SHOCK, Vol. 41, Supplement 1, pp. 30–34, 2014

ROLE OF TRANSFUSED RED BLOOD CELLS FOR SHOCK AND COAGULOPATHY WITHIN REMOTE DAMAGE CONTROL RESUSCITATION

Philip C. Spinella* and Allan Doctor*†

**Division of Critical Care, Department of Pediatrics, Washington University in St Louis, St Louis, Missouri and †Department of Biochemistry, Washington University in St Louis, St Louis, Missouri*

Received 13 Sep 2013; first review completed 4 Oct 2013; accepted in final form 1 Nov 2013

ABSTRACT—The philosophy of damage control resuscitation (DCR) and remote damage control resuscitation (RDCR) can be summarized by stating that the goal is to prevent death from hemorrhagic shock by "staying out of trouble instead of getting out of trouble." In other words, it is preferred to arrest the progression of shock, rather than also having to reverse this condition after significant tissue damage and organ injury cascades are established. Moreover, to prevent death from exsanguination, a balanced approach to the treatment of both shock and coagulopathy is required. This was military doctrine during World War II, but seemed to be forgotten during the last half of the 20th century. Damage control resuscitation and RDCR have revitalized the approach, but there is still more to learn about the most effective and safe resuscitative strategies to simultaneously treat shock and hemorrhage. Current data suggest that our preconceived notions regarding the efficacy of standard issue red blood cells (RBCs) during the hours after transfusion may be false. Standard issue RBCs may not increase oxygen delivery and may in fact decrease it by disturbing control of regional blood flow distribution (impaired nitric oxide processing) and failing to release oxygen, even when perfusing hypoxic tissue (abnormal oxygen affinity). Standard issue RBCs may assist with hemostasis but appear to have competing effects on thrombin generation and platelet function. If standard issue or RBCs of increased storage age are not optimal, then are there alternatives that will allow for an efficacious and safe treatment of shock while also supporting hemostasis? Studies are required to determine if fresh RBCs less than 7 to 10 days provide an outcome advantage. A resurgence in the study of whole blood stored at 4°C for up to 10 days also holds promise. Two randomized controlled trials in humans have indicated that following transfusion with either whole blood stored at 4°C or platelets stored at 4°C there was less clinical bleeding than when blood was reconstituted with components or when platelets were stored at 22°C. Early reversal of shock is essential to prevent exacerbation of coagulopathy and progression of cell death cascades in patients with severe traumatic injuries. Red blood cell storage solutions have evolved to accommodate the needs of non–critically ill patients yet may not be optimal for patients in hemorrhagic shock. Continued focus on the recognition and treatment of shock is essential for continued improvement in outcomes for patients who require damage control resuscitation and RDCR.

KEYWORDS—Trauma, transfusion, prehospital, hemorrhage, shock, red blood cells

INTRODUCTION

Over time, the composition of blood products and fluids used to resuscitate patients with traumatic hemorrhagic shock has changed dramatically. Whole blood was the primary resuscitation fluid during the first half of the 20th century. This preference was driven by the British and US Medical Department leadership's belief that whole blood best addressed both shock and coagulopathy early in the resuscitation (1). With the development of robust whole-blood fractionation techniques, individual blood components (red blood cells [RBCs], plasma, and platelets) became available and an approach to treating specific deficiencies (in anemia and coagulation) with specific components emerged as a means to improve resource utilization as well as target therapy. For example, patients with isolated anemia could be treated with RBCs while reserving plasma and platelets for another patient. In addition, during the 1960s, the use of crystalloids and colloids became the primary early resuscitation fluid for hemorrhagic shock. During the latter half of the 20th century, Advanced Trauma Life Support guidelines recommended that patients with traumatic hemorrhagic shock receive crystalloids initially to restore circulating blood volume then receive RBCs to improve oxygen-carrying capacity, with plasma and platelets indicated only to rectify documented coagulopathy or thrombocytopenia and ongoing bleeding. This approach to hemorrhagic shock, with crystalloid for volume resuscitation, RBCs for oxygen-carrying capacity, and "catch-up" therapy for coagulopathy, represented a fundamental departure from the experience and doctrine of World War II, which emphasized early prevention of coagulopathy (1). Moreover, during this period, RBC storage solutions were composed to progressively extend storage duration, with safety criteria focused on infectious risk and efficacy criteria limited to posttransfusion RBC circulation (rather than function, e.g., oxygen delivery).

