Published in final edited form as:

Semin Perinatol. 2012 August ; 36(4): 248–259. doi:10.1053/j.semperi.2012.04.005.

Effect of Processing and Storage on RBC function *in vivo*

Allan Doctor, MD^{1,2} [Associate Professor] and Phil Spinella, MD¹

¹Department of Pediatrics and Biochemistry Washington University in St Louis School of Medicine St Louis, MO

²Department of Molecular Biophysics Washington University in St Louis School of Medicine St Louis, MO

Abstract

Red Blood Cell (RBC) transfusion is indicated to improve oxygen delivery to tissue, and for no other purpose. We have come to appreciate that donor RBCs are fundamentally altered during processing and storage, in a fashion that both impairs oxygen transport efficacy and introduces additional risk by perturbing both immune and coagulation systems. The protean biophysical and physiologic changes in RBC function arising from storage are termed the 'storage lesion'; many have been understood for some time; for example, we know that the oxygen affinity of stored blood rises during the storage period¹ and that intracellular allosteric regulators, notably 2,3-bisphosphoglyceric acid (DPG) and ATP, are depleted during storage. Our appreciation of other storage lesion features has emerged with improved understanding of coagulation, immune and vascular signaling systems. Herein we review key features of the 'storage lesion'. Additionally, we call particular attention to the newly appreciated role of RBCs in regulating linkage between regional blood flow and regional O₂ consumption by regulating the bioavailability of key vasoactive mediators in plasma, as well as discuss how processing and storage disturbs this key signaling function and impairs transfusion efficacy.

Goal of Red Blood Cell (RBC) Transfusion

Red Blood Cell (RBC) transfusion is indicated to improve oxygen delivery to tissue, and for no other purpose. We have come to appreciate that donor RBCs are fundamentally altered during processing and storage, in a fashion that both impairs oxygen transport efficacy and introduces additional risk by perturbing both immune and coagulation systems. The protean biophysical and physiologic changes in RBC function arising from storage are termed the 'storage lesion'; many have been understood for some time; for example, we know that the oxygen affinity of stored blood rises during the storage period¹ and that intracellular allosteric regulators, notably 2,3-bisphosphoglyceric acid (DPG) and ATP, are depleted during storage. Our appreciation of other storage lesion features has emerged with improved understanding of coagulation, immune and vascular signaling systems. In this review, we will call particular attention to the newly appreciated role of RBCs in regulating regional blood flow (and thus O₂ delivery) as well as discuss how disturbance of this key signaling

© 2012 Elsevier Inc. All rights reserved.

Corresponding Author: Allan Doctor, MD Associate Professor of Pediatrics, and Biochemistry & Molecular Biophysics Washington University School of Medicine Director, Division of Pediatric Critical Care St. Louis Children's Hospital Campus Box 8208 McDonnell Pediatric Research Building 660 South Euclid Avenue St. Louis, MO 63110 tel: (314) 454-0021 fax: (314) 286-2894 doctor@wustl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

function (by processing and storage for transfusion) impairs transfusion efficacy (improving O₂ delivery).

Alterations to RBCs during Processing and Storage ('the Storage Lesion')

Many recent reports summarize the changes that occur with RBC storage²⁻⁷ (FIGURE 1). These reports document increased potassium, lactate, and free hemoglobin with increased RBC storage time^{8, 9}. Additionally, RBCs lose deformability with increased duration of storage⁸, limiting passage through the microcirculation, which is further impaired by increased RBC aggregation and adhesion to endothelium^{8, 10-12}. As noted above, the concentration of 2,3 DPG decreases with storage time⁸, the resultant increase in oxygen affinity limits oxygen unloading from hemoglobin during systemic perfusion¹³. RBC storage also impacts recipient immune function. Stored RBCs induce alterations in multiple cytokines after incubation with plasma or whole blood samples, including increased IL-6, IL-8, phospholipase A2, and superoxide anions, and decreased TNF-alpha concentrations^{9, 14}. The clinical consequences of this phenomena was first reported in the 1970's when Opelz et al. reported improved renal allograft success in patients transfused pre-renal transplant¹⁵. Subsequently, additional reports indicating an immune suppressive effect of RBC transfusions have documented an association with increased cancer reoccurrence, live births in women with a history of spontaneous abortions, and increased postoperative infection rates^{16, 17}.

Of note, the genesis of RBC microparticles during RBC storage may be related to the influence of transfusion upon donor immune and coagulation systems^{18, 19}. The oxidative injury that occurs with storage leads to formation of RBC membrane microparticles and release of bioactive lipids from its membrane²⁰. Such RBC microvesicles contain CD47 antigens associated with macrophage inhibition²⁰. Other data suggests that increased generation of procoagulant phospholipids occurs during RBC storage^{21, 22}. These bioactive lipids, such as lysophosphatidylcholine, have also been demonstrated to be pro-inflammatory and are associated with an increased risk of acute lung injury²³. The immune dysfunction secondary to transfusion has been termed Transfusion Related Immune Modulation (TRIM) and has been well described^{24, 25}. Interestingly, the transfusion of RBCs of increased storage duration has also been associated with Transfusion Related Acute Lung Injury (TRALI) via pro-inflammatory mechanisms¹⁷.

RBC Transfusion Efficacy as a function of RBC Storage Duration

While transfusion of RBCs increases the hematocrit (Hct) and therefore increases oxygen (O₂) content, it does not necessarily follow that O₂ delivery to tissue is likewise increased. The purpose of transfusion is not to merely improve O₂ content, but instead – to improve O₂ delivery. As outlined above, fundamental changes occur to RBCs during storage that alter their biological function and their ability to deliver O₂⁷. Beyond their role as O₂ carriers, RBCs play a fundamental role in the O₂ dependent regulation of vascular tone by exporting vasodilatory signals that regulate regional blood flow. This key RBC regulatory function is modified by processing and storage, resulting in compromised RBC-dependent vasoregulation, which impairs regional O₂ delivery^{8, 26}.

In general, animal models evaluating transfusion efficacy demonstrate adverse effects of storage upon O₂ delivery²⁷⁻²⁹. For example, in an animal shock model, human RBCs of decreased storage age improved O₂ consumption whereas older RBCs could not²⁹. In humans, several groups have measured O₂ delivery and consumption before and after RBC transfusion in critically ill patients and have reported a disconcerting lack of benefit directly attributable to RBC transfusion³⁰⁻³⁹. Though it is difficult to ascertain the RBC transfusion efficacy and there are many difficulties with measures of global or tissue-specific cellular

respiration, available evidence suggests that transfusion of stored RBCs may have adverse effects on microcirculatory flow and oxygen utilization.

Most storage lesion effects appear to worsen with increased storage duration. When older RBCs (21-42 days) have been compared to fresh RBCs (0-4 days of storage): inflammatory response, mediators of oxidative injury, and risk of hyper-coagulation appear increased^{8, 14, 21, 22, 40, 41}.

RBCs stored for a longer time also appear more likely to activate recipient neutrophils that have been primed by recipient disease (such as sepsis or multiple trauma)²³, a mechanism posited for transfusion related acute lung injury (TRALI)^{17, 42}. In a study of critically ill adults, transfusion of RBCs stored for > 14 days was associated with a decrease in tissue oxygenation, whereas transfusion of RBCs stored < 14 days did not change tissue O₂ saturation⁴³. In a study of rats in shock, human RBCs stored for 3 days increased O₂ consumption whereas human RBCs stored for 28 days did not²⁹. RBCs of increased storage age have also been associated with decreased perfusion and O₂ consumption in critically ill adults⁴. These results were not confirmed in a similar study involving 22 patients which did not reveal any improvement in perfusion or oxygen consumption with RBCs of increased or decreased age potentially because it was not adequately powered to detect a difference in O₂ consumption⁴⁴. Conflicting results have also been reported for patients with traumatic brain injury, where one study reports no improvement in the partial pressure of O₂ in brain tissue with RBCs > 19 days of storage and another found no relationship between RBC age and cerebral oxygenation^{45, 46}. Other small studies have not measured a decrease in O₂ consumption or tissue saturations with the transfusion of RBC stored for longer times⁴⁶. Of note, evaluation of blood product efficacy and safety is best informed by distinctly separating analysis of outcome in critically ill and non-critically ill populations, since the risk-benefit ratio for blood product administration is dependent on the baseline physiologic state and differs between critically ill and non-critically ill patients⁴⁷. Specifically, patients with marginally compensated organ failure are less tolerant of additional insult, including adverse effects attributable to transfusion¹⁷.

