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The risks of red cell storage

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"Transfuse two units packed red blood cells" may be among the most common orders a physician will write in his or her career, especially in the intensive care or surgical fields. Approximately 40% of critically ill individuals receive at least one unit of packed red blood cells in the intensive care unit, with a mean of five units per patient—half of human blood volume[1].

Although blood is a vital therapy for many patients, recent lessons from the bedside suggest that red blood cell transfusions may harbor a dark side. Red blood cell transfusion is associated with increased morbidity and mortality in the critically ill population[1]. A higher number of red blood cell units transfused seems to increase the risk of acute lung injury, and the age of red blood cells under storage is associated with respiratory failure requiring prolonged ventilator support[1,2].

Insights gained from these bedside studies are spurring researchers to understand at the mechanistic level how transfusion may drive organ injury in susceptible individuals. The process is complex and may involve multiple mechanisms, including lysis of red blood cells (hemolysis), oxidative stress and blood clot formation driven by microparticles that form over time in stored blood. At first glance, it would appear that the most practical solution is less blood, and younger blood. However, allocation of stored red blood cells, with nearly 15 million units of blood transfused each year in the US, is not a simple matter when one considers the precarious balance between the supply from healthy donors and the growing demand resulting from medical and surgical advances. A better understanding of red blood cell storage at the basic level can lead to rational strategies to prolong shelf life or identify factors to reduce risk in susceptible individuals.

Ever since the 1960s, massive red blood cell transfusions have been recognized as a risk factor for acute lung injury—a lesson first appreciated during the Vietnam War[3,4]. However, data from the 1990s provided reason for additional concern, calling into question the routine practice of transfusion when hemoglobin concentrations drop below 10 g dl^{-1}. A randomized controlled trial showed that a restrictive strategy (transfusion for a hemoglobin concentration of <7 g dl^{-1}) was as efficacious and possibly superior to a liberal strategy (transfusion for a hemoglobin concentration of <10 g dl^{-1}) (ref. 1). The incidence of cardiopulmonary complications, in particular pulmonary edema and acute respiratory

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distress syndrome, was lower in the restrictive strategy group compared with the liberal strategy group ($P < 0.01$ and $P = 0.06$, respectively).

In 2008, a highly publicized retrospective analysis by Koch *et al.*[2] raised serious questions about the shelf life of blood. The authors examined subjects undergoing coronary artery bypass grafting, heart-valve surgery or both[2]. They found that subjects transfused with red blood cell units older than the median storage duration, for longer than 14 days, were more likely to die in the hospital, required a ventilator for longer and had higher rates of sepsis and renal failure than subjects receiving newer blood[2]. Although the pool of subjects was high (6,000 subjects), the data were obtained from only one center and had several limitations, namely that the clinical characteristics of the patients and the ABO type of red blood cells received were not entirely similar between the two groups of patients[5].

The findings were supported by several earlier but smaller studies[2]—but not all studies suggest increased mortality, and uncertainty remains regarding the clinical importance of red blood cell storage duration and exactly what defines ‘newer’ versus ‘older’ blood, as red blood cells can be stored up to 42 days.

These studies at the bedside have propelled investigators at the bench to examine closely how stored red blood cells, under standard blood bank conditions, may promote or perpetuate microcirculatory perturbations or organ dysfunction.

To lay the groundwork for a mechanistic understanding, researchers have carefully documented what storage does to red blood cells. The changes include reduced deformability and increased osmotic fragility. Oxidative stress increases with prolonged storage, resulting in lipid peroxidation, protein oxidation and reduced integrity of the erythrocyte membrane— all contributing to the formation of exocytic microvesicles or ‘microparticles’ and hemolysis. It remains to be determined whether these changes translate into functional perturbations in the susceptible host. A number of candidate pathways are currently being explored (Fig. 1).