The advancements in blood banking that enabled increased storage duration dramatically improved blood product access and utilization. These advancements are likely to have saved many lives, but we have now come to appreciate the importance of functional criteria in evaluating efficacy (oxygen delivery) and (noninfectious) safety of blood products. Mounting evidence suggests that the efficacy and safety of RBCs progressively diminish during storage, quite possibly to clinically meaningful thresholds within currently accepted durations (2). The relevance of this "storage lesion" upon clinical outcomes is a subject of intense study at present. This problem

Address reprint requests to Philip C. Spinella, MD, Washington University in St Louis, One Children's Place, St. Louis, MO 63110. E-mail: spinella_p@kids.wustl.edu

DOI: 10.1097/SHK.0000000000000089

is challenging for translational investigators because it appears that both the nature and severity of illness, as well as both transfusion recipient- and donor-specific factors, affect the clinical relevance of the RBC storage lesion. More simply put, the "two-hit" hypothesis suggests that illness severity increases patient vulnerability to the adverse effects of transfusion with RBCs of increased storage duration. Observational data support this concept, most clearly for patients with traumatic injuries (3, 4).

The current blood supply and RBC allocation methods are ideal for non–critically ill patient populations such as those with oncologic disorders requiring chemotherapy. These patients have single deficits in either RBCs or platelets and are not in shock when transfused. As such, they do not require RBCs or platelets with maximal efficacy at the moment of transfusion (although not well studied, some evidence suggests that some storage lesion features "normalize" in the first few days following transfusion). Bleeding trauma victims lose all blood components concurrently; we now recognize the importance of early attention to coagulopathy as well as the loss of blood volume and oxygen-carrying capacity. Because these patients are at highest risk of death in the first 6 to 12 h following injury, they would benefit from a blood product that provides oxygen-carrying and hemostatic capacity of the highest efficacy, with the least adverse effects (3). Current "component-based" approaches and blood banking allocation practices do not meet this need optimally. At many institutions, the oldest RBC units in stock (possibly with reduced efficacy and noninfectious safety relative to those of shorter storage duration) are dispensed to severe trauma patients, with unknown consequences.

In the past decade, however, there is renewed interest in the doctrine developed during World War II, in which circulating volume, oxygen-carrying capacity, and coagulopathy were equally addressed early in resuscitation. Holcomb and colleagues (5) have termed this *damage control resuscitation* (DCR). Prehospital application of DCR is termed *remote damage control resuscitation* (RDCR) (6).

Role of shock in acute traumatic coagulopathy

Shock is defined as a pathophysiologic state that occurs when oxygen delivery is insufficient to maintain aerobic respiration in tissue (7). Reduced perfusion often accompanies shock but is not essential for its development. The consequence of persistent shock is cell death due to inadequate energy production. Before cell death, the shock state is associated with a broadly cascading array of noxious chemical and signaling mediators, which lead to endothelial and vascular wall injury endothelium and the subendothelial matrix; these events are hypothesized to be a direct cause of the coagulopathy seen in trauma victims (acute traumatic coagulopathy). Hess and colleagues (8) have documented in animal and human observational studies that reduced perfusion is associated with rapid development of a coagulopathic and hyperfibrinolytic state. While the exact mechanisms of acute traumatic coagulopathy are poorly understood, it is apparent that tissue hypoxia itself, is also an important inciting factor. As such, to successfully treat coagulopathy, it is essential to identify and correct diminished oxygen delivery to tissue throughout trauma resuscitation.

Methods to reverse shock

Very simply, hemorrhagic shock can be treated by physical control of bleeding, medical treatment of coagulopathy, and restoration of oxygen delivery. Oxygen delivery can be augmented by increasing blood flow to tissue and/or blood oxygen content. Cardiac output can be increased by maintaining adequate preload, cardiac contractility, and afterload. Tissue blood flow is further regulated by the interaction of perfusion pressure and vascular resistance at the regional level; it is essential that maneuvers to augment hemodynamics globally do not interfere with control of regional blood flow. Blood oxygen content can be augmented by increasing the amount and/or the oxygen saturation of hemoglobin; because of poor solubility of oxygen in water (particularly at body temperature), increasing the partial pressure of oxygen contributes little to rectifying poor oxygen content in blood.