RBC Vasoactivity: Relevance to O₂ Delivery and Transfusion Efficacy

Oxygen delivery is a function of blood O₂ content and flow, but the latter (flow) is the far more important determinant. Specifically, the ability to increase O₂ content is somewhat limited (varying linearly with Hb concentration and % O₂ saturation), whereas regional blood flow (a function of vessel radius to the 4th power) may be increased or decreased by several orders of magnitude. In fact, under most circumstances, it is principally the volume and distribution of blood flow that vary to maintain dynamic coupling between O₂ delivery and metabolic demand. Of note, classic experiments demonstrate that it is Hb O₂ saturation (rather than free plasma or tissue PO₂) that is coupled to blood flow to maintain this physiology *in vivo*^{48, 49}. These and other studies provide evidence that RBCs act as both O₂ sensors and transducers of vasoconstrictor and vasodilator responses⁵⁰⁻⁵³. RBCs have been demonstrated to recapitulate this response *in vitro*, actuating graded vasodilation across the physiological O₂ gradient⁵². As such, remarkably, RBCs dilate blood vessels in concert with, and proportional to, O₂ delivery^{54, 55}. That this coupling is directly linked to Hb O₂ saturation rather than to PO₂, indicates that, in addition to service as an O₂ transport protein, Hb is a key physiological effector in resolution of tissue hypoxia^{49, 56-59}.

Control of NO transport and bioavailability is fundamental to RBC-based blood flow autoregulation and acts by effecting reflex hypoxic vasodilation (HVD) in the circulation. It should be noted, however, that *endothelium-derived* NO does not *directly* mediate the HVD that underlies blood flow regulation^{51, 60}. In fact, because of substrate (O₂) limitation, NO

production by eNOS is most likely attenuated by hypoxia⁶¹⁻⁶³. Moreover, NOS inhibitors do not block the acute change in blood flow that is coupled to Hb desaturation⁴⁸. Rather, it appears that NO is transported by RBCs to effect HVD at a time and place remote from the original site of NO synthesis (the NO groups deployed by RBCs do, however, originally arise from eNOS⁵⁴ and perhaps, other NOS isoforms⁶⁴ or nitrite⁶⁵). RBCs are therefore vascular control elements (FIGURE 2). Thus, it is increasingly appreciated that the transition in Hb conformation that occurs in the course of O₂ loading and delivery during arterio-venous (A-V) transit *also* governs transactions between RBCs and circulating NO groups in plasma: oxygenated (R-state) Hb sequesters, and deoxygenated (T-state) Hb releases, net NO bioactivity. As such, Hb R- to T-state cycling drives processing of circulating NO groups (through RBCs) to plasma or cellular thiols, to form *vasoactive* NO adducts known as S-nitrosothiols (SNOs). This system provides functional coupling between regional blood flow and biochemical cues of perfusion insufficiency, which may include hypoxia, hypercarbia and acidosis (FIGURE 3). As described below, the system is vulnerable to disruption during periods of oxidative stress and sustained alterations in PO₂.

Evidence for RBC Vasoactivity

Multiple lines of evidence indicate that NO is transported by RBCs to distal tissues. This RBC paracrine function is governed by O₂-linked transitions in Hb conformation, and Hb O₂ saturation thereby couples export of vasodilatory signals to tissue PO₂. Importantly, the only known endogenous NO compounds that retain bioactivity in the presence of Hb are S-nitrosothiols (SNOs). The data corroborating this biology is extensive. For example, Stamler et al.^{52, 55} and Datta et al.⁶⁶ demonstrated that RBCs dilate pre-constricted aortic rings at low PO₂ (1% O₂) but constrict at high PO₂ (95% O₂). More broadly, vasoconstriction and vasodilation by RBCs is graded across the physiological range of PO₂ encountered in the microcirculation⁵². In addition, Kubes' group⁶⁷⁻⁶⁹ has demonstrated bioactivity of inhaled NO in the mesenteric circulation that is commensurate with SNO production, and Cannon et al.⁷⁰ observed that NO gas inhalation lowers forearm vascular resistance in human volunteers who had undergone pharmacological NOS inhibition. McMahon and colleagues⁷¹ identified inhaled NO-based activity, at least in part, with SNO-Hb. Moreover, Reynolds and colleagues⁷² and Diesen and colleagues⁷³ have shown that NO added to RBCs potentiates vasodilatory activity to the extent that SNO-Hb is generated. Sonveaux et al.⁷⁴ have reported that the lifetime of infused SNO-Hb is increased by high PO₂ and destabilized at low PO₂, in direct contrast to NO itself. Additional studies support the conclusion that SNOs are impervious to inactivation by RBCs^{73, 75}. It therefore appears that NO bioactivity is preserved by RBCs in the form of stable SNOs that can be 'exported' to peripheral tissues on demand. Notably, hypoxic vasodilation by RBCs is entirely independent of endothelium^{54, 73, 76-78} and is facilitated by the presence of extra-erythrocytic low-molecular-weight thiols.

Expanding the NO Paradigm: RBCs Control NO Bioactivity in Blood

Originally, the "classic" NO paradigm was based on several tenets: 1) NO[•] is the primary bioactive product of NOS; 2) NO life-time and fate is explained by the diffusion of NO[•] "gas" in solution and by its terminal reactions with Hb (and superoxide); and 3) the heme center of guanyl cyclase is the primary NO[•] receptor. More recent evidence suggests that NO-based signaling is significantly more complex. In particular, the landscape has been fundamentally altered by appreciation that NO acts principally through covalent binding to cysteine thiols to form S-nitrosothiols (SNOs), rather than by signaling exclusively via sGC. Approximately one thousand SNO-proteins have now been identified in cells and tissues, indicating the central role of SNOs in NO-based signaling⁷⁹⁻⁸¹.

It is now understood that through context-responsive reactions between NO and Hb (that is, reactions coupled to PO₂ and influenced by CO₂ and pH), RBCs exert functional control over NO group bioavailability in blood, and thereby perform crucial roles as both sensors of O₂ supply and effectors in vascular signaling at local, organ, and system levels^{54-56, 82}. Thus, RBCs shuttle 3 gases in the respiratory cycle (NO/O₂/CO₂) to control delivery of O₂. Both the complexity of the biological chemistry of NO and the specificity of NO-group reactions in signaling are well exemplified in the case of Hb. Through tightly regulated reactions at its heme center and a reactive cysteine thiol (β cys⁹³), in the course of circulatory transit, Hb captures (binds NO at β -hemes), activates (converts β -heme-NO into β cys⁹³-SNO) and deploys NO groups (transnitrosylates receptor thiols, see details below). Heme-redox coupled activation of the NO group, a requirement for the oxidative chemistry that subserves conversion of heme-NO into β cys⁹³-SNO, is governed by O₂-coupled allosteric transitions in Hb^{54, 82-84}. The O₂-linked change in Hb conformation also initiates export of SNO from RBCs by promoting NO transfer to receptor thiols, including those associated with the erythrocytic membrane protein AE-1 (Band 3)⁸⁵ and thence to extra-erythrocytic thiols^{50, 75} to form plasma or other cellular SNOs, which are vasoactive at concentrations as low as 1 to 5 nM^{54, 55}. By governing the bioavailability of NO in the microcirculation through regulated sequestration, transport and delivery of NO groups, RBCs maintain appropriate coupling between regional blood flow and both O₂ availability in the lung and O₂ need in the periphery.

