Published in final edited form as:

Transfusion. 2011 April ; 51(4): 874–880. doi:10.1111/j.1537-2995.2011.03095.x.

New frontiers in transfusion biology: Identification and significance of mediators of morbidity and mortality in stored red cell concentrates

Katie Grimshaw¹, Julie Sahler¹, Sherry L. Spinelli², Richard P. Phipps^{1,2,3}, and Neil Blumberg²

¹Department of Microbiology and Immunology; University of Rochester School of Medicine and Dentistry, Rochester, NY

²Department of Pathology and Laboratory Medicine; University of Rochester School of Medicine and Dentistry, Rochester, NY

³Department of Environmental Medicine; University of Rochester School of Medicine and Dentistry, Rochester, NY

Abstract

Red cell transfusions are associated with inflammation and thrombosis, both arterial and venous, the mechanisms of which are not understood. Although a necessary life saving procedure in modern medicine, transfusions have rarely been subjected to modern assessments of efficacy and safety, including randomized trials. Storage of red blood cells induces changes, including the release of free hemoglobin and the accumulation of biologically active soluble mediators and microparticles. These mediators likely play a direct role in the inflammatory and pro-thrombotic properties of red cell transfusions. Methods such as leukoreduction, washing of red cells and rejuvenation may improve the quality of red cell transfusions.

Keywords

red cell transfusion; transfusion; adverse events; cytokines; prostanoids; platelets

Red cell transfusions in modern medicine

Blood transfusions are amongst the oldest therapeutics of the modern era of medical practice and are the most commonly employed procedure amongst discharge codes recorded for hospital inpatients.¹ Estimates are that 3–4 million patients a year are transfused in the USA, and that most of us will receive a transfusion at some point in our lives. Despite this unique prevalence, and perhaps because of its time-honored place in medical practice, transfusions have rarely been subjected to modern assessments of efficacy and safety, including randomized trials. Indeed, as in most clinical research, when randomized trials have been performed, surrogate endpoints of clinical benefit have been employed (*e.g.* hematocrit

Correspondence to Neil Blumberg at: Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, Box 608, Rochester, NY 14642, Telephone 585/275-3189, neil_blumberg@urmc.rochester.edu.

Disclosure of Conflicts of Interest

NB has received lecture honoraria, consulting fees and research support from Pall Biomedical and Fenwal, manufacturers of leukoreduction filters, as well as from Caridian, manufacturers of cell washing equipment and supplies. The other authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

increments) rather than clinically more important outcomes (*e.g.* reduced morbidity and mortality from anemia).

Having early on demonstrated life saving potential in catastrophic emergencies such as peripartum hemorrhage and trauma surgery, transfusions in clinical practice are often characterized by a maxim of “it might help and it can’t hurt.” This is particularly true subsequent to the great scientific triumphs of the 20th century in transfusion medicine: pre-transfusion testing for red cell compatibility and infectious disease testing for hepatitis, HIV and other viruses, which made transfusion much safer than previously. However, data supporting the efficacy and safety of our current practices are virtually non-existent. Red cells are often not purified, and are contaminated with donor white cells, platelets and plasma, all of which are modulated by storage-induced changes. Changes in red cell properties during storage have been known for decades, but the exact significance of these changes is still largely uncertain.² The most numerous contaminating cell by two orders of magnitude is the donor platelet. While leukoreduction removes as much as 99.9% of contaminating white cells and platelets, approximately 20% of transfusions in the USA are still non-leukoreduced. Moreover, leukoreduction may be performed prestorage or after varying lengths of storage, during which white cells and platelets may release harmful mediators into the stored unit which would be retained after leukoreduction.

Adverse outcomes

Many of the recently described associations between transfusion and adverse clinical outcomes likely involve innate immunity and hemostasis. Examples include acute lung injury,³ bacterial infection,^{4,5} multi-organ failure,⁶ myocardial infarction⁷ and other thrombotic conditions.^{8,9} There are controversial randomized trials demonstrating that leukoreduction mitigates some of these effects of red cell transfusions, particularly in infection⁴ and multi-organ failure.¹⁰ There is evidence that stored supernatant, even from leukoreduced red cells, can contribute to adverse events in recipients,¹¹ presumably through interactions with recipient white cells, platelets, endothelial cells and structural cells.^{12,13} Given the roles of the platelet as an innate immune and inflammatory cell, as well as its involvement in hemostasis and thrombosis, our lab is investigating how transfused leukoreduced red cells and soluble supernatant mediators influence the recipient’s platelets and clinical outcomes.

