

Thromboelastographic and Hemostatic Characteristics in Pediatric Patients With Sickle Cell Disease

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• **Context.**—Patients with sickle cell disease suffer from a variety of vaso-occlusive events that may be related to activation of the hemostatic system. Thromboelastography assesses the functionality of this system from a global standpoint and has demonstrated some utility in detecting hypercoagulable states in varied clinical settings, but it has not been systematically evaluated in patients with sickle cell disease.

Objective.—To characterize the findings of thromboelastography in patients with sickle cell disease during periods of steady state and illness, to compare these results with those of healthy controls, and to correlate these profiles with other measured hemostatic parameters.

Design.—In this cross-sectional study, we obtained thromboelastographic and other hemostatic data on specimens from 46 patients with sickle cell disease (35 with hemoglobin SS, 7 with hemoglobin SC, and 4 with hemoglobin S- β thalassemia) and 20 healthy race-matched controls. Data were obtained from patients with sickle cell disease at baseline conditions ($n = 41$) and in the setting of acute illness ($n = 5$).

Patients with sickle cell disease (SCD) suffer from a variety of clinical events associated with small and large vessel occlusion, including vaso-occlusive painful episodes, strokes, and acute chest syndrome. Such events may be related to the well-described complex derangements of plasma and cellular hemostatic mechanisms that occur in SCD, which may impart a thrombogenic tendency to this disorder. Reported changes include increases in thrombin generation and subsequent fibrinolytic activation,¹ activation of platelets²⁻⁴ and cellular elements,^{5,6} increased levels of antiphospholipid antibodies,^{7,8} decreased levels of circulating anticoagulants and contact factors,^{7,9} and increases in circulating levels of tissue factor and in levels of endothelial cells expressing a tissue factor phenotype.^{1,5} In addition, loss of normal red blood cell membrane asym-

Results.—Patients with hemoglobin SS had lower reaction time and higher angle, maximum amplitude, and coagulation index values on thromboelastography than the control group. Hemoglobin SC patients had higher angle, maximum amplitude, and coagulation index values than controls. Hemoglobin S- β thalassemia patients showed no significant differences compared with controls. Five hemoglobin SS patients with recent or current illness demonstrated increased maximum amplitude and coagulation index compared with hemoglobin SS patients at baseline conditions.

Conclusions.—Patients with sickle cell disease demonstrated a significant hypercoagulable state in thromboelastography profiles, with the degree of abnormality dependent on the type of sickle cell disease and perhaps the presence of acute illness. Continued follow-up of this patient cohort, as well as further study of larger and more homogeneous patient groups, is required to adequately assess the utility of thromboelastography in predicting complications of sickle cell disease.

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metry with presentation of anionic phosphatidylserine on the sickle red blood cell surface occurs and is associated with increases in procoagulant activity.¹⁰⁻¹² Measurement of such hemostatic perturbations appears to have some predictive value for clinical disease phenotype. For example, increases in fibrinolytic activity have been reported to correlate with frequency of painful episodes,¹³ and higher levels of thrombin generation and expression of phosphatidylserine on red blood cells have been reported in patients with increased risk of stroke¹⁴ and during vaso-occlusive episodes.

Thromboelastography (TEG) as a method of assessing global hemostatic and fibrinolytic function has existed for more than 50 years.¹⁵ Thromboelastography has been used primarily in the settings of liver transplant and cardiac surgery, with proven utility for monitoring hemostatic and fibrinolytic derangements inherent in these scenarios.^{16,17} However, its utility in assessing hypercoagulable or prothrombotic states has only recently been studied. In particular, its use in evaluating the properties of the hemostatic system in sickle cell patients has not been reported. Because properties such as platelet function, robustness of the coagulation cascade, and tendency toward fibrinolysis are reflected in various components of a TEG profile, TEG has the potential to serve as a useful tool for the identification of sickle cell patients at risk for vaso-occlusive or thrombotic events.