S-nitrosylation (SNO synthesis) in RBCs

The chemical basis of S-nitrosylation reactions and the molecular determinants of targeting specificity are reviewed in detail elsewhere^{56, 80, 86}. In brief, hemoglobin processes NO[•], low-molecular-weight SNO or nitrite (NO⁻²) into S-nitroso-Hb (SNO-Hb), and thus has been analogized to a SNO reactor⁸⁷⁻⁹². As described below, the locus of NO binding to the Hb globin chain is the highly conserved β cys⁹³, which conforms to both acid-base and hydrophobic motifs for S-nitrosylation^{87, 89, 92-95}. Importantly, as a result of conformation-dependent variation in the β cys⁹³ microenvironment, the likelihood of NO ligation to β cys⁹³ thiol is increased in R-structure (oxygenated Hb) and diminished in T-structure (deoxygenated Hb)^{54, 55, 82}. Therefore, S-nitrosylation is favored upon Hb oxygenation, whereas NO group (NO/NO⁺) release is promoted upon Hb deoxygenation⁸⁹. By contrast, NO binding to heme (at physiological ratios of NO/Hb) is favored in T state and disfavored in R. Subsequent binding of O₂ to heme as RBCs transit the lung, which promotes Hb R structure thus facilitates NO group transfer from heme to thiol (auto-S-nitrosylation).

Control of NO Entry into RBCs

Although free Hb readily inactivates NO[•], Hb packaging in RBCs limits NO[•] consumption by a factor of ~1,000⁹⁶⁻⁹⁹. Specifically, four successive barriers separate eNOS-generated NO[•] from intra-erythrocytic Hb: 1) the endothelial cell membrane, 2) an abluminal RBC-free zone, created by velocity gradients at the vascular edge of the migrating RBC column⁹⁹⁻¹⁰¹, 3) an unstirred layer of plasma surrounding moving RBCs¹⁰², and 4) the RBC membrane and cytoskeleton – which together provide a dynamically responsive diffusion barrier to NO[•]^{97, 103, 104}. It is generally accepted that, due to its solubility properties, the neutral and (relatively) nonpolar NO[•] radical freely permeates membranes. However, it has been recently reported that dynamic chemical and physical variation of the RBC membrane alters, and in fact may control, the rate of NO[•] uptake by RBCs^{97, 104}. This variation appears to arise from conformation-dependent interactions between Hb and key RBC membrane proteins that modulate NO[•] uptake (FIGURE 4), rather than from an O₂-linked change in the physical nature of the lipid bilayer itself.

NO Group Processing in RBCs

As described above, after formation of HbFeNO, the O₂-linked transition from T- to R-structure promotes intra-molecular transfer of the NO group from β -heme to β -thiol (Cys β^{93}), thereby forming HbCys β^{93} SNO (SNO-Hb)^{65, 84}. Once formed in R-state Hb, Cys β^{93} SNO is stable and protected from solvent through confinement in a hydrophobic pocket^{54, 55, 105-107}. Additionally cycling of NO between Cys β^{93} and heme *in vivo* is supported by measurements of FeNO and SNO as a function of PO₂ (FIGURE 5)^{50, 52, 55}.

NO Group Delivery by RBCs

The described arterio-venous gradient of SNO-Hb^{50, 52, 54, 66, 108-110} is consistent with dynamic processing of NO by circulating RBCs, in which O₂ loading in the lung is accompanied by Hb S-nitrosylation and NO-group export is facilitated by O₂ delivery when steep O₂ gradients are encountered in the periphery (hypoxic vasodilation)⁵⁴. The R $\rightarrow$ T transition in Hb conformation that occurs with O₂ delivery shifts Cys β^{93} SNO from its sequestered hydrophobic niche to exposure in aqueous solvent^{54, 55, 106}, making the NO group available for transnitrosylation of target protein or small-molecular-weight thiol^{54, 75, 85, 111}. Importantly, NO is transferred in this reaction as a nitrosonium ion (NO⁺)^{54, 56} and is therefore protected from (Fe(II)) heme recapture and/or inactivation (as well as from pharmacological NO[•] chelators)¹¹².

Hb deoxygenation (and/or heme oxidation) enables release of NO bioactivity by RBCs, which results in vasodilation^{50, 54, 55, 66, 85, 113}. Export of NO bioactivity from RBCs occurs, at least in part, via low-mass thiols in plasma^{50, 52, 73, 75} (FIGURE 6), although a role for direct cell-cell contact in the microcirculation may be envisaged. In either case, signaling is believed to proceed via a transnitrosylation cascade; the RBC membrane protein AE-1, with which Hb is known to undergo conformation-dependent docking^{114, 115}, appears to initiate this cascade. SNO-Hb docking on AE-1 is followed by NO-group transfer from SNO-Cys β^{93} to cysteine thiol(s) of the cytoplasmic domain of AE-1; moreover, this transfer is required for RBC-induced vasodilation⁸⁵. The identity of the acceptor thiols that ferry the NO signal *in vivo* from AE-1 to the vascular wall is unknown.

Vasodilation by SNO-Hb is readily detected at $< 10^{-8}$ M, which suggests that the endogenous SNO-Hb pool ($10^{-7} - 10^{-5.5}$ M) dispenses NO bioactivity in a tightly regulated fashion and with significant potency. By exploiting the Hb conformation-based NO delivery system, RBCs actuate graded dilation of blood vessels over rapid time scales - that differ greatly from the slow relaxation of native vascular smooth muscle under hypoxia (a response that occurs over minutes, and which may set background vascular tone). Thus, baseline tone appears to be regulated further by RBC-based HVD (acute changes, over seconds)^{51, 60, 66, 73}. Hypercarbia and acidosis are additional effectors of the T-conformation in Hb, and thereby also promote vasodilation by RBCs¹¹⁶. Furthermore, oxidation of hemes can elicit a T-like conformation in SNO-Hb to promote NO release^{54, 113}. Thus, ischemia and oxidative stress may provide multiple biochemical cues that can be employed by RBCs to correct perfusion insufficiency.

Dysregulated RBC Vasoactivity Arising from the Storage Lesion

Strong evidence is mounting in support of a causal relationship between acquired, NO-related RBC dysfunction and a host of morbidities that complicate critical illness¹¹⁷⁻¹²⁴. Recently, it has been observed that levels of SNO-Hb are altered in several disease states characterized by disordered tissue oxygenation^{66, 71, 74, 77, 78, 125-127}. In addition, where examined, RBCs from such patients exhibit impaired vasodilatory capacity^{50, 71, 78, 127}. These data suggest that altered RBC-derived vasoactivity contributes to human pathophysiology. Specifically, alterations in NO metabolism by RBCs have been reported in

congestive heart failure⁶⁶, diabetes^{78, 126}, pulmonary hypertension^{52, 71} and sickle cell disease^{127, 128}, all of which are conditions characterized by inflammation, oxidative stress and dysfunctional vascular control.

RBCs are progressively altered by processing and storage for transfusion (referred to as the “storage lesion”, as described above)⁷, and the interval between RBC donation and administration appears to be an independent risk factor for transfusion-associated morbidity and mortality^{3, 129-131}. Altered O₂ loading/unloading by stored RBCs is well documented¹³²⁻¹³⁴. It has been shown more recently that, in blood that has been collected, processed, and stored using blood-banking industry standard operating procedures, RBC SNO-Hb levels and RBC-dependent vasodilatory activity are profoundly depressed, and that these defects can be reversed by repletion of RBC SNO^{8, 72} (FIGURE 7). These observations provide mechanistic insight into clinical data that suggest impaired vasoregulation by administration of banked blood^{4, 132, 135-137}.

Summary

Trapping, sequestration, processing and delivery of NO groups by RBCs, which are coupled to Hb allostery, links regional blood flow to biochemical cues of perfusion insufficiency (metabolic demand). The respiratory cycle may thus be broadly viewed as comprised of three gases, NO, O₂ and CO₂, which are coordinately or covalently bound to Hb. RBCs therefore actively regulate regional blood flow (and thus, O₂ delivery) by coupling O₂ sensing by Hb to the formation and release of vasodilator SNO, conveying a PO₂-linked signal in the setting of perfusion lack. In addition to the multiple components of the ‘storage lesion’, which impact every aspect of RBC physiology, malfunction of this precise RBC vascular signaling system may undermine transfusion efficacy. Understanding of such should inform improvements in blood processing and storage, which are necessary to ameliorate the ‘storage lesion’.

