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Effect of Chronic Blood Transfusion on Biomarkers of Coagulation Activation and Thrombin Generation in Sickle Cell Patients at Risk for Stroke

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Abstract

Hypercoagulability in sickle cell disease (SCD) is associated with multiple SCD phenotypes, association with stroke risk has not been well described. We hypothesized that serum levels of biomarkers of coagulation activation correlate with high transcranial Doppler ultrasound velocity and decreases with blood transfusion therapy in SCD patients. Stored serum samples from subjects in the Stroke Prevention in Sickle Cell Anemia (STOP) trial were analyzed using ELISA and protein multiplexing techniques. 40 subjects from each treatment arm (Standard Care [SC] and Transfusion [Tx]) at three time points—baseline, study exit and one year post-trial and 10 each of age matched children with SCD but normal TCD (SNTCD) and with normal hemoglobin (HbAA) were analyzed. At baseline, median vWF, TAT and D-dimer levels were significantly higher among STOP subjects than either HbAA or SNTCD. At study exit, median hemoglobin level was significantly higher while median TCD velocity was significantly lower in Tx compared to SC subjects. Median vWF (409.6 vs. 542.9 μg/ml), TAT (24.8 vs. 40.0 ng/ml) and D-dimer (9.2 vs. 19.1 μg/ml) levels were also significantly lower in the Tx compared to the SC group at study exit. Blood levels of biomarkers coagulation activation/thrombin generation correlated positively with TCD velocity and negatively with number of blood transfusions. Biomarkers of coagulation activation/thrombin generation were significantly elevated in children with SCD, at high risk for stroke. Reduction in levels of these biomarkers correlated with reduction in stroke risk (lower TCD velocity), indicating a possible role for hypercoagulation in SCD associated stroke.
Red Blood Cell Transfusion Effect on Coagulation Biomarkers in SCD

Introduction

Sickle cell disease (SCD) is a significant public health problem, affecting over 100,000 people in the United States (US). It is a huge burden to the healthcare system and a significant source of health disparity [1–4]. The most common form, Hemoglobin SS or sickle cell anemia (SCA) is due to a point mutation in both human β-globin genes leading to the substitution of valine for glutamic acid at the 6th positions of both β-globin chains [5]. Hypercoagulability has been described in SCD [6, 7], and been associated with complications of SCD, such as acute chest syndrome (ACS) and vaso-occlusive episodes [8, 9]. Global activation and increased serum levels of pro-thrombotic factors and/or mediators have been described, which may be triggered by erythrocyte microparticles and inflammatory mediators [9, 10]. We have previously shown that children with SCD, at high risk for stroke, demonstrated by a high transcranial Doppler ultrasound (TCD) velocity, have significantly elevated serum levels of platelet derived growth factor AA [11] and total plasminogen activator inhibitor 1 [12]. Elevated baseline levels of both biomarkers were associated with high risk of incident stroke or decreased probability of stroke free survival, respectively [11, 12]. A high TCD velocity, ≥ 200cm/s [13], the severity of cerebral vasculopathy [14–16], and presence of extracranial vasculopathy [17] are among the recognized risk factors for development of stroke in children with SCD. The role of coagulation activation in SCD associated stroke is still not well-understood.

Chronic packed red blood cell (PRBC) transfusion is currently the main modality for primary [18] and secondary [19] stroke prevention in children with SCD. Although a beneficial regimen, chronic PRBC transfusion is associated with potential complications (iron overload and alloimmunization); and there is no safe time to discontinue it [20]. Further, when severe cerebral vasculopathy is established, chronic PRBC transfusion may not prevent occurrence of silent cerebral infarcts [21]. It is has been documented that inflammation [22, 23], vascular remodeling [24] and anemia leading to a hyperdynamic cerebral circulation [25, 26] are associated with an increased risk for developing cerebral vasculopathy and stroke in children with SCA. Anemia and inflammation are mitigated by chronic PRBC transfusion, resulting in decreased stroke risk and incidence [18, 22].

The conditions that could potentially initiate and/or propagate the development of cerebral arteriopathy in SCA remain elusive [27]. In addition, the mechanisms by which PRBC transfusions prevent stroke are not well-defined. This study seeks to delineate the possible role of coagulation activation in the pathobiology of stroke in SCD and the effects of red cell transfusion regimen on the coagulation pathway, especially thrombin generation. We hypothesize that serum levels of biomarkers of coagulation activation and thrombin generation are elevated in children with SCD and high TCD velocity. These levels are expected to be significantly reduced in those who received chronic PRBC transfusion compared to those who did not. Further, the lower levels of coagulation activation will be related to the number of transfusions received and hemoglobin levels.

