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Properties of stored RBCs: Understanding immune and vascular reactivity

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Blood transfusion is an integral component of life-saving fluid resuscitation for anemic, surgical, oncological, and severely injured patients. However, there have been well-publicized studies suggesting that blood may have harmful side-effects. One possible factor contributing to adverse effects of transfusion is the duration of red blood cell storage prior to transfusion. Our group is exploring possible mechanisms of a putative “storage lesion”, including measuring modulation of the immune system or changes in adhesion properties between endothelial cells and transfused red blood cells. The following review will explore changes that occur during red blood cell storage and how these might impact pathways that are important in transfusion recipients’ clinical outcome, as well as provide a broad overview of the research our group is performing on the topic.

Known physiologic effects of stored blood

There are several well described mechanisms by which stored or damaged red blood cells can contribute to immune activation and vascular reactivity. These mechanisms include release of hemoglobin, heme and iron from ruptured cells, the shedding of microvesicles with negatively charged surfaces from intact cells¹, the loss of lipid and protein products with biologic activities from damaged red cells², and the activation of red cell adhesins³. The accumulation of these products and activities over the time of blood storage means that they are presented to the body in high activities or concentrations at the time of transfusion.

Hemoglobin, heme and iron can all cause redox injuries and are increasingly toxic to mononuclear and endothelial cells as the iron is unpackaged. Iron-stimulated mononuclear cells secrete the cytokines TNF and IL-1 and the chemokine IL-8 in response to stimulation which further amplifies endothelial cell coagulant responses and immune responses by stimulating and localizing immune effector cells⁴. The endothelial cells in turn respond by shedding thrombomodulin and glycocalyx limiting the localization of coagulation and the diffusion of the immune response respectively⁵.

Microvesicles are normally shed from reticulocytes, healthy red cells, and effete red cells. Their loss from reticulocytes is part of the remodeling process that sees reticulocyte cell volume shrink from 140 fL to 90 fL over the first day or two in the circulation. Normal red cells use the microvesiculation process as a way of shedding oxidized and polymerized lipids. These surfaces are procoagulant and proinflammatory. Effete cells shed microvesicles as part of a process of programmed cell death. These microvesicles are marked by exposed phosphatidyl serine and are markedly procoagulant⁶.

Abnormal breakdown of red cell constituents during storage can lead to their delivery at high concentrations during transfusion. The lysophospholipid platelet activating factor (PAF) can activate oxidative attack which is not inhibited because stored red cells have low concentrations of glutathione, or by phospholipase released for white cells stored with the red cells. PAF has been described a potent second-hit effector in transfusion-related acute lung injury (TRALI)⁷. Beta-hemorphins are fragments of the beta globin chain with physiologic activity that can be both proinflammatory and cytotoxic.

Red cells have a guanidine triphosphate (GTP) requirement just as they have an ATP requirement. GTP directly inhibits a red cell transglutaminase with a factor XIII-like activity which can be leaked in storage-damaged red cells. GTP also provides energy for inhibiting G-protein coupled reactions, one of which is involved in the process by which the Lutheran blood group protein is activated to become an intracellular adhesin capable of binding damaged red cells to the walls of post-capillary venules.

All of these materials and activities are normal parts of the red cell life cycle, but they are concentrated in space and time by the red cell storage and transfusion process. These materials and activities are presented in abnormal concentrations to sick patients who have less reserve to handle the extra physiologic or chemical loads. The pathophysiology of red cell storage is necessarily complex and probably greatest in the sickest patients (Figure 1).

Transfusion-related immunomodulation (TRIM) and RBC storage

Originally TRIM was perceived to be due to adverse pro-inflammatory or immunosuppressive responses, which unlike allergic or hypersensitivity reactions, are not immediately apparent upon transfusion, but nevertheless manifest as a poor outcome resulting in increased morbidity or mortality⁸. With increasing evidence about the complex interactions between blood cells (RBCs, platelets, leukocytes), plasma factors (including the coagulation system), and endothelium, the concept of TRIM has broadened to encompass the whole spectrum of hemodynamic control of blood and the consequential effects of perturbations on organs and tissues⁹.