Reference List

1. Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: A systematic review of the literature. *Crit Care Med.* 2008; 36:2667–2674. [PubMed: 18679112]
2. Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, Jenkins D, Azarow K, Holcomb JB. Risks associated with fresh whole blood and red blood cell transfusions in a combat support hospital. *Crit Care Med.* 2007; 35:2576–2581. [PubMed: 17828033]
3. Ho J, Sibbald WJ, Chin-Yee IH. Effects of storage on efficacy of red cell transfusion: When is it not safe? *Crit Care Med.* 2003; 31:S687–S697. [PubMed: 14724467]
4. Marik PE, Sibbald WJ. Effect of stored blood transfusion on oxygen delivery in patients with sepsis. *JAMA.* 1993; 269:3024–3029. [PubMed: 8501845]
5. Napolitano LM, Corwin HL. Efficacy of blood transfusion in the critically ill: Does age of blood make a difference? *Crit Care Med.* 2004; 32:594–595. [PubMed: 14758188]
6. Raghavan M, Marik PE. Anemia, allogenic blood transfusion, and immunomodulation in the critically ill. *Chest.* 2005; 127:295–307. [PubMed: 15653997]
7. Tinmouth A, Fergusson D, Yee IC, Hebert PC. Clinical consequences of red cell storage in the critically ill. *Transfusion.* 2006; 46:2014–2027. [PubMed: 17076859]
8. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored rbc. *Proceedings of the National Academy of Sciences.* 2007; 104:17063–17068.
9. Karam O, Tucci M, Toledano BJ, Robitaille N, Cousineau J, Thibault L, Lacroix J, Le Deist F. Length of storage and in vitro immunomodulation induced by prestorage leukoreduced red blood cells. *Transfusion.* 2009; 49:2326–2334. [PubMed: 19624600]

10. Luk CS, Gray-Statchuk LA, Cepinkas G, Chin-Yee IH. Wbc reduction reduces storage-associated rbc adhesion to human vascular endothelial cells under conditions of continuous flow in vitro. *Transfusion*. 2003; 43:151–156. [PubMed: 12559009]
11. Hovav T, Yedgar S, Manny N, Barshtein G. Alteration of red cell aggregability and shape during blood storage. *Transfusion*. 1999; 39:277–281. [PubMed: 10204590]
12. Yedgar S, Hovav T, Barshtein G. Red blood cell intercellular interactions in oxidative stress states. *Clin Hemorheol Microcirc*. 1999; 21:189–193. [PubMed: 10711742]
13. Apstein CS, Dennis RC, Briggs L, Vogel WM, Frazer J, Valeri CR. Effect of erythrocyte storage and oxyhemoglobin affinity changes on cardiac function. *Am J Physiol*. 1985; 248:H508–515. [PubMed: 3985174]
14. Zallen G, Moore EE, Ciesla DJ, Brown M, Biffl WL, Silliman CC. Stored red blood cells selectively activate human neutrophils to release il-8 and secretory pla2. *Shock*. 2000; 13:29–33. [PubMed: 10638666]
15. Opelz G, Terasaki PI. Improvement of kidney-graft survival with increased numbers of blood transfusions. *N Engl J Med*. 1978; 299:799–803. [PubMed: 357971]
16. Hill GE, Frawley WH, Griffith KE, Forestner JE, Minei JP. Allogeneic blood transfusion increases the risk of postoperative bacterial infection: A meta-analysis. *J Trauma*. 2003; 54:908–914. [PubMed: 12777903]
17. Silliman CC, Moore EE, Johnson JL, Gonzalez RJ, Biffl WL. Transfusion of the injured patient: Proceed with caution. *Shock*. 2004; 21:291–299. [PubMed: 15179127]
18. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: Role of red blood cell breakdown. *Transfusion*. 2011; 51:844–851. [PubMed: 21496045]
19. 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*. 2011; 51:610–621. [PubMed: 20738825]
20. Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, Papassideri IS. Rbc-derived vesicles during storage: Ultrastructure, protein composition, oxidation, and signaling components. *Transfusion*. 2008; 48:1943–1953. [PubMed: 18564399]
21. Cardo LJ, Hmel P, Wilder D. Stored packed red blood cells contain a procoagulant phospholipid reducible by leukodepletion filters and washing. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*. 2008; 38:141–147. [PubMed: 18346937]
22. Sweeney J, Kouttab N, Kurtis J. Stored red blood cell supernatant facilitates thrombin generation. *Transfusion*. 2009; 49:1569–1579. [PubMed: 19413726]
23. Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil nadph oxidase. *J Lab Clin Med*. 1994; 124:684–694. [PubMed: 7964126]
24. Blajchman MA. Immunomodulation and blood transfusion. *American journal of therapeutics*. 2002; 9:389–395. [PubMed: 12237730]
25. Blumberg N. Deleterious clinical effects of transfusion immunomodulation: Proven beyond a reasonable doubt. *Transfusion*. 2005; 45:33S–39S. discussion 39S–40S. [PubMed: 16086785]
26. Raat NJ, Verhoeven AJ, Mik EG, Gouwerok CW, Verhaar R, Goedhart PT, de Korte D, Ince C. The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. *Crit Care Med*. 2005; 33:39–45. discussion 238–239. [PubMed: 15644646]
27. d'Almeida MS, Gray D, Martin C, Ellis CG, Chin-Yee IH. Effect of prophylactic transfusion of stored rbcs on oxygen reserve in response to acute isovolemic hemorrhage in a rodent model. *Transfusion*. 2001; 41:950–956. [PubMed: 11452165]
28. van Bommel J, de Korte D, Lind A, Siegemund M, Trouwborst A, Verhoeven AJ, Ince C, Henny CP. The effect of the transfusion of stored rbcs on intestinal microvascular oxygenation in the rat. *Transfusion*. 2001; 41:1515–1523. [PubMed: 11778066]
29. Fitzgerald RD, Martin CM, Dietz GE, Doig GS, Potter RF, Sibbald WJ. Transfusing red blood cells stored in citrate phosphate dextrose adenine-1 for 28 days fails to improve tissue oxygenation in rats. *Crit Care Med*. 1997; 25:726–732. [PubMed: 9187588]