In the latter half of the 20th century, resuscitation of patients with hemorrhagic shock was initiated with crystalloids to restore circulating volume (improving preload and cardiac output), followed by RBC transfusion to restore oxygen content (as well as further optimizing volume status). There are two flaws with this approach. First, enthusiasm for use of crystalloid-based volume resuscitation is now tempered by concern for creating dilutional coagulopathy, a proinflammatory state, and organ injury associated with severe interstitial edema (9). Second, although RBC transfusion will improve O_2 content, new evidence suggests that with progressive storage duration transfusion fails to improve O_2 delivery in a likewise fashion. This functional defect, which decouples O_2 delivery from content, appears attributable to the RBC storage lesion and is the focus of intense study.

Most physicians consider RBC units as the optimal resuscitative solution to treat hemorrhagic shock. It is commonly believed that because transfused RBC units increase hemoglobin content and blood oxygen content, that oxygen delivery to tissue is likewise improved. New data now suggest that, with increasing storage duration, oxygen delivery is progressively impaired by altered oxygen affinity, disfavorable rheology and adhesion, as well as by abnormal vascular signaling by RBCs that increases regional vascular resistance (and diminishes regional blood flow despite adequate perfusion pressure). This is of particular concern because RBCs of increased storage duration are routinely transfused to trauma patients (3).

Native RBC function

RBCs transport oxygen bound to hemoglobin; oxygen is released when tissue oxygen tensions fall below the hemoglobin-oxygen affinity threshold. Released oxygen, now in solution, diffuses along a pressure gradient until rebinding to O2 storage proteins in tissue (e.g. myoglobin, cytoglobin, or neuroglobin) or to heme proteins in mitochondria, whereupon it is consumed in the process of oxidative phosphorylation (e.g., tissue respiration). Moreover, RBCs also regulate oxygen delivery by linking nitric oxide bioavailability (and therefore vascular tone) to biochemical measures of perfusion sufficiency (principally P_{O_2} and pH). Red blood cell–mediated vasoregulation operates at the regional level, governing regional blood flow distribution, rather than by influencing global hemodynamics. In this paradigm, RBCs either export or sequester nitric oxide as a function of hemoglobin

oxygen content (10). In hypoxic tissue, native RBCs export nitric oxide, increasing regional flow and therefore augmenting oxygen delivery. As improved flow resolves hypoxia, RBCs traverse the same vascular bed with increasing hemoglobin oxygen content, which leads to nitric oxide sequestration (rather than export) by RBCs, and attenuation of regional flow (11). As such, coupling between tissue oxygen tension and hemoglobin oxygen content links tissue respiration to regional blood flow. This fundamental feature of human physiology (systemic hypoxic vasodilation) hinges upon normal RBC physiology, which is progressively impaired during storage (12). Studies in humans indicate that increasing regional flow is a more effective method of increasing oxygen delivery than increasing oxygen content with hemoglobin mass (13). Therefore, the treatment of shock requires attention to increasing regional flow, while maintaining adequate oxygen-carrying capacity.

Native RBCs also affect hemostasis (14). Anemia is associated with impaired hemostasis via reduced viscosity and increased platelet inhibition. Platelet inhibition occurs from anemia due to sheer stress-induced nitric oxide release and diminished nitric oxide sequestration. Compensatory mechanisms have been described in which sheer stress due to anemia induces the release of ADP from RBCs, which stimulates platelet aggregation (14).

Stored RBC Function

Stored RBCs have been found to have reduced capacity to both offload oxygen and support hypoxic vasodilation. In fact, the majority of the evidence indicates that RBCs of increased storage length may, in fact, reduce oxygen delivery and regional flow via the mechanisms displayed in Figures 1 and 2 (3, 12, 15–22).

As a result of allocation strategies that aim to limit waste, the RBC units typically issued to patients with hemorrhagic shock are of increased storage duration (23). Specifically, RBC units in inventory closest to expiration are transfused first to all patients (with few pediatric cardiac surgery exceptions). Likewise, interhospital allocation compounds this problem; as RBC units approach the 42-day storage limit in low-use centers, usually after 35 days of storage, RBCs are commonly transported to large trauma centers. As a result, patients with severe trauma are typically transfused with older RBCs, with unit age documented to be as high as a mean of 27 days in a US trauma center and at a median of 33 days in military settings (24, 25).

