelets. The difference was the result of the freeze–thaw–wash (FTW) recovery value of 70% for the previously frozen washed platelets. The in vivo recovery and function of the liquid-preserved and cryopreserved platelets in these patients were similar to values seen in studies in which liquid-preserved and cryopreserved platelets were transfused to aspirin-treated human volunteers and baboons [28,30,31]. Although in the patients in this study, the in vivo recovery values were higher for the liquid-preserved allogeneic platelets than for the washed previously frozen platelets, nonsurgical blood loss was lower in the patients who received the washed previously frozen platelets and they required fewer units of allogeneic RBCs and FFP [29].

In 2000, we modified the method of freezing platelets with 6% DMSO at 2–3 °C/min with storage in a −80 °C mechanical freezer. With the modified method, the

supernatant DMSO was removed before the platelets were frozen and the previously frozen platelets were not washed prior to transfusion [32–34]. When platelets are washed, the in vitro recovery is reduced by 20%. With the modified method, the thawed platelets are not washed but are diluted with 10 ml of 0.9% NaCl and stored without agitation at room temperature for 6 h. The freeze–thaw (F–T) recovery is approximately 90%, with 5–8% platelet microparticles, in vivo recovery is 25–30%, and the linear lifespan is 7 days. The diluted platelets have a bimodal population: one population is GPIb-normal and annexin V-reduced and the other is GPIb-reduced with increased annexin V binding [34]. It is not known whether the population of activated platelets and platelet microparticles might produce thromboembolic events.

Washed previously frozen platelets contain 400 mg of the residual DMSO compared to 600 mg for unwashed previously frozen platelets. After storage at room temperature undisturbed for 6 h, the diluted nonwashed platelets had a pH of 6.37. Diluting the thawed platelets with 0.9% NaCl instead of plasma reduces the occurrence of transfusion related acute lung injury (TRALI). Removing the supernatant DMSO before the platelets are frozen and diluting the thawed platelets with 0.9% NaCl reduces the postthaw processing time from 1.5 h to only 10 min [34].

The total number of platelets and the pH of the platelets following storage of the diluted platelets at room temperature for 6 h are used as quality control measurements for the platelets. The percentage of platelets that are GPIb-normal and annexin V-reduced correlated to the in vivo recovery and platelet lifespan. The percentage of platelets that are GPIb-reduced with increased annexin V binding and the percentage of platelet microparticles correlate with the ability of the platelets to function to reduce the bleeding time and nonsurgical blood loss [34]. Platelets that were frozen with 6% DMSO and washed before transfusion produced no untoward effects in patients after CPB surgery [29]. Studies are needed to determine whether the platelets processed by the modified method and not washed before transfusion might be associated with any untoward effects [34]. Hornsey and associates [35] have reported in vitro testing of buffy coat derived leukoreduced platelet concentrates treated with 6% DMSO with removal of the supernatant solution before storage at −80 °C without postthaw washing [1,2]. As reported by Lelkens and associates [2] the thawed platelets were resuspended in AB plasma and not 0.9% NaCl prior to testing [35]. Freeze–thaw loss of platelets was 23% and a bimodal population of platelets was observed by flow cytometry: one population with normal level of GPIba and a reduced level of annexin V binding and the other population with reduced level of GPIba and an increased level of annexin V binding. In vitro testing for platelet function using the DiaMed Impact R procedure showed that the surface coverage and aggregate size of the frozen platelets were similar to those observed for platelets stored at 22 °C for 2 days. The in vitro testing showed that the frozen platelets were activated and were capable of adhering to surfaces and forming aggregates under shear force [35].

Studies at the NBRL, Boston, MA that have been funded by the US Navy and the US Congress have demonstrated the effectiveness of the −80 °C mechanical freezer in freezing and storing RBC, platelets, and plasma as a supplement to liquid-preserved blood products [1,2]. Mechanical freezers maintained at −80 °C are needed for the storage of these safe and therapeutically effective frozen blood products for use by both the military and civilian communities to supplement the liquid preservation of RBC stored at 4 °C, platelets stored at +22 °C, and fresh frozen plasma and cryoprecipitate stored at −20 °C.

Studies at the NBRL have shown that previously frozen washed RBC have not been associated with the severe adverse events observed with the current FDA-approved blood products. Frozen RBC stored at −80 °C have been successfully deployed during the Vietnam War and the Persian Gulf War by the US Navy and the Department of Defense [1,36,37].