While there are no, and may never be any randomized trials of red cell transfusions in exsanguinating hemorrhage or life-threatening anemia, there is consensus that blood transfusion as currently practiced is effective in those settings. Whether it could be more effective and safer is uncertain, but seems likely. In contrast to life-threatening clinical settings, most red cell transfusions are given to treat mild to moderate anemia, in an effort to prevent the complications of severe anemia or improve quality of life when anemia cannot be treated pharmacologically. These are empirical practices with little or no data to support them. There is agreement that the clinical and laboratory information needed to decide on a “transfusion trigger” in any given patient is largely unknown.¹⁴ The only evidence demonstrating red cell transfusion’s efficacy in stable medical patients is the prevention of recurrent stroke in sickle cell disease.¹⁵

Efficacy of red cell transfusions

Conversely, there is evidence that red cells, as currently stored, do not acutely improve oxygen delivery to the tissues. This may be due, in part, to striking storage changes in important red cell regulatory molecules (Figure 1). Decreased 2,3-bisphosphoglycerate (2,3-BPG),¹⁶ which aids oxygen unloading from hemoglobin in tissues, and severe deficiencies of nitric oxide (NO),¹⁷ needed for normal vasodilatation in the microcirculation, have been

described along with rheologic and morphologic changes. Oxidative damage resulting from oxygen dissociation from hemoglobin generates superoxide which generates a hydroxyl radical upon interaction with iron.¹⁸ These free radicals have the potential to interact with membrane lipids, such as arachidonic acid to give rise to isoprostanes.¹⁹ In addition, activation of the complement cascade may occur in some stored blood products²⁰ which could lead to immune activation in the host upon transfusion. Studying the effects of storage on red cells and their ability to deliver oxygen to hypoxic tissue will be critical to assessing the efficacy of current red cell transfusion products. Rejuvenation of red blood cells has been shown to restore red cell morphology and 2,3-BPG levels to those of freshly drawn cells,²¹ which could improve oxygen delivery to tissue. However, permanent changes in red cell membrane integrity occur when ATP levels drop too low, increasing microparticle formation.²² Nevertheless, there are no human studies demonstrating the modifications of 2,3-BPG or NO can improve clinical outcomes, nor are modifications that improve red cell rheology known. Thus, the future investigative challenge here is great, but the benefits uncertain.

Safety of red cell transfusions

In recent years, observational studies have demonstrated that, with some exceptions (*e.g.* exsanguinating hemorrhage and sickle cell anemia), patients transfused according to current standards of practice experience striking dose dependent increases in morbidity and mortality compared with non-transfused patients, or patients receiving fewer transfusions.^{23,24} Sorting out cause and effect from ascertainment bias (*i.e.* sicker patients are more likely to be transfused or transfused more aggressively) has been difficult and controversial. Nonetheless, there is abundant evidence that cause and effect is involved, including animal model studies.²⁵ To some degree, red cell transfusions certainly play a role in the negative outcomes of some patients as the effects are simply too large to be explained by confounding and randomized trials. Randomized trials demonstrate that a cause and effect relationship between allogeneic transfusions and adverse clinical events exists as alternative strategies to unmodified allogeneic transfusions reduce morbidity and mortality. Conservative transfusion triggers²⁶ and autologous²⁷ or leukoreduced²⁸ transfusions reduce morbidity and mortality in some settings. However, even leukoreduced transfusions are associated with increased morbidity.²⁹

In one of the most influential randomized trials in transfusion medicine history, critical care patients who received transfusions when hemoglobin values were 7–8 g/dl rather than 9–10 g/dl had equivalent or better clinical outcomes, suggesting that transfusions should be used more parsimoniously.²⁶ However, this effect may or may not be generalized to patients with cardiac disease.³⁰ Multiple randomized trials, although controversial, demonstrate a reduction in post-operative infection in patients receiving leukoreduced red cell transfusions.³¹ Most convincingly, patients undergoing cardiac surgery have strikingly and indisputably reduced mortality and multi-organ failure when randomized to receive only leukoreduced transfusions.^{10,28} Patients with acute leukemia who receive leukoreduced, washed transfusions have better long-term survival suggesting that residual mediators in leukoreduced transfusions may harm patients.³²

