Published in final edited form as:

Transfusion. 2013 February ; 53(2): 297–305. doi:10.1111/j.1537-2995.2012.03822.x.

Low shear red cell oxygen transport effectiveness is adversely affected by transfusion and further worsened by deoxygenation in sickle cell disease patients on chronic transfusion therapy

Jon Detterich¹, Tamas Alexy², Miklos Rabai³, Ani Dongelyan⁴, Thomas Coates⁴, John Wood¹, and Herbert Meiselman³

¹Division of Cardiology, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA

²Department of Medicine, University of Minnesota, Minneapolis, MN

³Department of Physiology and Biophysics, University of Southern California Keck School of Medicine, Los Angeles, CA

⁴Division of Hematology, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA

Abstract

BACKGROUND—Simple chronic transfusion therapy (CTT) is a mainstay for stroke prophylaxis in sickle cell anemia, but its effects on hemodynamics are poorly characterized. Transfusion improves oxygen carrying capacity, reducing demands for high cardiac output. While transfusion decreases factors associated with vaso-occlusion, including percent HbS, reticulocyte count and circulating cell-free hemoglobin, it increases blood viscosity, which reduces microvascular flow. The hematocrit to viscosity ratio (HVR) is an index of red cell oxygen transport effectiveness that varies with shear stress and balances the benefits of improved oxygen capacity to viscosity-mediated impairment of microvascular flow. We hypothesized that transfusion would improve HVR at high shear despite increased blood viscosity, but would decrease HVR at low shear.

STUDY DESIGN AND METHODS—To test this hypothesis, we examined oxygenated and deoxygenated blood samples from 15 sickle cell patients on CTT immediately pre-transfusion and again 12–120 hours post-transfusion.

RESULTS—Comparable changes in hemoglobin, hematocrit, reticulocyte count and hemoglobin S with transfusion were observed in all subjects. Viscosity, hematocrit and high-shear HVR increased with transfusion while low shear HVR decreased significantly.

CONCLUSION—Decreased low-shear HVR suggests impaired oxygen transport to low-flow regions and may explain why some complications of sickle cell anemia are ameliorated by chronic transfusion therapy and others may be made worse.

Keywords

sickle cell disease; chronic transfusion therapy; blood viscosity; hematocrit to viscosity ratio

Correspondence: Jon Detterich, MD, Division of Cardiology, Children's Hospital Los Angeles, 4650 Sunset Blvd Mailstop 34, Los Angeles California, United States of America 90027. jdetterich@chla.usc.edu.
Reprint requests should be sent to Jon A. Detterich, M.D.

Conflict of Interest: The authors have no disclosures.

Introduction

Simple chronic transfusion therapy (CTT) is a mainstay of therapy for patients with sickle cell disease (SCD) who are at risk for stroke, have had a stroke or have chronic unremitting complications from SCD.¹⁻³ CTT for primary and secondary prevention of stroke is well accepted, but the mechanisms by which it provides protection are not well known. In addition, existing data suggest that CTT confers incomplete protection against silent stroke thought to be microvascular in origin⁴⁻⁶ and is not fully protective against progression of large vessel disease⁶; unfortunately, SCD patients on CTT are still at risk for acute pain crisis.⁷ Red blood cell (RBC) transfusions thus involve a balance between positive and negative factors: 1) decreased hemoglobin S% (HbS%) which improves cellular deformability of the RBC population; 2) increased hemoglobin concentration which increases oxygen carrying capacity; 3) the elevated hematocrit increases blood viscosity but if vascular reactivity (e.g., endothelial NO function) is adequate, the viscosity change may not markedly alter blood flow if there are compensatory decreases of vascular resistance; 4) in situations where vascular reactivity reserve is inadequate, decreased vascular resistance may not be able to compensate and tissue perfusion could be compromised.^{8,9} Changes in blood rheological properties (i.e., blood viscosity, RBC deformability) are thought to be important modulators of vascular response to transfusion and organ perfusion.^{10,11} Limited data exist for the target of CTT (i.e., hematocrit, blood hemoglobin content, HbS%), with current protocols based solely on clinical experience and laboratory observations of SS/AA RBC suspensions in protein-free isotonic buffer.^{2,3,12}

Many factors converge in sickle cell disease to cause abnormal vascular function, blood flow and organ perfusion, including chronic inflammation, chronic hemolysis and abnormal blood rheology. Abnormal blood rheology contributes to microcirculatory dysfunction due to increased red cell stiffness, increased extent and strength of RBC aggregation¹³⁻¹⁵ and abnormal blood viscosity; at equal hematocrit, SCD blood viscosity is increased compared to normal AA blood.^{12,16,17} At low shear or near stasis, blood viscosity increases as the cube of hematocrit and is somewhat less sensitive at higher shear (i.e., 4% increase per unit hematocrit increase). Blood viscosity at low shear is markedly increased with enhanced RBC aggregation, whereas aggregates are dispersed at high shear: the non-Newtonian flow behavior of blood is due to this shear-dependent RBC aggregation plus the ability of RBC to deform and align with flow streamlines.¹⁸⁻²⁰

