Red blood cell transfusion and its effect on microvascular dysfunction in shock states

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Among critically ill patients, red blood cell (RBC) transfusion is often prescribed for anemia in the absence of active or recent bleeding. The failure of RBC transfusion to improve physiological parameters and clinical outcomes in this setting may be explained by current understanding of the relationship between the RBCs and the microcirculation. It is now evident that the circulating RBCs contribute to microcirculatory hypoxic vasodilation by regulated nitric oxide (NO)-dependent vasodilation, thereby facilitating delivery of oxygen to oxygen-deprived tissue. The structural and functional changes in RBCs during storage, collectively known as the storage lesion, result in circulating RBCs that may not function as expected after transfusion. In recent years, there has been a significant focus on the dysfunctional interaction between stored RBCs and the microcirculation, with emphasis on understanding the mechanisms that drive erythrocyte NO-mediated vasodilation. The development of technology that allows noninvasive observation of the microcirculation in humans has allowed for direct observation of the microcirculation immediately before and after RBC transfusion. The current understanding of RBC NO-mediated vasodilation and the results of direct observation of the microcirculation in the setting of RBC transfusion are reviewed.

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Introduction

For the treatment of hemorrhagic shock, the benefit of allogeneic red blood cell (RBC) transfusion is indisputable. Such patients are at the threshold of impending circulatory collapse and death, and replacement of shed blood with stored RBCs partially restores both intravascular volume and critical oxygen delivery until the site of bleeding is controlled. Until a viable “blood substitute” capable of delivering oxygenation to tissues is available, RBC transfusion will remain the primary treatment for acute blood loss anemia. RBC transfusion, however, is often prescribed in the absence of active bleeding. Moderate anemia often accompanies shock states unrelated to active bleeding, such as septic shock or systemic inflammatory response syndrome (traumatic shock) after injury, and it is not uncommon for physicians to transfuse RBCs in this setting to treat moderate anemia with the goal of improving oxygen delivery and ultimately oxygen uptake at the cellular level.

Such clinical practice contradicts a relatively large body of research that demonstrates that transfusion in this setting does not improve oxygen uptake [1]. Similarly, many studies have suggested that RBC transfusion can, in fact, be harmful rather than beneficial. RBC transfusion has been demonstrated to be an independent predictor of mortality, infection, and multiorgan failure [2]. In the landmark multiinstitutional TRICC trial, wherein nonbleeding patients were randomized to a liberal (goal hemoglobin 10–12 g/dL) versus restrictive (goal hemoglobin 7–9 g/dL) transfusion practice, no survival benefit was observed in the liberal transfusion group [3].

The failure of RBC transfusion to improve physiological parameters and clinical outcomes in such settings may be explained by current understanding of the relationship between the RBC and the microcirculation. In particular, it is evident that the circulating RBCs contribute to microcirculatory hypoxic vasodilation by regulated nitric oxide (NO)-dependent vasodilation, thereby facilitating delivery of oxygen to oxygen-deprived tissue. The structural and functional changes in RBCs during storage, collectively known as the storage lesion, result in circulating RBCs that may not function as expected after transfusion. Although recent randomized trials failed to demonstrate any differences in outcome associated with the transfusion of relatively fresh versus relatively older RBCs [4,5], it is well known that the functional and morphological degradation of RBCs occurs relatively early and progressively during storage, and multiple studies have demonstrated that stored RBCs fail to promote hypoxic vasodilation [6–10].

In recent years, there has been a significant focus on the dysfunctional interaction between stored RBCs and the microcirculation, with emphasis on understanding the mechanisms that drive erythrocyte NO-mediated vasodilation. The development of technology that allows noninvasive observation of the microcirculation in humans has allowed for direct observation of the microcirculation immediately before and after RBC transfusion. In this overview, the current understanding of RBC NO-mediated vasodilation and the results of direct observation of the microcirculation in the setting of RBC transfusion are reviewed.

Storage-dependent changes in RBCs and effects on no signaling

How RBCs modulate hypoxic vasodilation can be generalized into 2 mechanisms involving: (i) changes in how hemoglobin scavenges NO and inhibits NO signaling and (ii) how RBCs stimulate formation of NO and subsequent signaling. This balance between inhibitory and stimulatory effects has emerged as a key aspect of our understanding of vascular NO homeostasis mechanisms [11]. The changes that occur in RBCs during storage dramatically change this balance toward to the inhibitory side through multiple mechanisms [12]. With respect to inhibition of NO, the central reaction is that of oxy- or deoxyhemoglobin (where iron is in the ferrous oxidation state) with NO formed by endothelial nitric oxide synthase (eNOS). With cell-free hemoglobin, this reaction is rapid (rate constant $\sim 10^7$ M$^{-1}$ s$^{-1}$), but is slowed significantly (rate constant $\sim 10^4$ M$^{-1}$ s$^{-1}$) when hemoglobin is encapsulated within the RBC, a consequence of diffusion barriers created by the RBC membrane [13,14]. This property of hemoglobin compartmentalization is critical in allowing eNOS-derived NO to affect local signaling.