Patients with acute coronary syndromes experience a dose-dependent increase in myocardial infarction and mortality if they are transfused with red cells in observational studies.⁷ Transfusions are also associated in epidemiologic studies with increased venous and arterial thrombosis.^{8,9} In a retrospective cohort study, transfusion of red blood cells stored for longer than 28 days was associated with increased incidence of deep vein thrombosis and mortality in critically ill trauma patients and this effect was independent of red blood cell volume.³³ Furthermore, only 1 to 2 units of red blood cells older than 28 days was sufficient to

increase mortality. Our group and others have described mediators, such as sCD40L (CD154),³⁴ IL-6³⁵ and IL-8,³⁶ whose levels in stored transfusions are associated with adverse outcomes such as fever,¹² rigors³⁷ and acute lung injury.¹³ In general, both down regulation of T and NK cell immunity and inappropriate inflammatory responses have been documented *in vitro* and *in vivo* after red cell transfusions.³⁸ While these results are not definitive, and much uncertainty remains, it is very likely that cells³⁹ and supernatant mediators⁴⁰ present in stored blood unfavorably affect clinical outcomes in some patient populations.

Harmful mediators in stored red cells

Randomized controlled clinical trials demonstrate that modifications of the stored red cells such as leukoreduction^{28,31} and removal of supernatant by washing of leukoreduced platelets improve important clinical outcomes such as inflammatory responses and overall mortality.^{32,41} Therefore, we believe that studies elucidating the compositional changes in stored red cells that contribute to the “toxicity” of transfusion have greatest potential for rapid translation into improving clinical outcomes.

Red cell free hemoglobin and lipid mediators (*e.g.* prostaglandins and isoprostanes) accumulate in microparticle and supernatant fractions of leukoreduced stored red cells. Accumulation of mediators could significantly alter recipient platelet and endothelial cell function as these mediators are linked to vascular disease and chronic inflammation. Free hemoglobin has been implicated in vascular pathology, lung injury, and thrombosis in patients with sickle cell anemia⁴² and is known to alter endothelial cell and vascular function. Other hemolytic diseases, particularly paroxysmal nocturnal hemoglobinuria also are characterized by thrombotic complications.^{43,44} Thus, it is reasonable to hypothesize that infused free hemoglobin is a key mediator of post-transfusion inflammatory and thrombotic complications.

Each red blood cell contains 270 million hemoglobin molecules per cell that comprise approximately 95% of red cell cytoplasmic proteins.⁴⁵ As red blood cells become senescent and damaged with storage they can easily be lysed, releasing free hemoglobin into the supernatant of stored units. Studies have shown that free hemoglobin increases during the storage of leukoreduced red cells and can exceed 500 mg/dl, far above what is seen in patients with sickle cell disease or paroxysmal nocturnal hemoglobinuria. Furthermore, concentrations of free hemoglobin as low as 5–10 mg/dl cause platelet activation *in vitro*, thus may contribute to platelet hyper reactivity after transfusion.⁴³

Haptoglobin, a plasma protein, binds free hemoglobin to inhibit its oxidative activity and leads to its degradation while preserving the iron moiety. Haptoglobin concentrations have also been found to decrease with storage of red cell concentrates.⁴⁶ The dual effects of increasing concentrations of free hemoglobin and decreasing concentrations of haptoglobin could potentially lead to increased oxidative activity of hemoglobin. Oxidation of hemoglobin generates reactive oxygen species that may then interact with ferrous compounds to generate free radicals.¹⁸

Isoprostanes are generated from the free radical induced peroxidation of arachidonic acid and can be quantified as markers of oxidative stress and cell damage.⁴⁷ Oxidative stress during storage could lead to the increased production of lipid mediators, such as prostaglandins and isoprostanes that might contribute to transfusion complications. New evidence suggests that isoprostanes may not only be markers of cellular damage, but may also play a role in the pathogenesis of several disease states, including atherosclerosis.⁴⁸ In addition, F2 isoprostanes have been demonstrated to activate platelets by inducing shape

change and aggregation.⁴⁹ Accumulation of isoprostanes in stored red cell concentrates is one area we are currently investigating.

