














ORIGINAL ARTICLE

Thrombo-inflammatory biomarkers and D-dimer in a biracial cohort study

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Funding information

This research project is supported by cooperative agreement U01 NS041588 cofunded by the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute on Aging (NIA), National Institutes of Health, Department of Health and Human Service. Additional funding was supplied by K08HL096841 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NINDS or the NIA. Representatives of the NINDS were involved in the review of the manuscript but were not directly involved in the collection, management, analysis, or interpretation of the data.

Abstract

Background: Higher D-dimer is a risk factor for cardiovascular diseases and venous thromboembolism. In the general population, D-dimer and other thrombo-inflammatory biomarkers are higher among Black individuals, who also have higher risk of these conditions compared to White people.

Objective: To assess whether Black individuals have an exaggerated correlation between D-dimer and thrombo-inflammatory biomarkers characteristic of cardiovascular diseases.

Methods: Linear regression was used to assess correlations of 11 thrombo-inflammatory biomarkers with D-dimer in a cross-sectional study of 1068 participants of the biracial Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort.

Results: Adverse levels of most biomarkers, especially fibrinogen, factor VIII, C-reactive protein, N-terminal pro-B-type natriuretic peptide, and interleukin (IL)-6, were associated with higher D-dimer. Several associations with D-dimer differed significantly by race. For example, the association of factor VIII with D-dimer was more than twice as large in Black compared to White participants. Specifically, D-dimer was

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Handling Editor: Pantep Angchaisuksiri

26% higher per standard deviation (SD) higher factor VIII in Black adults and was only 11% higher per SD higher factor VIII in White adults. In Black but not White adults, higher IL-10 and soluble CD14 were associated with higher D-dimer.

Conclusions: Findings suggest that D-dimer might relate to Black/White differences in cardiovascular diseases and venous thromboembolism because it is a marker of amplified thrombo-inflammatory response in Black people. Better understanding of contributors to higher D-dimer in the general population is needed.

KEYWORDS

biomarkers, cardiovascular diseases, D-dimer, race, thrombo-inflammation

Essentials

- Black people have higher thrombo-inflammatory responses, reflected partly by higher D-dimer.
- We examined the associations of several thrombo-inflammatory biomarkers with D-dimer.
- We found stronger associations of some biomarkers with D-dimer in Black people.
- These responses might underlie pathways involved in health disparities impacting Black people.

1 | INTRODUCTION

Higher D-dimer, a by-product of fibrin degradation,¹ is a predictor of cardiovascular and venous thromboembolism risk in healthy people.²⁻⁵ D-dimer also appears to be the best representation in the laboratory of the summative impact of the thrombo-inflammation and risk of poor outcomes in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).⁶⁻⁹

Several mechanisms may explain the correlation of elevated D-dimer with these diseases. D-dimer is a marker of fibrin formation; as such, elevated D-dimer reflects hypercoagulability.¹⁰ D-dimer is also higher with inflammatory processes.¹¹ However, much uncertainty exists about biological mechanisms underlying the association of D-dimer and health.^{12,13}

Black people have poorer cardiovascular health compared to White people,¹⁴ and they are also at increased risk of coronavirus disease 2019 (COVID-19).¹⁵⁻²⁰ Black individuals also have higher thrombo-inflammatory responses and higher D-dimer,^{21,22} suggesting a hypothesis that associations of thrombo-inflammatory factors with D-dimer might differ with race. Understanding the underpinnings of higher D-dimer in Black people might elucidate new reasons for the health disparities they experience.

Consequently, this report assessed the associations of levels of thrombo-inflammatory biomarkers with D-dimer in participants of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort. We determined whether correlations of biomarkers with D-dimer differed by race. Studied biomarkers were white blood cell count (WBC), platelet count, albumin, N-terminal pro-B-type natriuretic peptide (NT-proBNP), interleukin (IL)-6, IL-8, IL-10, C-reactive protein (CRP), fibrinogen, factor VIII, soluble CD14 (sCD14).