There are no multicenter randomized controlled trials examining the influence of RBC storage duration upon clinical outcomes in adult trauma patients in hemorrhagic shock. There are *in vitro*, animal, and nonrandomized studies in adult trauma patients that indicate that RBCs of increased storage duration are associated with reduced oxygen delivery, reduced perfusion, and perturbed vasoregulation, as well as impaired immune and coagulation function (2, 3, 25). A recent study by Kiraly and colleagues in adult trauma patients indicated that RBCs stored for more than 21 days reduced oxygen delivery compared with those stored for less than 21 days (26). Another prospective study performed in a severe pneumonia model in canines indicated that transfusion of older RBCs was associated with impaired vasoregulation and increased mortality (27). Figures 1 and 2 demonstrate the biologic changes that occur over storage time with RBCs and in the supernatant of RBC units. The effects of storage on RBC energetics, nitric oxide bioavailability, and on the extracellular solution have been thoroughly reviewed elsewhere (3, 12, 15–22). Although there is biologic plausibility that with increased RBC storage there is a reduction in efficacy and safety, it is not yet clear if these effects have clinical implications in patients with hemorrhagic shock.

Therefore, although there are no definitive data that indicate RBCs of increased storage duration increase the risk of morbidity

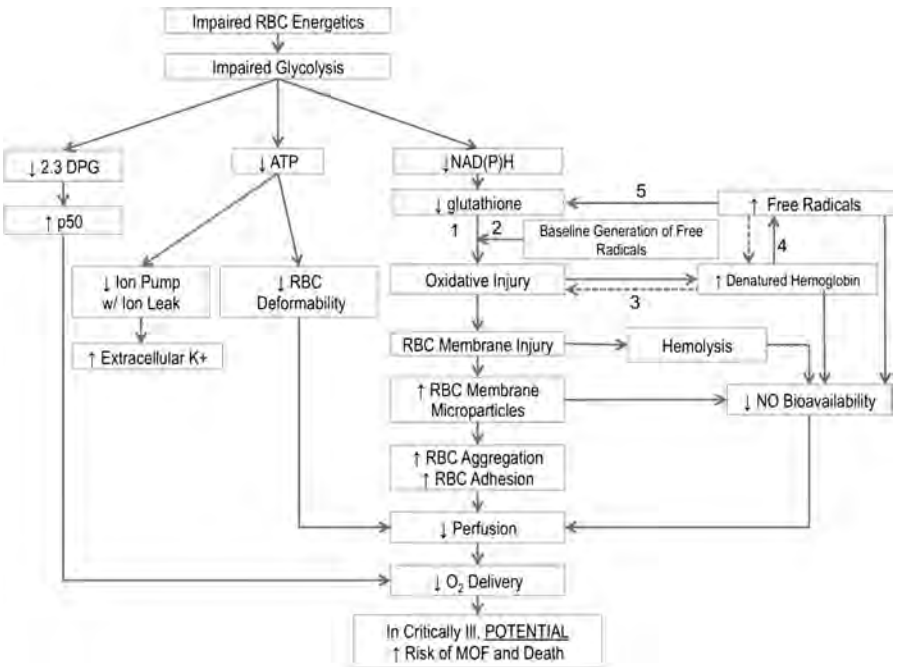

Fig. 1. **Effects of storage on RBC oxygen delivery capacity**. DPG indicates diphosphoglycerate; ATP, adenosine triphosphate; NAD(P)H, nicotinamide adenine dinucleotide phosphate; RBC, red blood cells; K, potassium; NO, nitric oxide; MOF, multiple organ failure.

