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Decreased Erythrocyte Deformability After Transfusion and the Effects of Erythrocyte Storage Duration

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Abstract

Background—Erythrocyte cell membranes undergo morphologic changes during storage, but it is unclear whether these changes are reversible. We assessed erythrocyte cell membrane deformability in patients before and after transfusion to determine the effects of storage duration and whether changes in deformability are reversible after transfusion.

Methods—Sixteen patients undergoing posterior spinal fusion surgery were studied. Erythrocyte deformability was compared between those who required moderate transfusion (≥ 5 units erythrocytes) and those who received minimal transfusion (0–4 units erythrocytes). Deformability was measured in samples drawn directly from the blood storage bags before transfusion, and in samples drawn from patients before and after transfusion (over 3 postoperative days). In samples taken from the blood storage bags, we compared deformability of erythrocytes stored for a long duration (≥ 21 days), those stored for a shorter duration (<21 days), and cell-salvaged erythrocytes. Deformability was assessed quantitatively using the elongation index (EI) measured by ektacytometry, a method which determines the ability for the cell to elongate when exposed to shear stress.

Results—Erythrocyte deformability was significantly decreased from the preoperative baseline in patients after moderate transfusion (EI decreased by 12 ± 4 to 20 ± 6 %) ($P = 0.03$) but not after minimal transfusion (EI decreased by 3 ± 1 to 4 ± 1 %) ($P = 0.68$). These changes did not reverse over 3 postoperative days. Deformability was significantly less in erythrocytes stored for ≥ 21 days (EI = 0.28 ± 0.02) than in those stored for <21 days (EI = 0.33 ± 0.02) ($P = 0.001$) or those drawn from patients preoperatively (EI = 0.33 ± 0.02) ($P = 0.001$). Cell-salvaged erythrocytes had intermediate deformability (EI = 0.30 ± 0.03) that was greater than that of erythrocytes stored ≥ 21 days ($P = 0.047$), but less than that of erythrocytes stored <21 days ($P = 0.03$).

Conclusions—The findings demonstrate that increased duration of erythrocyte storage is associated with decreased cell membrane deformability and that these changes are not readily reversible after transfusion.

Introduction

Blood transfusion, the most commonly performed procedure in United States hospitals,^a has been associated with adverse outcomes in some clinical situations. Although most outcome studies of transfusion are retrospective and thus subject to confounding variables, a growing body of evidence, albeit controversial, indicates that transfusion, especially with blood stored for a long duration, may not improve oxygen delivery^{1,2} and may be associated with increased morbidity and mortality.^{3,4} Of primary concern is the quality of transfused erythrocytes after storage, because multiple biochemical and biomechanical changes occur in the cells that adversely influence erythrocyte function.^{5,6} These changes, referred to as the “storage lesion” have been recognized for more than two decades.⁷ One particular erythrocyte characteristic that is susceptible to change during storage is the ability for the cell membrane to deform, which allows erythrocytes (6–8 μm in diameter) to traverse capillaries of similar, or even smaller diameter (5–10 μm).⁸

The various components of the storage lesion include depletion of nitric oxide,⁹ 2,3-diphosphoglycerate (DPG),¹⁰ and adenosine triphosphate (ATP);¹¹ increased free hemoglobin concentration from hemolysis;⁹ and increased erythrocyte aggregability.¹² Storage-related changes in cell membrane structure and function include membrane phospholipid vesiculation,¹³ protein oxidation,¹⁴ lipid peroxidation,¹⁵ and loss of cell membrane deformability.¹⁶ Although decreased deformability has been described after erythrocyte storage, the mechanism is poorly understood. Because nitric oxide and ATP levels are thought to maintain and enhance cell membrane deformability,^{17–19} and given that nitric oxide and ATP are quickly replenished after transfusion of erythrocytes,^{20,21} it is possible that the loss of deformability is reversible. In this study, we tested the hypotheses that longer duration of erythrocyte storage is associated with loss of cell membrane deformability and that this change is reversible after transfusion.