2 | METHODS

2.1 | Participants

The REGARDS study is a national, longitudinal cohort study investigating causes of racial and geographic disparities in stroke mortality and cognitive impairment in the United States. Between January 2003 and October 2007, 30 239 Black and White adults aged ≥ 45 years were enrolled in the study, with oversampling of Black people and those residing in the southeastern Stroke Belt region of the United States (North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Alabama, Louisiana, and Arkansas). Further details about the objectives and design of the REGARDS study were previously published.²³ Participants were self-reported non-Hispanic Black or White men and women, were noninstitutionalized, understood English, and were not undergoing treatment for active cancer. Extensive baseline data were obtained from participants by computer-assisted telephone interviews and in-home visits. Trained staff obtained physical measurements, medication inventory, blood, and urine specimens in participant homes in 2003 to 2007.

For this report, existing data on multiple baseline biomarkers was leveraged from a case-cohort study of stroke and cognitive impairment risk nested within the REGARDS study. To lessen sampling bias, only the cohort random sample (1100 participants) and not those from the case groups were included in the present study. To ensure appropriate representation by race, sex, and age categories, the random cohort was generated using a stratified sampling method.⁴ Individuals taking warfarin at baseline (direct oral anticoagulants were not in use) were excluded, leaving a sample size of 1068 participants. The institutional review boards of the University

of Alabama at Birmingham and University of Vermont approved the study. The participants gave written informed consent.

2.2 | Biomarker measurement

At baseline, REGARDS performed fasting phlebotomy in the morning, and serum and plasma were shipped overnight on ice packs to the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT, USA) for storage or further analysis.²⁴

D-dimer was measured with the STAR automated coagulation analyzer, (Diagnostica Stago, Parsippany, NJ, USA) using an immuno-turbidometric assay (Liatest D-DI; Diagnostica Stago). Fibrinogen was assessed with a BN II nephelometer (N Antiserum to Human Fibrinogen; Siemens Healthcare Diagnostics, Newark, DE, USA), and CRP was measured using a validated high-sensitivity particle-enhanced immunonephelometric assay on the BN II nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL, USA).

Measurements of platelet count and WBC were performed on an LH 755 Hematology Workcell (Beckman Coulter, Inc., Indianapolis, IN, USA). Serum albumin was measured using colorimetric reflectance density (Ortho Vitros 950 IRC Clinical Analyzer; Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). Serum NT-proBNP was measured by electrochemiluminescence immunoassay (Roche Elecsys 2010 analyzer; Roche Diagnostics Indianapolis, IN, USA). IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN, USA); IL-8 was measured with the Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc., St. Charles, MO, USA), and IL-10 with the Milliplex MAP Human Cardiovascular Disease Panel 3 (Millipore Corporation, Burlington, MA, USA) run as a singleplex assay. Factor VIII antigen (Enzyme Research Laboratories, South Bend, IN, USA) and sCD14 (R&D Systems) were measured using ELISA.

All assay analytical coefficients of variation were <5% to 10%.

2.3 | Covariates

Age, sex, race, income, and education were self-reported. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or self-reported use of hypertensive medications. Diabetes was defined as fasting glucose ≥ 126 mg/dL or random plasma glucose ≥ 200 mg/dL or self-reported use of insulin or oral diabetes medication. Coronary heart disease (CHD) was defined as self-reported myocardial infarction or coronary surgical procedures or evidence of prior myocardial infarction on the study electrocardiogram. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Statin use was determined by self-report and medication inventory conducted during the in-home examination.

2.4 | Statistical analysis

The outcome of interest was D-dimer, and the following biomarkers were considered as independent variables: CRP, WBC, platelet count, albumin, NT-proBNP, IL-6, IL-8, IL-10, fibrinogen, factor VIII, and sCD14. Biomarkers that did not fit a normal distribution were natural log transformed. The mean and standard deviation (SD) or geometric mean were tabulated for all biomarkers by age groups, sex, and race.

Linear regression with Bonferroni correction was used to estimate the adjusted difference in the mean level of log-transformed D-dimer associated with a 1-SD increment of each biomarker. Standardization of data in this manner allowed for comparison of the relative associations across biomarkers and in subgroups. Results were interpreted as the percentage difference in D-dimer per 1-SD increase in each biomarker, calculated as follows: percent difference in D-dimer = $(e^{\beta} - 1) \times 100$. The 95% confidence interval (CI) of the percentage difference was obtained by replacing the β coefficient with $\beta \pm 1.96^*$ (standard error) in the aforementioned formula. Complete case analysis was used for all regression models.

