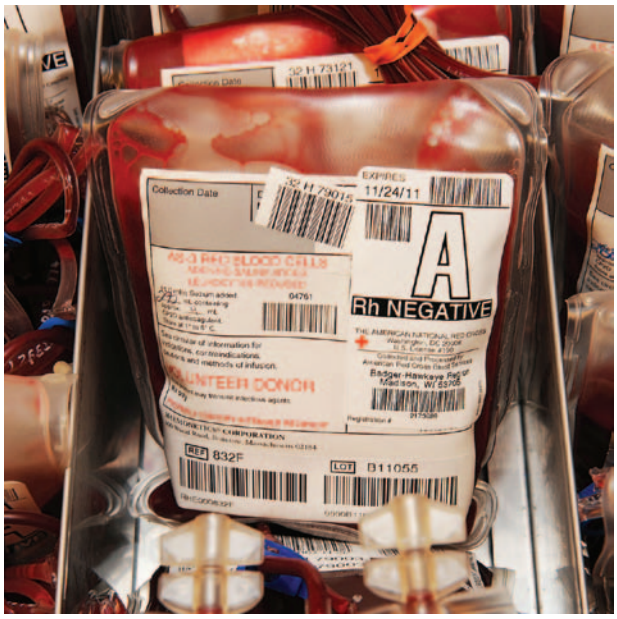
Understanding the Erythrocyte Storage Lesion

THE methodology to store erythrocytes for transfusion has markedly enhanced the supportive care capacity in hematology, surgery, trauma, and critical care medicine. However, the limitations of stored blood have come under recent scrutiny and great controversy. In this issue of ANESTHESIOLOGY, Lei *et al.*[1] at Massachusetts General Hospital show that transfusion with stored erythrocytes increases tissue injury of hemorrhagic shock more than transfusion with fresh erythrocytes, using an experimental rodent model of blood transfusion. Furthermore, they find that preexisting endothelial dysfunction caused by a high-fat diet increases the risk of this adverse response to transfused stored blood, modeling a common scenario under which human patients might receive transfusions for traumatic emergencies.



"... this [work and others] supports a credible hypothesis that the blood storage lesion effect on morbidity and mortality is seen primarily in patients with preexisting risk factors for cardiovascular disease during critical illness."

Over the years, several observational studies have hinted that critically ill patients are at greater risk of dying if they receive transfusions of multiple units of erythrocytes, suggesting an adverse property of stored erythrocytes. This finding is hotly debated, because it is essentially impossible to erase the confounding issue that the patients who require the most transfusions inherently are also the sickest and most likely to die. Do the sickest patients die because of blood transfusions or despite blood transfusions? This is a sensitive but crucial question.

Pathologic changes in blood during storage, often called the blood storage lesion, is a rapidly growing area of biomedical research. Before 2005, this was an esoteric subject, addressed in five peer-reviewed articles each year on average. Since that year, nearly 20 articles per year on the average fuel the debate with new research on the storage lesion. Stored erythrocytes undergo morphologic changes, metabolic alterations, and some degree of hemolysis during storage. During storage, erythrocytes undergo depletion of potassium, 2,3-diphosphoglycerate, adenosine triphosphate, lipids, and membrane, with increased erythrocyte rigidity and impaired oxygen delivery.[2] The stored units accumulate microvesicles derived from erythrocytes, free hemoglobin and biologically active lipids, demonstrating increased proinflammatory and procoagulant activity. Modern blood-bank preservative solutions provide glucose and other stabilizing substances to minimize these undesirable changes that might adversely affect the transfusion recipient.[3]

Despite these stabilizers, detectable hemolysis occurs in the storage bag and in the patient. The Food and Drug Administration mandates minimal standards that less than 1% of the erythrocytes lyse in the storage bag, and no more than 25% of the cells cleared from the recipient within 24 h after infusion, which likely correspond to cells lysed *in vivo*.[3] These round number thresholds are arbitrarily established long ago and not derived by any empirical scientific testing.

This debate more recently has been focused on a single hot button: duration of erythrocyte storage. Patients who received newer blood serve as experimental controls for those who receive older blood. Wang *et al.*[4] performed a meta-analysis of 21 independent observational studies evaluating associations of transfusion with erythrocytes stored for longer duration. Older blood was associated with a significantly increased risk of death (odds ratio, 1.16; 95% CI, 1.07–1.24). These authors provocatively estimate that if all cardiac surgery and trauma patients were transfused with

Photo: J. P. Rathmell.

Accepted for publication July 18, 2012. Dr. Kato receives research funding from the Division of Intramural Research of the National Heart, Lung and Blood Institute at the National Institutes of Health (Bethesda, Maryland; grant number 1 ZIA HL006014-03 and others).

 Anesthesiology 2012; 117:1159-61

◆ This Editorial View accompanies the following article: Lei C, Yu B, Shahid M, Beloiartsev A, Bloch KD, Zapol WM: Inhaled nitric oxide attenuates the adverse effects of transfusing stored syngeneic erythrocytes in mice with endothelial dysfunction after hemorrhagic shock. ANESTHESIOLOGY 2012; 117:1190–202.

fresher rather than older blood, one additional life would be saved for each 97 transfused with standard blood.