Methods

Patient and Anesthetic Management

After receiving IRB approval (Johns Hopkins Hospital) and written informed consent, we enrolled 16 patients scheduled for posterior cervical, thoracic and/or lumbar spinal fusion surgery. Exclusion criteria included sickle cell disease or other red blood cell disorders. Intraoperative care was standardized, and all patients received general anesthesia with a combination of midazolam, fentanyl, isoflurane, nitrous oxide, and vecuronium. Intraoperatively, both allogeneic and cell-salvaged erythrocyte units were given to maintain hemoglobin concentration above 9 g/dL. Duration of erythrocyte storage was not controlled and was based on the usual practice at our institution for which the average storage duration is approximately 18 days. The *a priori* definition of moderate transfusion was 5 units (~one-half of total blood volume) of either allogeneic or cell-salvaged erythrocytes given intraoperatively. Any intraoperative transfusion of 0–4 units was defined as minimal transfusion. In the first 3 postoperative days, erythrocytes were transfused at the discretion of the surgical team to maintain the hemoglobin concentration above 9 g/dL.

All patients had radial arterial catheters that were used for obtaining blood samples during and after surgery. Blood samples were drawn from the patient into heparinized syringes before incision, upon completion of surgery, and on the morning of postoperative days 1, 2, and 3. After the arterial catheter was removed, venous samples were obtained by phlebotomy. A sample was drawn directly from the blood storage bag for most of the blood

^aAgency for Healthcare Research and Quality.<http://archive.ahrq.gov/data/hcup/factbk7/factbk7b.htm#MostCommonProcedures> (accessed November 1, 2012)

units that were transfused during surgery (including cell-salvaged units). Samples were stored on ice until the end of surgery and then taken promptly to the laboratory where the deformability tests were performed. The duration of storage for each transfused unit was recorded. Duration of storage was calculated according to the expiration date of Adsol-preserved units; the storage duration limit was 42 days. Thirteen (87%) of 15 cell-salvaged blood units, and 36 (68%) of 53 allogeneic blood units, were tested for deformability.

Ektacytometry

To standardize measurements with regard to temperature, all blood samples were allowed to warm to room temperature, and measurements were made using prewarmed cuvettes (37°C) for all samples. Erythrocyte deformability was measured with a microfluidic slit-flow ektacytometer (Rheo Meditech, Seoul, South Korea). The principle behind this measurement has been described in detail.²² For deformability measurements, erythrocytes were suspended (final hematocrit ~ 0.5%) in a highly viscous polyvinylpyrrolidone solution (viscosity ~30 cP) with slow mixing and then loaded onto a sample reservoir of a microfluidics chip. During operation, a vacuum-generating mechanism allowed the sample to flow toward the waste reservoir through the micro-channel at a range of shear stresses (0.5 to 20 Pa), while the elliptical diffraction patterns of the flowing cells were generated by a laser beam (wavelength = 635 nm from a 1.5 mW laser diode) focused onto the micro-channel. Deformability is expressed as elongation index (EI), which was defined as $(L - W)/(L + W)$, where L and W are the major and minor axes of the ellipse, respectively, at various shear stress values. The EI values used for analysis were those measured at the shear stress level of 3 Pa, as suggested by Baskurt.²³

Data Analysis

The percentage change in the EI from the preoperative baseline measurement was used to assess erythrocyte deformability after surgery in patients who received minimal or moderate transfusion. Differences within groups and between groups were analyzed by repeated measures ANOVA with a Tukey-Kramer *post hoc* test for multiple comparisons of means. We compared mean EI for units with a long storage duration (≥ 21 days), units with a short storage duration (<21 days), and cell-salvage units to the EI of preoperative patient blood samples using a one-way ANOVA and unpaired Students t-tests. Dichotomous variables were analyzed by the Chi squared test. All data are presented as mean $\pm$ SD, and $P < 0.05$ was used to define significance.

Results

Intraoperatively, six patients received moderate transfusion (5–15 erythrocyte units), and 10 patients received minimal transfusion (0–3 erythrocyte units; Table 1). The mean duration of storage for erythrocyte units was 22 ± 4 days for units given to the moderately transfused patients, and 25 ± 5 days for units given to the minimally transfused patients ($P = 0.23$). In total, the 16 patients received 53 units of allogeneic and 13 units of cell-salvaged erythrocytes (~250 mL per unit) during surgery. Postoperatively, the average number of units transfused was 2.4 ± 1.3 in the moderate transfusion group and 0.8 ± 0.6 in the minimal transfusion group. The duration of storage for these units was 24 ± 10 and 32 ± 12 days, in the moderate and minimal transfusion groups, respectively ($P = 0.19$).