Sample, Materials and Methods

Sample and sample processing

This study is an ancillary to the Stroke Prevention Trial in Sickle Cell Anemia (STOP) [18] [NCT00000592] and is an addition to prior studies [11, 12] from our laboratory. It is a longitudinal cross-sectional analysis of stored serum samples from the STOP study.

In brief, the STOP study was a randomized phase III clinical trial of the efficacy of chronic PRBC transfusion for the prevention of stroke in children with SCD and high TCD velocity. A total of 130 children, ages 2–16 years, with SCA and high TCD velocity (≥ 200cm/s) were
randomly assigned to received either standard care (observation, n = 67) or chronic PRBC transfusion (n = 63). The study was terminated early because of the overwhelming evidence of benefit to the transfusion arm \[18\]. Chronic PRBC transfusion resulted in a 92% reduction in stroke incidence. An additional year of post-trial follow up period was carried out. During this period, subjects randomized to the standard care arm were allowed to cross-over and receive PRBC transfusion. Majority of the subjects in the standard care arm crossed over to receive transfusion, while some patients on the transfusion arm stopped receiving transfusion all together; there were subjects in between, who either receive less or more transfusions during this period. All the patients included in this study from the standard care arm crossed over to receive transfusion while none of those from the transfusion arm in this study, completely stopped receiving transfusion at the end of 1 year post-trial.

In this study, the experimental design included only subjects from the STOP study for whom we had serum samples at all 3 time points to ensure that comparisons between time points included the same subjects. Serum samples were obtained from the bio-repository of the STOP study currently maintained at the Medical University of South Carolina (MUSC). Two hundred and forty frozen serum samples were available from 40 subjects in each of the standard care [SC] and transfusion [Tx] arms at baseline or randomization, study exit and 1 year post-trial time points were analyzed using Enzyme Linked Immuno-Sorbent Assay (ELISA) and multiplex analysis. The sample size for this ancillary was smaller than that of the parent study, but sample size estimates based on either a t or F distribution indicated that the sample size of 40 was robust to type 1 error and adequate to achieve a power of 80%.

An additional 10 samples each from age matched children with Sickle cell disease and Normal TCD velocity (SNTCD) and healthy African American children with normal hemoglobin (HbAA) were assayed for comparison. The samples for the children with SCD and normal TCD, and healthy control were obtained at “steady state” (when subjects were in a normal state of health (not during acute illnesses), four months after the last transfusion (for SCD subjects), from SCD subject not taking hydroxyurea. These samples were obtained in 2008–2009 as part of a study to investigate vascular function in children with SCD.

The original study did not plan for studies that might require platelet poor serum and as such, samples were not rendered platelet poor before storage in the biorepository. Venous blood was drawn and allowed to clot at room temperature, and then shipped to the Medical College of Georgia (MCG)-Hemoglobinopathy laboratory on wet-ice within 48 hours where samples were centrifuged, serum was removed and frozen (−70°C), subsequent sample storage was done at-80°C. Sample processing for Healthy controls, sickle cell patients with normal TCD velocity and that of STOP study participants were similar. Furthermore, the dates on the sample storage tubes for STOP study subjects indicates that samples were for the most part obtained at or in close proximity to the time of TCD velocity measurement.

We assayed for serum levels of thrombin-antithrombin (TAT) complex based on manufacturer’s protocols with conscious awareness of sample types and sample preparation requirements (http://www.abcam.com/thrombin-antithrombin-complex-tat-human-elisa-kit-ab108907.html and http://www.abcam.com/Thrombin-Antithrombin-Complex-TAT-Human-ELISA-Kit-ab108907.pdf). In addition, we also assayed serum levels of von Willibrand factor (vWF), ADAMTS 13 (to enable us define vWF to ADAMTS 13 ratio for as index of functionality with regards to proteolytic cleavage of vWF by ADAMTS 13), D-dimer, P-selectin and fibrinogen.

Ethics statement

The original STOP study and consent forms and documents were approved by the institutional review boards (IRBs) of the Medical College of Georgia and New England Research Institute.
written signed informed consent was obtained from participants and their caregivers. Approval to conduct this study was obtained from the IRBs of the Medical University of South Carolina (MUSC), Morehouse School of Medicine (MSM) and Children’s Healthcare of Atlanta (CHOA). Informed consent had been obtained from participants and their caregivers during the original clinical trial to allow for subsequent use of the samples by other investigators without the need for re-consenting.