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Given the complexity of hemostatic derangements observed in SCD patients, and the likely important roles that such alterations play in the pathophysiology of SCD, TEG, as a global evaluator of hemostatic and fibrinolytic function, may prove useful in such patients. We hypothesized that sickle cell patients would show abnormal TEG profiles consistent with a thrombogenic state when compared with healthy controls and that they would demonstrate more extreme derangements with increasing disease severity and illness. Thus, we evaluated TEG and other hemostatic test results in SCD patients and a group of race-matched controls in an effort to assess its clinical utility.

MATERIALS AND METHODS

Human Subjects

This study was approved by the Baylor College of Medicine Institutional Review Board. All guardians of the subjects provided informed consent, and the subjects assented as appropriate. Between October 2002 and February 2003, SCD subjects were recruited from the patient population at the Texas Children's Hospital Hematology Clinic presenting for routine office and sick visits. Sickle cell disease patients included patients with hemoglobin (Hb) S- β thalassemia, Hb SC disease, and Hb SS disease. Control subjects were recruited from healthy patients presenting to our hospital for elective outpatient surgery between December 2002 and March 2003 and from unaffected siblings of SCD subjects.

Sickle cell disease patients and their guardians were interviewed to gather information on clinical history. Their medical records were also reviewed for this purpose. Sickle cell disease patients were classified as "ill" for the purposes of this study if symptoms consistent with a vaso-occlusive episode were present at the time of specimen acquisition. Sickle cell disease patients were classified as having "severe" disease if they (1) were on chronic SCD therapy, defined as chronic transfusion or hydroxyurea therapy; (2) had more than 3 admissions for vaso-occlusive painful episodes in the previous year; or (3) had a history of stroke or more than 1 episode of acute chest syndrome. Control subjects were excluded if they had any chronic systemic medical condition or if they had used any nonsteroidal anti-inflammatory drugs in the preceding week.

Blood Sampling and Processing

All blood samples for this study from SCD patients were obtained during the same phlebotomy required for performance of routine testing as ordered by each subject's clinician at the time of an office visit. Specimens for TEG and coagulation testing were drawn into tubes containing 3.2% buffered sodium citrate (0.129 mol/L) as the anticoagulant (9:1, vol/vol). Specimens for complete blood count, reticulocyte count, and hemoglobin profile were drawn into tubes containing EDTA.

Blood specimens from control subjects were obtained through a temporary intravenous access device that was placed immediately after anesthesia induction prior to an elective surgical procedure. Subjects were under anesthesia at the time of specimen acquisition, and the blood specimens were drawn from the intravenous line into plastic syringes and transferred to the collection tubes. Specimens were transported to the Special Coagulation Laboratory and processed within 60 minutes of venipuncture for either specimen storage or performance of assays. All samples were maintained at room temperature until testing or processing was completed. Thromboelastography testing was completed within 3 hours of phlebotomy. Coagulation specimens were centrifuged at 2500g for 20 minutes to obtain platelet-free plasma (platelet count, $<5 \times 10^3/\mu\text{L}$). Prothrombin time, activated partial thromboplastin time, fibrinogen level, and dimerized plasmin fragment D (D-dimer) testing were performed immediately on an STA-Compact machine (Diagnostica Stago Inc, Parsippany, NJ). Prothrombin time and partial thromboplastin time were per-