Of relevance is the proposed '2-insult' model of post-transfusion injury, in which the first insult (i.e. the patient's underlying inflammatory condition) primes the patient's immune cells or endothelium, and frank inflammation is triggered by a second inflammatory insult, resulting in full-scale activation, be it immune and/or thrombotic. Transfusion has been proposed as a potential second insult¹⁰. This model provides a feasible basis to begin to understand the biological mechanisms by which transfusion has been implicated as a risk factor for poor outcome, including transfusion-related acute lung injury (TRALI)¹¹. The blood component parameters that are implicated in TRIM include: 1) leukocyte content; 2) length of storage; 3) RBC component processing method; 4) storage solution composition; and 5) number of units transfused¹². Although the implementation of pre-storage leukoreduction of blood components has produced a marked decrease in the incidence of transfusion-related adverse reactions, it is likely to have only a partial benefit at best on TRIM, because storage-related changes to RBCs very likely contribute to TRIM⁹.

Effects of RBC storage on RBC-endothelial cell interaction

The primary clinical objective of RBC transfusion is the restoration of oxygen carrying capacity, however it is becoming increasingly apparent that transfusion has an equally important role in the restoration of microvascular function, including blood rheology and functional capillary density¹³⁻¹⁶. The adhesion of RBCs to the vascular endothelium has important consequences for RBC transfusion and blood rheology.

In healthy individuals, RBCs do not readily adhere to blood vessel walls, thus maintaining smooth blood flow. Adhesion of RBCs to vascular endothelium can disrupt local blood flow patterns, decrease oxygen delivery to peripheral tissues and in severe cases, lead to occlusion of microvessels¹⁷. In diseases that affect RBCs, such as malaria¹⁸, sickle cell disease¹⁹, beta-thalassemia²⁰, and diabetes mellitus²¹, the incidence of RBC adhesion to vascular endothelium is significantly increased and contributes to vascular dysfunction and morbidity. These diseases are characterized by pathological changes to the RBCs that include changes to cell constituents, membrane receptors and cytoskeletal disruptions that affect RBC shape, deformability and rheology. These disease-related RBC changes appear to bear at least some similarities to the changes that occur to RBCs during refrigerated storage prior to transfusion²².

Recent studies using *in vitro* flow perfusion models to simulate blood flow have demonstrated that stored RBCs adhere to vascular endothelial cells (ECs) and that the number of adherent RBCs increases with storage duration^{3,23,24}. Pre-storage leukocyte-reduction of the RBC units reduces the number of adherent RBCs, but does not eliminate the effect, suggesting that RBC adhesion is induced, at least in part, by storage-related changes, independent of leukocyte-induced effects. Pretreatment of the ECs with endotoxin to activate the ECs and mimic infection, results in increased strength of adhesion of RBCs to the activated ECs²⁵.

The identity of the biological mechanisms involved in the interaction of stored RBCs with ECs is yet to be elucidated. Exposure of the apoptotic marker, phosphatidylserine (PS), at the external RBC membrane has been proposed to be involved²⁴. Reports of PS exposure on stored RBCs vary between laboratories. It is our experience that only a very small, if not undetectable, proportion of stored RBCs express PS at the external membrane, which does not change with storage²⁶. Instead, the number of PS-enriched microparticles shed from RBCs increases with storage time^{27,28}, consistent with the notion that more than one mechanism is involved in the adhesion of stored RBCs to ECs.

Mature circulating RBCs express an array of cell adhesion molecules (e.g. CD44, CD47, CD58, CD147, CD239, CD242), but the function of these molecules on RBCs is not well understood²⁹. During refrigerated storage of RBC units, cell surface proteins are gradually lost and RBCs deemed to be “chronologically older” have lower levels of cell membrane receptors compared to “chronologically younger” RBCs²⁶. Changes to RBC membrane carbohydrates during storage of RBC units also occurs³⁰. These changes, together with oxidative and enzymatic changes to RBC membrane constituents, altered RBC shape, and decreased RBC deformability that occur during refrigerated storage of RBC units are all potential candidates for eliciting altered cell-cell interactions in the transfusion recipient.