5. Preservation and disinfection of blood products; blood substitutes; resuscitative solutions; hemostatic agents

Both government and commercial sources have provided funds to support research and development opportunities to study hemoglobin-based oxygen carriers (HBOCs); lyophilized platelets, lyophilized plasma, and enzymatic conversion of group A and group B RBC to group O RBC. Funding has also been provided for the production and testing of resuscitative fluids containing Ringer's ketone solution to provide substrate-mediated resuscitation rather than volume-mediated resuscitation; and hemostatic agents to reduce blood loss and accelerate wound healing, as well as for the development of instruments using membrane technology to prepare sterile, pyrogen-free large volume parenteral solutions from potable water.

In spite of all the funding for this research, the DOD of the US has recently decided to approve a very dangerous practice in Iraq and Afghanistan. Injured servicemen and women in these countries are being given fresh whole blood that has not been tested for the FDA mandated infectious disease markers prior to transfusion. This dangerous situation should be remedied as soon as possible. Meanwhile, the Netherlands military under the direction of Dr. Charles Lelkens is treating wounded servicemen and women in Iraq, Afghanistan, and Bosnia with tested frozen RBC, platelets, and plasma stored in −80 °C mechanical freezers [2]. If this small country can provide frozen blood products in these areas and not depend upon untested fresh whole blood, why cannot the United States provide our service men and women with safe and therapeutically effective frozen blood products that have been tested for infectious disease markers and collected from donors approved by FDA guidelines?

The past 45 years have seen many significant improvements in cryopreservation procedures. Removing supernatant glycerol from RBC and DMSO from platelets prior to freezing have simplified the postthaw processing [1,34,37]. Platelets diluted with 10 ml of 0.9% NaCl after thawing can be stored at room temperature without agitation for 6 h prior to use. With the functionally closed Haemonetics ACP215 instrument, deglycerolized RBC can

be stored at 4 °C in AS-3 (Nutricel) for 2 weeks. With this instrument, the volume of solution needed to deglycerolize RBC is now only 1.6 l compared to 3.2 l with the Haemonetics Blood Processor 115 and 6.8 l with the Huggins cytoglomerator [1,36,37].

The increased mortality and morbidity, and the occurrence of posttransfusion infectious diseases, TRALI and SIRS observed after transfusion of red blood cells stored in the liquid state at 4 °C for 42 days or platelets stored at room temperature for 5 days can be prevented by using previously frozen blood products stored in a −80 °C mechanical freezer [1,2,38–45].

Some have suggested that if RBC, platelets, and plasma were disinfected, this would eliminate the risk of transmission of infectious disease agents that are not detected with the current FDA mandated testing. Unfortunately, the disinfection of red blood cells, platelets, and plasma may alter their antigenicity so that repeated infusion of allogeneic disinfected blood products could reduce their safety and therapeutic effectiveness. Studies over the past 45 years using glycerol to freeze RBC and DMSO to freeze platelets have produced no alteration of the antigenicity of the RBC and platelets [1,46]. Likewise, attempts at enzymatic conversion of group A and group B RBC to universal donor O red blood cells have not yet been successful.

Extensive funding has been provided to companies trying to produce a hemoglobin-based oxygen carrier as a replacement for RBC. Natanson and associates have reported the mortality, myocardial infarction, and morbidity associated with the clinical use of HBOCs [47]. Moreover, to compare the safety and therapeutic effectiveness of HBOCs to red blood cells is disingenuous since HBOC's circulate for only 24 h and RBC have a 24-h posttransfusion survival value of at least 75% and normal lifespan of 3 months [48]. Even if the HBOCs were proven to be safe and therapeutically effective, they would provide oxygen to the brain, heart and kidney and exert a hemostatic effect for only 24 h and would not reduce the need for safe and therapeutically effective allogeneic RBC [43]. The severe adverse events that have been reported with HBOCs have been related to their vasoconstriction to impair blood flow to the brain, heart and kidneys, to activate the hemostatic mechanism and to generate oxygen free radicals [47,49–60].

6. Prevention of TRALI and SIRS

Severe adverse events associated with transfusions of RBC, platelets and plasma include acute lung injury (TRALI) and systemic inflammatory response syndrome (SIRS) [41,42,61–63]. TRALI is caused by the presence in RBC, platelets and plasma of antibodies to granulocytes and to WBC HLA antigens which occur at a higher incidence in female blood donors [41]. The cytokines and the biologically active substances in blood products following infusion activate the recipient's granulocytes that produce oxygen free radicals, and the severe adverse events related to the systemic inflammatory response syndrome (SIRS) [12,13,61–63].

