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Association of Pro-Inflammatory High Density Lipoprotein Cholesterol with Clinical and Laboratory Variables in Sickle Cell Disease

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Abstract

Background—Although cholesterol levels are known to be decreased in sickle cell disease (SCD), the level of pro-inflammatory high density lipoprotein cholesterol (proHDL) and its association with clinical complications and laboratory variables has not been evaluated.

Design and Methods—Plasma levels of total cholesterol, high density lipoprotein cholesterol (HDL), proHDL and selected clinical and laboratory variables were ascertained in a cohort of SCD patients and healthy African American control subjects in this single center, cross-sectional study.

Results—Although total cholesterol was significantly lower in SCD patients compared with control subjects, HDL and proHDL levels were similar in both SCD and control groups. In univariate analyses, proHDL was correlated with echocardiography-derived tricuspid regurgitant

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Study concept and design: Ataga, Hillery. *Acquisition of data*: Ataga, Hinderliter, Brittain, Jones, Xu, Pritchard, Hillery. *Analysis and interpretation of data*: Ataga, Hinderliter, Brittain, Cai, Kim, Pritchard, Hillery. *Drafting of the manuscript*: Ataga, Hinderliter, Hillery. Critical revision of the manuscript for important intellectual content: Ataga, Hinderliter, Brittain, Jones, Pritchard, Hillery. *Statistical analysis*: Cai, Kim. *Study supervision*: Ataga, Hillery.

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jet velocity. ProHDL was higher in SCD patients with suspected pulmonary hypertension (PHT) compared to patients without suspected PHT. ProHDL was positively correlated with lactate dehydrogenase, total bilirubin, direct bilirubin, indirect bilirubin, prothrombin fragment 1+2, D-dimer and thrombin-antithrombin complexes (TAT). In multivariable analyses, only higher lactate dehydrogenase and direct bilirubin levels were associated with higher levels of proHDL.

Conclusions—SCD is characterized by hypocholesterolemia. Although proHDL is not increased in SCD patients compared with healthy controls, it is significantly associated with markers of liver disease. In addition, proHDL is associated with tricuspid regurgitant jet velocity and markers of coagulation, although these associations are not significant in multivariable analyses.

Keywords

Sickle cell disease; Pro-inflammatory HDL; Cholesterol; Coagulation activation; Pulmonary vasculopathy

Introduction

Sickle cell disease (SCD) is an inherited disorder characterized by the presence of chronic hemolysis, ischemia-reperfusion injury and organ damage. Although somewhat controversial, it has been proposed that the clinical manifestations of SCD may fall into two partially overlapping phenotypes that are characterized by the presence of chronic hemolytic anemia and vaso-occlusive complications (1). While the risk of atherosclerosis is thought to be low in SCD (2) sickle cell anemia and other related hemoglobinopathies are complicated by the presence of vasculopathic complications, including stroke and pulmonary hypertension (PHT), which may occur, at least in part, as a result of increased hemolysis (1). Although cholesterol levels are reported to be low in patients with various anemias (3-9), the association of plasma lipid subsets with clinical complications and laboratory variables in SCD has not been extensively evaluated.

In this study, we compared levels of plasma lipids, including total cholesterol and high-density lipoprotein cholesterol (HDL) in SCD patients and healthy, African-American control subjects. As SCD is described as a chronic inflammatory state (10,11), we also determined the levels of pro-inflammatory HDL-cholesterol (proHDL) in this patient cohort. ProHDL is unable to perform the usual protective functions of HDL in the prevention of atherosclerosis, including the inhibition of LDL oxidation. Finally, we evaluated the association of selected lipid subsets (total cholesterol, HDL cholesterol and proHDL) with clinical complications and laboratory measures of hemolysis, activation of the coagulation system, inflammation, and N-terminal pro-brain natriuretic peptide (NT-proBNP) as a measure of elevated cardiac filling pressures.