In brief, blood viscosity increases dramatically in a non-linear fashion as hematocrit increases (Figure 1, left panel), with the increases being shear rate dependent. Conversely, oxygen content is a linear function of hematocrit (i.e., hemoglobin content). Given these two different sensitivities to hematocrit, one can predict disadvantages of either too a low hematocrit (i.e., low oxygen content) or too high a hematocrit (i.e., elevated viscosity).¹² An index to RBC oxygen transport effectiveness, termed the hematocrit-to-viscosity ratio (HVR), quantifies the effects of both variables.²¹ Figure 1 shows a schematic representation of the effects of hematocrit on HVR. Patients in group 'A' would be expected to have low viscosity but limited oxygen transport due to anemia, while patients in group 'C' would have high oxygen capacity due to elevated hematocrit but limited RBC transport due to high viscosity. This trade-off is reflected in the right hand panel, which demonstrates that HVR has an optimal level with respect to hematocrit. Since blood is a non-Newtonian fluid and its viscosity changes with shear stress, the shape of the HVR curve and the optimal hematocrit will vary with the vascular bed studied.

Simple transfusions increase hemoglobin and hematocrit, where improved oxygen carrying capacity is counterbalanced by cardiac output reduction in patients on CTT. That is, increased viscosity and vascular resistance lowers flow such that oxygen delivery is constant

following transfusion²². While large artery disease is prevented by CTT, increased viscosity from CTT may worsen microvascular disease. Alexy, et al. examined the *in vitro* rheologic behavior of SS and AA RBC mixtures suspended in sickle plasma to evaluate relations between viscosity, hematocrit, percent S hemoglobin and HVR. Their study demonstrated that there was an optimum hematocrit at high shear rates but that HVR was decreased at low rates of shear.²³

Given the results from *in vitro* mixtures of SS and AA red cells²³, we hypothesized that after a single transfusion, sickle cell disease patients on chronic transfusion therapy will have an improved HVR at high shear rates but a decreased HVR at low shear rates. The present study was designed to test this hypothesis using blood viscosity results for samples obtained from SCD subjects prior to and following transfusion.

Patients and Methods

Patients

This was a prospective, cross sectional evaluation of the hemorheologic effects of transfusion in 15 chronically transfused SCD patients. Subjects with homozygous HbSS sickle cell disease on chronic transfusion protocol were identified in the outpatient transfusion clinic at Children's Hospital Los Angeles or by their primary hematologist and were enrolled between August 2008 and January 2011. The exclusion criteria were age <10 years old, chronic transfusion therapy <1 year, sickle crisis within the previous four weeks defined as requiring inpatient admission, blood transfusion outside the CTT protocol or an emergency room visit with an increase in pain medications above their baseline usage for greater than 2 days. This group represents a sub-population of a larger project; the age criterion was based on other investigative protocols that are not included in the present study. All patients provided written informed consent, or for minor patients, parental consent and patient assent were obtained. The Committee for Clinical Investigation at Children's Hospital Los Angeles approved the protocol.

Every patient underwent a history and physical exam, and a venous blood sample was obtained on the morning of transfusion prior to the patient receiving any blood products (i.e., pre-transfusion). The standard clinical protocol used attempts to achieve a target percent hemoglobin S of less than 30% and a total hemoglobin of 11 g/dl; each patient received their transfusion according to these targets. Hemoglobin S concentrations were obtained prior to each transfusion as part of the standard clinical protocol. The patient then returned for a second visit 12 – 120 hours post-transfusion (mean 2.3 days, SD=1.4 days) when peri-transfusion changes in fluid and hormonal parameters should have dissipated and post-transfusion hematocrit stabilized. Blood was drawn during the second visit and thus both pre- and post-transfusion blood samples were obtained.

Hematologic Analysis

Venous blood was drawn into EDTA (1.5 mg/ml) *prior* to transfusion and at the *post* transfusion visit to evaluate complete blood count (CBC), markers of hemolysis (i.e., LDH, plasma free hemoglobin, arginine/ornithine ratio), inflammation (i.e., high sensitivity C reactive protein) and plasma amino acid profile. All of these measurements were performed by the clinical laboratory at Children's Hospital Los Angeles.