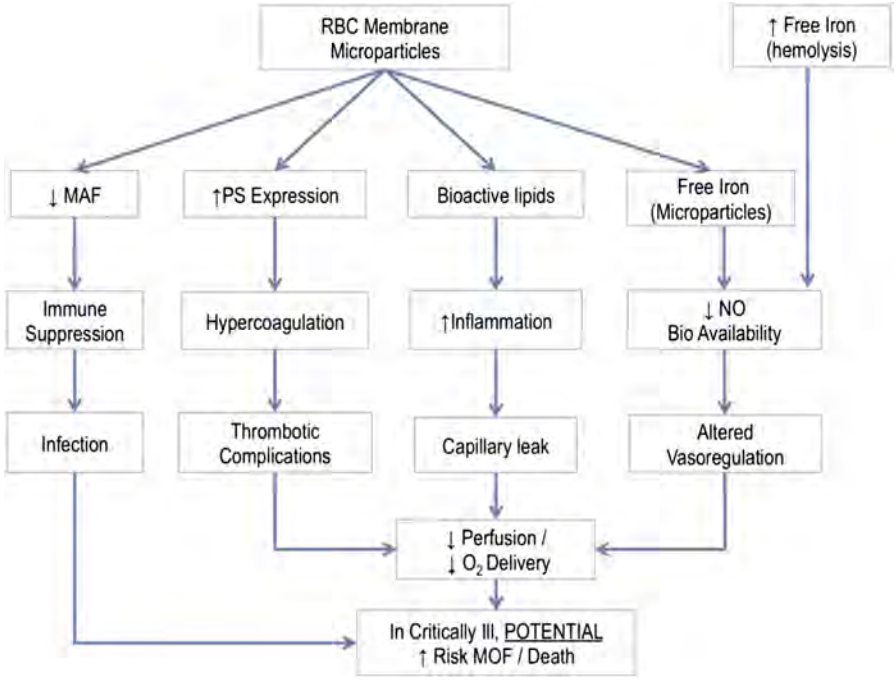

FIG. 2. **Effects of stored RBCs on the extracellular solution in RBC units**. PS indicates phosphatidylserine; MAF, macrophage activating factor; RBC, red blood cells; NO, nitric oxide; MOF, multiple organ failure.

and mortality in patients with hemorrhagic shock, it is important to also realize that the extension of the limit upon storage duration from 21 to 42 days was based on RBC circulation criteria alone, as an efficacy end point, rather than evaluation of RBC function or simply of patient outcomes. With the preponderance of animal and human data indicating reduced efficacy and safety of older RBCs in critically ill populations, particularly in severe trauma patients, it is appropriate, if possible, to preferentially use RBCs of reduced storage duration (<7–10 days) until there is evidence that older RBCs are equivalent to fresher ones. If fresher RBCs are not available, it is still important to transfuse available RBC units because impaired regulation of regional blood flow is a moot point if overall cardiac output and hemodynamics are inadequate to generate regional perfusion pressure. Also, if the patient survives the acute resuscitation, some of the adverse effects associated with the storage lesion appear to reverse in the 48 h following transfusion, such as loss of 2,3-DPG and ATP concentrations, (e.g., akin to improved function of transplanted organs over time) (28). Future research in reengineered RBCs, blood farming, and even the use of whole blood stored at 4-C for 10 days may increase the availability of blood products that will effectively and safely increase oxygen delivery in patients with traumatic hemorrhagic shock.

Stored RBCs also affect hemostasis. The literature indicates that RBC transfusions are predominantly procoagulant and associated with less bleeding in animal models. The hemostatic effects of RBCs have been documented according to viscoelastic measures, reduced bleeding times, and light transmission aggregometry. Red blood cell transfusions have also been associated with increased platelet function, which is thought to be due to increased viscosity, RBC release of ADP, stimulating platelet release of thromboxane A2, and P-selectin expression, as well as nitric oxide sequestration (14, 29, 30). Interestingly, when the effect of RBC storage duration on hemostasis has been evaluated, it appears that older RBCs are associated with increased thrombin generation (increased thrombin-antithrombin complex formation and reduced thromboelastography R time) and reduced platelet function, according to TEG and Platelet Function Assay-100 testing as well as collagen-stimulated single platelet disappearance measures (31–33).

CONCLUSIONS

The philosophy of DCR and RDCR can be summarized by stating that the goal is to prevent death from hemorrhagic shock by "staying out of trouble instead of getting out of trouble." In other words, it is preferred to arrest the progression of shock, rather than also having to reverse this condition after significant tissue damage and organ injury cascades are established. Moreover, to prevent death from exsanguination, a balanced approach to the treatment of both shock and coagulopathy is required. This was military doctrine during World War II, but seemed to be forgotten during the last half of the 20th century (1). Damage control resuscitation and RDCR have revitalized the approach, but there is still more to learn about the most effective and safe resuscitative strategies to simultaneously treat shock and hemorrhage.