With storage, however, several changes occur that result in RBCs that scavenge NO as quickly as cell-free Hb. The first change pertains to hemolysis, which increases with storage time. In fact, older stored
RBCs (35–42 days old) may typically have 50–100 μM cell-free hemoglobin concentrations; transfusion with as little as 5 μM cell-free hemoglobin is sufficient to scavenge NO and cause hypertension [10,15]. Second, storage results in the formation of microvesicles that contain hemoglobin, and in these microvesicles, the rate of NO scavenging by hemoglobin is the same as that by free hemoglobin [10,13]. Finally, even without RBC destruction and hemolysis, during storage, RBCs become smaller and denser and have altered shape and surface area. This results in cells that scavenge NO faster than “normal” biconcave RBCs [6,16]. In summary, during storage, there is a continuum of changes occurring in RBCs, from RBC shape to the formation of microvesicles and ultimately hemolysis, all of which lead to products that are more potent inhibitors of NO signaling (Fig. 1). Indeed, hypertension, oxidative stress, and inflammation associated with acute loss of NO bioavailability are observed after transfusion with older stored RBCs compared with fresh RBCs [7,8,17–21].

On the other side of the balance is the ability of RBCs and hemoglobin to sense local oxygen tensions and link this to increasing NO bioavailability. This paradigm has been discussed in the context of hypoxic blood flow and specifically coupling of hemoglobin deoxygenation with NO formation mechanisms [11]. Three mechanisms have been proposed, including nitrite reduction, S-nitrosohemoglobin, and ATP release and subsequent eNOS activation [5,6,22,23]. Although biochemically distinct, these proposed pathways share the need for hemoglobin deoxygenation. During storage, loss of 2,3-BPG leads to RBCs with increased oxygen affinity, and thus immediately upon transfusion, there is likely a mismatch between appropriate deoxygenation and activation of NO-dependent vasodilation. In addition, stored RBCs oxidize nitrite (a substrate for the nitrite reduction pathway), providing an additional pathway for preventing NO formation [16,24].

**Direct observation of microcirculation during RBC transfusion**

The development of orthogonal polarization spectral (OPS) microscopy, followed by the next-generation sidestream dark-field (SDF) imaging, has allowed for real-time visualization of the microcirculation, typically in the sublingual region when performed at the bedside. In addition, near-infrared spectrography (NIRS) has been used to measure tissue oxygenation saturation in muscle beds in a noninvasive manner (usually the musculature of the thenar eminence in humans). Using these techniques, one can observe microvesicles and free hemoglobin reacting with nitric oxide –1000-fold faster than intact RBCs, which results in rapid hypertensive and inflammatory microcirculatory responses after transfusion with older stored red cells. NO, nitric oxide.
modalities, several studies have demonstrated that the microcirculation is altered in sepsis, and persistence of microcirculatory derangement is associated with multiorgan failure and mortality. De Backer et al. used OPS imaging to compare the sublingual microcirculation between septic and nonseptic patients. In patients with severe sepsis, the density of all vessels was reduced relative to the nonseptic cohort, and these vessel density alterations were more severe in septic nonsurvivors [25]. More recently, Edul et al. similarly compared patients with septic shock with healthy volunteers using SDF imaging paired with analytical software, and found that the sublingual microcirculation in patients with septic shock is characterized by hypoperfusion and flow heterogeneity relative to healthy controls [26].

In the setting of sepsis and other nonhemorrhagic states, observation of the microcirculation in response to RBC transfusion has been examined in several studies. Sakr et al. were among the first to evaluate the effect of RBC transfusion on microvascular perfusion in septic patients [27]. Using OPS imaging, they identified that patients with relatively altered baseline microcirculatory perfusion had improvement in perfusion following transfusion. However, when the pretransfusion microcirculation appeared relatively normal, transfusion was followed by either no change or deterioration in microvascular perfusion. This negatively correlated response to transfusion related to baseline microcirculation has been observed in similar studies (Fig. 2). Creteur et al. used NIRS to evaluate the effect of RBC transfusion on muscle tissue oxygenation consumption [28]. They observed improvement in oxygen consumption among patients with altered microvascular reactivity at baseline, and deterioration in oxygen consumption among patients with preserved baseline microvascular reactivity.