This analysis has unleashed a volley of caveats regarding the intrinsic limitations of these retrospective analyses of observational data.[5] A patient receiving multiple transfusions is likelier to receive at least 1 unit of blood that has been stored longer than average, confounding the age of blood with the amount of blood and the severity of illness. There is no standard, accepted method that accurately scores the properties of the stored blood, even storage duration, when multiple units of blood are administered, partly because many factors are known to change in stored blood as part of the storage lesion. Assumptions during the analysis can introduce statistical artifact and biases that may either obscure or overestimate the association of storage duration to subsequent outcomes.[5] Randomized controlled trials certainly could produce data with fewer such issues.

Three randomized controlled trials have been initiated in cardiac surgery,[6] critically ill patients,[7] and in neonates,[8] but no final results are yet published. In an effort to reach answers more quickly and mechanistically, several investigators have conducted physiologic studies of stored blood in animal models and in humans. Many of the researchers have experimentally investigated vasodilatory dysfunction in recipients of stored blood products attributable to extracellular free hemoglobin. *In vitro*, extracellular free hemoglobin avidly scavenges nitric oxide, a signal transduction ligand that orchestrates a broad program of vascular homeostasis. Additional human and animal research studies have evaluated the contribution of nitric oxide scavenging by free hemoglobin to physiologic consequences of the blood storage lesion.[9] Alternatively, some investigators have emphasized oxidant stress as the principal mechanism mediating free hemoglobin toxicity to endothelial function and vascular smooth muscle tone.[10] Still others even implicate oxidant stress from free heme or iron released from hemoglobin.[11] Whatever the dominant mechanism, many diverse lines of investigation implicate hemolysis of stored blood as pathogenic.

Regardless of exact mechanism, a growing number of publications report evidence that stored blood impairs normal vasodilation and blood flow and implicate extracellular hemoglobin as the offending molecule.[12] Free hemoglobin and hemoglobin-containing erythrocyte microvesicles accumulate during blood storage and consume nitric oxide *in vitro*. Transfusion of these forms of extracellular hemoglobin induces vasoconstriction in rats.[13] Haptoglobin, the endogenous scavenger of free hemoglobin, in a guinea pig model blunts adverse effects of stored blood, including intravascular hemolysis, acute hypertension, vascular injury, and kidney dysfunction, supporting a role in vascular pathophysiology of extracellular hemoglobin among the other potentially pathogenic molecules released during hemolysis.[14] Transfusion of blood stored for 40 days into lambs induces pulmonary vasoconstriction and acute pulmonary hypertension preventable with inhaled nitric oxide.[15] The effectiveness of either haptoglobin or nitric oxide breathing is consistent with free hemoglobin as the pathogenic agent in stored blood, whether mediated by nitric oxide scavenging or oxidative stress.

It may be possible that the effect of the blood storage lesion on outcome is clinically significant only when combined with additive or synergistic risk factors. The Zapol group has found that known risk factors for vasomotor dysfunction, including diabetes,[16] high-fat diet, and hemorrhagic shock,[1] amplify the adverse effect of blood storage duration on vascular function and outcome in animal models. In clinical practice, this supports a credible hypothesis that the blood storage lesion effect on morbidity and mortality is seen primarily in patients with preexisting risk factors for cardiovascular disease during critical illness.

This hypothesis would help to explain why the effect of stored blood on vascular physiology is not apparent in healthy volunteers.[17] Future investigations may yield more informative results if focused on transfusion with stored blood under life-threatening conditions compounded by other vascular risk factors. Until then, clinical use of erythrocytes stored up to 42 days remains the standard of care.

Gregory J. Kato, M.D., Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. gkato@mail.nih.gov