To illustrate the data acquired by ektacytometry, representative curves were plotted to show the measured EI at each shear stress value for four different individual blood samples taken from a preoperative patient, an allogeneic blood unit stored for a short duration (14 days), a cell-salvaged blood unit, and a blood unit stored for a long duration (38 days; Fig. 1). The accepted standard for assessing deformability at 3 Pa of shear stress is indicated and is

approximately the halfway point to the maximum EI, which was measured at the highest level of shear stress (20 Pa). Of these four samples, the most deformable erythrocytes were from the preoperative patient blood sample, followed by erythrocytes stored for 14 days and the cell-salvaged erythrocytes; the least deformable erythrocytes were those stored for 38 days.

For patients who required moderate transfusion, the mean EI decreased significantly from that measured preoperatively (baseline) to that measured at the end of surgery (12 ± 4 % decrease in EI) ($P = 0.03$). This difference persisted in the moderately transfused patients without additional significant change on postoperative days 1, 2, and 3 (Fig. 2). The maximum decrease in EI from baseline was 20 ± 6 %, on postoperative day 3. This decrease in EI was statistically significant between and within the moderate and minimal transfusion groups ($P = 0.008$). The changes in EI for patients who required minimal transfusion were small (3 ± 1 to 4 ± 1 % decrease in EI), and not significantly different from preoperative measurements ($P = 0.68$).

The mean EI for samples drawn directly from the allogeneic blood units stored ≥ 21 days, units stored < 21 days, and cell-salvaged units, were compared to the mean EI from preoperative patient blood samples (Fig. 3). The differences among groups were significant ($P = 0.008$). When individual means were compared, the mean EI for erythrocytes stored ≥ 21 days ($EI = 0.28 \pm 0.02$) was significantly less than that for both erythrocytes stored < 21 days ($EI = 0.33 \pm 0.02$) ($P = 0.001$) and erythrocytes drawn preoperatively ($EI = 0.33 \pm 0.02$) ($P = 0.001$). The EI for cell-salvaged erythrocytes ($EI = 0.30 \pm 0.03$) was significantly greater than that for erythrocytes stored ≥ 21 days ($P = 0.047$) but less than that for both preoperative patient erythrocytes ($P = 0.01$), and erythrocytes stored < 21 days ($P = 0.03$).

The EI measured in samples taken from units of blood transfused intraoperatively was plotted against storage duration (Fig. 4). The preoperative patient blood samples (0 days' storage) are plotted on the left side of the figure, and each sample from the transfused blood units is shown on the right side. There was a statistically significant and progressive decrease in the EI with increasing storage duration, with a drop-off at approximately 20 days of storage. The majority (15 of 17, 88%) of samples drawn from blood units stored < 21 days had values higher than 0.30, whereas the majority (12 of 19, 63%) of units stored ≥ 21 days had measured values below 0.30 ($P = 0.002$).

Discussion

The results indicate that erythrocytes from moderately transfused patients are significantly less deformable postoperatively than those from minimally transfused patients. The finding of impaired deformability did not reverse over a 3-day period, contrary to our hypothesis. Additionally, erythrocytes stored ≥ 21 days were less deformable than erythrocytes stored for a shorter duration or fresh erythrocytes from preoperative blood samples. Cell-salvaged erythrocytes had intermediate deformability that was between that of fresh erythrocytes and that of erythrocytes stored for ≥ 21 days, suggesting that they may undergo some detrimental changes during the cell salvage processing procedure. Another explanation for this finding may be that a significant proportion of the cell-salvaged erythrocytes were actually a mixture of older and younger allogeneic transfused cells and native patient erythrocytes that were shed during bleeding and recovered by the cell-salvage system.