**ELISA assay**

Serum levels of vWF and TAT complex were assayed using ELISA at the Morehouse School of Medicine Proteomics core laboratory. Frozen serum samples were allowed to thaw slowly at room temperature, and along with the assay kits (Abcam PLC., Cambridge, MA), warm up to room temperature. All assays were carried out according to the manufacturer’s protocol and were done in duplicate. Absorbance was read using a Softmax Pro (Molecular Devices LLC, Sunnyvale, CA) with concentration of analytes calculated based on a linear curve generated using manufacturer provided standards, using an \( R^2 \) of 0.95 or greater. The concentration of vWF was read in mU/ml and then converted to ng/ml by multiplying with 9.8 to convert to \( \mu g/ml \) and then by 1000 to convert from \( \mu g/ml \) to ng/ml. This conversion was necessary to enable the calculation of vWF to ADAMTS 13 ratio.

**Multiplex assay**

Thawed serum samples (same as was used for ELISA assay) were allowed to warm up to room temperature before analysis. This assay measured serum levels of ADAMTS 13, D-dimer, P-selectin and fibrinogen. Samples for estimating ADAMTS 13, D-dimer and P-selectin were diluted 1:100, while they were further diluted to 1:40,000 to allow for the measurement of fibrinogen level. Assays were carried out in duplicates using a customized human cardiovascular disease panel 2 (ADAMTS 13, D-dimer, P-selectin) and 3 (fibrinogen) of antibody immobilized bead kit (Millipore Inc., Billerica, MA). The mean fluorescent intensity (MFI) was read on a BioRad Bioplex reader powered by Luminex (Bio-Rad Inc., Hercules, CA) and concentration calculated using an interpolated 5PL logistic curve generated using manufacturer supplied standard prepared via a serial 1:4 dilution. In addition to ELISA and multiplex data, values for TCD velocity, anthropometric and hematological data were exported to Microsoft Excel and analyzed using IBM SPSS 20 for Windows and GraphPad Prism.

**Data analysis and presentation**

Median anthropometric and hematological characteristics of subjects were calculated at each time point. This is presented in Table 1 along with their respective 10th and 90th percentile in brackets. Except for percentage fetal hemoglobin, where a parametric t-test was used (data was normally distributed), a non-parametric t-test was used in all other cases to compare median differences between SC and Tx groups at each time point. Serum levels of the biomarkers were compared at baseline and at study exit between HbAA, SNTCD, SC and Tx using non-parametric analysis of variance (ANOVA) with Dunn’s correction for multiple comparisons. The median serum levels of biomarkers were expressed using box and whisker plots with 10th and 90th percentiles, at baseline and study exit respectively. The total number of transfusions received during the 30 month study period (some of the SC subjects received transfusions for other indications) was determined. This number was then correlated with TCD velocity and serum levels of biomarkers of coagulation activation and thrombin generation at the study exit time point. A two-tailed partial correlation with adjustment for baseline variation in serum biomarker levels was used. Results were expressed as scatter plots with regression lines and their
respective correlation coefficients. Paired sample t-test was used for comparison of serum concentration of biomarkers and TCD velocity at baseline and study exit within each study arm (i.e. baseline vs. study exit for SC arm, and so on) and also expressed in a box and whisker plot (S1 and S2 Files.). Finally the median percentage change in serum levels of the biomarkers from baseline was calculated and then compared between SC and Tx. The results of this is presented in a box and whisker plot for the biomarkers with statistically significant difference.

Results

Clinical characteristics of subjects

The clinical characteristics of STOP study subjects whose samples were analyzed are shown in Table 1. Except for median hemoglobin level (7.6 [6.7, 8.9] vs. 7.2 [6.1, 8.5]) mg/dL, which was significantly higher among the Standard Care (SC) (p = 0.021) compared to the Transfusion (Tx) arm, there were no significant differences in baseline characteristics and TCD velocity between study arms. At study exit, median hemoglobin level was significantly higher (8.8 [7.2, 10.4] vs. 8.0 [6.9, 9.3]) mg/dL and median TCD velocity significantly lower (157 [133, 236] vs. 203 [144, 244]) cm/sec among the Tx arm compared to the SC arm, p = 0.006 and 0.002 respectively (Table 1 and Fig 1H). At the one year post-trial time point, those randomized to the Tx arm had significantly lower median TCD velocity (166 [121, 220] vs. 189 [120, 266]) cm/s and body mass index (16.1 [14.2, 21.2] vs. 18.4 [14.0, 26.1]) kg/m² compared to those randomized to SC, p = 0.034 and 0.031 respectively (Table 1).