Blood Viscosity

Blood viscosity measurements were made on venous blood samples at multiple shear rates using an automatic tube viscometer (RheologTM, Health Vector Co., Pennsauken, NJ) operating at 37 °C. This instrument has been described in detail elsewhere and has been

shown to yield viscometric data consistent with cone-plate and Couette viscometers.²⁴ In brief, the device uses a horizontal glass tube (~ 0.8 mm ID) having vertical fluid manometers at each end. The blood sample is introduced such that there is initially a pressure gradient (i.e., difference in height of blood in manometers), and then allowed to flow through the tube; the pressure gradient progressively decreases as the heights tend to equalize. The positions of the blood in each manometer are continuously recorded, thus providing pressure gradient and volumetric flow information as a function of time. The pressure-flow data are analyzed by computer software provided by the manufacturer to obtain apparent blood viscosity versus shear rate results; viscosity is reported at several preselected shear rates from 1 to 1,000 s⁻¹. The ratio of whole blood viscosity at 5 s⁻¹ to that at 300 s⁻¹ was used as an index of RBC aggregation; this ratio increases with enhanced aggregation.¹⁸

Prior to measuring blood viscosity, an aliquot of each sample was equilibrated with humidified room air or nitrogen containing 2.5% CO₂, using a hollow tube gas exchanger (Living Systems Instrumentation, Burlington, VT), yielding an average pH of 7.15±0.02, PO₂ of 206±7.2 or 45±9.3 mm Hg for room air and nitrogen respectively (i-STAT-1 Analyzer, i-STAT Corporation, East Windsor, NJ). Note that the design of the Rheolog™ viscometer allows introduction of blood and subsequent viscosity measurements at all shear rates without exposure to ambient air, and thus the oxygenation status of the equilibrated samples was unaltered while being tested. All blood equilibrations and viscosity measurements were completed within four hours after the samples were obtained.

Data Analysis

Numerical data in the text and tables are expressed as mean ± standard error of the mean or mean ± standard deviation (i.e., mean ±SE or ±SD). Hematologic responses to transfusion were evaluated by two-sided, paired T-tests. The Wilcoxon Rank Sum Test was used when data were not normally distributed. Linear regression was performed for correlations of continuous data. JMP (version 5.1.2, SAS Institute Inc., Cary, North Carolina) was used for all statistical analyses.

Results

Demographics and Laboratory Values

Table 1 presents gender-specific demographic characteristics for the patient population used in this study. The patients were well matched for age, BMI, years on transfusion therapy and elapsed time between pre- and post-transfusion blood sampling (Table 1) and were pooled for further analysis. Table 2 shows selected laboratory data: hematocrit and hemoglobin increased significantly with transfusion, while white blood cell count (WBC), platelet count, percent hemoglobin S and reticulocyte count decreased. A decrease of percent Hb S and an increase of hematocrit following transfusion occurred in each patient. Transfusion had no significant effect on LDH or the arginine/ornithine ratio, two markers of chronic hemolysis. There was generally a small, non-significant drop of plasma hemoglobin and hsCRP, a marker of inflammation, with a few patients showing slight post-transfusion increases of these parameters.

Blood Viscosity: Non-Newtonian Behavior

Figure 2 shows the effects of transfusion on whole blood viscosity under oxygenated and deoxygenated conditions. As anticipated, both pre- and post-transfusion bloods behaved as shear thinning, non-Newtonian fluids regardless of oxygenation status. An increase in shear rate from 1 s⁻¹ to 1000 s⁻¹ caused: 1) a 81% decrease in viscosity pre-transfusion and a 84% decrease post-transfusion under oxygenated conditions; 2) a 80% decrease in viscosity pre-

transfusion and an 86% decrease post-transfusion under deoxygenated conditions. Thus, the effects of shear rate on viscosity were similar for both conditions, with a slight tendency for enhanced non-Newtonian flow behavior for the higher hematocrit, post-transfusion samples. The aggregation index (i.e., ratio of viscosity at 5 s^{-1} to 300 s^{-1}) increased 11% under oxygenated conditions and 15% under deoxygenated conditions ($p < 0.05$, Table 2).

There was a significant increase of blood viscosity at all shear rates following transfusion for both the oxygenated and deoxygenated samples ($p < 0.001$). However, the increase of viscosity following transfusion was shear rate dependent. Under oxygenated conditions, there was a $7.9 \pm 1.6 \text{ mPa.s}$ increase at 1 s^{-1} compared with 0.6 ± 0.1 at $1,000 \text{ s}^{-1}$. Thus, the 21% increase in hematocrit (Table 2) increased viscosity by 34% at low shear compared to only 16% at high shear. Results were similar but of larger magnitude for the deoxygenated samples, especially at low shear: an increase of $11.5 \pm 2.4 \text{ mPa.s}$ at 1 s^{-1} and 0.7 ± 0.2 at $1,000 \text{ s}^{-1}$ and hence a 56% increase at low shear and a 19% increase at high shear.