The biochemical changes that occur during blood storage, such as depletion of nitric oxide, 2,3-DPG, and ATP, are rapidly reversed after stored erythrocytes are transfused back into the circulation. Nitric oxide, which is thought to contribute to the maintenance of normal deformability,^{17,18} is replenished rapidly (within hours),²⁴ and ATP concentrations recover

within 2 days.²⁰ The effects of storage on cell membrane properties are less well understood. The cell membrane undergoes multiple storage-related changes, including oxidative damage to the spectrin-actin-protein complex,¹⁴ which binds the phospholipid bilayer of the red cell cytoskeleton and leads to microvesiculation of the cell membrane.^{25,26} Lipid peroxidation also occurs,¹⁵ and all of these changes have been associated with loss of membrane deformability.¹⁹ Our findings suggest that the structural cell membrane changes associated with storage, unlike the biochemical changes, may be irreversible. The further decline in deformability over the first 3 postoperative days in the moderately transfused patients, although not a statistically significant change from the “end-of-surgery” measurement, may be related to the greater number of postoperative transfused blood units (2.8 units) compared to the minimally transfused patients (0.8 units), and the fact that the average age of these units was 24 days.

One particular feature of the storage lesion is the presence of spherocytes and echinocytes, which become prevalent after 2 weeks of storage.²⁶ These shape changes are associated with loss of deformability as measured by filterability,^{5,27} ektacytometry,²⁸ and increased osmotic fragility,²⁹ and this decreased deformability is associated with decreased erythrocyte survival.³⁰ Storage has also been associated with an increased inflammatory response,³¹ increased endothelial adhesion³² and a decrease in microvascular flow.³³ These changes are thought to become significant even after short (7-14 days) duration of storage.³⁴ Animal^{2,35} and human³⁶ studies have shown that transfusion with erythrocytes that have been stored for a long duration delivers less oxygen to the intestinal mucosa than transfusion with fresh erythrocytes. These studies illustrate that blood cells with lengthy storage times do not improve oxygen delivery at the tissue level and may predispose patients to tissue hypoxia.

Whether longer storage duration is related to adverse clinical outcomes is a topic of significant controversy. A meta-analysis that was published recently included 21 studies and more than 400,000 patients and had as its primary outcome death within 30 days or during the hospitalization.³ Overall, transfusion of blood that had been stored for longer periods was associated with increased mortality (odds ratio = 1.16, $P = 0.0001$). The studies included in the meta-analysis generally defined older blood as that which had been stored for longer than 14–21 days, and younger blood as that stored for less than 8–14 days. In the subgroup analyses, multiple organ dysfunction and pneumonia were increased in adult patients as a function of blood storage duration, and the increased mortality was recognized in trauma patients and in those undergoing both cardiac and noncardiac surgery. Limitations of the meta-analysis were that only three of the 21 studies were randomized clinical trials, trials that were randomized included small numbers of patients, and very old blood (4–6 weeks storage) was not used in most of the studies included.

Larger, more definitive, randomized clinical trials on blood storage duration and clinical outcomes are continuing and include the RECESS and the ABLE trials.^{37,38} Recently, the first large randomized trial to be completed showed no difference in outcomes in 377 neonatal patients randomized to receive fresh blood (average of 5.1 days old) or standard blood (average of 14.6 days old).³⁹ Death rate was no different (16% in each group), and the incidence of infections and length of stay were also similar between groups. Of interest is that all of these patients received blood units of relatively shorter storage duration (compared to our study), because the investigators perceived “ethical” problems in giving blood units of longer storage duration to neonatal patients, although that was not the institutional standard of care.

Since cell-salvaged erythrocytes are not stored and are thought to be fresh, we anticipated that deformability measurements of such cells would be similar to those of preoperative

patient blood samples. The finding of intermediate deformability was thus intriguing, and we can only speculate about the reasons. As previously mentioned, some of these cells may actually have been allogeneic transfused cells that were shed during bleeding, but those cells should make up a small proportion of salvaged cells, unless there is massive transfusion. Shear forces during suctioning from the surgical field as well as the effect of centrifugation during processing may cause changes in cell membrane integrity and structure.⁴⁰ Although morphologic abnormalities are seen consistently in salvaged blood cells,⁴¹ 24-hour survival for salvaged cells is not altered,⁴² and 2,3-DPG concentrations⁴³ and the oxyhemoglobin dissociation curve⁴⁴ remain normal after cell salvage. When all of these factors are considered, transfusion of cell-salvaged blood is still preferable to stored allogeneic blood, especially when cost, risk of immunologic challenge and viral transmission, and the detrimental effects of storage are considered.