Design and Methods

Patients and Study Design

The study patients represent a cohort followed at the Sickle Cell Clinic at the University of North Carolina (UNC), Chapel Hill. The data were collected as part of a study to investigate

the pathophysiology of PHT in SCD (12). Consecutive SCD patients seen in the clinic for routine follow up, who agreed to participate, were evaluated. Patients with SCD were assessed while in the non-crisis, "steady state;" had not experienced an episode of acute chest syndrome in the 4 weeks preceding enrollment; and had no clinical evidence of congestive heart failure. The control subjects were of African descent, had no known medical conditions, were not taking any medications, and were recruited by advertisement. Only control subjects who were not overweight or obese (i.e. had a body mass index [BMI] < 25) were enrolled. The study was approved by the Institutional Review Board at UNC, Chapel Hill and all subjects gave written informed consent to participate.

Study Measurements

Measurement of Lipid Profiles and other Laboratory Variables

Total cholesterol was quantified using a cholesterol oxidase/esterase kit from Wako Chemical, Inc. (Richmond, VA). HDL was isolated from whole plasma with a solution of dextran-sulfate-$MgCl_2$ (10 g/l, 0.5 M) (Berkeley HeartLab Inc., Alameda, CA), which precipitates apoB-containing lipoproteins. HDL was quantified using an HDL cholesterol E kit (Wako Diagnostics, Richmond, VA). ProHDL was determined using a modified method of a previously published cell-free assay (13). Briefly, HDL was incubated with $CuCl_2$ (5 μmol/l, final concentration) for 1 hour at 37°C in a 384-well microtiter plate (MJ Research Inc., Waltham, MA). After incubation, 10 μl of 2′,7′-dichlorodihdrofluorescein (H_2DCF) solution (0.2 mg/ml) was added to the HDL-Cu^{2+} mixture in a total volume of 50 μL. Rates of fluorescence (Excitation at 485 nm; Emission at 530 nm) were determined over the next 2 hours at 30 minute intervals using a Spectra Max Gemini EM fluorescence plate reader (Molecular Devices, Sunnyvale, CA). The results for proHDL are presented as slopes of the increase in dichlorofluorescein fluorescence over time.

Commercially available enzyme-linked immunosorbent assay kits were used to measure human soluble vascular cell adhesion molecule-1 (sVCAM-1), D-dimer and thrombin anti-thrombin complexes (TAT) (R&D systems, Minneapolis, Minnesota, USA), and prothrombin fragment 1+2 (F1+2) (Dade Behring, Marburg, Germany). Samples were assayed in duplicate and as per manufacturer's instructions. Measurements of routine laboratory tests were obtained at the McClendon Clinical Laboratory at UNC Hospitals.

SCD-Related Clinical Complications

Clinical complications in SCD patients were ascertained at the time of evaluation and defined using accepted definitions (14-16). Tricuspid regurgitant jet velocity (TRV) was measured by Doppler echocardiography as previously described (17). The estimated pulmonary artery systolic pressure (PASP) was calculated using the modified Bernoulli equation, and PHT was suspected if the PASP value, adjusted for age, sex, and body mass index exceeded the upper limits of normal in the reference ranges (18). All the echocardiograms were interpreted by a cardiologist blinded to all patient data. Only associations of total cholesterol, HDL and proHDL with the selected clinical and laboratory variables were performed because the study subjects were not required to be fasting. While fasting is recommended to minimize the influence of postprandial hyperlipidemia, serum

total cholesterol and HDL can be measured in fasting or non-fasting individuals (19). We have also observed differences in proHDL levels between non-fasted transgenic sickle cell (Berk) and control mice (Pritchard KA Jr, unpublished data).

Statistical Analyses

The normality assumption was not satisfied for continuous laboratory variables based on Shapiro-Wilk normality tests. Continuous variables were compared using Wilcoxon rank-sum test. Categorical variables were compared using Pearson's chi-square test for two groups or Kruskal-Wallis one-way analysis of variance for more than two groups. The association of continuous variables with lipid variables was explored using Spearman rank correlations. Multiple regression analyses were conducted to investigate the association of each lipid variable with clinical and laboratory variables. Because the lipid variables were skewed, the bootstrap method was used with 10,000 replications to estimate the p value and 95% confidence interval (20). A backward selection procedure was used for variable selection. The deletion criterion was based on a p value greater than 0.05 and the variable with the largest p value was deleted first at each step. The final model included only those variables which were statistically significant at 0.05 level. Reported p values are for individual tests, unadjusted for multiple comparisons because of the exploratory nature of this study. All analyses were performed using SAS (version 9.2, SAS Institute, Inc. Cary, NC).