Blood Viscosity: Oxygenation Effects

Figure 3 presents the effects of deoxygenation on whole blood viscosity for the pre-transfusion and post-transfusion periods. In the pre-transfusion samples, there was a mean increase of $3.2 \pm 0.5 \text{ mPa.s}$ at 1 s^{-1} and 0.4 ± 0.06 at $1,000 \text{ s}^{-1}$, and thus a 19% increase at low shear and a 13% increase at high shear. Transfusion somewhat widened the disparity between oxygenated and deoxygenated samples, but only at low shear rates: transfusion increased viscosity by $6.7 \pm 1.6 \text{ mPa.s}$ at 1 s^{-1} and 0.5 ± 0.1 at $1,000 \text{ s}^{-1}$, and thus a 27% increase at low shear and a 13% increase at high shear. All increases in viscosity were significant at all shear rates ($p < 0.002$).

Hematocrit to Viscosity Ratio (HVR)

The effects of oxygenation status on HVR (i.e., RBC oxygen transport effectiveness) as a function of shear rate are shown in Figure 4 for pre-transfusion (panel A) and post-transfusion (panel B) blood samples. Regardless of oxygenation status, HVR fell with decreasing shear rate. Deoxygenation-induced changes of HVR were shear rate dependent: decreases at low shear rate were 16% and 17% in pre- and post-transfusion samples, while at high shear rate the decreases were 10% and 9% for these samples (Figure 4). Thus, at a given rate of shear, the decreases resulting from deoxygenation were comparable for pre- and post-transfusion samples. The changes of HVR subsequent to transfusion, expressed as a percent of pre-transfusion levels, are shown in Figure 5 for the nine shear rates used in this study for both oxygenated and deoxygenated conditions. It is important to note that *transfusion significantly impaired HVR* at low shear ($1, 2$, and 5 s^{-1}) under both oxygenated and deoxygenated conditions, with an effect size ranging from 17.6% (1 s^{-1}) to 8.2% (5 s^{-1}) ($p < 0.05$). HVR trended lower (5.5% and 7.9%) at a shear rate of 10 s^{-1} but did not reach significance. There was a non-significant tendency towards improved HVR as shear rates were increased further (i.e., 50 to $1,000 \text{ s}^{-1}$, see Figure 5).

Low shear (1 s^{-1}) changes of HVR following transfusion were inversely correlated with the change in aggregation index for both deoxygenated and oxygenated conditions ($p = 0.0001$, $r^2 = 0.75$ and 0.76 respectively). Pooling the pre and post oxygenated and deoxygenated transfusion data indicated a strong inverse correlation between low shear HVR and both Hct and Hb ($P < 0.001$, $r^2 = 0.33$ – 0.48); these correlations did not exist at high rates of shear.

Discussion

The present study addressed the impact of transfusion on RBC oxygen transport effectiveness (i.e., hematocrit to viscosity ratio, HVR) in chronically transfused SCD

patients by measuring whole blood viscosity at native hematocrit. Since native plasma was the RBC suspending medium, cellular interactions such as reversible RBC aggregation (i.e., rouleaux formation), as well possible effects of inflammation and hemolysis, were preserved in the present study. In contrast, studies using red cell mixtures suspended in saline fail to accurately describe low-shear HVR.¹²

The major findings of this study are: 1) whole blood viscosity, especially at lower rates of shear, is made significantly higher by blood transfusion independent of oxygenation status; 2) RBC oxygen transport effectiveness, indexed as HVR, is *decreased* at low shear rate but is not significantly affected at mid and high shear rates (Figure 5). That is, a single transfusion as part of CTT therapy significantly *decreases* HVR at low shear rates (i.e., 1, 2, 5 s⁻¹) regardless of oxygenation status, whereas the same transfusion results in no significant change in HVR at high shear (i.e., 150–1,000 s⁻¹). There is a transition region somewhere in the range of 10–50 s⁻¹ where there is no obvious beneficial or detrimental acute effect of transfusion in the population used in this study.

The decreases in low shear HVR (Figures 4, 5) reflect viscosity increases due to increased hematocrit and the greater effect of RBC aggregation as hematocrit is increased. The increase in aggregation index and hematocrit after transfusion (Table 2) correlated with the *transfusion-induced* change in viscosity and HVR, whereas the pre-level, post-level and change of HbS% did not, suggesting that the increase of hematocrit has a stronger influence on low shear viscosity and hence HVR than the decrease of %HbS. Whether the admixture of donor AA red cells with the pre-transfusion heterogeneous suspension of AA and SS red cells further increases aggregation is unclear and could not be directly determined in our study. There are reports indicating that aggregation is decreased with reduced RBC deformability or when poorly deformable cells are added to a red cell mixture, however, this prior study did not include SS RBC.²⁵ Furthermore, there is conflicting evidence describing both increased and decreased RBC aggregation behavior in sickle cell disease.^{13–16}

The unaltered HVR values at high shear must reflect a near “balance” of two factors: an increase of viscosity with hematocrit offset by improved deformability of the RBC population (i.e., transfused AA RBC more deformable) since at equal hematocrit, suspensions of more deformable RBC are less viscous at high shear¹⁰. This suggestion is supported by our observation that %HbS is inversely correlated with high shear HVR, while the aggregation index, markers of inflammation and markers of hemolysis do not appear to play a role at high shear.