Biomarkers of coagulation activation

Despite differences in baseline hemoglobin levels, the subjects in the two treatment arms were well-matched on baseline levels of coagulation biomarkers, with no statistically significant differences in the serum levels of all biomarkers of coagulation activation or thrombin generation.
STOP subjects in both SC and Tx groups had significantly higher median levels of vWF, TAT, and D-Dimer than those with SCD and Normal TCD (SNTCD) or healthy controls (HbAA) (Fig 1A, 1C and 1E). While there was no difference in ADAMTS 13 levels between groups (Figure E in S1 File), VWF/ADAMTS 13 ratio was significantly higher at baseline for randomized subjects than for either SNTCD or HbAA groups (Figure G in S1 File). Additionally, the median level of sP-selectin among SC arm was significantly higher than among HbAA (Figure C in S1 File).

At study exit, subjects randomized to the Tx arm had significantly lower median vWF levels than those in the SC arm (409.6 [322.3, 446.2] vs. 542.9 [479.6, 597.9]) μg/ml, p < 0.0001. But there was no statistically significant difference in median vWF levels between Tx arm and either SNTCD or HbAA subjects at study exit (Fig 1B). As was the case at baseline, the serum vWF level at exit was significantly higher among the SC arm than either SNTCD or HbAA, p < 0.0001. Similarly, median serum level of TAT complex was significantly lower among the Tx arm compared to the SC arm (24.8 [18.2, 38.1] vs. 40.0 [25.7, 51.4]) ng/ml, p < 0.001. However, the Tx arm TAT complex level was still significantly higher than either SNTCD or HbAA; p = 0.002 and 0.007 respectively (Fig 1D). Further, serum D-dimer levels were significantly lower among the Tx arm at study exit than among the SC arm (9.2 [2.8, 35.6] vs. 19.1 [5.7, 37.7]) μg/ml, p < 0.001. There was no significant difference in the serum D-dimer levels between Tx arm and either SNTCD or HbAA respectively (Fig 1F).

Change in serum levels of biomarkers from baseline, by treatment group

Since both the SC and Tx groups showed significant decrease in both TCD velocity and some of the biomarkers of coagulation activation from baseline values, we compared the percentage reduction in TCD velocity and biomarkers of coagulation activation at study exit compared to baseline in SC and TX groups (Fig 2A–2E). The percentage reduction in vWF levels from baseline was significantly greater in the Tx group compared to SC (44.5 [33.5, 54.4] vs. 24.8 [2.4, 35.2], p < 0.0001). A similar pattern was observed for percentage reduction in TAT (30.9 [-18.3, 54.3] vs. -15.5 [-100.2, 28.2], p < 0.0001) and D-dimer (47.1 [-223.0, 80.8] vs. 3.7 [-98.8, 71.3], p < 0.001). The pattern of percentage reduction in serum levels of these biomarkers was similar to that of percentage reduction in TCD velocity in response to blood transfusion (27.2 [4.2, 42.2] vs. 6.7 [-8.9, 33.8], p < 0.0001).

Relationship of blood transfusion, TCD velocity and hematological variables to biomarker levels

We used partial correlation to test the associations between the number of blood transfusions received, TCD velocity and hematological variables (white cell, red cell and platelet counts, also Hb and HbF level) to levels of biomarkers of coagulation activation and thrombin generation at study exit, adjusted for baseline levels. Figs 3 and 4 shows the result of those tests that were statistically significant. Fig 3A and 3B shows that there was a significant positive correlation between TCD velocity and both serum vWF and TAT levels (r = 0.36, p = 0.0028) and (r = 0.27, p = 0.025), respectively. There was a trend to a positive correlation (r = 0.22, p = 0.079) between serum D-dimer levels and TCD velocity (Fig 3C). These results show a relationship between biomarkers of coagulation activation and TCD velocity.
At baseline, vWF level was significantly correlated with hemoglobin F (r = -0.32, p = 0.006) and hemoglobin S (r = 0.38, p = 0.001) levels. Further, there was a significant negative correlation between TAT levels and WBC count (r = -0.27, p = 0.020).