Regression models were further adjusted for socioeconomic (income and education) or clinical (hypertension, diabetes, CHD, BMI, statin use) confounders. Differences in the association of each biomarker with D-dimer by race were tested by separately adding interaction terms between each biomarker and race. A *P* value for the interaction term of $< .05$ indicated statistically significant differences, and in this case, stratified results were presented. Stratified models controlled only for age and sex, since other factors did not alter relationships in the overall model, and inclusion of unnecessary variables may result in loss of precision. Statistical analysis, here, was not adjusted for multiple comparisons.

R versions 3.5.1 and 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses.

3 | RESULTS

Table 1 shows that most biomarkers were higher in Black compared to White participants, including D-dimer. Albumin, WBC, NT-proBNP and sCD14 were lower in Black than White participants.

Associations of biomarkers with D-dimer are shown in Table 2. Biomarkers that were most strongly associated with D-dimer included fibrinogen, factor VIII, CRP, NT-proBNP, and IL-6. In the age-sex-race adjusted model, D-dimer was 18% higher per SD higher fibrinogen, 20% higher per SD higher factor VIII and 21% higher per SD higher NT-proBNP. It was 23% higher per SD CRP and 24% higher per SD higher IL-6. There was an inverse association between albumin and D-dimer; D-dimer was 10% lower with each SD higher albumin. There was no confounding of these associations after adjustment for socioeconomic or clinical variables.

There were no differences in the strong associations of IL-6, CRP, or WBC with D-dimer by race, while associations of IL-10,

TABLE 1 Biomarker levels by race

	Missing n	Race	
		Black n = 541	White n = 527
D-dimer, $\mu\text{g/mL}^a$	66	0.55	0.44
WBC, $\times 10^9$ cells/L	369 ^b	5.6 (2.0)	6.2 (1.9)
Platelet count, $\times 10^9$ cells/L	396 ^b	232 (68)	232 (66)
Albumin, g/dL	332 ^b	4.1 (0.3)	4.2 (0.3)
NT-proBNP, pg/mL ^a	59	76	93
IL-6, pg/mL ^a	70	3.5	2.9
IL-8, pg/mL ^a	63	2.8	2.7
IL-10, pg/mL ^a	63	9.4	9.2
CRP, mg/L ^a	67	2.5	1.9
Fibrinogen, mg/dL	62	412 (105)	378 (101)
Factor VIII, %	61	130 (48)	118 (42)
sCD14, pg/mL	75	1812 (422)	1971 (395)

Note: Mean (SD) except geometric mean for D-dimer, CRP, NT-proBNP, IL-6, IL-8, and IL-10.

Abbreviations: CRP, C-reactive protein; IL, interleukin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sCD14, soluble CD14; WBC, white blood cell count.

^aGeometric means for natural log-transformed biomarkers (did not fit a normal distribution).

^bThese analytes were measured as participants were enrolled in an ancillary study that was funded after the first ≈ 8000 participants were enrolled, thus the higher number of missing.

factor VIII and sCD14 with D-dimer differed significantly by race (Table 3, Figure 1). The association of factor VIII with D-dimer was more than twice as great in Black compared to White participants. Specifically, each SD higher factor VIII was associated with a 26% greater D-dimer in Black participants, while this difference in D-dimer was only 11% in White participants (P interaction = .009). In Black participants, each SD higher IL-10 was associated with a 15% greater D-dimer, while each SD higher sCD14 was associated with a 13% greater D-dimer; these biomarkers were not associated with D-dimer in White adults (P interaction = .001 for IL-10 and .02 for sCD14). There was no confounding of these associations after adjustment for socioeconomic or clinical variables. These associations are further displayed visually in Figure 2.