Our inability to demonstrate an optimum hematocrit for HVR at high shear rates, as shown schematically in Figure 1, is directly related to the range of relevant parameters in our experimental protocol (Table 2). Using whole blood obtained prior to and following a defined transfusion cycle within a CTT protocol, the increase of hematocrit (6.2 ± 0.7 units) and the decrease of %HbS (39.2 to 33.6%) were much smaller than the range utilized in the *in vitro* mixture studies by Schmalzer, et al. and by Alexy and co-workers.^{26,27} Thus, neither an optimal HVR nor unfavorable effects beyond an optimal value were evident. In order to achieve a wider range of variables, it is suggested that future work in this area compare SCD patients prior to the start of CTT (i.e., low hematocrit, high %HbS) with those who have been on a chronic transfusion protocol for an extended period of time.

Our results are consistent with the *in vitro* study by Alexy and co-workers²³, in that these investigators also showed a decrease of low-shear HVR subsequent to increasing hematocrit and decreasing percent HbS when simulating transfusion via mixing AA and SS RBC. The present study extends their observations to a more realistic situation where changes of blood composition are achieved by CTT and where the post-transfusion properties of blood are

modulated by the response of individual SCD subjects. A shear-dependent effect of transfusion has also been reported by Jan, et al. who studied non-CTT SS subjects acutely receiving 1–3 units of RBC based on various clinical indications: transfusion only improved the oxygen dependence of viscosity at shear rates $>5 \text{ s}^{-1}$.²⁸ A direct comparison to their results is problematic since hematocrit-viscosity pairs were not reported and the subjects were not participating in an ongoing, standardized transfusion protocol.²⁸

Post-capillary venules are low shear vessels with decreased volumetric flow rates^{10,29,30} that are further reduced in SCD, thereby predisposing patients to vaso-occlusive crisis.^{1,2} Thus, an optimal hematocrit for red cell oxygen transport effectiveness (i.e. HVR) is likely dependent on RBC interactions at low shear. The shear rate is high in arterioles and the geometry of the vasculature is small at the terminal arteriole and capillary level, and thus flow will be dependent on the cell's ability to deform under high shear and their ability to navigate small vascular channels. A decreased number of reticulocytes, which are known to be less deformable, as well as lower HbS% levels, improve the average deformability of the RBC population and tend to reduce blood viscosity in SCD; these changes are most likely the mechanisms by which transfusion might improve high shear blood flow. It is possible that for tissues with low oxygen tension and low perfusion pressure, the native hematocrit may represent an optimum, and that transfusion would fail to improve or might further compromise RBC transport potential. This is consistent with the finding that transfusion is not useful for treatment of sickle pain crisis and that even a modest increase of hematocrit may be detrimental.⁷ It is also consistent with reports that *in vivo* reduction of RBC aggregation and hence of low shear viscosity may have a beneficial effect on oxygen delivery.^{31,32}

Note that our results were obtained in a system in which flow is occurring in 8003m diameter, 10 cm long straight tubes. However, flow in the microcirculation is complex involving varying diameters, converging and diverging flow, reduced hematocrit due to the Fahraeus effect and endothelial-RBC interactions^{15,30,33–35}, and thus direct clinical application of our results requires caution. Further, while HVR is a useful proxy for RBC transport to the microcirculation, it does not provide specific information regarding oxygen diffusion from RBC to vessel walls or from vessel walls to tissues. Studies in which oxygen delivery is measured by near infrared or another independent, non-derived parameter would provide greater insight into the physiological relevance of the HVR index. The present study is limited by the small sample size and cross sectional nature of the study. Also, studying patients who were already on chronic transfusion therapy for stroke, high TCD or recurrent pain crises has an inherent selection bias toward a more severe vascular phenotype.

In overview, our results suggest that local flow conditions, hematocrit and RBC aggregation are important parameters to consider when evaluating possible effects of transfusion therapy in SCD. They suggest the acute rheologic effects of transfusion on hematocrit and RBC aggregation might be detrimental to blood flow in low shear regions of the circulation. However, detailed understanding of the specific roles of hematocrit, RBC aggregation and %HbS is, at present, problematic. Therapies in which HbS% is decreased to very low levels, while minimizing increases in hematocrit and aggregation, would be logical pursuits. Long term, repeated measures starting prior to initiation of CTT would better delineate chronic changes associated with changes in blood rheology.