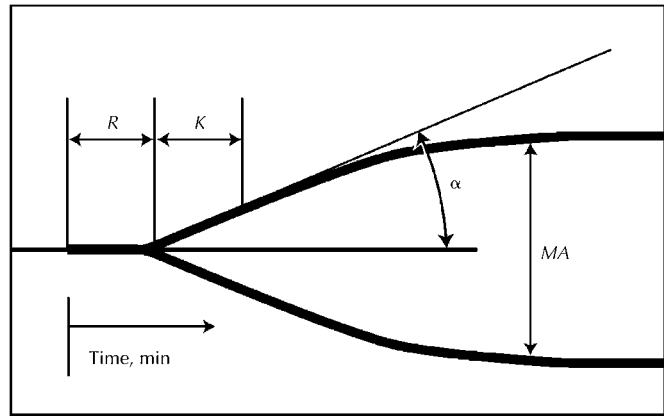


Figure 1. Schematic diagram of a typical thromboelastography tracing with primary parameters of interest. Assay time is tracked on the x-axis, and tracing amplitude is depicted on the y-axis. R indicates reaction time; K, time from the end of R until the tracing amplitude reaches 20 mm; α , angle; and MA, maximum amplitude.

formed on subjects with SCD but not on controls. Remaining plasma was aliquoted and frozen at -20°C . Protein C, protein S, and some fibrinogen and D-dimer assays were performed in batches on frozen plasma specimens. Frozen plasmas were thawed at 37°C for 5 minutes prior to testing.

Complete blood count and reticulocyte counts were measured using an ADVIA 120 machine (Bayer Inc, Tarrytown, NY). Hemoglobin profiles to calculate Hb S and Hb F percentage were obtained using a Primus High Performance Liquid Chromatography machine (Kansas City, Mo). All assays were performed according to standard clinical laboratory methods employed at Texas Children's Hospital.

Thromboelastography

The TEG assay was performed according to the manufacturer's guidelines using a Thromboelastograph Coagulation Analyzer model 5000 (Haemoscope Corporation, Skokie, Ill). Citrated whole blood specimens were allowed to equilibrate at room temperature for 30 minutes. Then, 20 μL of CaCl_2 (0.2 mol/L) were added to the TEG specimen cup. The specimen was gently inverted to adequately mix the sample. A 340- μL sample of citrated whole blood was added to the TEG cup, and the assay was then run for 60 minutes.

The TEG instrument measures the clot-formation properties of a whole blood specimen. With each sample run, a tracing is produced, which typically appears as an initially straight segment that splays into 2 separate curves (Figure 1). The x-axis represents the assay time and the y-axis the amplitude of the tracing. This tracing is characterized by 5 primary parameters of interest:

Reaction Time.—Reaction time (R) is the time in minutes from the start of a sample run until the first detectable levels of fibrin clot formation (this is the point when the tracing begins to splay and is considered to be attained when the splay amplitude reaches 2 mm). The R is prolonged by coagulation factor deficiencies and anticoagulants.

K Time.—The K time is the time in minutes from the end of R until the tracing amplitude reaches 20 mm. It is a measure of the speed of clot strengthening and is prolonged by low fibrinogen levels, anticoagulants, and platelet dysfunction.

Angle.—Angle, or α , refers to the size in degrees of the angle formed by the tangent line to the TEG tracing measured at R. Like K, it measures the dynamics of clot strengthening, and its value is influenced by similar hemostatic characteristics.

Maximum Amplitude.—Maximum amplitude (MA) is the width in millimeters of the widest gap in the TEG tracing, reflecting the maximum strength of the final hemostatic plug. It is dependent on the amount of fibrin formation and the number

Table 1. Characteristics for 46 Patients With Sickle Cell Disease (SCD) and 20 Race-Matched Controls*

	Controls	Hb S-β Thalassemia	Hb SC	Hb SS, Baseline	Hb SS, Ill
Number	20	4	7	30	5
Mean age, y (range)	6.5 (0.3–16.7)	10.0 (1.7–18.0)	4.6 (0.2–17.1)	8.2 (0.1–20.1)	11.8 (4.6–17.3)
Ethnicity	19 Black 1 Hispanic	2 Black 2 Hispanic	7 Black	25 Black 5 Hispanic	5 Black
Male, No. (%)	12 (60)	3 (75)	8 (86)	14 (47)	2 (40)
Severe disease	0	0	0	16	4
Chronic therapy	0	0	0	7	2

* Seventeen controls had specimens obtained prior to elective surgery, and 3 controls were siblings of SCD patients. Patients were classified as “severe” if there was a history of stroke, 2 or more episodes of acute chest syndrome, or 3 or more admissions for painful episodes during the preceding year. Current chronic transfusion or hydroxyurea therapy constituted “chronic therapy.” Hb S-β indicates the group of patients with hemoglobin S-β thalassemia; Hb SC, patients with hemoglobin SC disease; and Hb SS, patients with hemoglobin SS disease.