Because lung injury is a major contributor to increased mortality in critically ill individuals, investigators are reexamining the relationship of blood transfusion to lung injury. Transfusion-related acute lung injury, or TRALI, is an uncommon clinical syndrome characterized by new-onset acute lung injury during or immediately after transfusion. As the understanding of TRALI is evolving, it remains unclear whether some of the observed clinical associations can be attributed to unrecognized or milder forms. Yet, several aspects of its proposed mechanisms are relevant to understanding the potential *in vivo* effects of the storage lesion.

One proposed mechanism of TRALI that is gaining wider appreciation requires at least two independent events[6]. The first event occurs in the host before transfusion through infection or another trigger. This causes endothelial activation with increased expression of adhesion molecules and chemokines, leading to neutrophil sequestration in the lungs. The second event is transfusion of factors that accumulate during blood storage, providing additional signals for neutrophil-mediated endothelial damage and lung injury.

The ‘two-hit’ process is consistent with clinical observations that the risk of developing lung injury rises dramatically when more than one predisposing event occurs[4]. This combination of events is perhaps mediated by platelet-neutrophil interactions, as suggested by a recent study in a mouse model of TRALI[7]. Indeed, platelets triggering neutrophils to generate reactive oxygen species and leading to vascular damage is a plausible mechanism, as platelet activation occurs in people with acute lung injury[8]. Just how stored red blood cells promote platelet-leukocyte interactions is still unclear, but one possibility is hemostatic activation of platelets after encounter with microparticles derived from red blood cells. Microparticles, characterized by exposure of phosphatidylserine on their surface, provide a procoagulant surface leading to thrombin generation[9]. Hemostatic activation of platelets may spatially link thrombosis and inflammation to amplify microvascular damage.

Although approximately half of the observed hemolysis in stored blood is enclosed in microparticles, the other half is in the form of free hemoglobin in plasma. We have previously shown that, in individuals with sickle cell anemia, free hemoglobin from hemolyzed erythrocytes reduces nitric oxide (NO) bioavailability, resulting in detrimental effects[10]. We also observe that free hemoglobin in plasma and microparticles from red blood cell units will increase the rate of NO scavenging by endothelium—and that this will occur at very low concentrations of free heme (as little as 6 μM)[10,11].

NO has a fundamental role in vascular health by regulating vasodilation and inhibiting both platelet aggregation and endothelial adhesion molecule expression. It is therefore likely that reduced NO bioavailability from microparticles and free hemoglobin in plasma can augment thrombosis, microcirculatory perturbations or injury in susceptible hosts. The key role of the NO pathway is illustrated by human clinical trials of diaspirin–cross-linked hemoglobin and the NO synthase inhibitor L-NMMA. The studies were halted owing to concerns over increased multiple organ dysfunction (in particular, one case of fatal lung injury) or increased mortality in the treatment groups.

Still one other pathway is under scrutiny. Aging red blood cells and cell-free plasma hemoglobin are potent inducers of oxidative stress. Hemoglobin mediates fenton, autooxidation, peroxidase and nitration chemistries and may directly activate vascular oxidases and uncouple endothelial NO synthase to generate reactive oxygen species. A recent study examining the effects of cell-free hemoglobin infusion in a canine model showed extensive lipid peroxidation in the kidneys associated with nonheme iron deposition[12].

Hemoglobin in microparticles and released from hemolysis may represent a common factor driving multiple pathways such as oxidative stress, NO depletion and platelet activation. These processes may aggravate ongoing inflammation to produce vascular damage and lung injury in transfused susceptible hosts—and perhaps also drive damage in other susceptible organs, such as the kidneys. Whether therapies targeting these pathways ameliorate vascular damage awaits confirmation.