Fig. 2. Representative figures from studies demonstrating negative correlations between pretransfusion microcirculatory perfusion and the change in perfusion after RBC transfusion. (A) The change in capillary perfusion after transfusion as observed with OPS imaging is negatively correlated with baseline capillary perfusion [27]. (B) The change in oxygen uptake as measured by NIRS is negatively correlated with baseline oxygen uptake [28]. NIR VO2, oxygen uptake as measured by NIRS. (C) The change in small vessel perfusion is negatively correlated with baseline small vessel perfusion [29]. PPV, percentage of perfused vessels. (D) The change in percentage of perfused capillaries is negatively correlated with percentage of perfused capillaries before transfusion [30]. PPC, percentage of perfused capillaries.
More recently, Sadaka et al. performed an observational study whereby both NIRS and SDF imaging were used to evaluate the effect of RBC transfusion on tissue oxygenation and microcirculatory perfusion in severely septic patients [29]. Once again, the primary observation was improvement in oxygen consumption in patients with altered oxygen consumption at baseline and deterioration in oxygen consumption in patients with preserved baseline oxygen consumption. Our own work with relatively stable, nonbleeding polytrauma patients also supports the existence of this dichotomous or divergent response to transfusion [30]. Patients with trauma with relatively altered baseline capillary perfusion tended to demonstrate improvement in perfusion following transfusion, whereas those with relatively normal perfusion at baseline tended to demonstrate either no change or, in fact, a decline in capillary perfusion.

It appears that transfusion may not be beneficial, and potentially deleterious, in patients with preserved microcirculatory perfusion. The mechanism, however, is not entirely clear. Some studies have demonstrated that endogenous RBC morphology and rheology are altered in critically ill patients. For patients with relatively severe RBC deformity due to critical illness, it is possible that exogenous stored RBCs may provide erythrocytes with relatively better RBC deformity resulting in augmentation of microcirculatory perfusion. This concept is supported by the work of Friedlander et al., who demonstrated that transfusion of stored RBCs was positively correlated with improvement in RBC deformability in a septic patient cohort [31].

Another plausible explanation is the effect of transfusion on intravascular volume. It is possible that those patients with relatively altered baseline microcirculation might have been relatively volume depleted compared with those with preserved baseline microcirculation, despite the lack of evidence for any difference in volume status from common global clinical and laboratory indices such as arterial pressure or base deficit. The increase in intravascular volume due to transfusion might have restored the microcirculation in these patients, thus partially explaining the observations described above. Ospina-Tascon et al. evaluated the effect of both crystalloid (Ringers) and colloid (albumin) administration on microvascular perfusion using SDF imaging, and observed that in the early phase of sepsis (within 24 h of diagnosis), microcirculatory perfusion improved with the administration of fluids [32]. Although the mechanism for this seems intuitively related to increase in driving pressure with respect to mean arterial pressure and/or central venous pressure due to fluid administration, this was not borne out by their observations, as the positive microcirculatory response to fluids was observed in both patients who did and did not exhibit improvement in systemic hemodynamics. Nonetheless, it remains possible that the phenomenon of microcirculatory improvement following transfusion in some patients may be a result of the augmentation of intravascular volume due to transfusion.

The effect of RBC storage age has also been studied with respect to the microcirculation. Kiraly et al. evaluated peripheral tissue oxygenation as measured by near-infrared spectroscopy during RBC transfusion [33]. They observed that patients transfused with blood stored for 21 days or more had a statistically significant decline in tissue oxygen saturation compared with those transfused with blood stored for less than 21 days. We similarly demonstrated that transfusion of older RBC units was associated with the inhibition of microvascular perfusion as evidenced by a decline in both functional capillary density (as observed with SDF microscopy) and tissue oxygen saturation (as measured by NIRS) associated with increased RBC storage age [34]. Recently, Damiani et al. performed a similar study evaluating both pretransfusion and posttransfusion sublingual SDF microscopy and thenar eminence NIRS with respect to both RBC storage age and the concentration of free hemoglobin in each transfused RBC unit and recipient plasma following transfusion [35]. They observed that transfusion of relatively older RBC units was associated with an increase in plasma-free hemoglobin, and increased plasma-free hemoglobin concentrations were associated with decreased microvascular density. These observations provide bedside evidence for the body of work that implicates NO scavenging by free hemoglobin following stored RBC transfusion, which ultimately is an inhibitor of vasodilation.