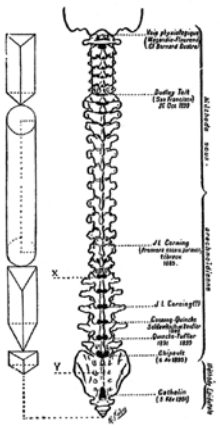
References

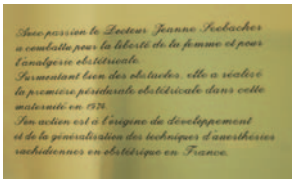
1. Lei C, Yu B, Shahid M, Beloiartsev A, Bloch KD, Zapol WM: Inhaled nitric oxide attenuates the adverse effects of transfusing stored syngeneic erythrocytes in mice with endothelial dysfunction after hemorrhagic shock. Anesthesiology 2012; 117:1190-202
2. Kim-Shapiro DB, Lee J, Gladwin MT: Storage lesion: Role of red blood cell breakdown. Transfusion 2011; 51:844–51
3. Hess JR: An update on solutions for red cell storage. Vox Sang 2006; 91:13–9
4. Wang D, Sun J, Solomon SB, Klein HG, Natanson C: Transfusion of older stored blood and risk of death: A meta-analysis. Transfusion 2012; 52:1184–95
5. Triulzi DJ, Yazer MH: Clinical studies of the effect of blood storage on patient outcomes. Transfus Apher Sci 2010; 43:95–106
6. Steiner ME, Assmann SF, Levy JH, Marshall J, Pulkrabek S, Sloan SR, Triulzi D, Stowell CP: 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; 43:107–16
7. Lacroix J, Hébert P, Fergusson D, Tinmouth A, Blajchman MA, Callum J, Cook D, Marshall JC, McIntyre L, Turgeon AF; ABLE study group: The Age of Blood Evaluation (ABLE) randomized controlled trial: Study design. Transfus Med Rev 2011; 25:197–205
8. Fergusson D, Hutton B, Hogan DL, LeBel L, Blajchman MA, Ford JC, Hebert P, Kakadekar A, Kovacs L, Lee S, Sankaran K, Shapiro S, Smyth JA, Ramesh K, Bouali NR, Tinmouth A, Walker R: The age of red blood cells in premature infants (ARIPI) randomized controlled trial: Study design. Transfus Med Rev 2009; 23:55–61
9. Gladwin MT, Kim-Shapiro DB: Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Curr Opin Hematol 2009; 16:515–23

10. Boretti FS, Buehler PW, D'Agnillo F, Kluge K, Glaus T, Butt OI, Jia Y, Goede J, Pereira CP, Maggiorini M, Schoedon G, Alayash AI, Schaer DJ: Sequestration of extracellular hemoglobin within a haptoglobin complex decreases its hypertensive and oxidative effects in dogs and guinea pigs. J Clin Invest 2009; 119:2271–80
11. Hod EA, Spitalnik SL: Stored red blood cell transfusions: Iron, inflammation, immunity, and infection. Transfus Clin Biol 2012; 19:84–9
12. Roback JD: Vascular effects of the red blood cell storage lesion. Hematology Am Soc Hematol Educ Program 2011; 2011:475–9
13. Donadee C, Raat NJ, Kanias T, Tejero J, Lee JS, Kelley EE, Zhao X, Liu C, Reynolds H, Azarov I, Frizzell S, Meyer EM, Donnenberg AD, Qu L, Triulzi D, Kim-Shapiro DB, Gladwin MT: Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. Circulation 2011; 124:465–76
14. Baek JH, D'Agnillo F, Vallelian F, Pereira CP, Williams MC, Jia Y, Schaer DJ, Buehler PW: Hemoglobin-driven pathophysiology is an *in vivo* consequence of the red blood cell storage lesion that can be attenuated in guinea pigs by haptoglobin therapy. J Clin Invest 2012; 122:1444–58
15. Baron DM, Yu B, Lei C, Bagchi A, Beloiartsev A, Stowell CP, Steinbicker AU, Malhotra R, Bloch KD, Zapol WM: Pulmonary hypertension in lambs transfused with stored blood is prevented by breathing nitric oxide. ANESTHESIOLOGY 2012; 116:637–47
16. Yu B, Lei C, Baron DM, Steinbicker AU, Bloch KD, Zapol WM: Diabetes augments and inhaled nitric oxide prevents the adverse hemodynamic effects of transfusing syngeneic stored blood in mice. Transfusion 2012; 52:1410–22
17. Berra L, Coppadoro A, Yu B, Lei C, Spagnolli E, Steinbicker AU, Bloch KD, Lin T, Sammy FY, Warren HS, Fernandez BO, Feelisch M, Dzik WH, Stowell CP, Zapol WM: Transfusion of stored autologous blood does not alter reactive hyperemia index in healthy volunteers. ANESTHESIOLOGY 2012; 117:56–63

ANESTHESIOLOGY REFLECTIONS FROM THE PIERRE VIARS MUSEUM

Epidural Anesthesia at the Pitié-Salpêtrière Hospital: From Fernand Cathelin (1901) to Jeanne Seebacher (1974)





The discovery of the epidural technique for locoregional anesthesia has been attributed to two independent French physicians, Jean Athanase Sicard (1872–1929) and Fernand Cathelin (1873–1945). However, for many French historians, it seems that the credit of the discovery should be attributed to Cathelin and that Sicard was something of an opportunist, being aware of the research conducted by Cathelin at the Pitié-Salpêtrière hospital. An image from the book of Cathelin published in 1903 is shown (*left*) and represents the main historical steps in the development of epidural injections from 1885 to 1901. In 1974, in the Pitié-Salpêtrière, the anesthesiologist Jeanne Seebacher (*top right*) introduced epidural anesthesia in obstetrics for the first time in France. In the obstetric department, a memorial tablet celebrates that event (*bottom right*): "With passion, Jeanne Seebacher struggled for the freedom of women and for obstetric analgesia. Overcoming many obstacles, she performed the first obstetric epidural anesthesia in 1974 in France in this hospital. Her action was the first step toward a widespread use of epidural and intrathecal anesthesia for obstetrical analgesia in France."

Jean-Bernard Cazalaà, M.D., President of Club d'Histoire de l'Anesthésie et de la Réanimation (French Association for the History of Anesthesiology and Critical Care), France (www.char-fr.net), and Musée Viars, CHU Pitié-Salpêtrière, Paris, France.