4 | DISCUSSION

In this general population study, there was a greater basal thrombo-inflammatory state in Black than White individuals. Moreover, several biomarkers were more strongly associated with higher D-dimer in Black compared to White participants. These findings were not explained by the evaluated socioeconomic or clinical factors. Findings illustrate that thrombo-inflammatory responses are greater in Black than White individuals, and this might partly explain their higher rate of cardiovascular diseases, venous thromboembolism, and COVID-19.

In accordance with these findings, in previous studies Black women had higher CRP and IL-6 than White women, and higher fibrinogen than both White and Hispanic women.²⁵⁻²⁷ Black men and women also have higher D-dimer levels compared to their White counterparts.^{25,28} Cardiovascular markers such as NT-proBNP were also higher in Black compared to White women and men.²²

There is little known on the correlations of the studied biomarkers with D-dimer concentration, with no previous data in a general population sample.^{29,30} Further, we are not aware of other reports on racial differences in inflammatory and hemostatic correlates of D-dimer. The association of factor VIII with D-dimer in this study was larger in Black than White adults. As an example, from the regression equation, a 65-year-old Black man with factor VIII 300% would be predicted to have D-dimer of 1.1 $\mu\text{g/mL}$, while the same White man would have D-dimer of 0.61 $\mu\text{g/mL}$ (noting that with this assay the cutoff for ruling out venous thromboembolism is 0.50 $\mu\text{g/mL}$ ³¹). In the circulation, factor VIII is carried by von Willebrand factor (VWF), and levels are highly correlated; both factors increase with endothelial damage.³²

Elevated factor VIII in healthy people is an established risk marker for cardiovascular disease and venous thromboembolism via effects of VWF.^{33,34} Upon vascular injury, VWF binds to the exposed subendothelial matrix that activates, adheres, and aggregates platelets via VWF. This in turn leads to thrombin generation and fibrin formation, which generates fibrin degradation products such as D-dimer.^{1,35,36} In previous research, part of the strong association of D-dimer with future venous thromboembolism risk was explained by adjustment for factor VIII.²⁸ Vessel injury or endotheliopathy also play an important role in the pathogenesis of COVID-19.³⁷ Postmortem histological analyses have revealed the presence of SARS-CoV-2 viral elements in the endothelium.³⁸ This viral infection and subsequent endothelial dysfunction might trigger inflammatory and procoagulant responses, including factor VIII/VWF elevation.^{37,39,40} The stronger correlation of factor VIII and D-dimer in Black individuals may suggest more enhanced fibrin formation from endothelial damage either subclinically in healthy people or perhaps even during SARS-CoV-2-induced endothelial damage. Ultimately, these differences may lead to poorer vascular outcomes and a more severe COVID-19-associated coagulopathy in Black compared to White individuals. We are not aware of any studies comparing the level of coagulopathy or elevation of D-dimer in SARS-CoV-2-infected patients by racial group, and this line of research would be very helpful to confirm our hypothesis.

Our findings similarly suggest that higher sCD14 might be part of heightened thrombo-inflammatory response in Black people, even though we observed that they have lower sCD14 levels. sCD14 is the soluble form of a pattern recognition receptor involved in innate immunity. It is induced by IL-6 and secreted by the liver.⁴¹ Higher sCD14 level is a race-specific risk marker for future cardiovascular disease events in Black persons.⁴² sCD14 level is higher in patients with pulmonary embolism,⁴³ and sCD14 is elevated in patients with COVID-19 compared to controls consistent with acute

TABLE 2 Overall percent difference in D-dimer level per SD increment of each biomarker

Biomarker	SD of biomarker	% Difference in D-dimer (95% CI) ^{a,b}		
		Model 1	Model 2	Model 3
Platelet count	67 × 10 ⁹ cells/L	2 (-4 to 9)	1(-5 to 8)	2 (-4 to 9)
WBC	1.98 × 10 ⁹ cells/L	12 (6 to 19)	12 (6 to 19)	10 (4 to 17)
Albumin	0.35 g/dL	-10 (-15 to -4)	-10 (-15 to -4)	-9 (-15 to -3)
NT-proBNP	1.27 pg/mL	21 (15 to 29)	21 (15 to 28)	20 (13 to 27)
In IL-6	0.62 pg/mL	24 (17 to 30)	24 (18 to 31)	21 (15 to 28)
In IL-8	0.62 pg/mL	10 (5 to 16)	10 (4 to 15)	9 (4 to 15)
In IL-10	1.00 pg/mL	6 (1 to 11)^c	6 (1 to 11)^c	6 (1 to 11)^c
In CRP	1.20 mg/L	23 (17 to 29)	23 (17 to 29)	20 (14 to 27)
Fibrinogen	105 mg/dL	18 (12 to 24)	18 (12 to 23)	16 (10 to 22)
Factor VIII	46%	20 (14 to 26)	20 (14 to 26)	18 (12 to 24)
sCD14	610 pg/ml	7 (2 to 12)^c	7 (2 to 12)^c	7 (1 to 13)^c