Microparticles are small anucleate vesicles that contain membrane-bound and cytoplasmic proteins from the cell which they were derived. In several settings, microparticles from various cell types, including leukocytes, platelets and endothelial cells, have been shown to modulate hemostasis, inflammation and thrombosis.⁵⁰ Therefore, it is likely that red cell microparticles also contribute in the same manner.⁵¹ Indeed, the concentration of red cell microparticles increases steadily over time in storage^{52,53} and may modify platelet-white cell⁵⁴ and platelet-endothelial cell interactions⁵⁵ in transfused patients. Microparticles are potential candidates for intracellular communication and are important mediators of inflammation and regulation of immune responses. Moreover, leukoreduction does not eliminate microparticles present in plasma thus even early in storage, microparticles are present from many cell types.

Platelets

Given that most red cell transfusions are now leukoreduced, we believe it appropriate to focus on the role of red cells themselves, microparticles and supernatant soluble mediators as possible factors in post-transfusion inflammation and thrombosis. Mounting evidence suggests that red cell transfusions modify hemostasis and thrombosis, and this is supported by their association with substantial increases in both venous and arterial thrombosis in epidemiologic studies.⁹ Therefore, it is likely that stored red cells and supernatant mediators alter recipient platelet function.⁵⁶ Indeed, an *in vitro* transfusion model demonstrated that red blood cell transfusions affect platelet function in healthy adults.⁵⁷ Specifically, red cell transfusion increased platelet aggregation and expression of the membrane-bound platelet activation marker, P-selectin. Furthermore, an *in vivo* transfusion model suggests synergistic injury in a “two-hit” model in rats.⁵⁸ In this model, aged erythrocytes were transfused (“second hit”) into rats, which had received a “first hit” of lipopolysaccharide. This “two-hit” TRALI model was characterized by both pulmonary and systemic coagulopathy when aged erythrocytes were used, but not washed or fresh erythrocytes, suggesting that soluble mediators in the supernatants were the cause of inflammation. Additionally we recently published preliminary evidence that both leukoreduction and washing may reduce the incidence of TRALI and TACO in transfusion recipients.⁵⁹

Platelets are the most numerous cell type in the recipient’s peripheral blood, other than red cells, and are intimately involved in hemostasis and thrombosis. Activated platelets themselves produce both immunomodulatory and proinflammatory mediators which exert large effects on circulating cells and the endothelium. Other cells, particularly recipient endothelial and white cells are likely also important to inflammatory and thrombotic events after transfusion. Platelets are known to interact with endothelial and white blood cells, and any findings regarding transfusion effects on platelets will have important implications for our understanding of recipient vascular,⁶⁰ endothelial and white blood cell responses to transfusion.⁶¹

Addressing the scientific unknowns

Identifying and understanding the effects of storage related changes in red cells and supernatant mediators will lead to strategies to correct or mitigate storage changes that render red cell transfusions more dangerous or less effective (Figure 2). For example, if inappropriate recipient platelet activation is caused by the stored, washed, leukoreduced red cells, but not by fresh red cells, modifications of storage conditions to maintain red cell “normalcy” such as rejuvenation might be indicated. Alternatively, if contaminating supernatant free hemoglobin, isoprostanes or microparticles are implicated in effects on

recipient platelets, saline washing or other techniques for minimizing these contaminants would be the strategy to employ in translational research and clinical trials. The effects of free hemoglobin are particularly important as blood substitutes consisting of modified hemoglobin are in investigational clinical use.⁶² Clinical cohort correlation studies will yield insights into the relative contributions of red cell storage time, free hemoglobin, isoprostanes and prostaglandins to changes in laboratory markers of inflammation and thrombosis such as soluble CD40L, C-reactive protein and D-dimer, and to clinical outcomes such as organ dysfunction and thrombosis.

Conclusion

Red cell transfusions evolved largely prior to modern concepts of randomized clinical trials, thus the true efficacy and safety of current practices are largely unknown. Furthermore, most transfusions are not given for active symptoms or signs of life threatening anemia, but as prophylaxis, skewing the risk to benefit ratio in the direction of increased risk with uncertain benefit. Precise measures of red cell transfusion efficacy are not available, and there is evidence that oxygen delivery by stored red blood cells may be neither optimum nor predictable. Red cell supernatant mediators, microparticles, and free hemoglobin may contribute to red cell damage and adverse effects on recipient platelets and endothelial cells.