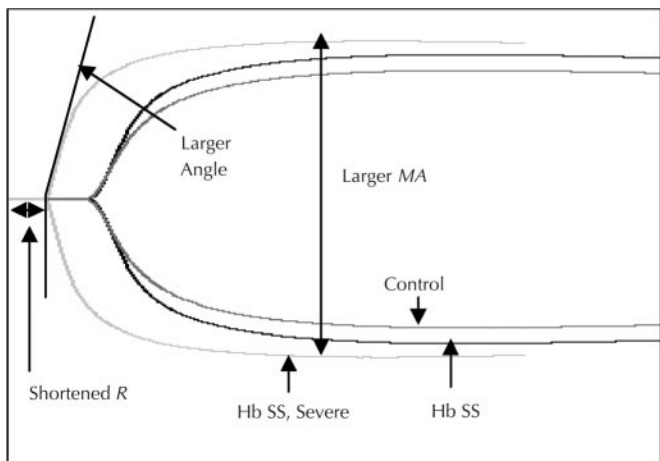


Figure 2. Characteristic thromboelastography profiles representative of findings in healthy subjects compared with 2 patients with sickle cell anemia. The innermost curve is from a control subject, the middle curve from a patient with sickle cell anemia (Hb SS), and the outermost from a patient with Hb SS and a history of stroke (Hb SS, severe). Compared with the control subject, the Hb SS subjects have shorter reaction time (R), larger angle, and larger maximum amplitude (MA) values. Hb indicates hemoglobin.

and function of platelets, and it corresponds to the elastic shear modulus of the clot.

Coagulation Index.—Coagulation index (CI) is a linear transformation obtained from the TEG manufacturer¹⁸ that takes into account each of the other TEG parameters to generate a global measure of coagulability ($CI = -0.2454R + 0.0184K + 0.1655MA - 0.0241\alpha - 5.0220$). Normal values are designated as lying be-

tween -3.0 and $+3.0$, equivalent to 3 SDs about the mean of 0. Values above 3.0 are regarded as consistent with a hypercoagulable state, and values below -3.0 are thought to indicate hypo-coagulability.¹⁸

Statistical Analysis

SPSS version 11.5 (SPSS Inc, Chicago, Ill) was used for all data analysis. Analysis of variance (ANOVA) and the Student *t* test were used to determine if significant differences existed between group means of each parameter. Analysis of covariance (ANCOVA) was used to test hypotheses of differing variable relationships between patient subgroups and to adjust for covariate differences between groups. Fisher least significant difference pairwise multiple comparison test¹⁹ was used to perform subgroup comparisons of means in post hoc testing of significant ANOVA and ANCOVA results. Pearson correlation coefficients were calculated to assess associations between TEG and hemostatic parameters. *P* values $<.05$ were regarded as significant.

RESULTS

Subject characteristics are summarized in Table 1. Mean ages did not differ significantly between subject groups ($P = .18$). All subjects classified as being ill, having severe disease, or receiving chronic therapy, as defined in the “Materials and Methods” section, had Hb SS disease.