When leukoreduced liquid-preserved RBC are washed, the level of antibodies to granulocytes and WBC HLA antigens which activate granulocytes in the recipient's is reduced [41,42]. Washing the RBC also reduces the level of cytokines and biologically active substances involved in the activation of the recipient's granulocytes that generate oxygen free radicals and produce the systemic inflammatory response syndrome (SIRS) [12,13,61–63]. The incidence of both TRALI and SIRS could be reduced by washing the leukoreduced RBC stored at 4 °C.

The procedure to deglycerolize the RBC produces leukoreduced and washed RBC which is the ideal RBC product to reduce or eliminate TRALI and SIRS. The enzymatic conversion of group A and group B red blood cells to group O RBC could be supplemented simply by freezing group O Rh positive and group O Rh negative RBC. Nonrejuvenated group O positive and group O negative RBC can be frozen without prior biochemical modification. Indated and outdated group O Rh positive and group O Rh negative RBC can be biochemically modified to increase the RBC 2,3 DPG, ATP and p50 levels before freezing. Biochemically modified universal donor RBC have acceptable 24-h posttransfusion survivals and normal or improved oxygen transport function [64–70]. RBC that are frozen do not have to be leukoreduced because the addition and removal of 40% W/V glycerol from the RBC produce a leukoreduced RBC which after washing have a total number of 1×10^7 WBC per unit [1,71–76].

At the international symposium on the diagnosis and prevention of TRALI, attendees from Poland suggested that TRALI is associated with storage of liquid-preserved RBC at 4 °C for longer than 2 weeks and the storage of platelets at room temperature (22 ± 2 °C) for longer than 48 h [41]. Participants at the International Forum on TRALI recommended that females should be excluded as plasma donors because they have a higher incidence of antibodies against granulocytes and WBC HLA antigens [41]. We now recommend that AB plasma from male donors should be frozen and stored at −80 °C. With all due respect to the investigators making these suggestions, what is needed are studies comparing the incidence of TRALI and SIRS associated with transfusions of liquid-preserved leukoreduced RBCs, previously frozen washed leukoreduced RBCs and leukoreduced washed liquid-preserved RBCs [42]. The NBRL, over a 45-year period, predominantly transfused multiple units of washed previously frozen leukoreduced RBC from male donors and never observed any incidence of either TRALI or SIRS [77].

The Netherlands military has been actively freezing universal donor group O RH positive and group O Rh negative RBC, single donor leukoreduced frozen group O platelets, and frozen AB plasma [2]. These frozen blood products have been collected from donors meeting FDA regulations that were safely transported and stored in −80 °C mechanical freezer without breakage. These blood products have been tested for the mandated infectious disease markers and have eliminated the need for fresh whole blood to treat patients suffering traumatic injuries with therapeutically effective outcomes and without adverse events [2].

An abstract reported at the American Association of Blood Banks (AABB) annual meeting held in New Orleans, LA from October 24 to 27, 2009 by F. Noorman, R. Strelitski, and C.C. Lelkens on "Frozen −80 °C Red Cells, Plasma and

Platelets in Combat Casualty Care" concluded that "fully tested frozen blood products, readily available after thawing, proved to be an effective and safe blood support for combat casualty care. A 1:1 red cells to plasma ratio appeared to increase survival in massively transfused patients when only −80 °C frozen blood products were used" [78].

"Since 2004 the Netherlands military mainly uses −80 °C frozen blood products to cover operation needs. Here we described our experience with these products based on data collected from two Netherland-deployed blood bank facilities in Afghanistan, during the past 33 months. Apheresis leukodepleted group O platelets in 5% DMSO/plasma were frozen as a concentrate (+15 ml) at −80 °C. After thawing, the platelets were resuspended in thawed AB plasma for use within 6 h. Apheresis leukodepleted AB plasma was thawed from −30 °C, repacked and frozen down to −80 °C before the final thawing procedure. Red cells from leukodepleted group O whole blood were frozen at −80 °C in 40% (W/V) glycerol. After thawing and deglycerolization, the red cells were stored for no longer than 14 days at 4 °C in AS-3, before use. All previously frozen blood products were in compliance with international regulations and guidelines. All frozen products were produced in the Netherlands, shipped at −80 °C (dry ice), stored in theater at −80 °C and were thawed on demand. Occasionally, standard liquid red cells were sent from the Netherlands as a supplement, to cover periods of higher usage. During the past 33 months, 533 patients (85% Afghan) were transfused with 533 units of standard liquid red cells and 3380 frozen blood products (1360 red cell units, 1425 plasma units and 595 apheresis platelet units). On one location, where all blood products were provided by the Netherlands Military Blood Banks, blood usage and survival were further analyzed. It showed that >90% of the transfused patients were trauma patients of which 15% (30 out of 209) required more than 10 red cells units within 24 h. In these massively transfused patients survival improved from 44% ($n = 16$) to 85% ($n = 14$) after the introduction of a new transfusion policy in November 2007 using 1:1 red cell to plasma ratio, with or without platelets. No shortages or transfusion reactions were reported" [78].