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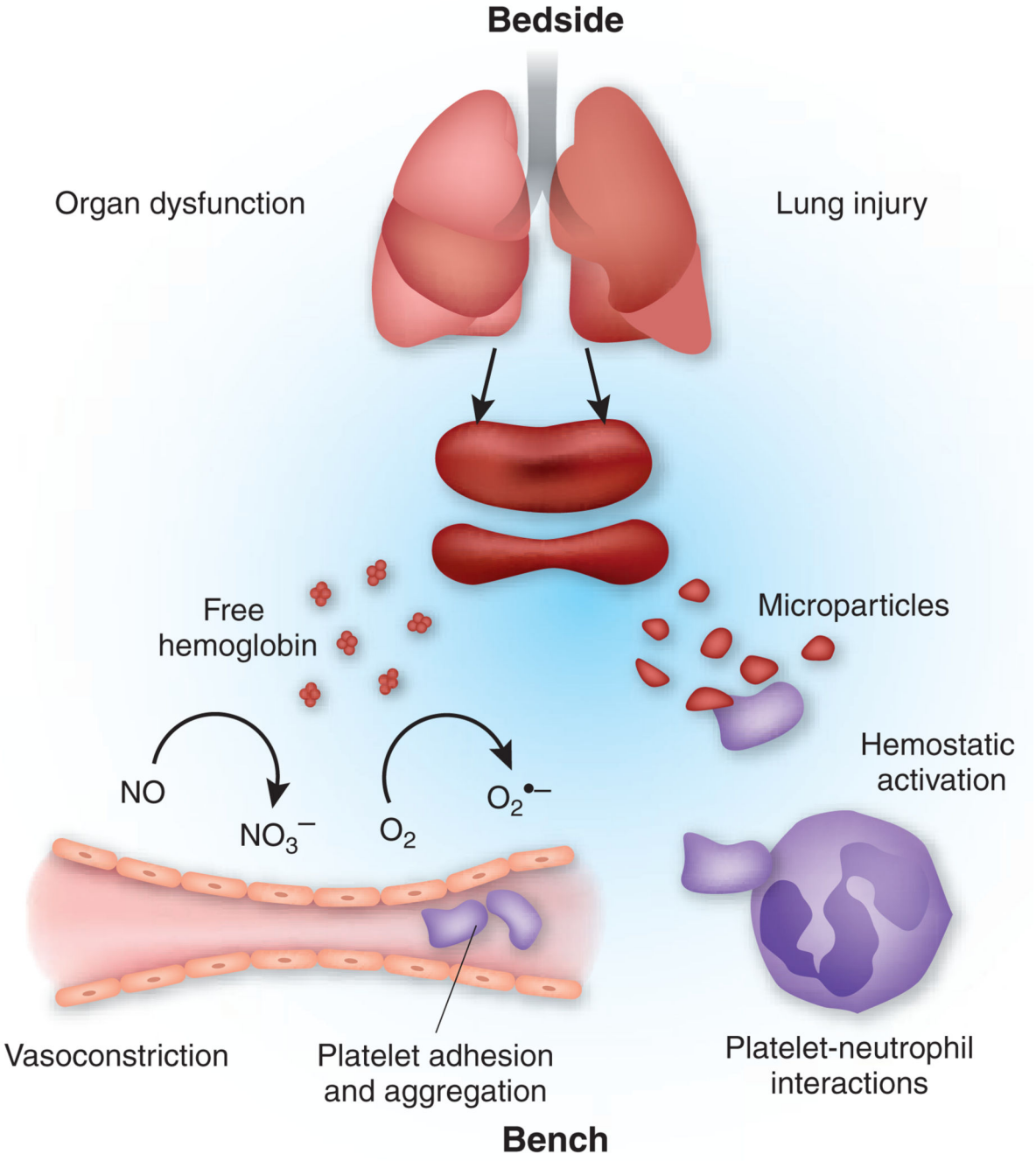

Figure 1.

Several proposed mechanisms by which stored red blood cells can perpetuate microcirculatory perturbations and organ dysfunction. Red blood cells undergo hemolysis and microparticle formation. Free hemoglobin and microparticle hemoglobin scavenge NO, resulting in the loss of tonic vasodilation and generation of reactive oxygen species. The reduction in NO bioavailability promotes both platelet adhesion and aggregation in an

already compromised microcirculation. Microparticles with exposed phosphatidylserine on their surface may promote hemostatic activation and platelet-neutrophil aggregation.