Figure 2 depicts representative TEG tracings from a single control subject and 2 subjects with Hb SS disease. Subjects with SCD differed significantly from race-matched controls with respect to all TEG parameters (Table 2, ANOVA *P* value $<.001$ for all TEG parameters). Subjects with Hb SS disease exhibited significant differences compared with controls on all parameters (*R*, angle, *MA*, and *CI*), whereas subjects with Hb SC disease demonstrated fewer

Table 2. Comparison of Thromboelastography Parameters Between Studied Subgroups*

	<i>R</i> , min	Angle (α), °	<i>MA</i> , mm	<i>CI</i>
Control	8.8 (5.3–14.8)	61.0 (47.6–70.7)	60.2 (55.0–64.7)	1.36 (-0.6–2.1)
Hb S-β thalassemia	8.5 (5.3–11.8)	60.1 (40.9–70.1)	63.6 (53.8–69.6)	2.03 (0.10–3.00)
Hb SC	8.0 (5.7–11.0)	68.2† (58.9–74.9)	67.1† (61.7–76.0)	2.51† (1.70–3.70)
Hb SS, baseline	4.9† (2.4–10.6)	73.1† (56.7–79.4)	66.5† (58.4–76.1)	3.04† (1.4–4.5)
Hb SS, ill	5.4† (3.4–8.4)	76.2† (70.8–79.4)	73.1† (71.6–75.1)	3.94† (3.4–4.4)
ANOVA <i>P</i> value	$<.001$	$<.001$	$<.001$	$<.001$

* Displayed values are mean and range. Last row represents analysis of variance (ANOVA) *P* value comparing all groups. *R* indicates reaction time; *MA*, maximum amplitude; *CI*, coagulation index; Hb S-β, the group of patients with hemoglobin S-β thalassemia; Hb SC, patients with hemoglobin SC disease; and Hb SS, patients with hemoglobin SS disease.

† Denotes *P* values of $<.05$ when comparing subgroup to controls.

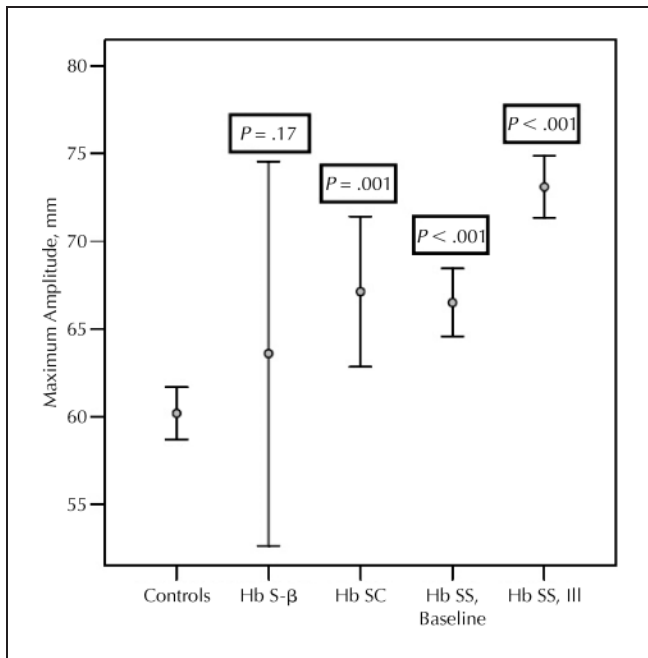


Figure 3. Comparison of subgroups on maximum amplitude. Bars depict 95% confidence intervals for subgroup mean estimates, along with P values comparing each subgroup with controls. Maximum amplitude values among hemoglobin (Hb) SC and Hb SS (baseline and ill) subjects were significantly higher compared with control subjects. $P = .004$ between Hb SS baseline versus ill subgroups.

differences (angle, MA, and CI). Subjects with Hb S-β thalassemia did not differ from controls on any TEG parameters (Table 2). Figure 3 illustrates an example of these comparisons using MA data. Hemoglobin SS subjects with an acute illness (including 4 patients with painful episodes and 1 patient with pneumonia) demonstrated higher MA ($P = .004$) and CI values ($P = .03$) than well Hb SS subjects. Acutely ill Hb SS subjects had a higher white blood cell count than Hb SS subjects at baseline (mean, $16.1 \times 10^3/\text{mm}^3$ vs $11.0 \times 10^3/\text{mm}^3$, respectively, $P < .001$), but no other measured parameters were significantly different between these 2 groups.