RBC-EC adhesion models

In vitro and *in vivo* models of endothelial perfusion provide a way to identify the mechanisms of stored RBC adhesion to endothelium, although they are inevitably challenged by the complexity of systemic biology. Vascular ECs respond differently to different shear stresses or no stress (i.e. static conditions)¹³. In order to be physiologically relevant, *in vitro* models of blood flow need to be conducted under continuous perfusion conditions at controlled shear stresses that mimic physiological blood flow of blood vessels or the microvasculature¹⁷. The general set-up of a flow perfusion system requires ECs to be cultured to a confluent monolayer on an appropriate surface such as a microvolume perfusion chamber or capillary and then mounted on an inverted microscope fitted with a CCD camera and imaging software capable of video analysis or automated time-sequence image capture. Alternatively, dissected intact microvessels can be used as an *ex vivo* vessel

chamber model³¹. The perfusion medium and cell suspension are perfused across the ECs at an appropriate flow rate controlled by a programmable pump (e.g. a syringe pump).

Intravital microscopy (IVM) is the most relevant technique currently available to study live-animal *in vivo* microvascular blood flow³². Rat and mouse models have been described. IVM has predominantly been used to study intravascular leukocyte trafficking, thrombus formation and sickle cell vaso-occlusion³³⁻³⁵. Preliminary studies have used the rat IVM model to investigate intravascular blood flow following transfusion of stored rat RBCs^{36,37}. Stored RBCs caused increased microcirculatory disturbance and increased adhesion compared to fresh RBCs. Pre-storage leukocyte-depletion reduced RBC-vascular adhesion³⁷. Since rodent RBCs have a much shorter life-span than human RBCs, a significant challenge in conducting these experiments is the need to establish appropriate blood processing and storage conditions to replicate banked human RBC units¹³. Species differences in terms of physiology, metabolism and cell biology need to be carefully considered before extrapolating findings from animal blood transfusion models to humans. Both the *in vivo* and *in vitro* models are technically demanding, require highly skilled and trained operators and specialized equipment. These requirements have limited the number of laboratories that have established these technologies, but nevertheless they provide a powerful means to investigate the dynamics of microvascular function and blood transfusion.

The challenge now before us is 1) to better understand the biological mechanisms of adhesion of stored RBCs to vascular endothelium, 2) to identify strategies that mitigate these effects and 3) ultimately to determine whether such strategies improve clinical outcome following transfusion. There is no doubt that the biology is complex and that multifaceted mechanisms operate both in terms of storage-related changes to RBCs (i.e. the “storage lesion”) and the nature of the adhesive interactions between stored RBCs and vascular endothelium.

Clinical evidence of a RBC storage lesion

There is conflicting evidence regarding the association between RBC storage age and adverse clinical outcomes²². Studies in critically ill (traumatic injury, cardiac surgery, and general intensive care) patients have reported an independent association between RBCs of increased storage age and increased risk of infection, deep vein thrombosis, cancer recurrence, organ failure, ICU length of stay, and mortality in both adults and children³⁸⁻⁵². The RBCs transfused were exclusively prestorage leukocyte reduced in a few of these reports^{44,49,51,52}. When patient study groups were categorized by a maximum RBC storage duration, RBCs greater than 14-28 days were associated with worse outcomes^{41,44,46,49-51}. When RBC age was evaluated as a continuous variable in a study of 4993 adult patients with cardiovascular disease, each day of RBC storage had an odds ratio of 1.02 for mortality⁵¹. Other studies evaluating cardiac surgery patients with low risk of adverse outcomes or non-critically ill patients have not reported an association between RBC storage age and adverse outcomes⁵³⁻⁵⁸. All of these retrospective analyses, are confounded by at least one of these factors: selection bias, confounding by indication, definition of exposure, lack of power, inability to adjust for unmeasured confounders and to adequately adjust for the number of RBC transfusions.