Laboratory and clinical scientific studies to define the mechanisms of postulated immunomodulatory, haemostatic, thrombotic and inflammatory effects of red cell transfusions should, in addition to yielding new insights, help in designing modifications of red cell preparation and storage that minimize the morbidity experienced by transfused patients. Modifications of stored red cells for transfusion that may be suggested by these mechanistic studies could include removal of contaminating plasma supernatant and/or rejuvenation of red cells metabolically prior to transfusion. It is likely that the supernatant of stored red cells and storage damaged red cells both contribute to the pro-thrombotic and pro-inflammatory properties of transfusions.

The challenge for transfusion medicine research in the 21st century is that we simply do not know which patients truly benefit from transfusions even though this powerful therapy is widely used across virtually all clinical specialties. Additionally, we do not know how transfusions should be prepared in terms of storage, purity and immunologic safety. We have only a rudimentary understanding of how transfusions alter the immunologic, biochemical, vascular, organ specific and haemostatic functions of the recipient. Answers to these questions can derive from observational, epidemiologic, mechanistic laboratory and animal model studies correlated with clinical outcomes, and randomized trials. In summary, it could plausibly be stated that we know less about the benefits and risks of blood transfusions than of any other commonly used medical therapy. Recognizing that transfusions have novel deleterious biologic effects on the recipient has been the first step toward future investigations that will elucidate the mechanisms and methods for mitigation of these undesirable side effects, and also improve therapeutic efficacy.

Acknowledgments

Grant Support

Supported in part by NIH grants ES01247, HL078603, HL095467, HL100051.

References

1. Health Care Cost and Utilization Project (HCUP). Facts and Figures [monograph on the internet]. Rockville, MD: Agency for Healthcare Research and Quality; 2007. Available from: http://www.hcup-us.ahrq.gov/reports/factsandfigures/2007/exhibit3_1.jsp
2. Schrier SL, Sohmer PR, Moore GL, Ma L, Junga I. Red blood cell membrane abnormalities during storage: correlation with in vivo survival. *Transfusion*. 1982; 22:261–5. [PubMed: 7101417]
3. Popovsky MA, Moore SB. Mechanism of transfusion-related acute lung injury. *Blood*. 1991; 77:2299. [PubMed: 1709383]
4. Tartter PI, Quintero S, Barron DM. Perioperative blood transfusion associated with infectious complications after colorectal cancer operations. *Am J Surg*. 1986; 152:479–82. [PubMed: 3777324]
5. Tartter PI, Mohandas K, Azar P, Endres J, Kaplan J, Spivack M. Randomized trial comparing packed red cell blood transfusion with and without leukocyte depletion for gastrointestinal surgery. *Am J Surg*. 1998; 176:462–6. [PubMed: 9874434]
6. van Hilten JA, van de Watering LM, van Bockel JH, van de Velde CJ, Kievit J, Brand R, van den Hout WB, Geelkerken RH, Roumen RM, Wesselink RM, Koopman-van Gemert AW, Koning J, Brand A. Effects of transfusion with red cells filtered to remove leucocytes: randomised controlled trial in patients undergoing major surgery. *BMJ*. 2004; 328:1281. [PubMed: 15142885]
7. Rao SV, Jollis JG, Harrington RA, Granger CB, Newby LK, Armstrong PW, Moliterno DJ, Lindblad L, Pieper K, Topol EJ, Stamler JS, Califf RM. Relationship of blood transfusion and clinical outcomes in patients with acute coronary syndromes. *JAMA*. 2004; 292:1555–62. [PubMed: 15467057]
8. Nilsson KR, Berenholtz SM, Garrett-Mayer E, Dorman T, Klag MJ, Pronovost PJ. Association between venous thromboembolism and perioperative allogeneic transfusion. *Arch Surg*. 2007; 142:126–32. discussion 33. [PubMed: 17309963]
9. Khorana AA, Francis CW, Blumberg N, Culakova E, Refaai MA, Lyman GH. Blood transfusions, thrombosis, and mortality in hospitalized patients with cancer. *Arch Intern Med*. 2008; 168:2377–81. [PubMed: 19029504]
10. van de Watering LM, Hermans J, Houbiers JG, van den Broek PJ, Bouter H, Boer F, Harvey MS, Huysmans HA, Brand A. Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: a randomized clinical trial. *Circulation*. 1998; 97:562–8. [PubMed: 9494026]
11. Blumberg N, Heal JM, Liesveld JL, Phillips GL, Rowe JM. Platelet transfusion and survival in adults with acute leukemia. *Leukemia*. 2008; 22:631–5. [PubMed: 17805333]
12. Blumberg N, Gettings KF, Turner C, Heal JM, Phipps RP. An association of soluble CD40 ligand (CD154) with adverse reactions to platelet transfusions. *Transfusion*. 2006; 46:1813–21. [PubMed: 17002639]
13. Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, Gettings KF, McLaughlin NJ, Silliman CC. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood*. 2006; 108:2455–62. [PubMed: 16772606]
14. Carson JL, Duff A, Berlin JA, Lawrence VA, Poses RM, Huber EC, O'Hara DA, Noveck H, Strom BL. Perioperative blood transfusion and postoperative mortality. *JAMA*. 1998; 279:199–205. [PubMed: 9438739]
15. Adams RJ, Brambilla DJ, Granger S, Gallagher D, Vichinsky E, Abboud MR, Pegelow CH, Woods G, Rohde EM, Nichols FT, Jones A, Luden JP, Bowman L, Hagner S, Morales KH, Roach ES, Study S. Stroke and conversion to high risk in children screened with transcranial Doppler ultrasound during the STOP study. *Blood*. 2004; 103:3689–94. [PubMed: 14751925]
16. 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]
17. Bonaventura J. Clinical implications of the loss of vasoactive nitric oxide during red blood cell storage. *Proc Natl Acad Sci U S A*. 2007; 104:19165–6. [PubMed: 18048331]