Acknowledgments

The authors would like to acknowledge Rosalinda B. Wenby for her technical expertise and discussions about hemorheology methodology. We would also like to thank the hematology division at Children's Hospital Los Angeles for their support of our work, particularly Susan Claster, M.D., Vasili Berdoukas, M.D., and Thomas Hofstra, M.D.

This work was supported by a grant from the National Institutes of Health, National Heart Lung and Blood Institute (NIH# 1 U54 HL090511-01)(T.C.): Sickle Cell Scholar Award; (5 RC1 HL099412-01)(J.D.), and by the Children's Hospital Los Angeles General Clinical Research Center (NIH #RR00043-43)(J.D.)

References

1. Vichinsky E. New therapies in sickle cell disease. *Lancet*. 2002; 360:629–31. [PubMed: 12241949]
2. Claster S, Vichinsky EP. Managing sickle cell disease. *BMJ*. 2003; 327:1151–5. [PubMed: 14615343]
3. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, Abboud M, Gallagher D, Kutlar A, Nichols FT, Bonds DR, Brambilla D. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. *N Engl J Med*. 1998; 339:5–11. [PubMed: 9647873]
4. Hillery CA, Panepinto JA. Pathophysiology of stroke in sickle cell disease. *Microcirculation*. 2004; 11:195–208. [PubMed: 15280092]
5. Bishop S, Matheus MG, Abboud MR, Cane ID, Adams RJ, Jackson SM, Kalpatthi R. Effect of chronic transfusion therapy on progression of neurovascular pathology in pediatric patients with sickle cell anemia. *Blood Cells Mol Dis*. 2011; 47:125–8. [PubMed: 21724428]
6. Hulbert ML, McKinstry RC, Lacey JL, Moran CJ, Panepinto JA, Thompson AA, Sarnaik SA, Woods GM, Casella JF, Inusa B, Howard J, Kirkham FJ, Anie KA, Mullin JE, Ichord R, Noetzel M, Yan Y, Rodeghier M, DeBaun MR. Silent cerebral infarcts occur despite regular blood transfusion therapy after first strokes in children with sickle cell disease. *Blood*. 2011; 117:772–9. [PubMed: 20940417]
7. Serjeant G. Blood transfusion in sickle cell disease: a cautionary tale. *Lancet*. 2003; 361:1659–60. [PubMed: 12747923]
8. Martini J, Carpentier B, Negrete AC, Frangos JA, Intaglietta M. Paradoxical hypotension following increased hematocrit and blood viscosity. *Am J Physiol Heart Circ Physiol*. 2005; 289:H2136–43. [PubMed: 16006543]
9. Vazquez BY, Cabrales P, Tsai AG, Intaglietta M. Nonlinear cardiovascular regulation consequent to changes in blood viscosity. *Clin Hemorheol Microcirc*. 2011; 49:29–36. [PubMed: 22214675]
10. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. *Semin Thromb Hemost*. 2003; 29:435–50. [PubMed: 14631543]
11. Fan FC, Chen RY, Schuessler GB, Chien S. Effects of hematocrit variations on regional hemodynamics and oxygen transport in the dog. *Am J Physiol*. 1980; 238:H545–22. [PubMed: 7377327]
12. Schmalzer EA, Lee JO, Brown AK, Usami S, Chien S. Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels. Implications for transfusion. *Transfusion*. 1987; 27:228–33.
13. Obiefuna PC. Rouleaux formation in sickle cell traits. *J Trop Med Hyg*. 1991; 94:42–4. [PubMed: 1995933]
14. Obiefuna PC, Photiades DP. Sickle discocytes form more rouleaux in vitro than normal erythrocytes. *J Trop Med Hyg*. 1990; 93:210–4. [PubMed: 2348499]
15. Tripette J, Alexy T, Hardy-Dessources MD, Mouguel D, Beltan E, Chalabi T, Chout R, Etienne-Julan M, Hue O, Meiselman HJ, Connes P. Red blood cell aggregation, aggregate strength and oxygen transport potential of blood are abnormal in both homozygous sickle cell anemia and sickle-hemoglobin C disease. *Haematologica*. 2009; 94:1060–5. [PubMed: 19644138]
16. Usami S, Chien S, Scholtz PM, Bertles JF. Effect of deoxygenation on blood rheology in sickle cell disease. *Microvasc Res*. 1975; 9:324–34. [PubMed: 236484]
17. Morris CL, Rucknagel DL, Joiner CH. Deoxygenation-induced changes in sickle cell-sickle cell adhesion. *Blood*. 1993; 81:3138–45. [PubMed: 8499647]
18. Baskurt, OgK; Neu, B.; Meiselman, HJ. Red blood cell aggregation. Boca Raton: CRC Press; 2012.
19. Kaul DK, Fabry ME, Nagel RL. The pathophysiology of vascular obstruction in the sickle syndromes. *Blood Rev*. 1996; 10:29–44. [PubMed: 8861277]