Results

Demographics and Laboratory Characteristics

The demographic and laboratory characteristics of all the study subjects are shown in Table 1. One hundred and seventeen patients with SCD (HbSS: 91; HbSC: 13; HbSβ^0 thalassemia: 5; and HbSβ^+ thalassemia: 8) and 11 healthy, African American, control subjects (HbAA: 11) were evaluated. There were no significant differences in age and gender distribution when SCD patients were compared to control subjects. As expected, patients with SCD had significantly higher WBC counts, platelet counts, reticulocyte counts, hemoglobin F, lactate dehydrogenase, and total and indirect bilirubin compared with control subjects, while hemoglobin was significantly lower in SCD patients compared with control subjects.

Plasma Lipids in Sickle Cell Disease Patients and Healthy Controls

The median level of total cholesterol was significantly lower in SCD patients than in control subjects (102.5 mg/dL [interquartile range {IQR}: 86.5, 112.5 mg/dL] vs. 125.4 mg/dL [IQR: 111.0, 152.7 mg/dL], p = 0.0036). However, there were no statistically significant differences in the levels of HDL (42 mg/dL [IQR: 34.0, 52.9 mg/dL] vs. 49.0 mg/dL [IQR: 44.8, 58.0 mg/dL], p = 0.075) and proHDL (3.1 fluorescence units {FU} [IQR: 2.2, 4.2 FU] vs. 3.4 FU [IQR: 2.0, 4.8 FU], p = 0.61) when SCD patients were compared with control subjects. When the four SCD genotypes were compared, there was a trend for a difference in the level of total cholesterol (SS: 102.2 mg/dL [IQR: 86.4, 120.0 mg/dL] vs. Sβ^0: 153.4 mg/dL [IQR: 152.1, 154.1 mg/dL] vs. Sβ^+: 103.5 mg/dL [IQR: 82.5, 141.7 mg/dL] vs. SC: 91.0 mg/dL [IQR: 86.5, 123.3 mg/dL], p = 0.055), but no differences were seen in the levels

of HDL (SS: 43.3 mg/dL [IQR: 34.0, 53.8 mg/dL] vs. $S\beta^0$: 36.4 mg/dL [IQR: 31, 45.6 mg/dL] vs. $S\beta^+$: 36.1 mg/dL [IQR: 32, 56 mg/dL] vs. SC: 42 mg/dL [IQR: 40.0, 50.5 mg/dL], p = 0.75) or proHDL (SS: 3.3 Fluorescence Units {FU} [IQR: 2.2, 4.3 FU] vs. $S\beta^0$: 2.0 FU [IQR: 1.6, 3.0 FU] vs. $S\beta^+$: 2.6 FU [IQR: 1.6, 3.3 FU] vs. SC: 3.2 FU [IQR: 2.4, 4.0 FU], p = 0.60). When SCD patients were grouped based on presumed disease severity ($SS/S\beta^0$ thalassemia vs. $SC/S\beta^+$ thalassemia), there were no statistically significant differences in the levels of total cholesterol, HDL, or proHDL (Supplementary data, Table 1S).

In patients with SCD, proHDL was correlated with HDL (r = 0.68 [95% Confidence Interval {CI}: 0.57, 0.77], p < 0.0001), but there was no correlation with total cholesterol (r = −0.043 [95% CI: −0.23, 0.14], p = 0.65).

Association of Pro-Inflammatory HDL Cholesterol, Total Cholesterol and HDL Cholesterol with Demographic and Clinical Variables in Patients with Sickle Cell Disease

No significant correlations were observed between age and proHDL (r = 0.17, p = 0.067), total cholesterol (r = 0.14, p = 0.12) or HDL (r = 0.14, p = 0.12). There were also no significant correlations between BMI and proHDL (r = 0.001, p = 0.99) or HDL (r = −0.092, p = 0.33), although there was a trend towards a significant correlation between BMI and total cholesterol (r = 0.18, p = 0.053). Total cholesterol level was higher in female SCD patients than in male patients (104.1 mg/dL [IQR: 91.6, 126.8 mg/dL] vs. 93.6 mg/dL [IQR: 83.8, 109.7 mg/dL], p = 0.016) but there were no gender differences in proHDL or HDL.