An important consideration when analyzing the current literature is the potential effect of severity of illness on the association between RBC age and clinical outcomes. If the two-hit hypothesis applies, patients with increased severity of illness would be at highest risk of poor outcomes secondary to exposure to RBCs of increased storage age⁹. A close examination of the reported literature would indicate that any adverse outcomes associated

with RBCs of increased storage duration most likely occurs in patients with increased severity of illness.

Interestingly, there is also a potential risk of transfusing RBC units of decreased storage duration. The risk of contracting infections caused by untested pathogens and certain intracellular viruses like CMV and HSV⁵⁹⁻⁶², transfusion-associated microchimerism^{63,64} and, in immunocompetent patients, the very rare risk of transfusion-associated graft versus host disease⁶⁵⁻⁶⁷ is increased with the use of RBC units of decreased storage duration. The risk benefit ratio on outcomes according to RBC storage duration may differ according to the patient population studied. The few ongoing randomized controlled clinical trials examining this question in adults and premature neonates, as well as the planned trial in children will hopefully provide answers to these questions.

Clinical trials to assess the RBC storage lesion

Currently there are three large multicenter randomized controlled trials studies that are being performed and one in preparation that will analyze the effect of RBC storage age on outcomes in critically ill patients. The Age of BLOOD Evaluation (ABLE) study, which is being conducted by Dr. Jacques Lacroix, is a RCT in which 2500 adult patients expected to require mechanical ventilation for greater than 48 hours are being randomized to either being transfused RBCs ≤ 7 days of storage time or to standard issue RBCs (mean storage time of 14-21 days); the primary outcome is 90-day mortality. The second ongoing adult (≥ 12 years of age) trial is the RED CELL Storage duration Study (RECESS), which is being conducted by The Transfusion Medicine Hemostasis Clinical Trial Network⁶⁸. In this study, approximately 1800 patients who have had complex cardiac surgery with sternotomy will be randomized to either RBCs ≤ 10 days or RBCs ≥ 21 days. The primary outcome is a change in composite multiple organ dysfunction score from preoperative baseline. There is also an ongoing trial called Age of Red Blood Cells in Premature Infants (ARIP) which is being conducted by Dr. Dean Fergusson⁶⁹. In this study of low birth weight infants (< 1250 grams), 450 patients will be randomized to either RBCs of ≤ 7 days of storage or to standard practice in neonatal ICU, which frequently means that the same RBC unit will be used repeatedly over time. The primary endpoint is a composite outcome of necrotizing enterocolitis, intraventricular hemorrhage, bronchopulmonary dysplasia and retinopathy of prematurity at 30 and 90 days. The Age of Blood in Children (ABC) study is a randomized controlled trial in pediatric patients (3 days to 16 years of age) which is being planned by Drs. Tucci, Spinella, and Lacroix. It will randomize 1502 critically ill children to either RBCs ≤ 7 days or standard issue RBCs (mean storage time of 14-21 days), and the primary outcome will be new or progressive multiple organ dysfunction at 28 days post enrollment.

There are mechanism studies and repository development projects that will start in early 2011 for both the ABLE and RECESS trials. In the ABLE trial, our research group, with Dr Mitchell Cohen, will enroll 200 patients and examine markers of inflammation, endothelial function, coagulation, T cell function, panel of microparticles, and the incidence and magnitude of transfusion associated microchimerism. These parameters will be measured pre-transfusion, day 2, day 6, day 28 and day 180 (day 180 for immune function and microchimerism only). Our hypotheses are that the transfusion of RBCs of increased storage duration will be associated with immune suppression, hypercoagulability, and increased microparticles, whereas patients transfused RBCs of decreased storage duration will be at increased risk of transfusion associated microchimerism at 6 months post transfusion. Remaining plasma and PBMC samples from these 200 patients will be maintained in a sample repository for future analysis. In the RECESS trial, our research group with Drs. Allan Doctor, Jerrold Levy, and Marie Steiner will develop a repository of RBC, plasma, and PBMC samples from 500 patients at time points; pre-transfusion, day 2, day 6, day 28