30. Shah DM, Gottlieb ME, Rahm RL, Stratton HH, Barie PS, Paloski WH, Newell JC. Failure of red blood cell transfusion to increase oxygen transport or mixed venous po2 in injured patients. *J Trauma*. 1982; 22:741–746. [PubMed: 7120526]
31. Steffes CP, Bender JS, Levison MA. Blood transfusion and oxygen consumption in surgical sepsis. *Crit Care Med*. 1991; 19:512–517. [PubMed: 2019137]
32. Conrad SA, Dietrich KA, Hebert CA, Romero MD. Effect of red cell transfusion on oxygen consumption following fluid resuscitation in septic shock. *Circ Shock*. 1990; 31:419–429. [PubMed: 2397567]
33. Dietrich KA, Conrad SA, Hebert CA, Levy GL, Romero MD. Cardiovascular and metabolic response to red blood cell transfusion in critically ill volume-resuscitated nonsurgical patients. *Crit Care Med*. 1990; 18:940–944. [PubMed: 2394117]
34. Gilbert EM, Haupt MT, Mandanas RY, Huaranga AJ, Carlson RW. The effect of fluid loading, blood transfusion, and catecholamine infusion on oxygen delivery and consumption in patients with sepsis. *The American review of respiratory disease*. 1986; 134:873–878. [PubMed: 3777684]
35. Lorente JA, Landin L, De Pablo R, Renes E, Rodriguez-Diaz R, Liste D. Effects of blood transfusion on oxygen transport variables in severe sepsis. *Crit Care Med*. 1993; 21:1312–1318. [PubMed: 8370294]
36. Boyd O, Grounds RM, Bennett ED. A randomized clinical trial of the effect of deliberate perioperative increase of oxygen delivery on mortality in high-risk surgical patients. *JAMA*. 1993; 270:2699–2707. [PubMed: 7907668]
37. Gattinoni L, Brazzi L, Pelosi P, Latini R, Tognoni G, Pesenti A, Fumagalli R. A trial of goal-oriented hemodynamic therapy in critically ill patients. Svo2 collaborative group. *N Engl J Med*. 1995; 333:1025–1032. [PubMed: 7675044]
38. Mink RB, Pollack MM. Effect of blood transfusion on oxygen consumption in pediatric septic shock. *Crit Care Med*. 1990; 18:1087–1091. [PubMed: 2209035]
39. Hayes MA, Timmins AC, Yau EH, Palazzo M, Hinds CJ, Watson D. Elevation of systemic oxygen delivery in the treatment of critically ill patients. *N Engl J Med*. 1994; 330:1717–1722. [PubMed: 7993413]
40. Biffl WL, Moore EE, Offner PJ, Ciesla DJ, Gonzalez RJ, Silliman CC. Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: Abrogation by poststorage washing but not prestorage leukoreduction. *J Trauma*. 2001; 50:426–431. discussion 432. [PubMed: 11265021]
41. Cardo LJ, Wilder D, Salata J. Neutrophil priming, caused by cell membranes and microvesicles in packed red blood cell units, is abrogated by leukocyte depletion at collection. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis*. 2008; 38:117–125. [PubMed: 18343726]
42. Silliman CC, Ambruso DR, Boshkov LK. Transfusion-related acute lung injury. *Blood*. 2005; 105:2266–2273. [PubMed: 15572582]
43. 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*. 2009; 67:29–32. [PubMed: 19590304]
44. Walsh TS, McArdle F, McLellan SA, Maciver C, Maginnis M, Prescott RJ, McClelland DB. Does the storage time of transfused red blood cells influence regional or global indexes of tissue oxygenation in anemic critically ill patients? *Crit Care Med*. 2004; 32:364–371. [PubMed: 14758149]
45. Leal-Noval SR, Munoz-Gomez M, Murillo-Cabezas F. Optimal hemoglobin concentration in patients with subarachnoid hemorrhage, acute ischemic stroke and traumatic brain injury. *Current opinion in critical care*. 2008; 14:156–162. [PubMed: 18388677]
46. Smith MJ, Stiefel MF, Magge S, Frangos S, Bloom S, Gracias V, Le Roux PD. Packed red blood cell transfusion increases local cerebral oxygenation. *Crit Care Med*. 2005; 33:1104–1108. [PubMed: 15891343]
47. Spinella PC, Doctor A, Blumberg N, Holcomb JB. Does the storage duration of blood products affect outcomes in critically ill patients? *Transfusion*. 2011; 51:1644–1650. [PubMed: 21831180]

48. Frandsen U, Bangsbo J, Sander M, Hoffner L, Betak A, Saltin B, Hellsten Y. Exercise-induced hyperaemia and leg oxygen uptake are not altered during effective inhibition of nitric oxide synthase with n g -nitro-l-arginine methyl ester in humans. *J Physiol.* 2001; 531:257–264. [PubMed: 11179408]
49. Ross JM, Fairchild HM, Weldy J, Guyton AC. Autoregulation of blood flow by oxygen lack. *AJP - Legacy.* 1962; 202:21–24.
50. Doctor A, Platt R, Sheram ML, Eischeid A, McMahon T, Maxey T, Doherty J, Axelrod M, Kline J, Gurka M, Gow A, Gaston B. Hemoglobin conformation couples erythrocyte s-nitrosothiol content to o₂ gradients. *Proceedings of the National Academy of Sciences.* 2005; 102:5709–5714.
51. Gonzalez-Alonso J, Richardson RS, Saltin B. Exercising skeletal muscle blood flow in humans responds to reduction in arterial oxyhaemoglobin, but not to altered free oxygen. *The Journal of Physiology Online.* 2001; 530:331–341.
52. McMahon TJ, Moon RE, Luschinger BP, Carraway MS, Stone AE, Stolp BW, Gow AJ, Pawloski JR, Watke P, Singel DJ, Piantadosi CA, Stamler JS. Nitric oxide in the human respiratory cycle. *Nat.Med.* 2002; 8:711–717. [PubMed: 12042776]
53. Wang X, Tanus-Santos JE, Reiter CD, Dejam A, Shiva S, Smith RD, Hogg N, Gladwin MT. Biological activity of nitric oxide in the plasmatic compartment. *Proc.Natl.Acad.Sci.U.S.A.* 2004; 101:11477–11482. [PubMed: 15258287]
54. Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: A dynamic activity of blood involved in vascular control. *Nature.* 1996; 380:221–226. [PubMed: 8637569]
55. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, Piantadosi CA. Blood flow regulation by s-nitrosohemoglobin in the physiological oxygen gradient. *Science.* 1997; 276:2034–2037. [PubMed: 9197264]
56. Singel DJ, Stamler JS. Chemical physiology of blood flow regulation by red blood cells: Role of nitric oxide and s-nitrosohemoglobin. *Annual Review of Physiology.* 2005; 67:99–145.
57. Singel DJ, Stamler JS. Blood traffic control. *Nature.* 2004; 430:297–297. [PubMed: 15254518]
58. Duling BR, Berne RM. Oxygen and the local regulation of blood flow; possible significance of longitudinal gradients in arterial blood oxygen tension. *Circulation Research.* 1971; 28(Suppl 1): 65–69. [PubMed: 5541124]
59. Duling BR, Berne RM. Longitudinal gradients in periarteriolar oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow. *Circulation Research.* 1970; 27:669–678. [PubMed: 5486243]
60. Gonzalez-Alonso J, Mortensen SP, Dawson EA, Secher NH, Damsgaard R. Erythrocytes and the regulation of human skeletal muscle blood flow and oxygen delivery: Role of erythrocyte count and oxygenation state of haemoglobin. *The Journal of Physiology.* 2006; 572:295–305. [PubMed: 16439430]
61. Kantrow SP, Huang YC, Whorton AR, Grayck EN, Knight JM, Millington DS, Piantadosi CA. Hypoxia inhibits nitric oxide synthesis in isolated rabbit lung. *AJP - Lung Cellular and Molecular Physiology.* 1997; 272:L1167–L1173.
62. Leone AM, Palmer RM, Knowles RG, Francis PL, Ashton DS, Moncada S. Constitutive and inducible nitric oxide synthases incorporate molecular oxygen into both nitric oxide and citrulline. *Journal of Biological Chemistry.* 1991; 266:23790–23795. [PubMed: 1721054]
63. Rengasamy A, Johns RA. Determination of km for oxygen of nitric oxide synthase isoforms. *Journal of Pharmacology and Experimental Therapeutics.* 1996; 276:30–33. [PubMed: 8558447]
64. Mamone G, Sannolo N, Malorni A, Ferranti P. In vitro formation of s-nitrosohemoglobin in red cells by inducible nitric oxide synthase. *FEBS Lett.* 1999; 462:241–245. [PubMed: 10622703]
65. Angelo M, Singel DJ, Stamler JS. An s-nitrosothiol (sno) synthase function of hemoglobin that utilizes nitrite as a substrate. *Proceedings of the National Academy of Sciences.* 2006; 103:8366–8371.
66. Datta B, Tufnell-Barrett T, Bleasdale RA, Jones CJ, Beeton I, Paul V, Frenneaux M, James P. Red blood cell nitric oxide as an endocrine vasoregulator: A potential role in congestive heart failure. *Circulation.* 2004; 109:1339–1342. [PubMed: 15023874]