20. Kaul DK, Liu XD, Chang HY, Nagel RL, Fabry ME. Effect of fetal hemoglobin on microvascular regulation in sickle transgenic-knockout mice. *J Clin Invest*. 2004; 114:1136–45. [PubMed: 15489961]
21. Bogar L, Juricskay I, Kesmarky G, Kenyeres P, Toth K. Erythrocyte transport efficacy of human blood: a rheological point of view. *Eur J Clin Invest*. 2005; 35:687–90. [PubMed: 16269018]
22. Detterich J, Kato RM, Dongelyan A, Bush A, Meiselman HJ, Coates TD, Wood JC. Acute Cardiovascular and Hematologic Changes After a Single Transfusion Demonstrate Sex Differences in Chronically Transfused Sickle Cell Anemia Patients. *ASH Annual Meeting Abstracts*. 2011; 118:2138.
23. Alexy T, Pais E, Armstrong JK, Meiselman HJ, Johnson CS, Fisher TC. Rheologic behavior of sickle and normal red blood cell mixtures in sickle plasma: implications for transfusion therapy. *Transfusion*. 2006; 46:912–8. [PubMed: 16734807]
24. Alexy T, Wenby RB, Pais E, Goldstein LJ, Hogenauer W, Meiselman HJ. An automated tube-type blood viscometer: validation studies. *Biorheology*. 2005; 42:237–47. [PubMed: 15894822]
25. Reinhart WH, Singh A. Erythrocyte aggregation: the roles of cell deformability and geometry. *Eur J Clin Invest*. 1990; 20:458–62. [PubMed: 2121506]
26. Alexy T, Pais E, Armstrong JK, Meiselman HJ, Johnson CS, Fisher TC. Rheologic behavior of sickle and normal red blood cell mixtures in sickle plasma: implications for transfusion therapy. *Transfusion*. 2006; 46:912–8. [PubMed: 16734807]
27. Schmalzer EA, Lee JO, Brown AK, Usami S, Chien S. Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels. Implications for transfusion. *Transfusion*. 1987; 27:228–33. [PubMed: 3590284]
28. Jan K, Usami S, Smith JA. Effects of transfusion on rheological properties of blood in sickle cell anemia. *Transfusion*. 1982; 22:17–20. [PubMed: 7064201]
29. Surgenor, DM.; Bishop, CW. *The red blood cell*. 2. New York: Academic Press; 1974.
30. Bishop JJ, Popel AS, Intaglietta M, Johnson PC. Effect of aggregation and shear rate on the dispersion of red blood cells flowing in venules. *Am J Physiol Heart Circ Physiol*. 2002; 283:H1985–96. [PubMed: 12384477]
31. Ballas SK, Files B, Luchtman-Jones L, Benjamin L, Swerdlow P, Hilliard L, Coates T, Abboud M, Wojtowicz-Praga S, Grindel JM. Safety of purified poloxamer 188 in sickle cell disease: phase I study of a non-ionic surfactant in the management of acute chest syndrome. *Hemoglobin*. 2004; 28:85–102. [PubMed: 15182051]
32. Gibbs WJ, Hagemann TM. Purified poloxamer 188 for sickle cell vaso-occlusive crisis. *Ann Pharmacother*. 2004; 38:320–4. [PubMed: 14742772]
33. Bishop JJ, Popel AS, Intaglietta M, Johnson PC. Rheological effects of red blood cell aggregation in the venous network: a review of recent studies. *Biorheology*. 2001; 38:263–74. [PubMed: 11381180]
34. Secomb TW, Pries AR. Information transfer in microvascular networks. *Microcirculation*. 2002; 9:377–87. [PubMed: 12375175]
35. Pries AR, Secomb TW. Rheology of the microcirculation. *Clin Hemorheol Microcirc*. 2003; 29:143–8. [PubMed: 14724335]

Figure 1.

Schematic representation of the effects of hematocrit on blood viscosity and the hematocrit to blood viscosity ratio (HVR). The HVR is less than maximal in area A due to low hemoglobin level and in area C due to elevated blood viscosity; a maximal HVR is achieved at an “optimal hematocrit”.