Echocardiography-derived TRV was significantly correlated with proHDL (r = 0.28, p = 0.016), but no correlations were observed between TRV and either total cholesterol (r = −0.11, p = 0.35) or HDL (r = −0.031, p = 0.79). ProHDL was higher in patients with suspected PHT (3.6 FU [IQR: 2.7, 5.0 FU] vs. 2.9 FU [2.0, 4.0 FU], p = 0.0099) and was lower in patients with a history of priapism (2.7 FU [IQR: 2.0, 4.0 FU] vs. 3.7 FU [IQR: 2.7, 4.6 FU], p = 0.035) than in patients without these complications (Table 2). Total cholesterol was lower in patients with suspected PHT than in those not suspected to have PHT (95.9 mg/dL [IQR: 80.1, 109.5 mg/dL] vs. 104.9 mg/dL [IQR: 90.2, 123.9 mg/dL], p = 0.011). There was also a trend for lower levels of total cholesterol in patients with histories of priapism (86.3 mg/dL [IQR: 80.1, 108.5 mg/dL] vs. 102.8 mg/dL [IQR: 86.5, 120.3 mg/dL], p = 0.063) and leg ulcers (93.6 mg/dL [IQR: 83.8, 109.5 mg/dL] vs. 103.1 [IQR: 90.9, 123.8 mg/dL], p = 0.084). HDL was lower in patients with a history of priapism than in those without this complication (38.0 mg/dL [IQR: 33.0, 45.7 mg/dL] vs. 48.2 mg/dL [IQR: 37.4, 55.6 mg/dL], p = 0.015).

Correlation of Pro-Inflammatory HDL Cholesterol, Total Cholesterol and HDL Cholesterol with Markers of Hemolysis, Coagulation Activation, Endothelial Injury and Inflammation in Patients with Sickle Cell Disease

Lipid subsets were evaluated for correlations with markers of hemolysis (hemoglobin, reticulocyte count, lactate dehydrogenase, as well as total and indirect bilirubin), coagulation activation (F1+2, D-dimer and TAT), endothelial injury (sVCAM-1), inflammation (white blood cell count, absolute neutrophil count and absolute monocyte count) and other selected laboratory variables (platelet count, fetal hemoglobin, direct bilirubin and NT-proBNP) in

our patient cohort. ProHDL was directly correlated with lactate dehydrogenase (r = 0.31, p = 0.0008), total bilirubin (r = 0.23, p = 0.013), direct bilirubin (r = 0.48, p < 0.0001), indirect bilirubin (r = 0.21, p = 0.028), F1+2 (r = 0.33, p = 0.0062), D-dimer (r = 0.27, p = 0.0053) and TAT (r = 0.22, p = 0.023) (Table 3; Supplementary data - Figures 1A-G). However, no correlations were observed between proHDL and hemoglobin, reticulocyte count, fetal hemoglobin, white blood cell count, or sVCAM-1. There was a modest correlation between total cholesterol and fetal hemoglobin (r = 0.19, p = 0.045), with inverse correlations between total cholesterol and lactate dehydrogenase (r = −0.20, p = 0.031), total bilirubin (r = −0.22, p = 0.019), indirect bilirubin (r = − 0.20, p = 0.034), and sVCAM-1 (r = −0.34, p = 0.0002). There appeared to be a positive correlation between total cholesterol and hemoglobin (r = 0.16, p = 0.085), although this did not achieve statistical significance. Finally, HDL was correlated with platelet count (r = −0.21, p = 0.025) and direct bilirubin (r = 0.39, p < 0.0001).