and day 180. There will be over 20,000 aliquots of samples available in the repository when completed. We will also study 170 patients within the RECESS trial and another 50 healthy volunteers for inflammatory and coagulation parameters, nitric oxide measures, a panel of microparticles, and immune function. An additional hypothesis in this study is that RBCs of increased storage duration will be associated with decreased nitric oxide availability and decreased vasoregulation capability. We also hypothesize that increased microparticle counts in patients transfused RBCs of increased storage duration will be associated with immune suppression and a hypercoagulable state. An added benefit of these two repository and mechanism studies is that they are being lead by the same team of investigators (Norris and Spinella) which ensures that samples will be collected at the same time intervals and sample processing and analysis will be the same. This coordination will allow for comparisons of similar parameters measured between general ICU patients in the ABLE study to post-op cardiac surgery patients in the RECESS trial.

Possible methods of ameliorating the red cell storage lesion

Much of the current work focuses on defining whether or not the RBC storage lesion is significant in clinical practice, and if so, defining the mechanistic pathways affected by storage of RBCs. Once clearly identified, there would be great interest in reversing or preventing a RBC storage lesion. Potential ways to store RBCs better include improved storage solutions and optimal physical conditions of storage. Improved storage solutions can provide critical nutrients and buffer capacity to allow the RBCs to retain functionality after storage. Thus, additional phosphate and bicarbonate can keep pH and ATP concentrations higher resulting in better glutathione-mediated protection of lipids and less microvesiculation. Storing RBCs in the cold and in the absence of oxygen can reduce metabolism generally and eliminate a major source of oxidative injury. While removing oxygen from blood is difficult, building better refrigerators that stored red cells at 0°C rather than 3°C has already been accomplished and will reduce RBC metabolism and catabolism by half⁷⁰. Such refrigerators are already widely used for green leafy vegetable storage in homes in Japan and Korea.

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References

1. Bosman GJ, Lasonder E, Luten M, et al. The proteome of red cell membranes and vesicles during storage in blood bank conditions. *Transfusion*. 2008; 48:827–35. [PubMed: 18346024]
2. Silliman CC, Clay KL, Thurman GW, et al. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. *J Lab Clin Med*. 1994; 124:684–94. [PubMed: 7964126]
3. Anniss AM, Sparrow RL. Storage duration and white blood cell content of red blood cell (RBC) products increases adhesion of stored RBCs to endothelium under flow conditions. *Transfusion*. 2006; 46:1561–7. [PubMed: 16965584]
4. McFaul SJ, Bowman PD, Villa VM, et al. Hemoglobin stimulates mononuclear leukocytes to release interleukin-8 and tumor necrosis factor alpha. *Blood*. 1994; 84:3175–81. [PubMed: 7949190]
5. Pati S, Matijevic N, Doursout MF, et al. Protective effects of fresh frozen plasma on vascular endothelial permeability, coagulation, and resuscitation after hemorrhagic shock are time dependent and diminish between days 0 and 5 after thaw. *J Trauma*. 2010; 69(Suppl 1):S55–63. [PubMed: 20622621]
6. Greenwalt TJ. The how and why of exocytic vesicles. *Transfusion*. 2006; 46:143–52. [PubMed: 16398744]