At study exit, there was significant correlation between vWF and hemoglobin S (HbS) (r = 0.27, p = 0.026), HbF (r = 0.35, p = 0.003), Hb level (r = -0.40, p = 0.001) or reticulocyte count (r = 0.25, p = 0.037). Similarly, serum TAT complex showed significant correlation with HbS (r = 0.26, p = 0.028), HbF (r = 0.38, p = 0.001), Hb (r = -0.35, p = 0.003) or reticulocyte count (r = 0.38, p = 0.001). We also observed that serum D-dimer levels were significantly correlated with HbS (r = 0.44, p < 0.0001), HbF (r = -0.37, p = 0.002), Hb (r = -0.45, p < 0.0001) or reticulocyte count (r = 0.40, p = 0.001). There was no significant correlation between the other biomarkers tested and HbS, HbF, Hb levels or with any other hematological marker; except for that between serum sP-selectin and HbS (r = 0.25, p = 0.036) or platelet count (r = 0.024, p = 0.048). These result shows that clinical parameters related to SCD severity and hemolysis were correlated to serum levels of biomarkers of coagulation activation and thrombin generation.

When we tested whether serum levels of biomarkers of coagulation activation correlated with the number of blood transfusions received from study entry to 1 year post-trial, we noted a strong negative correlation with serum vWF (r = -0.39, p = 0.0028), TAT (r = -0.53, p < 0.0001) and D-dimer (r = -0.55, p < 0.0001) levels. The pattern was similar to the correlation we observed between TCD velocity and number of blood transfusions received over the same period (Fig 4A–4D respectively).

Discussion

In this study, we report the effects of packed red blood cell (PRBC) transfusion on biomarkers of coagulation activation and thrombin generation in children with SCD who had a high TCD velocity and increased stroke risk. We had previously reported that elevated levels of PDGF-AA were associated with increased risk of incident stroke [11] and that frequent PRBC transfusion was associated with reduction in blood levels of biomarkers of inflammation and endothelial activation [12].

We found that children with SCD at high risk for stroke had significantly elevated serum levels of biomarkers of coagulation activation and thrombin generation at baseline, essentially a worse hypercoagulable state, compared to age-matched African American children with SCD.
and normal TCD velocity or healthy African American children without SCD (Fig 1). The serum levels of these biomarkers tracked with TCD velocity. At study exit, there were significantly lower levels of vWF, TAT and D-dimer, along with lower TCD velocity among Transfused subjects compared to the Standard Care group. Further, correlation analysis showed that TCD velocity was positively correlated with both vWF and TAT levels. Our data suggests that coagulation activation might be an important contributor to cerebrovascular complications in SCD.

In individuals without SCD, elevated vWF levels have been associated with increased risk for stroke, as both acute stroke patients and those with carotid atherosclerosis had elevated blood levels compared to healthy controls [28]. Further, elevated D-dimer levels are associated with increased risk for stroke and cardiovascular events [29]. Thus, our finding of significantly elevated vWF, D-dimer and TAT levels among children with SCD who had a higher risk for stroke was not surprising because of these well-established association in non-SCD related cases of cerebrovascular and cardiovascular diseases [28, 29].

A similar relationship has been described in sickle cell patients with acute chest syndrome or pulmonary hypertension. Patients with SCD who had pulmonary hypertension, a history of
stroke, retinopathy and increased pain frequency had one or a combination of high serum pro-
thrombin fragment F2.1, TAT or D-dimers in addition to elevated levels of inflammatory bio-
markers [8, 9]. In another study, coagulation activation was associated with SCD related small
vessel cerebral vasculopathy [30].

The STOP study showed that chronic PRBC transfusions reduce TCD velocity and the rate
of strokes in high risk children with SCA [18]. We found that PRBC transfusions also signifi-
cantly reduced biomarkers of the hypercoagulable state. The serum levels of vWF, TAT and D-
dimer levels were negatively correlated with the number of PRBC transfusions, suggesting a
dose-response relationship. In addition, we found that the number of PRBC transfusion was
also negatively correlated with TCD velocity. Taken together, our results indicate that throm-
bogensis and a hyperactive coagulation system are likely to play a role in the pathobiology of
cerebrovascular disease in children with SCD.