67. Fox-Robichaud A, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, Kubes P. Inhaled no as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *Journal of Clinical Investigation*. 1998; 101:2497–2505. [PubMed: 9616221]
68. Fox-Robichaud A, Payne D, Kubes P. Inhaled no reaches distal vasculatures to inhibit endothelium- but not leukocyte-dependent cell adhesion. *AJP - Lung Cellular and Molecular Physiology*. 1999; 277:L1224–L1231.
69. Kubes P, Payne D, Grisham MB, Jourdeuil D, Fox-Robichaud A. Inhaled no impacts vascular but not extravascular compartments in postischemic peripheral organs. *American Journal of Physiology*. 1999; 277:H676–H682. [PubMed: 10444494]
70. Cannon RO III, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, Gladwin MT. Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. *J Clin Invest*. 2001; 108:279–287. [PubMed: 11457881]
71. McMahon TJ, Ahearn GS, Moya MP, Gow AJ, Huang YC, Luchsinger BP, Nudelman R, Yan Y, Krichman AD, Bashore TM, Califf RM, Singel DJ, Piantadosi CA, Tapon VF, Stamler JS. A nitric oxide processing defect of red blood cells created by hypoxia: Deficiency of s-nitrosohemoglobin in pulmonary hypertension. *Proceedings of the National Academy of Sciences*. 2005; 102:14801–14806.
72. 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. *Proceedings of the National Academy of Sciences*. 2007; 104:17058–17062.
73. Diesen DL, Hess DT, Stamler JS. Hypoxic vasodilation by red blood cells: Evidence for an s-nitrosothiol-based signal. *Circulation Research*. 2008; 103:545–553. [PubMed: 18658051]
74. Sonveaux P, Kaz AM, Snyder SA, Richardson RA, Cardenas-Navia LI, Braun RD, Pawloski JR, Tozer GM, Bonaventura J, McMahon TJ, Stamler JS, Dewhirst MW. Oxygen regulation of tumor perfusion by s-nitrosohemoglobin reveals a pressor activity of nitric oxide. *Circulation Research*. 2005; 96:1119–1126. [PubMed: 15879309]
75. Palmer LA, Doctor A, Chhabra P, Sheram ML, Laubach VE, Karlinsey MZ, Forbes MS, Macdonald T, Gaston B. S-nitrosothiols signal hypoxia-mimetic vascular pathology. *J Clin Invest*. 2007; 117:2592–2601. [PubMed: 17786245]
76. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO III, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med*. 2003; 9:1498–1505. [PubMed: 14595407]
77. Crawford JH, Chacko BK, Pruitt HM, Piknova B, Hogg N, Patel RP. Transduction of no-bioactivity by the red blood cell in sepsis: Novel mechanisms of vasodilation during acute inflammatory disease. *Blood*. 2004; 104:1375–1382. [PubMed: 15150083]
78. James PE, Lang D, Tufnell-Barret T, Milsom AB, Frenneaux MP. Vasorelaxation by red blood cells and impairment in diabetes: Reduced nitric oxide and oxygen delivery by glycated hemoglobin. *Circulation Research*. 2004; 94:976–983. [PubMed: 14963010]
79. Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H. Biological significance of nitric oxide-mediated protein modifications. *Am J Physiol Lung Cell Mol Physiol*. 2004; 287:L262–L268. [PubMed: 15246980]
80. Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. Protein s-nitrosylation: Purview and parameters. *Nature Reviews Molecular Cell Biology*. 2005; 6:150–166.
81. Xue Y, Liu Z, Gao X, Jin C, Wen L, Yao X, Ren J. Gps-sno: Computational prediction of protein s-nitrosylation sites with a modified gps algorithm. *PLoS ONE*. 5:e11290. [PubMed: 20585580]
82. Gow AJ, Stamler JS. Reactions between nitric oxide and haemoglobin under physiological conditions. *Nature*. 1998; 391:169–173. [PubMed: 9428761]
83. Gow AJ, Luchsinger BP, Pawloski JR, Singel DJ, Stamler JS. The oxyhemoglobin reaction of nitric oxide. *Proceedings of the National Academy of Sciences*. 1999; 96:9027–9032.
84. Luchsinger BP, Rich EN, Gow AJ, Williams EM, Stamler JS, Singel DJ. Routes to s-nitrosohemoglobin formation with heme redox and preferential reactivity in the beta subunits. *Proceedings of the National Academy of Sciences*. 2003; 100:461–466.

85. Pawloski JR, Hess DT, Stamler JS. Export by red blood cells of nitric oxide bioactivity. *Nature*. 2001; 409:622–626. [PubMed: 11214321]
86. Foster MW, McMahon TJ, Stamler JS. S-nitrosylation in health and disease. *Trends in Molecular Medicine*. 2003; 9:160–168. [PubMed: 12727142]
87. Stamler JS. S-nitrosothiols and the bioregulatory actions of nitrogen oxides through reactions with thiol groups. *Current Topics in Microbiology & Immunology*. 1995; 196:19–36. [PubMed: 7634823]
88. Stamler JS. Redox signaling: Nitrosylation and related target interactions of nitric oxide. *Cell*. 1994; 78:931–936. [PubMed: 7923362]
89. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. *Science*. 1992; 258:1898–1902. [PubMed: 1281928]
90. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, Loscalzo J. S-nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds. *Proceedings of the National Academy of Sciences*. 1992; 89:444–448.
91. Stamler JS, Lamas S, Fang FC. Nitrosylation. The prototypic redox-based signaling mechanism. *Cell*. 2001; 106:675–683. [PubMed: 11572774]
92. Stamler JS, Toone EJ, Lipton SA, Sucher NJ. (s)no signals: Translocation, regulation, and a consensus motif. *Neuron*. 1997; 18:691–696. [PubMed: 9182795]
93. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. *Physiological Reviews*. 2001; 81:209–237. [PubMed: 11152758]
94. Stamler JS, Hausladen A. Oxidative modifications in nitrosative stress. *Nature Structural Biology*. 1998; 5:247–249.
95. Stamler JS. S-nitrosothiols in the blood: Roles, amounts, and methods of analysis. *Circulation Research*. 2004; 94:414–417. [PubMed: 15001539]
96. Carlsen E, Comroe JH. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. *J Gen Physiol*. 1958; 42:83–107. [PubMed: 13575776]
97. Huang KT, Han TH, Hyduke DR, Vaughn MW, Van Herle H, Hein TW, Zhang C, Kuo L, Liao JC. Modulation of nitric oxide bioavailability by erythrocytes. *Proceedings of the National Academy of Sciences*. 2001; 98:11771–11776.
98. Liu X, Miller MJ, Joshi MS, Sadowska-Krowicka H, Clark DA, Lancaster JR. Diffusion-limited reaction of free nitric oxide with erythrocytes. *Journal of Biological Chemistry*. 1998; 273:18709–18713. [PubMed: 9668042]
99. Vaughn MW, Huang KT, Kuo L, Liao JC. Erythrocytes possess an intrinsic barrier to nitric oxide consumption. *Journal of Biological Chemistry*. 2000; 275:2342–2348. [PubMed: 10644684]
100. Liao JC, Hein TW, Vaughn MW, Huang KT, Kuo L. Intravascular flow decreases erythrocyte consumption of nitric oxide. *Proceedings of the National Academy of Sciences*. 1999; 96:8757–8761.
101. Vaughn MW, Kuo L, Liao JC. Effective diffusion distance of nitric oxide in the microcirculation. *American Journal of Physiology*. 1998; 274:H1705–H1714. [PubMed: 9612383]
102. Barry PH, Diamond JM. Effects of unstirred layers on membrane phenomena. *Physiological Reviews*. 1984; 64:763–872. [PubMed: 6377340]
103. Han TH, Qamirani E, Nelson AG, Hyduke DR, Chaudhuri G, Kuo L, Liao JC. Regulation of nitric oxide consumption by hypoxic red blood cells. *Proceedings of the National Academy of Sciences*. 2003; 100:12504–12509.
104. Han TH, Liao JC. Erythrocyte nitric oxide transport reduced by a submembrane cytoskeletal barrier. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 2005; 1723:135–142.
105. Brunori M, Taylor JF, Antonini E, Wyman J, Rossi-Fanelli A. Studies on the oxidation-reduction potentials of heme proteins. Vi. Human hemoglobin treated with various sulfhydryl reagents. *Journal of Biological Chemistry*. 1967; 242:2295–2300. [PubMed: 6026227]
106. Chan NL, Rogers PH, Arnone A. Crystal structure of the s-nitroso form of liganded human hemoglobin. *Biochemistry*. 1998; 37:16459–16464. [PubMed: 9843411]