7. Silliman CC, Voelkel NF, Allard JD, et al. Plasma and lipids from stored packed red blood cells cause acute lung injury in an animal model. *J Clin Invest*. 1998; 101:1458–67. [PubMed: 9525989]
8. Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood*. 2001; 97:1180–95. [PubMed: 11222359]
9. 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]
10. Aiboshi J, Moore EE, Ciesla DJ, Silliman CC. Blood transfusion and the two-insult model of post-injury multiple organ failure. *Shock*. 2001; 15:302–6. [PubMed: 11303730]
11. Silliman CC. The two-event model of transfusion-related acute lung injury. *Crit Care Med*. 2006; 34:S124–31. [PubMed: 16617256]
12. Sparrow RL, Patton KA. Supernatant from stored red blood cell primes inflammatory cells: influence of prestorage white cell reduction. *Transfusion*. 2004; 44:722–30. [PubMed: 15104654]
13. 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]
14. 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]
15. Lipowsky HH. Microvascular rheology and hemodynamics. *Microcirculation*. 2005; 12:5–15. [PubMed: 15804970]
16. Somani A, Steiner ME, Hebbel RP. The dynamic regulation of microcirculatory conduit function: features relevant to transfusion medicine. *Transfus Apher Sci*. 2010; 43:61–8. [PubMed: 20580315]
17. Shiu YT, McIntire LV. In vitro studies of erythrocyte-vascular endothelium interactions. *Ann Biomed Eng*. 2003; 31:1299–313. [PubMed: 14758921]
18. Cooke BM, Mohandas N, Coppel RL. Malaria and the red blood cell membrane. *Semin Hematol*. 2004; 41:173–88. [PubMed: 15071793]
19. Parise LV, Telen MJ. Erythrocyte adhesion in sickle cell disease. *Curr Hematol Rep*. 2003; 2:102–8. [PubMed: 12901140]
20. Hovav T, Goldfarb A, Artmann G, et al. Enhanced adherence of beta-thalassaemic erythrocytes to endothelial cells. *Br J Haematol*. 1999; 106:178–81. [PubMed: 10444184]
21. Wautier JL, Wautier MP. Erythrocytes and platelet adhesion to endothelium are mediated by specialized molecules. *Clin Hemorheol Microcirc*. 2004; 30:181–4. [PubMed: 15258342]
22. Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. *Vox Sang*. 2009; 96:93–103. [PubMed: 19152602]
23. Luk CS, Gray-Statchuk LA, Cepinkas G, Chin-Yee IH. WBC reduction reduces storage-associated RBC adhesion to human vascular endothelial cells under conditions of continuous flow in vitro. *Transfusion*. 2003; 43:151–6. [PubMed: 12559009]
24. Relevy H, Koshkaryev A, Manny N, et al. Blood banking-induced alteration of red blood cell flow properties. *Transfusion*. 2008; 48:136–46. [PubMed: 17900281]
25. Aniss AM, Sparrow RL. Variable adhesion of different red blood cell products to activated vascular endothelium under flow conditions. *Am J Hematol*. 2007; 82:439–45. [PubMed: 17133424]
26. Sparrow RL, Healey G, Patton KA, Veale MF. Red blood cell age determines the impact of storage and leukocyte burden on cell adhesion molecules, glycophorin A and the release of annexin V. *Transfus Apher Sci*. 2006; 34:15–23. [PubMed: 16377250]
27. Xiong Z, Cavaretta J, Qu L, et al. Red blood cell microparticles show altered inflammatory chemokine binding and release ligand upon interaction with platelets. *Transfusion*. in press.
28. Veale MF, Healey G, Sparrow RL. Effect of additive solutions on red blood cell (RBC) membrane properties of stored RBCs prepared from whole blood held for 24 hours at room temperature. *Transfusion*. in press.
29. Telen MJ. Erythrocyte adhesion receptors: blood group antigens and related molecules. *Transfus Med Rev*. 2005; 19:32–44. [PubMed: 15830326]