When subjects receiving chronic therapy were excluded from analysis (resulting $n = 23$), well Hb SS subjects with severe disease had a lower MA than subjects without se-

vere disease (62.7 mm vs 68.1 mm, $P = .02$). Disease severity was not associated with differences in the other TEG parameters. Among Hb SS subjects with severe disease, chronic therapy was not associated with significant differences in TEG parameters (data not shown).

Fibrinogen levels did not differ significantly between the SCD groups and controls (although severely affected Hb SS subjects had slightly lower fibrinogen levels than the non-severely affected Hb SS group, 0.214 g/dL vs 0.282 g/dL, respectively [$6.3 \mu\text{mol/L}$ vs $8.3 \mu\text{mol/L}$], $P = .02$). However, D-dimer, protein C, and protein S levels differed between controls and "well" subjects with Hb SS disease (Table 3). When hemostatic data were incorporated into a model that adjusted for differences in D-dimer, protein C, and protein S levels as well as age, all significant TEG differences between SCD and control groups persisted. No significant interactions between hemostatic variables and SCD subtype were identified that influenced TEG parameters.

Pearson correlation coefficients (r) were performed between TEG parameters and other parameters (including age, hemoglobin, reticulocyte count, white blood cell count, platelet count, percentage Hb S, percentage Hb F, D-dimer, protein C level, and protein S level). These calculations were performed only on the data from the subgroup of Hb SS subjects who were in baseline condition ($n = 30$). Thromboelastography parameters did not correlate significantly with age or Hb F levels (data not shown). Following are all statistically significant results ($P < .05$). Hemoglobin ($r = 0.46$, $P = .01$), protein C ($r = 0.71$, $P < .001$), and protein S ($r = 0.44$, $P = .04$) correlated positively with R . Percentage Hb S ($r = -0.42$, $P = .02$) and reticulocyte count ($r = -0.44$, $P = .02$) correlated negatively with R . White blood cell count correlated positively with MA and CI ($r = 0.55$, $P = .004$, and $r = 0.43$, $P = .02$, respectively), as did D-dimer ($r = 0.38$, $P = .04$, and $r = 0.42$, $P = .02$, respectively). Platelet counts correlated positively with angle values ($r = 0.46$, $P = .01$).

COMMENT

Hypercoagulability, as detected by increased angle, increased MA, and decreased R values on TEG assays, has been reported in a wide variety of medical and surgical conditions.²⁰⁻²⁸ To our knowledge, this is the first report assessing the utility of TEG in detecting hemostatic abnormalities in SCD patients. We have shown that SCD pa-

Table 3. Comparison of Hemostatic Parameters Between Studied Subgroups*

	Fibrinogen, g/dL	D-dimer, mg/L	Functional Protein C, % activity	Functional Protein S, % activity
Control (n = 18)	0.283 (0.171-0.403)	0.21 (0.09-0.53)	106 (62-225)	78 (53-108)
Hb S-β thalassemia (n = 6)	0.337 (0.314-0.360)	1.30 (0.19-3.69)	80 (57-91)	58 (46-84)
Hb SC (n = 6)	0.309 (0.270-0.383)	0.52 (0.23-1.05)	82 (39-117)	65 (48-81)
Hb SS, baseline (n = 23)	0.265 (0.175-0.432)	1.28† (0.22-4.00)	66† (23-120)	53† (26-107)
Hb SS, ill (n = 5)	0.340‡	1.17 (0.61-1.76)	66 (32-100)	40† (24-46)
ANOVA P value	.33	.001	.007	.002

* Displayed values are mean and range. Last row represents analysis of variance (ANOVA) P value comparing all groups. Hb S-β indicates the group of patients with hemoglobin S-β thalassemia; Hb SC, patients with hemoglobin SC disease; and Hb SS, patients with hemoglobin SS disease.