Note: For each biomarker, interactions with race were assessed, with significance indicated by color. Model 1 (demographic): adjusted for age, sex and race; model 2 (socioeconomic): adjusted for model 1 variables +education and income; model 3 (clinical): adjusted for model 1 variables +hypertension, diabetes, heart disease, body mass index, and statin use. Bold blue font indicates significant difference by race in all three models (*P* interaction <.05).

Abbreviations: CI, confidence interval; CRP, C-reactive protein; IL, interleukin; In, natural log-transformed; NT-ProBNP, N-terminal pro-B-type natriuretic peptide; sCD14, soluble CD14; SD, standard deviation; WBC, white blood cell count.

^aBonferroni adjustment not applied to 95% CI.

^bMost had a Bonferroni adjusted *P* value <.05 (not platelet count, In IL-10 and sCD14).

^cNominal (non-Bonferroni adjusted) *P* values for In IL-10 and sCD14 <.05.

TABLE 3 Differences in associations of biomarkers with D-dimer by race

Biomarker	SD of biomarker	% Difference in D-dimer (95% CI)
In IL-10 - race interaction (<i>P</i> = .001)		
Black	1.03 pg/mL	15 (8 to 23)
White	0.98 pg/mL	-3 (-9 to 4)
Factor VIII - race interaction (<i>P</i> = .009)		
Black	48%	26 (19 to 35)
White	42%	11 (3 to 20)
sCD14 - race interaction (<i>P</i> = .02)		
Black	575 pg/mL	13 (6 to 23)
White	638 pg/mL	2 (-4 to 8)

Note: The *P* values shown are for the interaction of the listed biomarker with listed category on D-dimer concentration. Only biomarkers with *P* interaction <.05 were included. Models are only adjusted for age and sex because adjustment for other factors did not substantially change percent differences in D-dimer.

Abbreviations: CI, confidence interval; IL, interleukin; In, natural log-transformed; sCD14, soluble CD14; SD, standard deviation.

inflammatory response induced by this virus.⁴⁴ We are not aware of data on race-specific levels in SARS-CoV-2 infection and venous thromboembolism.

Epidemiological research has not shown consistent associations of IL-10 and cardiovascular risk. Smaller studies in older adults⁴⁵

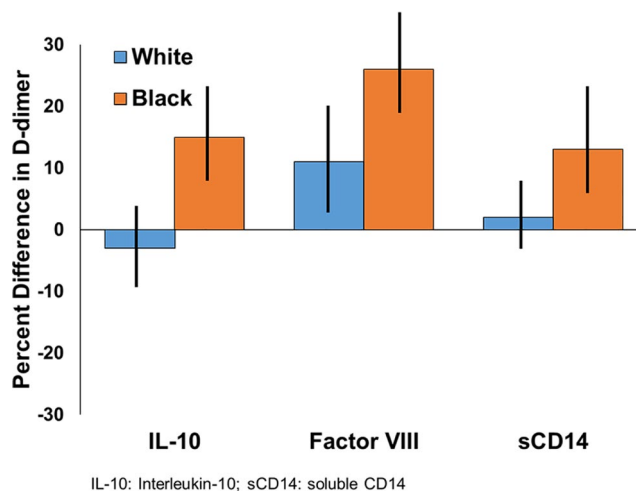


FIGURE 1 Difference in associations of biomarkers with D-dimer. The difference in association of each biomarker with D-dimer is shown by race, including biomarkers with interaction *P* value <.05 for race difference. Values shown are percent difference in D-dimer per SD increment of each biomarker, thus results may be compared across biomarkers as to their magnitude. Error bars indicate 95% confidence intervals. Models are adjusted for age and sex. IL, interleukin; sCD14, soluble CD14

and postmenopausal women with coronary atherosclerosis^{45,46} showed that higher IL-10 correlated with increased risk of cardiovascular events. On the other hand, larger and more diverse cohort