Certain limitations in our study should be recognized. First, the duration of follow-up after transfusion was limited to 3 days; therefore, we are unable to determine whether additional changes to cell membrane deformability occur after this time. The posttransfusion trend, however, was in the direction of less deformability, so return to baseline with longer follow-up seems unlikely. Second, we did not randomize or control the duration of blood storage for the units that were transfused. Thus, we did not have patients who received erythrocytes of exclusively shorter or longer storage duration, and we are unable to clearly delineate whether storage duration or the number of units transfused was the primary variable associated with decreased deformability in postoperative patient blood samples. Third, we did not include blood units with very short duration of storage, simply because they are not commonly given to adults in our institution, and they were not issued to the patients in our study. Lastly, we were unable to sample every unit of transfused blood to obtain deformability measurements. Most units given intraoperatively were sampled and tested, but none of the units transfused postoperatively was tested; however, the latter is a relatively small number.

In summary, erythrocytes sampled from patients who have undergone moderate transfusion (one-half of total blood volume) have decreased deformability compared to fresh erythrocytes, and this abnormality is not reversed during the subsequent 3 days. In addition, our findings suggest that storage of erythrocytes for more than 21 days has a detrimental effect on the cell membrane that causes a loss of the cell's normal ability to deform under shear stress. Our findings also suggest that both the "dose" of blood as well as the "age of blood" are important when considering the rheological properties of circulating erythrocytes after transfusion. Reliable evidence indicates that loss of erythrocyte deformability may render the cells less effective in delivering oxygen at the tissue level, as they must traverse capillaries that are similar, or even smaller in diameter to the erythrocytes themselves. The clinical implications of these findings will not be fully evident until the ongoing large, randomized clinical trials are completed, but our findings add to the growing body of evidence that allogeneic transfusion, especially with erythrocytes that have long storage times, may not fully restore the ability of circulating blood to deliver oxygen to tissues.