18. Winterbourn CC. Free-radical production and oxidative reactions of hemoglobin. *Environ Health Perspect.* 1985; 64:321–30. [PubMed: 3007097]
19. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin F₂-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc Natl Acad Sci U S A.* 1990; 87:9383–7. [PubMed: 2123555]
20. Yazdanbakhsh K. Controlling the complement system for prevention of red cell destruction. *Curr Opin Hematol.* 2005; 12:117–22. [PubMed: 15725901]
21. Usry RT, Moore GL, Manalo FW. Morphology of stored, rejuvenated human erythrocytes. *Vox Sang.* 1975; 28:176–83. [PubMed: 1119131]
22. Bosman GJ, Werre JM, Willekens FL, Novotný VM. Erythrocyte ageing in vivo and in vitro: structural aspects and implications for transfusion. *Transfus Med.* 2008; 18:335–47. [PubMed: 19140816]
23. Blumberg N. Deleterious clinical effects of transfusion immunomodulation: proven beyond a reasonable doubt. *Transfusion.* 2005; 45:33S–9S. discussion 9S–40S. [PubMed: 16086785]
24. Tinmouth A, Fergusson D, Yee IC, Hébert PC, Investigators A, Group CCCT. Clinical consequences of red cell storage in the critically ill. *Transfusion.* 2006; 46:2014–27. [PubMed: 17076859]
25. Gianotti L, Pyles T, Alexander JW, Babcock GF, Carey MA. Impact of blood transfusion and burn injury on microbial translocation and bacterial survival. *Transfusion.* 1992; 32:312–7. [PubMed: 1585434]
26. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med.* 1999; 340:409–17. [PubMed: 9971864]
27. Heiss MM, Mempel W, Jauch KW, Delanoff C, Mayer G, Mempel M, Eissner HJ, Schildberg FW. Beneficial effect of autologous blood transfusion on infectious complications after colorectal cancer surgery. *Lancet.* 1993; 342:1328–33. [PubMed: 7901637]
28. Bilgin YM, van de Watering LM, Eijssman L, Versteegh MI, Brand R, van Oers MH, Brand A. Double-blind, randomized controlled trial on the effect of leukocyte-depleted erythrocyte transfusions in cardiac valve surgery. *Circulation.* 2004; 109:2755–60. [PubMed: 15148271]
29. Weinberg JA, McGwin G, Griffin RL, Huynh VQ, Cherry SA, Marques MB, Reiff DA, Kerby JD, Rue LW. Age of transfused blood: an independent predictor of mortality despite universal leukoreduction. *J Trauma.* 2008; 65:279–82. discussion 82–4. [PubMed: 18695462]
30. Deans KJ, Minneci PC, Suffredini AF, Danner RL, Hoffman WD, Ciu X, Klein HG, Schechter AN, Banks SM, Eichacker PQ, Natanson C. Randomization in clinical trials of titrated therapies: unintended consequences of using fixed treatment protocols. *Crit Care Med.* 2007; 35:1509–16. [PubMed: 17440420]
31. Blumberg N, Zhao H, Wang H, Messing S, Heal JM, Lyman GH. The intention-to-treat principle in clinical trials and meta-analyses of leukoreduced blood transfusions in surgical patients. *Transfusion.* 2007; 47:573–81. [PubMed: 17381614]
32. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. *BMC Blood Disord.* 2004; 4:6. [PubMed: 15588315]
33. Spinella PC, Carroll CL, Staff I, Gross R, Mc Quay J, Keibel L, Wade CE, Holcomb JB. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. *Crit Care.* 2009; 13:R151. [PubMed: 19772604]
34. Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet.* 2001; 357:2023–4. [PubMed: 11438137]
35. Stack G, Snyder EL. Cytokine generation in stored platelet concentrates. *Transfusion.* 1994; 34:20–5. [PubMed: 8273124]
36. Davenport RD, Strieter RM, Standiford TJ, Kunkel SL. Interleukin-8 production in red blood cell incompatibility. *Blood.* 1990; 76:2439–42. [PubMed: 1702325]

37. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG. The role of the plasma from platelet concentrates in transfusion reactions. *N Engl J Med*. 1994; 331:625–8. [PubMed: 8052271]
38. Blumberg, N.; Heal, JM. Transfusion immunomodulation. In: Hillyer, CD.; Silberstein, LE.; Ness, PM., et al., editors. *Blood Banking and Transfusion Medicine*. Philadelphia, PA: Churchill Livingstone Elsevier; 2007. p. 701-12.
39. Blajchman MA, Bardossy L, Carmen R, Sastry A, Singal DP. Allogeneic blood transfusion-induced enhancement of tumor growth: two animal models showing amelioration by leukodepletion and passive transfer using spleen cells. *Blood*. 1993; 81:1880–2. [PubMed: 8461473]
40. Davenport RD, Kunkel SL. Cytokine roles in hemolytic and nonhemolytic transfusion reactions. *Transfus Med Rev*. 1994; 8:157–68. [PubMed: 8081078]
41. Vo TD, Cowles J, Heal JM, Blumberg N. Platelet washing to prevent recurrent febrile reactions to leucocyte-reduced transfusions. *Transfus Med*. 2001; 11:45–7. [PubMed: 11328571]
42. Gladwin MT, Vichinsky E. Pulmonary complications of sickle cell disease. *N Engl J Med*. 2008; 359:2254–65. [PubMed: 19020327]
43. Villagra J, Shiva S, Hunter LA, Machado RF, Gladwin MT, Kato GJ. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood*. 2007; 110:2166–72. [PubMed: 17536019]
44. Parker CJ. Paroxysmal nocturnal hemoglobinuria: an historical overview. *Hematology Am Soc Hematol Educ Program*. 2008:93–103. [PubMed: 19074065]
45. Telen, MJ.; Kaufman, RE. Part II: normal hematologic system, the mature erythrocyte. In: Greer, JP.; Forerster, J.; Lukens, JN., et al., editors. *Wintrobe's Clinical Hematology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2004. p. 217-48.
46. Nishiyama T, Hirasaki A, Odaka Y, Iwasaki T, Kobayashi O, Seto K. Concentrations of haptoglobin and free hemoglobin in preserved whole blood. *Masui*. 1994; 43:736–9. [PubMed: 8015163]
47. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med*. 1995; 332:1198–203. [PubMed: 7700313]
48. Praticò D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat Med*. 1998; 4:1189–92. [PubMed: 9771755]
49. Yin K, Halushka PV, Yan YT, Wong PY. Antiaggregatory activity of 8-epi-prostaglandin F2 alpha and other F-series prostanoids and their binding to thromboxane A2/prostaglandin H2 receptors in human platelets. *J Pharmacol Exp Ther*. 1994; 270:1192–6. [PubMed: 7932171]
50. Diamant M, Tushuizen ME, Sturk A, Nieuwland R. Cellular microparticles: new players in the field of vascular disease? *Eur J Clin Invest*. 2004; 34:392–401. [PubMed: 15200490]
51. Tissot JD, Rubin O, Canellini G. Analysis and clinical relevance of microparticles from red blood cells. *Curr Opin Hematol*. 2010; 17:571–7. [PubMed: 20960973]
52. Lawrie AS, Albany A, Cardigan RA, Mackie IJ, Harrison P. Microparticle sizing by dynamic light scattering in fresh-frozen plasma. *Vox Sang*. 2009; 96:206–12. [PubMed: 19175566]
53. Thomas S, Beard M, Garwood M, Callaert M, Cardigan R. Platelet concentrates produced from whole blood using the Atrius processing system. *Vox Sang*. 2009; 97:93–101. [PubMed: 19392785]
54. Xiong Z, Cavaretta J, Qu L, Stolz DB, Triulzi D, Lee JS. Red blood cell microparticles show altered inflammatory chemokine binding and release ligand upon interaction with platelets. *Transfusion*. 2010
55. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost*. 2009; 101:439–51. [PubMed: 19277403]
56. Matheu FA, McFaul SJ. Supernates from stored red blood cells inhibit platelet aggregation. *Transfusion*. 2010; 50:1196–202. [PubMed: 20158688]