30. Sparrow RL, Veale MF, Healey G, Payne KA. Red blood cell (RBC) age at collection and storage influences RBC membrane-associated carbohydrates and lectin binding. *Transfusion*. 2007; 47:966–8. [PubMed: 17524084]
31. Woollard KJ, Suhartoyo A, Harris EE, et al. Pathophysiological levels of soluble P-selectin mediate adhesion of leukocytes to the endothelium through Mac-1 activation. *Circ Res*. 2008; 103:1128–38. [PubMed: 18818407]
32. Weigert R, Sramkova M, Parente L, et al. Intravital microscopy: a novel tool to study cell biology in living animals. *Histochem Cell Biol*. 2010; 133:481–91. [PubMed: 20372919]
33. Deane JA, Hickey MJ. Molecular mechanisms of leukocyte trafficking in T-cell-mediated skin inflammation: insights from intravital imaging. *Expert Rev Mol Med*. 2009; 11:e25. [PubMed: 19691914]
34. Chang J, Patton JT, Sarkar A, et al. GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood*. 2010; 116:1779–86. [PubMed: 20508165]
35. Furie B, Furie BC. In vivo thrombus formation. *J Thromb Haemost*. 2007; 5(Suppl 1):12–7. [PubMed: 17635703]
36. Arslan E, Sierko E, Waters JH, Siemionow M. Microcirculatory hemodynamics after acute blood loss followed by fresh and banked blood transfusion. *Am J Surg*. 2005; 190:456–62. [PubMed: 16105536]
37. Chin-Yee IH, Gray-Statchuk L, Milkovich S, Ellis CG. Transfusion of stored red blood cells adhere in the rat microvasculature. *Transfusion*. 2009; 49:2304–10. [PubMed: 19624601]
38. Keller ME, Jean R, LaMorte WW, et al. Effects of age of transfused blood on length of stay in trauma patients: A preliminary report. *Journal of Trauma-Injury Infection & Critical Care*. 2002; 53:1023–5.
39. Napolitano LM, Corwin HL. Efficacy of blood transfusion in the critically ill: Does age of blood make a difference? *Crit Care Med*. 2004; 32:594–5. [PubMed: 14758188]
40. Purdy FR, Tweeddale MG, Merrick PM. Association of mortality with age of blood transfused in septic ICU patients. *Can J Anaesth*. 1997; 44:1256–61. [PubMed: 9429042]
41. Spinella PC, Carroll C, Staff I. Increased deep vein thrombosis and in hospital mortality with the transfusion of old RBCs for patients with traumatic injuries. *Vox Sang*. 2009; 96:44. [PubMed: 19121197]
42. Weinberg JA, McGwin G Jr, Marques MB, et al. Transfusions in the less severely injured: does age of transfused blood affect outcomes? *J Trauma*. 2008; 65:794–8. [PubMed: 18849793]
43. Zallen G, Offner PJ, Moore EE, et al. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *Am J Surg*. 1999; 178:570–2. [PubMed: 10670874]
44. Weinberg JA, McGwin G Jr, Vandromme MJ, et al. Duration of red cell storage influences mortality after trauma. *J Trauma*. 2010; 69:1427–32. [PubMed: 21150522]
45. Edgren G, Kamper-Jorgensen M, Eloranta S, et al. Duration of red blood cell storage and survival of transfused patients (CME). *Transfusion*. 2010; 50:1185–95. [PubMed: 20158690]
46. Koch CG, Li L, Sessler DI, et al. Duration of red-cell storage and complications after cardiac surgery. *N Engl J Med*. 2008; 358:1229–39. [PubMed: 18354101]
47. Basran S, Frumento RJ, Cohen A, et al. The association between duration of storage of transfused red blood cells and morbidity and mortality after reoperative cardiac surgery. *Anesth Analg*. 2006; 103:15–20. table of contents. [PubMed: 16790618]
48. Mynster T, Nielsen HJ. Storage time of transfused blood and disease recurrence after colorectal cancer surgery. *Dis Colon Rectum*. 2001; 44:955–64. [PubMed: 11496075]
49. Gauvin F, Spinella PC, Lacroix J, et al. Association between length of storage of transfused red blood cells and multiple organ dysfunction syndrome in pediatric intensive care patients. *Transfusion*. 2010
50. Karam O, Tucci M, Bateman ST, et al. Association between length of storage of red blood cell units and outcome of critically ill children: a prospective observational study. *Crit Care*. 2010; 14:R57. [PubMed: 20377853]
51. Eikelboom JW, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. *Am Heart J*. 2010; 159:737–43. e1. [PubMed: 20435180]