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References

1. Fitzgerald RD, Martin CM, Dietz GE, Doig GS, Potter RF, Sibbald WJ. Transfusing red blood cells stored in citrate phosphate dextrose adenine-1 for 28 days fails to improve tissue oxygenation in rats. *Crit Care Med*. 1997; 25:726–32. [PubMed: 9187588]
2. van Bommel J, de Korte D, Lind A, Siegemund M, Trouwborst A, Verhoeven AJ, Ince C, Henny CP. The effect of the transfusion of stored RBCs on intestinal microvascular oxygenation in the rat. *Transfusion*. 2001; 41:1515–23. [PubMed: 11778066]
3. Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: a meta-analysis. *Transfusion*. 2012; 52:1184–95. [PubMed: 22188419]
4. Tinmouth A, Fergusson D, Yee IC, Hebert PC. Clinical consequences of red cell storage in the critically ill. *Transfusion*. 2006; 46:2014–27. [PubMed: 17076859]
5. Berezina TL, Zaets SB, Morgan C, Spillert CR, Kamiyama M, Spolarics Z, Deitch EA, Machiedo GW. Influence of storage on red blood cell rheological properties. *J Surg Res*. 2002; 102:6–12. [PubMed: 11792145]
6. Laczko J, Feo CJ, Phillips W. Discocyte--echinocyte reversibility in blood stored in CPD over a period of 56 days. *Transfusion*. 1979; 19:379–88. [PubMed: 473341]
7. Wolfe LC. The membrane and the lesions of storage in preserved red cells. *Transfusion*. 1985; 25:185–203. [PubMed: 3890284]
8. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. *Semin Thromb Hemost*. 2003; 29:435–50. [PubMed: 14631543]
9. Gladwin MT, Kim-Shapiro DB. Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. *Curr Opin Hematol*. 2009; 16:515–23. [PubMed: 19701085]
10. Young JA, Lichtman MA, Cohen J. Reduced red cell 2,3-diphosphoglycerate and adenosine triphosphate, hypophosphatemia, and increased hemoglobin-oxygen affinity after cardiac surgery. *Circulation*. 1973; 47:1313–8. [PubMed: 4541156]
11. Dern RJ, Brewer GJ, Wiorkowski JJ. Studies on the preservation of human blood. II. The relationship of erythrocyte adenosine triphosphate levels and other in vitro measures to red cell storageability. *J Lab Clin Med*. 1967; 69:968–78. [PubMed: 6025497]
12. Hovav T, Yedgar S, Manny N, Barshtein G. Alteration of red cell aggregability and shape during blood storage. *Transfusion*. 1999; 39:277–81. [PubMed: 10204590]
13. Wagner GM, Chiu DT, Yee MC, Lubin BH. Red cell vesiculation--a common membrane physiologic event. *J Lab Clin Med*. 1986; 108:315–24. [PubMed: 3760672]
14. Wagner GM, Chiu DT, Qiu JH, Heath RH, Lubin BH. Spectrin oxidation correlates with membrane vesiculation in stored RBCs. *Blood*. 1987; 69:1777–81. [PubMed: 3580578]
15. Brunauer LS, Moxness MS, Huestis WH. Hydrogen peroxide oxidation induces the transfer of phospholipids from the membrane into the cytosol of human erythrocytes. *Biochemistry*. 1994; 33:4527–32. [PubMed: 8161507]
16. Karger R, Lukow C, Kretschmer V. Deformability of Red Blood Cells and Correlation with ATP Content during Storage as Leukocyte-Depleted Whole Blood. *Transfus Med Hemother*. 2012; 39:277–82. [PubMed: 22969698]
17. Bor-Kucukatay M, Wenby RB, Meiselman HJ, Baskurt OK. Effects of nitric oxide on red blood cell deformability. *Am J Physiol Heart Circ Physiol*. 2003; 284:H1577–84. [PubMed: 12521942]
18. Korbut R, Gryglewski RJ. Nitric oxide from polymorphonuclear leukocytes modulates red blood cell deformability in vitro. *Eur J Pharmacol*. 1993; 234:17–22. [PubMed: 8472757]
19. Card RT. Red cell membrane changes during storage. *Transfus Med Rev*. 1988; 2:40–7. [PubMed: 2980078]
20. Heaton A, Keegan T, Holme S. In vivo regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-1, AS-3 and CPDA-1 red cells. *Br J Haematol*. 1989; 71:131–6. [PubMed: 2492818]
21. Reynolds JD, Ahearn GS, Angelo M, Zhang J, Cobb F, Stamler JS. S-nitrosohemoglobin deficiency: a mechanism for loss of physiological activity in banked blood. *Proc Natl Acad Sci USA*. 2007; 104:17058–62. [PubMed: 17940022]