Figure 2. Effects of transfusion on whole blood viscosity-shear rate relations. All blood samples behaved as non-Newtonian fluids with viscosity increasing as shear rate is decreased. Blood viscosity also increases with hematocrit (see Figure 1) and thus post-transfusion levels are higher than those for pre-transfusion blood regardless of oxygenation status. ($p < 0.001$ at all shear rates for both deoxygenated and oxygenated blood)

Figure 3.

Effects of deoxygenation on whole blood viscosity-shear rate relations. Viscosity increased over the entire shear rate range subsequent to deoxygenation ($p < 0.002$), with the effects most prominent at lower shear rates. Pre-transfusion, deoxygenation increased viscosity by 19% at 1 s^{-1} and 13% at $1,000 \text{ s}^{-1}$; post-transfusion increases due to deoxygenation were somewhat greater at 1 s^{-1} (27%) but identical at the highest shear.

Figure 4.

Effects of deoxygenation on the hematocrit to blood viscosity ratio (HVR) for pre-transfusion (Panel A) and post-transfusion (Panel B) blood samples. Regardless of oxygenation status, HVR increased with shear rate owing to the non-Newtonian behavior of blood and the decrease of viscosity with increasing shear rate (Figure 2). Both pre- and post-transfusion blood samples exhibit a shear rate dependent decrease of HVR with deoxygenation.

Figure 5.

The effects of shear rate on HVR for the range used in the present study, with results expressed as percent change from appropriate pre-transfusion values; negative percent changes indicate a worsening of HVR. Note that *transfusion significantly impaired HVR* at low shear (1, 2, and 5 s⁻¹) under both oxygenated and deoxygenated conditions, with an effect size ranging from 17.6% (1 s⁻¹) to 8.2% (5 s⁻¹) (* = p<0.05). HVR tended to be lower at 10 s⁻¹ (** = 0.05<P<0.1); no significant effects were observed at higher rates of shear.

Table 1

Demographic Data

Demographics	Male (n=7)	Female (n=8)	P-Value
Age ($\pm$ SD)	17.7 $\pm$ 5.5	17.8 $\pm$ 4.4	0.99
% African American	86	75	
Height in cm ($\pm$ SD)	166.8 $\pm$ 22.3	155.9 $\pm$ 13.5	0.29
Weight in kg ($\pm$ SD)	61.0 $\pm$ 21.7	57.1 $\pm$ 17.5	0.71
BMI kg/m ² ($\pm$ SD)	21.1 $\pm$ 3.4	23.1 $\pm$ 4.7	0.36
BSA m ² ($\pm$ SD)	1.67 $\pm$ 0.41	1.56 $\pm$ 0.30	0.57
Days Since Last Transfusion ($\pm$ SD)	25.9 $\pm$ 8.9	21 $\pm$ 4.9	0.23
Years on Transfusion Therapy ($\pm$ SD)	7.4 $\pm$ 4.5	7.5 $\pm$ 3.4	0.98
Days Between Study Visits ($\pm$ SD)	2 $\pm$ 1.4	2.4 $\pm$ 1.5	0.63
Reason for Transfusions			
CVA	2	2	
Abnormal TCD	3	4	
recurrent Acute Chest	1	1	
Pulmonary Hypertension	1	1	

Data are mean $\pm$ one standard deviation. BSA = body surface area, BMI = body mass index, CVA = cerebrovascular accident, TCD = transcranial doppler

Table 2

Laboratory Data Pre and Post Transfusion

Laboratory Value	Pre	Post	Change after Transfusion
WBC (K/uL)	15±4.3	13.2±3.4	-1.8±0.7 *
Platelet (K/uL)	331.3±104.2	298.6±100.6	-32.7±10.3 *
Hematocrit (%)	28.4±2.1	34.6±3.2	6.2±0.7 *
Hemoglobin (g/dL)	9.6±0.7	11.9±1.1	2.3 ±0.2 *
Hemoglobin S (%)	39.2±20.7	33.6±17.7	-5.6±1.3 *
Reticulocyte Count (%)	12.1±6.8	8.8±5.6	-3.9±0.9 *
LDH (U/L)	1021.9±320.6	1012.3±319.2	-9.7±37.7
hsCRP (mg/L)	5.7±6.2	3.5±3.1	-1.7±2.1
Arg/Orn	1.1±0.5	1.0±0.3	-0.1±0.1
Plasma Free Hemoglobin (mg/dL)	22.1±16.5	19.4±15.8	-2.0±6.0
Oxygenated Aggregation Index	2.24±0.22	2.52±0.27	0.25±0.07 *
Deoxygenated Aggregation Index	2.37±0.18	2.73±0.33	0.36±0.09 *

Data are mean ± one standard deviation. WBC = white blood cells, LDH = lactate dehydrogenase, hsCRP = high sensitivity C-reactive protein, Arg/Orn = arginine/ornithine ratio.

* P<0.05