

To the editor:

Elevated D-dimer levels in African Americans with sickle cell traitRakhi P. Naik,¹ James G. Wilson,² Lynette Ekuwwe,³ Stanford Mwasongwe,³ Qing Duan,⁴ Yun Li,⁴ Adolfo Correa,⁵ and Alexander P. Reiner⁶¹Division of Hematology, Department of Medicine, Johns Hopkins University, Baltimore, MD; ²Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS; ³Jackson Heart Study Field Center, Jackson State University, Jackson, MS; ⁴Departments of Genetics, Biostatistics, and Computer Science, University of North Carolina, Chapel Hill, NC; ⁵Departments of Medicine and Pediatrics, University of Mississippi Medical Center, Jackson, MS; and ⁶Department of Epidemiology, University of Washington School of Public Health, Seattle, WA

Microvascular and macrovascular thrombosis is thought to contribute significantly to the pathophysiology of complications in sickle cell disease (SCD).^{1,2} The mechanism of hypercoagulability in SCD is multifactorial, including activation of coagulation at the surface of sickled cells, endothelial dysfunction, and inflammation.¹⁻⁴ D-dimer levels and other markers of coagulation activation are elevated in SCD and have been shown to correlate to stroke, retinopathy, and vaso-occlusive crises.⁵⁻⁷ Increased inflammatory and endothelial adhesion markers have also been observed in SCD, contributing to leukocyte and coagulation activation.⁸⁻¹⁰ Although recent evidence suggests that, similar to SCD,^{11,12} African Americans with sickle cell trait (SCT) are at increased risk of venous thromboembolism (VTE), and particularly pulmonary embolism, compared with noncarriers,^{13,14} only a few prior studies evaluating circulating coagulation and inflammatory markers have been performed in SCT.^{15,16} Using data from a large population-based cohort, the Jackson Heart Study (JHS), we investigated the association of SCT carrier status with available biomarkers in African Americans.

The JHS is a prospective, population-based cohort designed to investigate risk factors for cardiovascular disease among African Americans. The design and methods of this study have been previously described in detail.¹⁷ A total of 5301 African Americans ≥ 21 years of age were recruited to participate in the study. All participants included in this analysis provided written, informed consent for use of genetic data, and the study was approved by the institutional review boards of the participating centers.

Genotype data for rs334 encoding the sickle hemoglobin mutation (*HBB* p.Glu7Val) was performed in a blinded fashion by whole-exome sequencing in all consenting JHS individuals, and SCT was defined as the presence of 1 abnormal allele. Individuals found to have hemoglobin SS or SC were excluded from analysis. The most common α -thalassemia mutation (3.7-kb deletion) was imputed into the JHS data set using African (YRI) 1000 Genomes phase 1 data as the reference panel and MacH-ADMIX. The

deletion variant was modeled in the analysis as a continuous variable based on imputed allele dosage.

All participants were asked to provide blood samples at baseline and follow-up visits, and venipuncture was performed in accordance with the National Committee for Clinical Laboratory Standards. Commercially available enzyme-linked immunosorbent assay (ELISA) techniques were used to measure the following plasma markers: (1) for coagulation activation, fibrinogen and D-dimer were measured by immunoturbidimetric assay (STALiatest); (2) for endothelial adhesion, P- and E-selectin were measured by ELISA (R&D Systems); and (3) for inflammation, high-sensitivity C-reactive protein (CRP) was measured by latex particle immunoturbidimetric assay (Kamiya Biomedical Company). White blood cell (WBC) and platelets counts were also measured. All markers were measured using baseline samples, except for P- and E-selectin, which were performed at the third follow-up visit.

Linear regression was used to evaluate the relationship of SCT to continuous biomarker values. The association between SCT carrier status and biomarker levels was also analyzed using multinomial logistic regression comparing quartile measurements, with the lower 25th percentile as the reference category. All models were adjusted for age, sex, percentage African ancestry, current smoking, body mass index (BMI), and estimated glomerular filtration rate (eGFR). An interaction analysis between SCT and α -thalassemia on D-dimer levels was also performed. Statistical analyses were performed using Stata, version 12 (StataCorp).

After excluding participants with no available genotype data for SCT ($n = 2087$), with no available biomarker data ($n = 3$), with missing covariate data ($n = 346$), or with hemoglobin SS or SC ($n = 3$), we included 2871 total African Americans (241 SCT carriers and 2630 noncarriers) in our analysis. Overall, the mean age was 54.9 years, 62% were women, and 14% were current smokers. Participants with SCT had a lower mean eGFR than those without. There was no significant

Table 1. Median baseline biomarker levels in the JHS, by SCT carrier status

Biomarker	Participants, n	Median concentration (IQR)		P
		SCT carriers	Noncarriers	
Fibrinogen, mg/dL	2608	415 (352-493)	406 (355-463)	.08
CRP, mg/L	2866	0.31 (0.14-0.66)	0.26 (0.11-0.57)	.45
D-dimer, $\mu\text{g/mL}$	2584	0.55 (0.35-0.86)	0.38 (0.25-0.60)	<.0001
WBC, $\times 10^3/\mu\text{L}$	2527	5.2 (4.2-6.3)	5.3 (4.4-6.6)	.29
Platelet count, $\times 10^3/\mu\text{L}$	2808	243 (208-288)	245 (207-289)	.19
P-selectin, ng/mL	2055	31.9 (25.9-39.7)	32.5 (26.0-40.3)	.92
E-selectin, ng/mL	2065	40.8 (27.8-55.9)	41.3 (29.7-55.6)	.30

Table 2. Association of SCT with quartile D-dimer measurements in the JHS

Quartile	Range, $\mu\text{g/mL}$	Noncarriers, n (%)	SCT carriers, n (%)	OR	SE	95% CI	P	Linear P
1	0.01-0.25	600 (25)	24 (11)	1.00 (ref)				
2	0.26-0.38	653 (27)	44 (19)	2.003	0.583	1.312-3.545	.017	
3	0.39-0.62	579 (24)	52 (25)	2.444	0.728	1.363-4.382	.003	
4	0.63-11.98	537 (23)	95 (45)	5.347	1.517	3.067-9.323	<.0001	4.0×10^{-11}

Multinomial logistic regression adjusted for age, sex, current smoking, BMI, eGFR, and ancestry. ref, reference; SE, standard error.

association of SCT with age, sex, current smoking status, BMI, or genetic ancestry.