† Denotes P values of <.05 when comparing subgroup to controls.

‡ Only 1 value is available.

tients demonstrate a hypercoagulable TEG pattern when compared with healthy controls. The degree of abnormality appears to vary depending on the SCD type, because the differences compared with controls were more distinct in Hb SS disease than in the other hemoglobin variants. We speculate that this may reflect differences in the extent of erythrocyte sickling between the different disorders. This may in turn engender different degrees of membrane transposition of phosphatidylserine, leading to variable levels of procoagulant activity, as has been previously described.²⁹ Although previous work shows that Hb F levels correlate inversely with levels of phosphatidylserine-positive erythrocytes and markers of thrombin formation,³⁰ in our study, TEG parameters did not correlate significantly with either subject age or Hb F levels.

Consistent with previous studies in adults, our pediatric cohort of SCD patients exhibited elevated D-dimer and decreased protein C and protein S levels compared with controls. However, even after adjusting for these differences, significant differences in TEG parameters persisted. Unfortunately, complete blood count data were not available for the control group, so the possible confounding effects of differences in hematocrit or white blood cell count between SCD subjects and controls have not been assessed. Future studies should include such measurements in the control group.

The measured TEG parameters correlated with markers of a hypercoagulable state: decreased protein C and protein S levels correlated with short R, elevated D-dimer and fibrinogen levels correlated with increased MA, and increased platelet count correlated with increased angle. These findings are consistent with the concept of a prothrombotic tendency existing in SCD patients and suggest that the differences we have observed in these patients carry clinical significance. Continued follow-up of these patients for vaso-occlusive and thrombotic complications is required to properly assess the prognostic utility of the abnormalities we have detected, because others have reported that such abnormalities predict serious clinical events.³¹

In the group of Hb SS subjects not receiving chronic therapy, disease severity was not associated with hypercoagulable TEG parameters. The finding of lower MA values in the severely affected group is somewhat surprising, but it may be partially explained by the slightly lower fibrinogen levels in this group. Our results thus far do not support TEG's utility in classifying SCD patients into higher- versus lower-risk categories for disease severity. However, our study population was relatively small. It was also diverse in terms of the specific complications that resulted in categorization as severe versus nonsevere disease as well as the indications for chronic therapy. Such differences (along with the small subgroup sizes) may obscure associations between certain subgroups and more significant TEG abnormalities, such that continued recruitment to this study of similarly affected "severe" patients is required for more definitive determination. Although acute illness appears to accentuate some TEG abnormalities in our study, this result must be interpreted with caution because of the small number of ill subjects studied. Because this observation may possibly be explained by higher leukocyte counts in ill subjects, these results require confirmation in larger patient groups. A further limitation of this study is that we could not recruit patients who acutely presented with stroke or acute chest syndrome. However,

further follow-up of this cohort of subjects is ongoing to more reliably assess TEG's utility in predicting risk of sickle-related complications such as these.

In conclusion, our work shows that TEG detects significant differences in SCD patients versus controls and that these differences are consistent with a hypercoagulable condition. The exact hemostatic mechanisms underlying these differences are unknown and require elucidation, because measured hemostatic parameters either did not differ between SCD subjects and controls (fibrinogen) or did not account for the entire difference in a given TEG parameter when these hemostatic differences were statistically taken into account (protein C, protein S, and D-dimer). Our results support the need for continued follow-up of this patient cohort in order to determine whether TEG abnormalities predict thrombotic or vaso-occlusive complications. Moreover, further subject recruitment, focused on studying specific patient subgroups with comparable clinical phenotypes (eg, patients with active stroke, patients with active acute chest syndrome, patients receiving hydroxyurea for recurrent painful episodes) will better define the clinical utility of TEG in predicting disease severity.

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