22. Shin S, Ku Y, Park MS, Suh JS. Slit-flow ektacytometry: laser diffraction in a slit rheometer. *Cytometry B Clin Cytom.* 2005; 65:6–13. [PubMed: 15786510]
23. Baskurt OK. Deformability of red blood cells from different species studied by resistive pulse shape analysis technique. *Biorheology.* 1996; 33:169–79. [PubMed: 8679963]
24. Tsai AG, Hofmann A, Cabrales P, Intaglietta M. Perfusion vs. oxygen delivery in transfusion with “fresh” and “old” red blood cells: the experimental evidence. *Transfus Apher Sci.* 2010; 43:69–78. [PubMed: 20646963]
25. Knight JA, Voorhees RP, Martin L, Anstall H. Lipid peroxidation in stored red cells. *Transfusion.* 1992; 32:354–7. [PubMed: 1585442]
26. Rumsby MG, Trotter J, Allan D, Michell RH. Recovery of membrane micro-vesicles from human erythrocytes stored for transfusion: a mechanism for the erythrocyte discocyte-to-spherocyte shape transformation. *Biochem Soc Trans.* 1977; 5:126–8. [PubMed: 892138]
27. Kirkpatrick UJ, Adams RA, Lardi A, McCollum CN. Rheological properties and function of blood cells in stored bank blood and salvaged blood. *Br J Haematol.* 1998; 101:364–8. [PubMed: 9609536]
28. Baskurt OK, Meiselman HJ. Determination of red blood cell shape recovery time constant in a Couette system by the analysis of light reflectance and ektacytometry. *Biorheology.* 1996; 33:489–503. [PubMed: 9093444]
29. Beutler E, West C. Storage of red cell concentrates in CPD-A2 for 42 and 49 days. *The J Lab Clin Med.* 1983; 102:53–62.
30. Card RT, Mohandas N, Mollison PL. Relationship of post-transfusion viability to deformability of stored red cells. *Br J Haematol.* 1983; 53:237–40. [PubMed: 6821653]
31. Dong A, Sunkara M, Panchatcharam M, Salous A, Selim S, Morris AJ, Smyth SS. Synergistic effect of anemia and red blood cells transfusion on inflammation and lung injury. *Adv Hematol.* 2012; 2012:924042. [PubMed: 22919395]
32. Relevy H, Koshkaryev A, Manny N, Yedgar S, Barshtein G. Blood banking-induced alteration of red blood cell flow properties. *Transfusion.* 2008; 48:136–46. [PubMed: 17900281]
33. Tsai AG, Cabrales P, Intaglietta M. Microvascular perfusion upon exchange transfusion with stored red blood cells in normovolemic anemic conditions. *Transfusion.* 2004; 44:1626–34. [PubMed: 15504169]
34. Barshtein G, Manny N, Yedgar S. Circulatory risk in the transfusion of red blood cells with impaired flow properties induced by storage. *Transfus Med Rev.* 2011; 25:24–35. [PubMed: 21134624]
35. Raat NJ, Verhoeven AJ, Mik EG, Gouwerok CW, Verhaar R, Goedhart PT, de Korte D, Ince C. The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. *Crit Care Med.* 2005; 33:39–45. discussion 238–9. [PubMed: 15644646]
36. Marik PE, Sibbald WJ. Effect of stored-blood transfusion on oxygen delivery in patients with sepsis. *JAMA.* 1993; 269:3024–9. [PubMed: 8501845]
37. Lacroix J, Hebert P, Fergusson D, Tinmouth A, Blajchman MA, Callum J, Cook D, Marshall JC, McIntyre L, Turgeon AF. The Age of Blood Evaluation (ABLE) randomized controlled trial: study design. *Transfus Med Rev.* 2011; 25:197–205. [PubMed: 21550205]
38. RECESS Study. <http://clinicaltrials.gov/ct2/show/NCT00991341>
39. Fergusson DA, Hebert P, Hogan DL, LeBel L, Rouvinez-Bouali N, Smyth JA, Sankaran K, Tinmouth A, Blajchman MA, Kovacs L, Lachance C, Lee S, Walker CR, Hutton B, Ducharme R, Balchin K, Ramsay T, Ford JC, Kakadekar A, Ramesh K, Shapiro S. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial. *JAMA.* 2012; 308:1443–51. [PubMed: 23045213]
40. Reeder GD. Autotransfusion theory of operation: a review of the physics and hematology. *Transfusion.* 2004; 44:35S–9S. [PubMed: 15585003]
41. Yawn DH. Blood salvage for cardiovascular surgery. *Perfusion.* 1990; 5:31–7. [PubMed: 10149500]

42. Kent P, Ashley S, Thorley PJ, Shaw A, Parkin A, Kester RC. 24-hour survival of autotransfused red cells in elective aortic surgery: a comparison of two intraoperative autotransfusion systems. *Br J Surg.* 1991; 78:1473–5. [PubMed: 1773329]
43. Noon GP, Solis RT, Natelson EA. A simple method of intraoperative autotransfusion. *Surg Gynecol Obstet.* 1976; 143:65–70. [PubMed: 936052]
44. Kalra M, Beech MJ, al-Khaffaf H, Charlesworth D. Autotransfusion in aortic surgery: the Haemocell System 350 cell saver. *Br J Surg.* 1993; 80:32–5. [PubMed: 8428286]

Fig. 1.

Representative curves plotting the elongation index (EI) at increasing levels of shear stress as measured by ektacytometry. Erythrocytes with a greater EI are more deformable and thereby can transition from the normally round-shaped cell to an elongated, elliptical-shaped cell when subjected to shear stress.

Fig. 2.