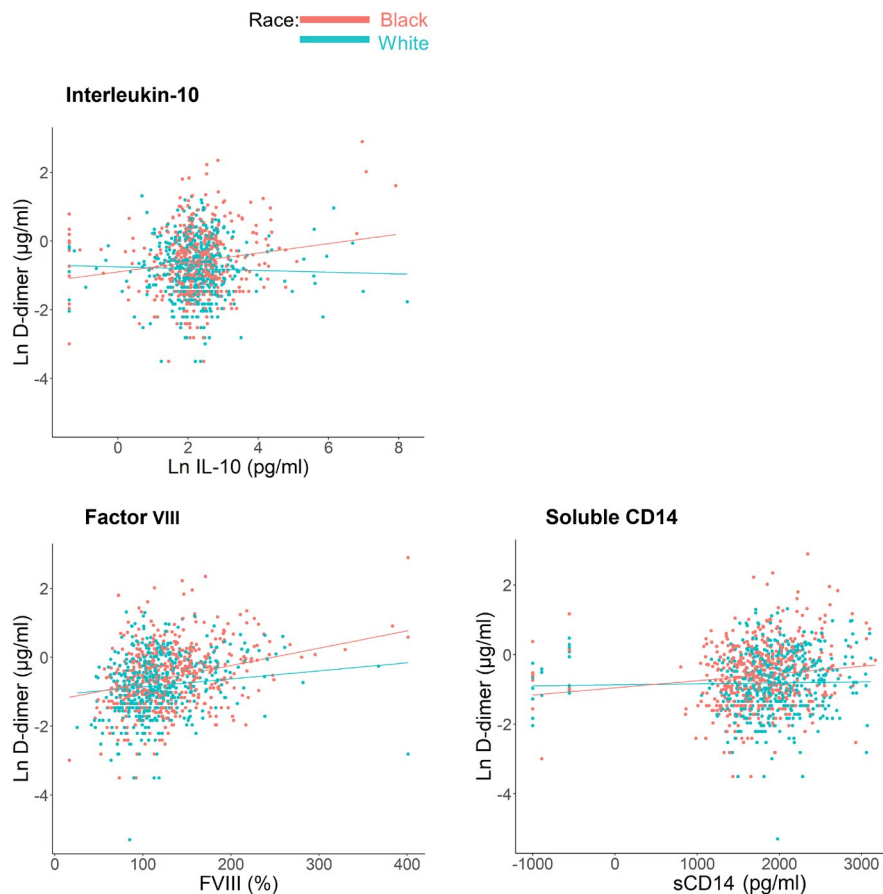


FIGURE 2 Partial regression plots showing the association of thrombo-inflammatory biomarkers with log D-dimer, after adjustment for age and sex, in Black and White participants. FVIII, factor VIII; IL, interleukin; sCD14, soluble CD14

studies have shown no association of IL-10 and cardiovascular outcomes.^{45,47,48} There are also conflicting findings on the association of IL-10 and venous thromboembolism. In case-control studies, higher IL-10 level was associated with lower risk of venous thromboembolism,^{49,50} yet a prospective analysis of a Norwegian cohort found no evidence for a relationship between IL-10 and venous thromboembolism.⁵¹ IL-10 is elevated in patients with COVID-19 and appears to be part of the cytokine storm in a subset of patients with very severe disease.⁵²⁻⁵⁴

The inconsistency of the epidemiology study findings on IL-10 calls into question the pathophysiological interpretation of higher IL-10 blood levels. Much remains unknown. Preclinical animal studies demonstrated that IL-10 was a potent anti-inflammatory cytokine. However, clinical trials revealed that IL-10 had immunostimulatory properties in humans. This dual effect of IL-10 is poorly understood.⁵⁵ Under basal conditions, IL-10 signaling is dominated by the activation of signal transducer and activator of transcription (STAT)-3, which confers anti-inflammatory properties to IL-10. Cellular priming by type I interferon modifies IL-10 signaling toward STAT1 activation, leading to type 1 T helper -like inflammation.⁵⁵ IL-10 was correlated with D-dimer in Black but not White participants in this study, suggesting a hypothesis that IL-10 may have proinflammatory properties in Black individuals.