This study by the Netherlands military demonstrates the safety and therapeutic effectiveness of previously frozen deglycerolized RBC in the resuscitation of massively transfused patients. Previously frozen leukoreduced washed RBC together with frozen plasma with or without frozen platelets all stored in a −80 °C mechanical freezer improved the survival of massively transfused patients.

It is imperative that civilian and military blood banking communities change their methods of collection and preservation of blood products if they are really interested in providing patients with the safest and most therapeutically effective blood products and in avoiding risks associated with transfusion.

The primary concern of the blood banking community appears to be the extension of the length of storage of the blood products. This practice may make inventory management easier, but it certainly will not improve patient outcomes or provide patients with the safest and most therapeutically effective blood products. Of course, cost must be a consideration but, in the long run, the recipient's health should be paramount. With regard to the mortality and morbidity associated with the severe adverse reactions to transfused blood products, it is important to note that Medicare has informed hospitals that they will no longer pay for long stays in hospitals when proper care could have prevented the increased hospitalization. A concerted effort must be made to determine why the transfused blood products are causing mortality and morbidity associated with posttransfusion infection, TRALI and SIRS, and what can be done to eliminate the causes. Our studies have shown that the severe adverse events observed after the transfusion of blood products stored under current FDA guidelines can be prevented by using washed liquid-preserved RBC that have been stored at 4 °C for no more than 2 weeks in combination with washed previously frozen red blood cells that have satisfactory survival and function. Washing liquid-preserved red blood cells with 0.9% NaCl and 0.2 g% glucose does not adversely affect their posttransfusion survival and function [13,79–82]. The health of the patient must be of greater importance than making blood collection and inventory management easier.

7. Summary

There have been several retrospective studies reporting severe adverse events of mortality and morbidity associated with blood transfusions. Mortality and morbidity associated with posttransfusion infection, TRALI, and SIRS have been reported in patients undergoing cardiac surgery, after massive transfusions for severe traumatic injuries, and after transfusions for elective and emergency indications. After 35 days of storage at 4 °C in additive solutions, RBC have 24-h posttransfusion survival values of 75% but do not function satisfactorily. For RBC to function satisfactorily shortly after transfusion, they should be stored at 4 °C for no more than 2 weeks. Yet while the FDA requires a 24-h posttransfusion survival value of 75%, they make no requirement for the function of the transfused RBC. It has been shown that red blood cells that circulate and function immediately or shortly after transfusion exert a very important hemostatic effect to reduce the bleeding time and nonsurgical blood loss in anemic and thrombocytopenic patients. Greater restoration of hemostasis is seen with viable and functional RBC transfusions than with platelets or plasma even though the platelets and plasma proteins may have satisfactory viability and function.

The FDA, HHS, and the blood banking community have focused on further testing of blood products to reduce the rate of disease transmission. Another consideration is the disinfection of red blood cells, platelets, and plasma. What is more important is that, they should be looking at the quality of the blood products being transfused. We know that the length of storage of the blood products affects their survival and function and that transfusing nonviable compatible RBC, antibodies to granulocytes and WBC HLA antigens and biologically active substances affects the patient's clinical outcome. One of the easiest ways to prevent

the severe adverse effects that have been observed is to ensure that the transfused blood products survive and function at an optimum level and that the levels of antibodies to granulocytes and WBC HLA antigens and biologically active substances are reduced. The best way to ensure this is to store liquid-preserved human red blood cells at 4 °C in additive solutions for no more than 2 weeks and leukoreduced platelets at room temperature for no more than 2 days. These liquid-preserved blood products can be used in conjunction with frozen RBC, platelets, and plasma stored in −80 °C mechanical freezers.

The reluctance of the blood banking community to consider any changes that could improve the safety and therapeutic effectiveness of blood products brings to mind two very relevant quotations: one by Maurice Maeterlinck is that "At every crossway on the road that leads to the future, every progressive spirit is opposed by a thousand men appointed to guard the past." The other by Winston Churchill is that "Occasionally man will stumble on the truth but will manage to pick himself up and continue on as if nothing had happened." It is time now to investigate the current blood banking procedures and to seek ways to improve them.

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