Current data suggest that our preconceived notions regarding the efficacy of standard issue RBCs during the hours after transfusion may be false. Standard issue RBCs, which are the RBCs of highest storage duration, may not increase oxygen delivery and may in fact decrease it by disturbing control of regional blood flow distribution (impaired nitric oxide processing) and failing to release oxygen, even when perfusing hypoxic tissue (abnormal oxygen affinity). Standard issue RBCs may assist with hemostasis but appear to have competing effects on thrombin generation and platelet function.

If standard issue or RBCs of increased storage age are not optimal, then are there alternatives that will allow for an efficacious and safe treatment of shock while also supporting hemostasis? Studies are required to determine if fresh RBCs less than 7 to 10 days provide an outcome advantage.

In patients with severe traumatic injury, early reversal of shock is essential to prevent exacerbation of coagulopathy and progression of cell death cascades. Red blood cell storage solutions have evolved to accommodate the needs of non–critically ill patients yet may not be optimal for patients in hemorrhagic shock. Continued focus on the recognition and treatment of shock is essential for continued improvement in outcomes for patients who require DCR and RDCR.

REFERENCES

1. Churchill E: *Surgeon to Soldiers: Diary and Records of the Surgical Consultant Allied Force Headquarters, World War II*. Philadelphia, PA: Lippincott, 38–48, 1972.
2. Hess JR: Red cell changes during storage. *Transfus Apheresis Sci* 43(1):51–59, 2010.
3. Spinella PC, Doctor A, Blumberg N, Holcomb JB: Does the storage duration of blood products affect outcomes in critically ill patients? *Transfusion* 51(8): 1644–1650, 2011.
4. Steiner ME, Stowell C: Does red blood cell storage affect clinical outcome? When in doubt, do the experiment. *Transfusion* 49(7):1286–1290, 2009.
5. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Mehta S, Cox ED, Gehrke MJ, Beilman GJ, Schreiber M, et al.: Damage control resuscitation: directly addressing the early coagulopathy of trauma. *J Trauma* 62(2):307–310, 2007.
6. Gerhardt RT, Strandenes G, Cap AP, Rentas FJ, Glassberg E, Mott J, Dubick MA, Spinella PC; THOR Network and RemTORN Study Groups. Remote damage control resuscitation and the Solstrand Conference: defining the need, the language, and a way forward . *Transfusion* 53(Suppl 1):9S–16S, 2013.
7. Antonelli M, Levy M, Andrews PJ, Chastre J, Hudson LD, Manthous C, Meduri GU, Moreno RP, Putensen C, Stewart T, et al.: Hemodynamic monitoring in shock and implications for management. International Consensus Conference, Paris, France, 27–28 April 2006. *Intensive Care Med* 33(4):575–590, 2007.
8. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB, Yukioka T, et al.: The coagulopathy of trauma: a review of mechanisms. *J Trauma* 65(4):748–754, 2008.
9. Cotton BA, Guy JS, Morris JA Jr, Abumrad NN: The cellular, metabolic, and systemic consequences of aggressive fluid resuscitation strategies. *Shock* 26(2): 115–121, 2006.
10. Doctor A, Stamler JS: Nitric oxide transport in blood: a third gas in the respiratory cycle. *Compr Physiol* 1(1):541–568, 2011.
11. Doctor A, Spinella P: Effect of processing and storage on red blood cell function *in vivo*. *Semin Perinatol* 36(4):248–259, 2012.
12. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Telen MJ, Mulherin MA, Zhu H, Buck RD, Califf RM, et al.: Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci U S A* 104(43):17063–17068, 2007.
13. Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, Tejero J, Hemann C, Hille R, Stuehr DJ, et al.: Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. *Proc Natl Acad Sci U S A* 104(45):17593–17598, 2007.