Median levels of D-dimer were higher (0.55 $\mu\text{g/mL}$; interquartile range [IQR], 0.35-0.86) among SCT carriers compared with noncarriers (0.38 $\mu\text{g/mL}$; IQR, 0.25-0.60; $P < .0001$) (Table 1). There were no significant differences in fibrinogen, CRP, WBC, platelet count, or P- and E-selectin levels between individuals with and without SCT. After adjusting for age, sex, BMI, smoking, eGFR, and African ancestry, ln D-dimer levels remained significantly higher in participants with SCT than noncarriers ($\beta = 0.315$; 95% confidence interval [CI], 0.221-0.409). Coinheritance of α -thalassemia and SCT significantly interacted to decrease D-dimer levels (interaction $P = .018$).

Of the participants with SCT, 24 (11%) had D-dimer levels in the first quartile, 44 (19%) in the second quartile, 52 (25%) in the third quartile, and 95 (45%) in the fourth quartile. SCT was significantly associated with second to fourth quartile measurements of D-dimer compared with the first quartile (Table 2). Specifically, the odds of SCT carriers being in the second and third quartiles of D-dimer concentration were twofold higher than the first quartile, with a fourth quartile odds ratio (OR) of 5.35 (95% CI, 3.07-9.32). A D-dimer level greater than 0.5 $\mu\text{g/mL}$, the conventional cutoff for predicting VTE,¹⁸ was found in 54% ($n = 116$) of SCT participants compared with 33% ($n = 782$) of noncarriers (OR, 2.56 [95% CI, 1.89-3.48]).

In this cohort of over 2000 African Americans, we found that SCT carriers had significantly higher D-dimer levels than noncarriers. There was a graded increase in risk of SCT carriers being in the second to fourth quartiles, with the highest risk for the top quartile of D-dimer measurements. The association was independent of known venous thrombosis risk factors such as age, sex, smoking, and BMI. In addition, this relationship was independent of baseline eGFR, which is strongly associated with SCT and has been demonstrated to influence D-dimer measurements.^{19,20}

Although elevated D-dimer levels have long been observed in SCD, the relationship between SCT and D-dimer levels has not been clearly established. Two small case-control studies have shown an association of SCT to D-dimer, but these analyses were limited by sample size and lack of adjustment for confounders.^{15,16} A recent, larger prospective study by Folsom et al evaluating the relationship of D-dimer with future VTE noted higher D-dimer levels among SCT carriers, but this association was not further quantified.²¹ That study also found that D-dimer elevations conferred a stepwise increase in risk of future VTE, but that the presence of SCT did not fully modify or explain the association of D-dimer with VTE.

In the current study, we have expanded on the findings from prior studies and find that the presence of SCT is associated with the highest of D-dimer levels, independent of comorbidities, and that coinheritance of α -thalassemia trait interacts to decrease D-dimer levels in SCT carriers. Coinheritance of α -thalassemia mutations, which are known to result in decreased sickle hemoglobin percentage, has similarly been shown to have a protective effect on urinary-concentrating defects in carriers of SCT.²² Our finding, therefore, suggests a dose-dependent relationship between sickle hemoglobin and D-dimer generation, and

infers potential causality between sickle hemoglobin percentage and coagulation activation in SCT. This dose-dependent variability in D-dimer levels may also relate to the differential risk of VTE among individuals with SCT. Future studies are required to investigate this relationship.

The strengths of our study are the large sample size of African Americans and the robust phenotypic and genotypic data. However, our study does have limitations. We relied on imputation for the α -thalassemia genotype, and additional studies will be needed to validate this relationship. In addition, we did not have hemoglobin electrophoresis data and did not have available information on VTE outcomes.

In conclusion, D-dimer levels are significantly higher among SCT carriers compared with noncarriers. Coinheritance of α -thalassemia, which decreases sickle hemoglobin levels in SCT, may interact to significantly decrease D-dimer levels among African Americans with SCT.

Acknowledgments: The authors thank Gina Peloso and Sekar Kathiresan for providing genotypic data; and thank the investigators, staff, and participants of the JHS for their valuable contributions.

The JHS is supported in part by the National Institutes of Health, National Heart, Lung, and Blood Institute grants K08HL125100 (R.P.N.) and R01HL071862 (A.P.R.); National Institute on Minority Health and Health Disparities contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, and HHSN268201300050C); and National Human Genome Research Institute grant R01HG006703 (Y.L.).

The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute, the National Institutes of Health, or the US Department of Health and Human Services.

Contribution: R.P.N. and A.P.R. designed the study; J.G.W., L.E., S.M., Q.D., Y.L., A.C., and A.P.R. provided the data; A.P.R. performed the statistical analysis; R.P.N., A.P.R., and J.G.W. interpreted the data; and R.P.N., J.G.W., L.E., S.M., A.C., and A.P.R. wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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 DOI 10.1182/blood-2016-01-694422

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2016 127: 2261-2263

doi:10.1182/blood-2016-01-694422 originally published
online March 11, 2016

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