Erythrocytes drawn from patients who underwent moderate blood transfusion (5 units) exhibited a greater decrease in elongation index (EI) (loss of deformability) compared to erythrocytes drawn from patients who underwent minimal transfusion (0–3 blood units). The EI was significantly lower at the end of surgery than at the preoperative baseline ($P = 0.03$), and this decreased deformability persisted through postoperative day (POD) 3. The small decrease in deformability from baseline in the minimal transfusion group over the 3-day period was not significant. Overall, the differences between and within groups were statistically significant ($P = 0.008$). *Significantly different from preoperative patient blood and minimal transfusion group.

Fig. 3.

Comparison of erythrocyte elongation index (EI) in blood drawn preoperatively (preop), in cell salvage blood, and in allogeneic blood units stored for ≥ 21 days or <21 days. A greater EI indicates a more deformable erythrocyte cell membrane. The differences among the four groups were significant ($P = 0.008$). Deformability for erythrocytes stored for the longer duration (≥ 21 days) was significantly less than that for erythrocytes stored <21 days ($P = 0.001$) and erythrocytes drawn from patients preoperatively ($P = 0.001$). Deformability for cell salvage erythrocytes was significantly greater than that of erythrocytes stored ≥ 21 days ($P = 0.047$) and less than that of both preoperative patient blood ($P = 0.01$) and blood stored <21 days ($P = 0.03$). *Significantly less than preoperative patient blood and blood stored <21 days; #significantly less than preoperative patient blood and blood units of <21 day storage duration, and significantly greater than blood units of ≥ 21 day storage duration.

Fig. 4.

Comparison of erythrocyte deformability in preoperative patient blood, blood units stored for a short duration (12–20 days), and blood units stored for a long duration (21–40 days). Loss of deformability as measured by ektacytometry is indicated by a decreased elongation index (EI). Erythrocyte deformability was significantly less in samples drawn from blood units of longer storage duration than in samples drawn from preoperative patient blood or samples drawn from blood units with shorter storage duration. * $P = 0.001$ compared to preoperative patient blood and blood units stored 12–20 days.

Table 1

Demographic Data and Intraoperative Allogeneic and Cell Salvage Blood Transfusion Requirements

Patient Number	Age (yrs)	Sex	Surgical Procedure	Minimal or Moderate Transfusion	No. of Intraoperative Allogeneic RBC Units Transfused	No. of Intraoperative Cell Salvage Units* Transfused	Mean (range) Duration of Storage for Allogeneic RBCs (days)
#1	56	M	T7 - L4 Thoracolumbar fusion	Moderate	13	2	18 ± 8 (12-33)
#2	67	M	T11 - S2, Thoracolumbosacral fusion	Moderate	9	3	20 ± 5 (15-24)
#3	49	F	T - S1 Thoracolumbar fusion	Moderate	7	2	28 ± 5 (22-38)
#4	68	F	T3 - S1 Thoracolumbar fusion	Moderate	6	1	18 ± 1 (17-20)
#5	64	F	T11 - S1 Thoracolumbar fusion	Moderate	5	1	31 ± 2 (28-32)
#6	51	M	L2 - L5 Lumbar fusion	Moderate	4	1	25 ± 1 (20-26)
#7	58	F	7C - T1 Cervical thoracic fusion	Minimal	3	0	19 ± 8 (14-28)
#8	42	M	T11 - L5 Thoracolumbar fusion	Minimal	2	1	40 ± 0 (40, 40)
#9	35	F	T6 - T9 Thoracic fusion	Minimal	2	0	27 ± 2 (25-28)
#10	39	M	L2 - L5 Lumbar fusion	Minimal	1	2	18 ± 0 (18)
#11	63	M	L1 - L4 Lumbar fusion	Minimal	1	0	20 ± 0 (20)
#12	48	F	C4 - C7 Cervical fusion	Minimal	0	0	-
#13	68	F	T1 - T4, Thoracic fusion	Minimal	0	0	-
#14	45	F	T2 - L2 Thoracolumbar fusion	Minimal	0	0	-
#15	42	M	L5 - S1 Lumbar fusion	Minimal	0	0	-
#16	48	F	L2 - L5 Lumbar fusion	Minimal	0	0	-

* Each unit was 250 mL.

RBC = erythrocyte