107. Chiancone E, Currell DL, Vecchini P, Antonini E, Wyman J. Kinetics of the reaction of the “masked” and “free” sulfhydryl groups of human hemoglobin with p-mercuribenzoate. *Journal of Biological Chemistry*. 1970; 245:4105–4111. [PubMed: 5496995]
108. Funai EF, Davidson A, Seligman SP, Finlay TH. S-nitrosohemoglobin in the fetal circulation may represent a cycle for blood pressure regulation. *Biochemical & Biophysical Research Communications*. 1997; 239:875–877. [PubMed: 9367862]
109. Gladwin MT, Shelhamer JH, Schechter AN, Pease-Fye ME, Wacławiw MA, Panza JA, Ognibene FP, Cannon RO III. Role of circulating nitrite and s-nitrosohemoglobin in the regulation of regional blood flow in humans. *Proceedings of the National Academy of Sciences*. 2000; 97:11482–11487.
110. Nagababu E, Ramasamy S, Abernethy DR, Rifkind JM. Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction. *Journal of Biological Chemistry*. 2003; 278:46349–46356. [PubMed: 12952953]
111. Mayer B, Pfeiffer S, Schrammel A, Koesling D, Schmidt K, Brunner F. A new pathway of nitric oxide. Cyclic gmp signaling involving s-nitrosoglutathione. *Journal of Biological Chemistry*. 1998; 273:3264–3270. [PubMed: 9452441]
112. Crawford JH, White CR, Patel RP. Vasoactivity of s-nitrosohemoglobin: Role of oxygen, heme, and no oxidation states. *Blood*. 2003; 101:4408–4415. [PubMed: 12560216]
113. Pawloski JR, Swaminathan RV, Stamler JS. Cell-free and erythrocytic s-nitrosohemoglobin inhibits human platelet aggregation. *Circulation*. 1998; 97:263–267. [PubMed: 9462528]
114. Barvitenko NN, Adragna NC, Weber RE. Erythrocyte signal transduction pathways, their oxygenation dependence and functional significance. *Cellular Physiology and Biochemistry*. 2005; 15:1–18. [PubMed: 15665511]
115. Chu H, Breite A, Ciraolo P, Franco RS, Low PS. Characterization of the deoxyhemoglobin binding site on human erythrocyte band 3: Implications for o₂ regulation of erythrocyte properties. *Blood*. 2008; 111:932–938. [PubMed: 17942752]
116. Shimazutsu K, Uemura K, Auten K, Baldwin M, Belknap S, La Banca F, Jones M, McClaine D, Eubanks W, Stamler J, Reynolds J. Inclusion of a nitric oxide congener in the insufflation gas repletes s-nitrosohemoglobin and stabilizes physiologic status during prolonged carbon dioxide pneumoperitoneum. *Clin Transl Sci*. 2009; 2:405–412. [PubMed: 20443932]
117. Besarab A. The effects of normal as compared to low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *New England Journal of Medicine*. 1998; 339:584–590. [PubMed: 9718377]
118. Wu WC, Rathore SS, Wang Y, Radford MJ, Krumholz HM. Blood transfusion in elderly patients with acute myocardial infarction. *New England Journal of Medicine*. 2001; 345:1230–1236. [PubMed: 11680442]
119. Hart RG, Kanter MC. Hematologic disorders and ischemic stroke. *Stroke*. 1990; 21:1111–1121. [PubMed: 2202092]
120. Cirillo M, Laurenzi M, Trevisan M, Stamler J. Hematocrit, blood pressure, and hypertension. The gubbio population study. *Hypertension*. 1992; 20:319–326. [PubMed: 1516951]
121. Stephansson O, Dickman PW, Johansson A, Cnattingius S. Maternal hemoglobin concentration during pregnancy and risk of stillbirth. *JAMA*. 2000; 284:2611–2617. [PubMed: 11086368]
122. Hebert PC, Wells G, Blajchamn MA, Marshall J. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. *New England Journal of Medicine*. 1999; 340:409–417. [PubMed: 9971864]
123. Vincent JL, Baron JF, Reinhart K, Gattinoni L, Thijs L, Webb A, Meier-Hellmann A, Noillet G, Peres-Bota D, Investigators AAaBTiCC. Anemia and blood transfusion in critically ill patients. *JAMA*. 2002; 288:1499–1507. [PubMed: 12243637]
124. Ketcham EM, Cairns CB. Hemoglobin based oxygen carriers: Development and clinical potential. *Annals of Emergency Medicine*. 1999; 33:326–337. [PubMed: 10036348]
125. Liu L, Yan Y, Zeng M, Zhang J, Hanes MA, Ahearn G, McMahon TJ, Dickfeld T, Marshall HE, Que LG, Stamler JS. Essential roles of s-nitrosothiols in vascular homeostasis and endotoxic shock. *Cell*. 2004; 116:617–628. [PubMed: 14980227]

126. Milsom AB, Jones CJ, Goodfellow J, Frenneaux M, Peters JR, James P. Abnormal metabolic fate of nitric oxide in type 1 diabetes. *Diabetologia*. 2002; 45:1515–1522. [PubMed: 12436334]
127. Pawloski JR, Hess DT, Stamler JS. Impaired vasodilation by red blood cells in sickle cell disease. *Proceedings of the National Academy of Sciences*. 2005; 102:2531–2536.
128. Hammerman SI, Klings ES, Hendra KP, Upchurch GR Jr, Rishikof DC, Loscalzo J, Farber HW. Endothelial cell nitric oxide production in acute chest syndrome. *AJP - Heart and Circulatory Physiology*. 1999; 277:H1579–H1592.
129. Leal-Noval SR, Jara-Lopez I, Garcia-Garmendia JL, Marin-Niebla A, Herruzo-Aviles AMD, Camacho-Larana P, Loscertales J. Influence of erythrocyte concentrate storage time on postsurgical morbidity in cardiac surgery patients. *Anesthesiology*. 2003; 98:815–822. [PubMed: 12657840]
130. Purdy FR, Tweeddale MG, Merrick PM. Association of mortality with age of blood transfused in septic ICU patients. *Canadian Journal of Anesthesia*. 1997; 44:1256–1261. [PubMed: 9429042]
131. Zallen G, Offner PJ, Moore EE, Blackwell J, Ciesla DJ, Gabriel J, Denny C, Silliman CC. Age of transfused blood is an independent risk factor for postinjury multiple organ failure. *American Journal of Surgery*. 1999; 178:570–572. [PubMed: 10670874]
132. Tsai AG, Cabrales P, Intaglietta M. Microvascular perfusion upon exchange transfusion with stored red blood cells in normovolemic anemic conditions. *Transfusion*. 2004; 44:1626–1634. [PubMed: 15504169]
133. Valeri CR, Hirsch NM. Restoration in vivo of erythrocyte adenosine triphosphate 2,3-diphosphoglycerate potassium ion and sodium ion concentrations following the transfusion of acid-citrate-dextrose stored human red blood cells. *J Lab Clin Med*. 1969; 73:722–733. [PubMed: 5779258]
134. Valtis DJ. Defective gas transport function of stored red blood cells. *Lancet*. 1954; 266:119–124. [PubMed: 13118742]
135. Fortune JB, Feustel PJ, Saifi J, Stratton HH, Newell JC, Shah DM. Influence of hematocrit on cardiopulmonary function after acute hemorrhage. *J Trauma*. 1987; 27:243–249. [PubMed: 3560265]
136. Leal-Noval SR, Munoz-Gomez M, Arellano-Orden V, Marin-Caballeros A, Amaya-villar R, Marin A, Puppo-Moreno A, Ferrandiz-millon C, Flores-cordero JM, Murillo-Cabezas F. Impact of age of transfused blood on cerebral oxygenation in male patients with severe traumatic brain injury. *Crit Care Med*. 2008; 36:1290–1296. [PubMed: 18379257]
137. Sugerman HJ, Davidson DT, Vibul S, Delivoria-Papadopoulos M, Miller LD, Oski FA. The basis of defective oxygen delivery from stored blood. *Surg Gynecol Obstet*. 1970; 131:733–741. [PubMed: 5458535]

FIGURE 1.