Multivariable Analyses

Multiple regression analysis was conducted to investigate the association of total cholesterol, HDL and proHDL with selected clinical and laboratory variables in SCD patients. The initial model included clinical variables (history of stroke, avascular necrosis, history of leg ulcers, history of acute chest syndrome, history of smoking, suspected PHT and number of pain episodes in the previous year) and laboratory variables (absolute neutrophil count, absolute monocyte count, hemoglobin, platelet count, lactate dehydrogenase, total bilirubin, direct bilirubin, sVCAM-1, D-dimer, F1+2 and TAT). In the final model, using only significant covariates after the model selection, sVCAM-1 was significantly and inversely associated with total cholesterol (estimate: −0.015, p = 0.003); TAT was significantly associated with HDL (estimate: 0.068, p = 0.039); and direct bilirubin (estimate: 1.4, p = 0.047) and lactate dehydrogenase (estimate: 0.0011, p = 0.00024) were significantly associated with proHDL (Table 4). This means that for a continuous variable such as direct bilirubin, we expect an increase in proHDL by 1.4 FU for every 1 mg/dL increase in direct bilirubin, given the same level of lactate dehydrogenase.

Discussion

Patients with SCD have previously been reported to have lower total cholesterol and LDL levels compared with healthy, control subjects (3-9). Although it has been suggested that hypocholesterolemia is not due to increased erythropoiesis, but rather is a consequence of anemia (3), a study of patients with chronic anemia, including those with high erythropoietic activity, low erythropoietic activity and healthy, control subjects reported the presence of hypocholesterolemia only in patients with anemia and increased erythropoietic activity (21). In addition, significant inverse correlations were observed between serum levels of cholesterol and soluble transferrin receptor, a marker of high erythropoietic activity in the absence of iron deficiency, suggesting that hypocholesterolemia is associated with increased erythropoiesis. Although our findings of decreased total cholesterol levels in SCD patients and their association with measures of hemolysis in univariate analysis appear to confirm and extend these findings, no associations were observed between total cholesterol and any

measures of hemolysis in multivariable analyses, suggesting that hypocholesterolemia in SCD is not solely due to increased hemolysis.

While multiple studies show that HDL level is a strong predictor of cardiovascular risk (22-24), there is evidence that in some circumstances HDL may be dysfunctional (i.e. it fails to prevent the formation of and/or fails to inactivate biologically active LDL-derived oxidized phospholipids) or pro-inflammatory (i.e. it enhances the formation of biologically active oxidized phospholipids) (25-30). Elevated plasma concentrations of oxidized LDL are associated with coronary artery disease (31), and patients with acute coronary syndromes have higher levels of malondialdehyde-modified LDL than patients with stable coronary artery disease (32). High levels of proHDL have been observed in patients with inflammatory diseases such as systemic lupus erythematosus and rheumatoid arthritis (33). Although SCD is frequently referred to as a chronic inflammatory disease (10,11), we found no significant difference in the level of proHDL when SCD patients were compared with healthy African American controls. In addition, there were no associations between proHDL and any of the evaluated inflammatory markers in our study patients. The observed level of proHDL in this study, combined with the lower cholesterol levels in SCD patients compared with healthy, control subjects may contribute to the low incidence of atherosclerosis observed in SCD.

The association of proHDL with measures of hemolysis in univariate analysis was somewhat discordant. Although correlations were observed with lactate dehydrogenase, as well as total and indirect bilirubin, no significant associations were observed with hemoglobin or reticulocyte count. Furthermore, the observed association of proHDL with both lactate dehydrogenase and direct bilirubin in the final model of the multivariable analysis, combined with usual increases in the levels of lactate dehydrogenase and direct bilirubin in liver disease, suggest that proHDL may be associated with liver dysfunction. The liver plays a central role in lipoprotein metabolism and is responsible for both degradation and synthesis of lipoproteins (34). Inflammation and injury of the liver induces a variety of metabolic changes that can negatively impact lipoprotein metabolism. Thus during chronic states of inflammation and oxidative stress, such as those that are known to occur in SCD (35,36), the injured liver may be unable to metabolize HDL properly (37), which may explain the observed correlations between proHDL and both lactate dehydrogenase and direct bilirubin in our patient population.