52. Weinberg JA, McGwin G Jr, Griffin RL, et al. Age of transfused blood: an independent predictor of mortality despite universal leukoreduction. *J Trauma*. 2008; 65:279–82. discussion 82–4. [PubMed: 18695462]
53. Weiskopf RB, Feiner J, Hopf H, et al. Fresh blood and aged stored blood are equally efficacious in immediately reversing anemia-induced brain oxygenation deficits in humans. *Anesthesiology*. 2006; 104:911–20. [PubMed: 16645441]
54. van de Watering L, Lorinser J, Versteegh M, et al. Effects of storage time of red blood cell transfusion on the prognosis of coronary artery bypass graft patients. *Transfusion*. 2006; 46:1712–8. [PubMed: 17002627]
55. Vamvakas EC, Carven JH. Transfusion and postoperative pneumonia in coronary artery bypass graft surgery: Effect of the length of storage of transfused red cells. *Transfusion*. 1999; 39:701–10. [PubMed: 10413277]
56. Vamvakas EC, Carven JH. Length of storage of transfused red cells and postoperative morbidity in patients undergoing coronary artery bypass graft surgery. *Transfusion*. 2000; 40:101–9. [PubMed: 10644819]
57. Leal-Noval SR, Jara-Lopez I, Garcia-Garmendia JL, et al. Influence of erythrocyte concentrate storage time on postsurgical morbidity in cardiac surgery patients. *Anesthesiology*. 2003; 98:815–22. [PubMed: 12657840]
58. Yap CH, Lau L, Krishnaswamy M, et al. Age of transfused red cells and early outcomes after cardiac surgery. *Ann Thorac Surg*. 2008; 86:554–9. [PubMed: 18640333]
59. Vamvakas EC. Is human herpesvirus-8 transmitted by transfusion? *Transfus Med Rev*. 2010; 24:1–14. [PubMed: 19962570]
60. Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion*. 2009; 49(Suppl 2):1S–29S. [PubMed: 19686562]
61. Hladik W, Dollard SC, Mermin J, et al. Transmission of human Herpesvirus 8 by blood transfusion. *N Engl J Med*. 2006; 355:1331–8. [PubMed: 17005950]
62. Trottier, H.; Alfieri, C.; Robitaille, N., et al. Blood. American Society of Hematology (ASH); Baltimore, MD: 2010. Transfusion-related Epstein-Barr virus (EBV) infection among stem cell transplant recipients: A retrospective cohort study in children..
63. Utter GH, Reed WF, Lee TH, Busch MP. Transfusion-associated microchimerism. *Vox Sang*. 2007; 93:188–95. [PubMed: 17845255]
64. Reed W, Lee TH, Norris PJ, et al. Transfusion-associated microchimerism: a new complication of blood transfusions in severely injured patients. *Semin Hematol*. 2007; 44:24–31. [PubMed: 17198844]
65. Takahashi K, Juji T, Miyamoto M, et al. Analysis of risk factors for post-transfusion graft-versus-host disease in Japan. Japanese Red Cross PT-GVHD Study Group. *Lancet*. 1994; 343:700–2. [PubMed: 7907679]
66. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. *Anesth Analg*. 2009; 108:759–69. [PubMed: 19224780]
67. Ruhl H, Bein G, Sachs UJ. Transfusion-associated graft-versus-host disease. *Transfus Med Rev*. 2009; 23:62–71. [PubMed: 19056035]
68. Steiner ME, Assmann SF, Levy JH, et al. Addressing the question of the effect of RBC storage on clinical outcomes: The Red Cell Storage Duration Study (RECESS) (Section 7). *Transfus Apher Sci*. 2010
69. Fergusson D, Hutton B, Hogan DL, et al. The age of red blood cells in premature infants (ARIPI) randomized controlled trial: study design. *Transfus Med Rev*. 2009; 23:55–61. [PubMed: 19056034]
70. Moroff G, Holme S, Keegan T, Heaton A. Storage of ADSOL-preserved red cells at 2.5 and 5.5 degrees C: comparable retention of in vitro properties. *Vox Sang*. 1990; 59:136–9. [PubMed: 2124752]

Figure 1. Biological pathways and potential causes of a RBC storage lesion

The figure illustrates that there are many potential mechanisms of a potential storage lesion, impacting overlapping pathways of oxygen delivery, RBC rheology and physiology, and immune modulation.