14. Valeri CR, Khuri S, Ragno G: Nonsurgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, red blood cells, platelets, and plasma-clotting proteins. *Transfusion* 47(Suppl 4):206S–248S, 2007.
15. Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, Papassideri IS: Storage-dependent remodeling of the red blood cell membrane is associated with increased immunoglobulin G binding, lipid raft rearrangement, and caspase activation. *Transfusion* 47(7):1212–1220, 2007.
16. Messana I, Ferroni L, Misiti F, Girelli G, Pupella S, Castagnola M, Zappacosta B, Giardina B: Blood bank conditions and RBCs: the progressive loss of metabolic modulation. *Transfusion* 40(3):353–360, 2000.
17. Bosman GJ, Lasonder E, Luten M, Roerdinkholder-Stoelwinder B, Novotny VM, Bos H, De Grip WJ: The proteome of red cell membranes and vesicles during storage in blood bank conditions. *Transfusion* 48(5):827–835, 2008.
18. 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 U S A* 104(43):17058–17062, 2007.
19. Kucukakin B, Kocak V, Lykkesfeldt J, Nielsen HJ, Magnussen K, Rosenberg J, Gögenur I: Storage-induced increase in biomarkers of oxidative stress and inflammation in red blood cell components. *Scand J Clin Lab Invest* 71(4): 299–303, 2011.
20. Dumaswala UJ, Zhuo L, Jacobsen DW, Jain SK, Sukalski KA: Protein and lipid oxidation of banked human erythrocytes: role of glutathione. *Free Radic Biol Med* 27(9–10):1041–1049, 1999.
21. Lion N, Crettaz D, Rubin O, Tissot JD: Stored red blood cells: a changing universe waiting for its map(s). *J Proteomics* 73(3):374–385, 2010.
22. Spinella PC, Sparrow RL, Hess JR, Norris PJ: Properties of stored red blood cells: understanding immune and vascular reactivity. *Transfusion* 51(4):894–900, 2011.
23. Sayers M, Centilli J: What if shelf life becomes a consideration in ordering red blood cells? *Transfusion* 52(1):201–206, 2012.
24. Offner PJ, Moore EE, Biffl WL, Johnson JL, Silliman CC: Increased rate of infection associated with transfusion of old blood after severe injury. *Arch Surg* 137(6):711–716, 2002. discussion 716–717.
25. Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, Jenkins D, Azarow K, Holcomb JB; 31st Combat Support Hospital Research Working Group.: Risks associated with fresh whole blood and red blood cell transfusions in a combat support hospital. *Crit Care Med* 35(11):2576–2581, 2007.
26. Kiraly LN, Underwood S, Differding JA, Schreiber MA: Transfusion of aged packed red blood cells results in decreased tissue oxygenation in critically injured trauma patients. *J Trauma* 67(1):29–32, 2009.
27. Solomon SB, Wang D, Sun J, Kanias T, Feng J, Helms CC, et al.: Mortality increases after massive exchange transfusion with older stored blood in canines with experimental pneumonia. *Blood* 121(9):1663–1672, 2013.
28. 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* 71(1):131–136, 1989.
29. Spoerke NJ, Van PY, Differding JA, Zink KA, Cho SD, Muller PJ, Karahan ZA, Sondeen JL, Holcomb JB, Schreiber MA: Red blood cells accelerate the onset of clot formation in polytrauma and hemorrhagic shock. *J Trauma* 69(5):1054–1059, 2010. discussion 1059–1061.
30. Silvain J, Pena A, Cayla G, Brieger D, Bellemain-Appaix A, Chastre T, Vignalou JB, Beygui F, Barthelemy O, Collet JP, et al.: Impact of red blood cell transfusion on platelet activation and aggregation in healthy volunteers: results of the TRANSFUSION study. *Eur Heart J* 31(22):2816–2821, 2010.
31. Reinhart WH, Zehnder L, Schulzki T: Stored erythrocytes have less capacity than normal erythrocytes to support primary haemostasis. *J Thromb Haemost* 101(4):720–723, 2009.
32. Roeloffzen WW, Kluin-Nelemans HC, Bosman L, de Wolf JT: Effects of red blood cells on hemostasis. *Transfusion* 50(7):1536–1544, 2010.
33. Nepstad I, Reikvam H, Strandenes G, Hess J, Apelseth T, Hervig T: Comparison of *in vitro* responses in fresh whole blood and reconstituted whole blood after collagen stimulation. *Blood Transfusion* 12(1):50–55, 2014.