57. Silvain J, Pena A, Cayla G, Brieger D, Bellemain-Appaix A, Chastre T, Vignalou JB, Beygui F, Barthelemy O, Collet JP, Montalescot G. Impact of red blood cell transfusion on platelet activation and aggregation in healthy volunteers: results of the TRANSFUSION study. *Eur Heart J*. 2010; 31:2816–21. [PubMed: 20591842]
58. Vlaar AP, Hofstra JJ, Levi M, Kulik W, Nieuwland R, Tool AT, Schultz MJ, de Korte D, Juffermans NP. Supernatant of aged erythrocytes causes lung inflammation and coagulopathy in a “two-hit” in vivo syngeneic transfusion model. *Anesthesiology*. 2010; 113:92–103. [PubMed: 20508493]
59. Blumberg N, Heal JM, Gettings KF, Phipps RP, Masel D, Refaai MA, Kirkley SA, Fialkow LB. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. *Transfusion*. 2010; 50:2738–44. [PubMed: 20561296]
60. Wagner DD, Burger PC. Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2003; 23:2131–7. [PubMed: 14500287]
61. Aslam R, Speck ER, Kim M, Freedman J, Semple JW. Transfusion-related immunomodulation by platelets is dependent on their expression of MHC Class I molecules and is independent of white cells. *Transfusion*. 2008; 48:1778–86. [PubMed: 18522705]
62. Bunn HF. The use of hemoglobin as a blood substitute. *Am J Hematol*. 1993; 42:112–7. [PubMed: 8416284]

Figure 1. Free hemoglobin, isoprostanes and microparticles accumulate during storage of red blood cells

Stored red blood cell membranes become ruffled and more rigid as ATP levels decrease (1). Microparticles bud off from the red cell membrane and expose the negatively charged phosphatidylserine on their surface (2). Hemoglobin is released upon storage-induced hemolysis, increasing the concentration of free hemoglobin in the stored unit (3). Oxidative damage occurs as oxygen dissociates from free hemoglobin yielding superoxide and hydrogen peroxide through a series of reactions (4). Reactive oxygen species generate free radicals upon interaction with iron containing compounds (5), which interact with membrane lipids to form lipid radicals (6). Free radicals may also be formed independent of hemoglobin and contribute to the generation of lipid radicals (7). Isoprostanes are formed from the free radical induced peroxidation of membrane lipids (8). Microparticles, free hemoglobin and isoprostanes may adversely affect the transfused recipient by causing inflammation and thrombosis (9).

Figure 2. Proven (leukoreduction) and potential future methods for reducing morbidity and mortality following red blood cell transfusions
One or a combination of these methods may be employed to improve the quality of stored red blood cells.