One of the beneficial effects of PRBC transfusions in stroke prevention may be the improve-
ment of the procoagulant internal milieu. This assertion is supported by evidence from a num-
ber of studies. First, in a recent study, it was reported that PRBC transfusion in the STOP study
patients resulted in low serum free hemoglobin level [31]. Levels of serum free hemoglobin
have been found to be positively associated with levels of vWF multimers (which is procoagu-
lant in nature) because free hemoglobin binds to the vWFA2 receptors and prevents the cleav-
age of vWF by ADAMTS 13 [32]. Hemoglobin inhibition of ADAMTS 13, results in high
circulating concentration of vWF multimers. Secondly, there is evidence from multiple studies
that repeated sickling and unsickling of RBCs in SCD, results in an over exposure of membrane
phosphatidylin serine (PS). The subpopulation of PS exposed RBC in health individuals was
about 0.59±19% [33], but in SCD patients, the proportion was between 2–26%. This is thought
to create a hypercoagulable milieu that activates thrombin more powerfully than in non-SCD
individuals [33–36]. The benefit of chronic PRBC transfusion might in part be due to a reduc-
tion in the proportion of PS exposed RBCs in SCD patients receiving such therapy.

A potential limitation of our study is that the coagulation assays were not performed on
platelet poor serum, but on serum. This has a potential to impact external validity if the abso-
late numbers were compared to those that used platelet poor serum. However, the internal
validity of our group comparisons was maintained because all of our samples were handled in
the same manner. Furthermore, the uniformity of the protocol described for serum by the
ELISA assay kit manufacturer ensures that our findings can be reproduced if samples treated in
similar manner were used. Our samples were obtained from the STOP study bio-repository
which was fully established by 1999. While it could be suggested that this is an old repository
and there is risk for protein degradation, studies has shown that protein markers of hypercoag-
ulability are stable in frozen samples over a long period of storage at-80°C [37, 38]. In this
study, our samples were stored at -80°C, with care taken to avoid repeated freeze-thaw cycles
(maximum of two or less). Even with prolonged storage, the STOP study samples showed
much serum levels of biomarkers of coagulation activation and thrombin generation than that
of the more recently drawn SNTCD or HbAA samples. Finally, we noted that there was an
unexpected decrease in HbS levels at study exit compared to baseline for the SC group. We
hypothesize, that subjects in the SC group might have received transfusions that were not
reported or that those who received a high number of reported transfusions skew the median
HbS value.

In conclusion, we surmise that the observed higher levels of biomarkers of coagulation acti-
vation and thrombin generation among subjects with high TCD velocity, together with the
associated decrease with transfusion as TCD velocity decreased, support a role for hypercoagu-
lability and thrombotic phenomenon in the pathobiology of SCD-associated cerebrovascular
disease. Further investigation of the role of the coagulation system is warranted to delineate its
role in development of stroke and potential application as a predictive diagnostic biomarker of stroke risk in SCD patients.

Supporting Information

S1 File. Comparison of biomarker levels between standard care and transfusion arm at baseline and study exist. At study exit that, plasma sP-selectin level was significantly higher among Tx arm than either SNTCD (p = 0.03) or HbAA (p = 0.003). Similarly, plasma sP-selecting level was significantly higher among SC arm than among HbAA subjects, p = 0.02 (Figure D). For reasons we could not explain, at study exit, the plasma ADAMTS 13 level was higher among the SC arm than among HbAA subjects. At study entry, the median plasma vWF/ADAMTS 13 ratio was significantly higher among Tx arm than among either SNTCD (p = 0.0009) or HbAA (p = 0.02). Similarly, baseline median plasma vWF/ADAMTS 13 ratio was significantly higher among SC arm than among either SNTCD (p = 0.002) or HbAA, p = 0.02 (Figure G). But at study exit, there was no statistically significant difference in median plasma vWF/ADAMTS 13 ratio between groups (Figure H).

S2 File. Pairwise comparison of decrease in plasma levels of biomarkers of coagulation activation and TCD velocity from baseline. It shows that SC and Tx groups both had a decrease from baseline, with TCD velocity, plasma levels of vWF and TAT showing statistically significant decreases from baseline (Figures A-C). The decrease in plasma D-dimer levels from baseline was not statistically significant for either the Tx group, while the SC group actually showed an increased in D-dimer levels from baseline (Figure D).

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Author Contributions

Conceived and designed the experiments: HIH CSG BEG JMH. Performed the experiments: HIH. Analyzed the data: HIH BEG AH JV. Contributed reagents/materials/analysis tools: HIH RJA BEG. Wrote the paper: HIH RJA CSG JV AH JMH BEG. Data entry and data management: AH.
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