Taken together, our findings suggest that high levels of sCD14 and IL-10 are accompanied by greater fibrin formation in Black than White people, and any role of this in cardiovascular,

venous thromboembolism, or COVID-19 pathogenesis remains to be determined.

A limitation of this investigation is that results are not generalizable to nonincluded racial groups. Additionally, participants were aged ≥ 45 years; therefore, findings may not pertain to younger age groups. We did not measure VWF due to the pragmatic sample collection methods in REGARDS and platelet contamination of the plasma²⁴ and did not account for all factors that might affect biomarker clearance, which might differ by race. A key strength of this study was its measurement of a suite of disease-relevant biomarkers in a biracial sample. We expressed the data standardized to the SD of each biomarker, so the relative strength of associations of each biomarker with D-dimer can be compared. To our knowledge, this is the first study to analyze racial differences in the association of D-dimer and thrombo-inflammatory biomarkers in a general population sample.

Racial disparities in cardiovascular outcomes and COVID-19 have brought to light issues of structural racism and their detrimental consequences on the health of Black people. As the observations here were independent of socioeconomic or clinical risk factors, differences in thrombo-inflammatory responses may reflect unmeasured effects of variables embedded within systemic racism. A hypothesis has been presented that for COVID-19 the interaction of systemic racism and the social environment causes dysregulated inflammatory and physiological responses,⁵⁶ which in turn might increase susceptibility to COVID-19 severity and death. Similar factors could be in play for cardiovascular diseases and venous thromboembolism.

That systemic racism could drive poorer health in Black people deserves further study.

In summary, in this study Black individuals had a higher thrombo-inflammatory state than White individuals, consistent with prior research findings. Moreover, in Black individuals, some biomarkers were more strongly associated with D-dimer in Black than White people. This suggests that D-dimer might relate to Black/White differences in cardiovascular diseases, venous thromboembolism, and possibly COVID-19 because it is a marker of amplified thrombo-inflammatory response in Black people. Future research on racial disparities in risk of cardiovascular diseases, venous thromboembolism, and COVID-19 might benefit from considering the findings here and evaluating the interplay of multiple risk biomarkers together.

ACKNOWLEDGMENTS

The authors thank the other investigators, the staff, and the participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at: <https://www.uab.edu/soph/regardsstudy/>.

RELATIONSHIP DISCLOSURE

LBK reported personal fees from CSL Behring, Quercegen Pharmaceuticals, and DHHS Vaccine Injury Compensation Program. CC reported personal fees from BioCogniv. The other authors report no relevant relationships.

AUTHOR CONTRIBUTIONS

DKM: design of study, analysis/interpretation of data, writing/revising, and final approval. MG: design of study, interpretation of data, writing/revising, and final approval. I. Koh: design of study, analysis/interpretation of data, final approval. NZ: interpretation of data, revising, and final approval. SEJ: interpretation of data, revising, and final approval. MS: interpretation of data, revising, and final approval. L. Baumann: Interpretation of data, revising, final approval. KF: interpretation of data, revising, and final approval. CC: interpretation of data, revising, and final approval. NCO: interpretation of data, revising, and final approval. MC: design of study, interpretation of data, writing/revising, and final approval.

DATA AVAILABILITY STATEMENT

The data are not publicly available because they contain information that could compromise research participant privacy. The data that support the findings of this study are available on reasonable request from the corresponding author.

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How to cite this article: Kamin Mukaz D, Gergi M, Koh I, et al. Thrombo-inflammatory biomarkers and D-dimer in a biracial cohort study. *Res Pract Thromb Haemost.* 2021;5:e12632. doi:[10.1002/rth2.12632](https://doi.org/10.1002/rth2.12632)