Conclusions

Observation of the microcirculation’s response to transfusion, coupled with the understanding of stored RBC dysfunction related to NO signaling, provides further evidence to question the value of RBC transfusion that is frequently prescribed for moderate anemia in the absence of active bleeding. In
multiple studies, the microvascular response to the administration of fluids or RBCs was dissociated from the global macrohemodynamic response. In addition, in most studies, there was little if any indication from standard measurement of perfusion adequacy (base deficit, blood pressure, etc.) that baseline differences in microcirculatory perfusion were present.

These observations have important implications. It can be confidently stated that there is agreement on improvement in tissue perfusion being the main goal for fluid resuscitation. Will a moderately anemic patient who is mildly pressor-dependent benefit from RBC transfusion? In clinical practice, such patients often show clinical improvement following RBC transfusion, as observed by improvement in mean arterial pressure and liberation from pressor requirements. However, without opportunity to interrogate the microcirculation before and after transfusion, it remains unclear on whether concomitant improvement with respect to perfusion was achieved with RBC transfusion versus no effect or worsening of microcirculatory perfusion due to transfusion. Therefore, bedside monitoring of the microcirculation would be the ideal approach for determining the efficacy of resuscitation. However, this process is yet to be embraced in most centers. Although research studies have demonstrated that NIRS is valuable in terms of predicting outcome and measuring response to therapeutic interventions, this method has failed to find a foothold for regular clinical use. Similarly, sublingual microscopy is yet to reach the stage of user friendliness such that it is ready for routine application at the bedside. In addition, it remains unclear whether improving altered microcirculation by therapy such as transfusion will translate to improved outcome for the patient. Ultimately, however, the refinement of existing technology and development of new technology for microcirculatory monitoring will lead to better understanding of the response (or lack of) to therapies including transfusion, and perhaps microcirculatory monitoring will eventually become standard practice in the intensive care units of the developed world.

It has now been revealed that stored RBCs do not have the functional capacity of endogenous RBCs with respect to NO signaling in the microcirculation. Recently, 2 randomized trials have demonstrated that there is no clinical outcome difference with respect to the transfusion of relatively longer stored RBC units versus fresher units. Nonetheless, it is clear from laboratory studies that RBCs become relatively dysfunctional, even in the early days of storage, and in the setting of massive transfusion, which these studies did not address, it is plausible that replacement of large volumes of shed blood with exogenous dysfunctional RBCs contributes to the sequelae of massive transfusion including acute respiratory distress syndrome (ARDS) and multiorgan failure. The solution to this clinical challenge may reside in the refinement of RBC rejuvenation therapies that quickly restore the NO signaling capability of the stored RBC without untoward effects. Alternatively, refinements in storage techniques, such as anaerobic storage, may lead to prevention of RBC functional and morphological degradation, and retained NO signaling following transfusion.

At present, however, contemporary storage mediums and the lack of a clinically viable rejuvenation strategy are the status quo. There are little data to support using RBC transfusion to augment oxygen uptake on a global level. Nonetheless, for those using the monitoring of the microcirculation at the bedside, the data support, at a conceptual level, RBC transfusion to improve perfusion when baseline alterations are evident in moderately anemic patients.

Practice points

- RBC transfusion, in the setting of anemia without active bleeding, does not seem to improve oxygen uptake.
- The failure of RBC transfusion to improve physiological parameters and clinical outcomes in this setting may be explained by the relative dysfunction of stored allogeneic RBCs with respect to mediating nitric oxide-dependent vasodilation.
- From bedside observation of the microcirculation, it is evident that RBC transfusion in non-bleeding anemic patients may not be beneficial, and potentially deleterious, in patients with preserved pretransfusion microcirculatory perfusion.
- For those physicians using the monitoring of the microcirculation in clinical practice, the extant data support that RBC transfusion may improve microcirculatory perfusion in non-bleeding anemic patients, provided that pretransfusion microcirculation is altered.
Research agenda

- Development of technology better suited to clinical use that will allow physicians to detect altered microcirculatory physiology and evaluate the microcirculatory response to therapies such as transfusion
- Determination if clinical decision-making according to the management of the microcirculation leads to improved outcomes versus current clinical practice
- Advances in transfusion concerning improvements in cell storage mediums to better preserve RBC structure and function during storage, and/or development of viable rejuvenation technologies to improve stored RBC function before or during transfusion

Conflict of interest

None.

References