Interaction between donor RBCs and transfusion recipients as a consequence of receiving RBCs altered by processing and storage². The figure illustrates that the storage lesion impacts overlapping pathways of oxygen delivery, RBC rheology and physiology, as well as immune modulation. MOF = multiple organ failure; NO = nitric oxide.

FIGURE 2.

RBCs transduce regional O_2 gradients in tissue to control NO bioactivity in plasma by trapping or delivering NO groups as a function of Hb O_2 saturation. **A**) In this fashion, circulating NO groups are processed by Hb into the highly vasoactive (thiol-based) NO congener, S-nitrosothiol (SNO). By exporting SNOs as a function of Hb deoxygenation, RBCs precisely dispense vasodilator bioactivity in direct proportion to regional blood flow lack. **B**) O_2 delivery homeostasis requires biochemical coupling of vessel tone to environmental cues that matches perfusion sufficiency to metabolic demand. Because oxy- and deoxy-Hb process NO differently (see text), allosteric transitions in Hb conformation

afford context-responsive (O_2 - coupled) control of NO bioavailability, thereby linking the sensor and effector arms of this system. Specifically, Hb conformation governs the equilibria among deoxyHbFeNO (A; NO sink), oxySNOHb (B; NO store), and acceptor thiols including the membrane protein SNO-AE1 (C; bioactive NO source). Direct SNO export from RBCs or S-transnitrosylation from RBCs to plasma thiols (D) yields vasoactive SNOs, which influence resistance vessel caliber and close this signaling loop. Thus, RBCs either trap (A) or export (D) NO groups to optimize blood flow. C) NO processing in RBCs (panels A and B) couples vessel tone to tissue PO_2 ; this system subserves hypoxic vasodilation in the arterial periphery and thereby calibrates blood flow to regional tissue hypoxia.

FIGURE 3.

Hb O₂ saturation (Hb SO₂%) exerts coordinated governance of RBC SNO content, RBC vasoactivity and human peripheral blood flow. **A)** Human blood gas measurements of SNO and O₂. RBCs with (black) or without (red) added extracellular glutathione (GSH) were deoxygenated under inert gas⁵⁰. The natural logarithm of SNO content in RBCs (SNORBC) is linearly related to Hb O₂ saturation. GSH accelerated SNORBC decay, consistent with O₂-linked export of NO groups to extra-erythrocytic thiols. **B)** RBCs induce graded relaxation of systemic arteries (aortic ring bioassay) that is inversely related to Hb O₂ saturation across the physiological range, recapitulating hypoxic vasodilation⁵². Hb O₂ saturations spanned

from red (oxy, > 90% Hb SO₂) to blue (deoxy, < 40% Hb SO₂). C) Leg vascular conductance increases as blood O₂ content falls (hypoxic vasodilation)⁵¹. Hb O₂ saturation and thus arterial blood O₂ content were manipulated by CO exposure ± varying FiO₂ (fraction of inspired O₂; hypoxia versus hyperoxia). Neither vascular conductance nor blood flow correlated with blood PO₂ per se.

FIGURE 4.

Conformation-specific binding between Hb and the RBC membrane protein AE-1 affords O₂ responsive control of NO trapping¹⁰³. **A)** Under oxygenated conditions (normoxic RBC, at left), the RBC membrane constitutes a significant barrier to NO[•] entry via tight association between the sub-membrane cytoskeleton and the cytoplasmic domain of the Band 3 (AE-1) membrane protein (ankyrin successfully competes with oxyHb for binding to AE-1). Upon RBC deoxygenation (hypoxic RBC), oxyHb R-state is converted to deoxyHb T-state, which now successfully competes with ankyrin for the AE-1 cytoplasmic domain (affinity for AE-1 ranks as follows: deoxyHb > ankyrin > oxyHb). Proximal apposition of heme to the membrane and diminished cytoskeleton-membrane interaction allows increased NO entry and affords intraerythrocytic Hb greater access to extraerythrocytic NO. Moreover, if deoxyHb encounters high concentrations of NO[•], “super-T” αFe(II)NOHb may form (in which NO bound to the α subunit disrupts normal heme-globin linkage, locking Hb

in the deoxy or T conformation; see text for details). **B)** Increasing the proportion of intra-erythrocytic T-state Hb (by forming either deoxyHb or $\alpha\text{Fe(II)NO}$, both of which are T-state tetramers and bind avidly to AE-1) increases NO consumption by intact RBCs as measured by a competition assay. In this plot, increased consumption in treated vs control RBCs appears as an increased KRBC/KRBCcontrol ratio (y-axis). **C)** Increased NO uptake by NOpretreated hypoxic RBCs correlates with the formation of $\alpha\text{Fe(II)NO}$ (i.e. “super-T” Hb).

FIGURE 5.

O₂-dependent variation in SNO-Hb (■) and Hb[FeNO] (□) demonstrate the association between Hb conformation and intramolecular heme → thiol migration of NO groups in RBCs. **A - D**)⁵², Moles NO per mole Hb tetramer in arterial and mixed venous blood from humans breathing either 21% O₂ at 1 ATA (absolute atmospheres) (A and D), 21% O₂ at 0.56 ATA (equivalent to ~ 12% O₂ at 1 ATA) (B) or 100% O₂ at 3 ATA (D). Total Hb-bound NO equals the sum of the 2 bars for each condition. These data demonstrate O₂ - dependent shuttling of Hb-bound NO groups between heme and Cys-β93. (E and F), SNO content of blood Hb, presented as the fraction of Hb-NO (% SNO), correlates with Hb O₂ saturation (E), but not with pO₂ (F).

FIGURE 6.

PO₂ - regulated export of NO groups from RBCs can occur via NO group transfer from SNO-Hb to an extra-erythrocytic thiol reactant⁷⁵. Circulatory transit was simulated for human whole blood in a thin-film tonometer (under 5% CO₂, balance N₂, pH 7.4) after spiking the sample with the non-native, low-mass thiol, N-acetyl cysteine (NAC, 100 M in plasma). The concentration of S-nitroso-N-acetyl cysteine (SNOAC) that formed in plasma was measured by mass spectrometry, and confirmed by mass labeling with ^{15}N . The conversion of extra-erythrocytic NAC to SNOAC correlated with RBC O₂ and SNO content. Serum SNOAC formed as a function of Hb SO₂. **A**) Liquid chromatogram demonstrating co-elution of RBC-generated SNOAC with the ^{15}N -labeled SNOAC standard. **B**) Mass spectrum demonstrating paired signals from RBC-generated SNOAC and the ^{15}N -labeled SNOAC standard (m/z 194). **C**) Extra-erythrocytic SNOAC concentration follows oxyHb desaturation (by co-oximetry). **D**) RBC SNO content (black) decreased in tandem with HbO₂ desaturation (dashed blue). Note that, as SNO content in RBCs fell, extra-erythrocytic SNOAC (yellow symbols) accumulated. Note also that SNOAC levels were below the limits of detection when the HbO₂ saturation was above 80%.

FIGURE 7.

Impact of processing and storage upon RBC NO content and vasoactivity (both in isolated vascular rings⁸ and in a whole-animal cardiac preparation⁷²). In these projects, blood from healthy volunteers was leukofiltered, processed into standard additive solution and stored according to AABB standards. (A) RBC NO content was significantly depressed, (B) as was vasoactivity in a rabbit vascular ring preparation. (C) Representative tracings from a similar project demonstrate the degree of vasorelaxation as percent change in tension in rabbit aortic rings produced by fresh, stored (expired and day 1), or reinitrosylated (day 1) RBCs. (D) Hypoxic vasodilation by stored and reinitrosylated RBCs in vivo. Shown are changes in canine coronary artery bloodflow (mean $\pm$ SD; n7) produced by infusion of SNO-depleted or reinitrosylated RBCs. Increases in flow elicited by reinitrosylated RBCs were significantly greater than those produced by SNO-depleted RBCs, and the degree of change was greater under hypoxic (5% FiO_2) than normoxic (21% FiO_2) conditions.