The negative correlation between total cholesterol and sVCAM-1 in both the univariate and multivariable analyses suggests that hypocholesterolemia may contribute to endothelial cell injury in SCD. This finding was surprising, and is in contrast to the observation that basal VCAM-1 protein expression is higher in hyperlipidemic mice (ApoE [−/−]) than in wild-type mice (38). In addition, both VCAM-1 mRNA and protein levels are further increased by high fat diet, with a correlation of VCAM-1 mRNA and protein levels to plasma cholesterol, LDL and HDL, but not to triglyceride levels. Induction of VCAM-1 by high fat diet in blood vessel walls may be dependent on inflammation, initiated by modified lipoprotein particles such as oxidized phospholipids and short-chain aldehydes, which in turn activate VCAM-1 transcription via activation of NF-κB (39). It is possible, however, that extremes of cholesterol levels (i.e. too high or too low) may be detrimental to health by

causing endothelial cell injury. An alternative explanation is that the increased erythropoiesis associated with SCD may contribute to both lower cholesterol levels and increased endothelial injury for, as yet, unknown reasons.

SCD is also described as a hypercoagulable state (40). We observed an association between proHDL and F1+2, TAT and D-dimer in univariate analyses, suggesting that proHDL may promote coagulation activation in SCD. However, proHDL was not independently associated with markers of coagulation activation. The absence of significant associations between proHDL and markers of coagulation activation in the final model of the multivariable analysis may be a result of the association of proHDL with lactate dehydrogenase, a biomarker that has been reported to be associated with markers of coagulation activation (41). Oxidized LDL has been reported to significantly enhance tissue factor expression induced by the inflammatory mediator, bacterial lipopolysaccharide (LPS), in a time- and dose-dependent manner (42). In another study, low concentrations of oxidized LDL has been shown to enhance tissue factor expression in human monocyte-derived macrophages, whereas higher concentrations attenuate tissue factor expression both at baseline as well as following LPS stimulation (43). As proHDL enhances the formation of biologically active oxidized phospholipids, increased levels of proHDL likely contributes to coagulation activation by increasing levels of oxidized LDL.

Total cholesterol and proHDL were associated with suspected PHT in univariate analyses. In addition, proHDL was significantly correlated with echocardiography-derived TRV. This suggests that proHDL may contribute to the pathophysiology of pulmonary vasculopathy in SCD. The absence of a significant association between proHDL and suspected PHT in the final model of the multivariable analysis may be related to the association of proHDL with lactate dehydrogenase, as multiple studies have shown associations of both TRV and echocardiography-defined PHT with lactate dehydrogenase in SCD (44,45).

Our study has several limitations. Right heart catheterizations were not obtained to confirm the presence of PHT. Extensive tests were not obtained to assess liver function in study subjects. As with all cross-sectional studies, this analysis demonstrates associations, but cannot prove causation.

In summary, our study confirms and extends the findings of hypocholesterolemia in SCD, with an association of lower cholesterol levels with increased sVCAM-1, a marker of endothelial injury. The lack of association of markers of hemolysis with lipid variables in the final model of the multivariable analysis suggests that hemolysis and increased erythropoiesis are unlikely to be the sole causes of hypocholesterolemia in SCD. The level of proHDL is not increased in SCD compared to healthy control subjects. Higher proHDL levels are associated with TRV, suspected PHT and markers of coagulation activation in univariate analyses. The association of proHDL with direct bilirubin and lactate dehydrogenase in the final model of the multivariable analysis suggests that proHDL may be a biomarker of liver dysfunction in SCD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and Laboratory Characteristics of Study Subjects

Variable	N	Sickle Cell Disease Median (IQR) or N (%)	N	Healthy Controls Median (IQR) or N (%)	p Value
Age	117	38(29, 47)	11	37(26,49)	0.5515
Gender (Male)	117	41 (35%)	11	5 (45%)	0.4914
Genotype (SS)	117	91 (78%)	11	0 (0%)	<0.0001
Genotype (SC)		13 (11%)		0 (0%)	
Genotype ($S\beta^0$)		5 (4%)		0 (0%)	
Genotype ($S\beta^+$)		8 (7%)		0 (0%)	
Genotype (AA)		0 (0%)		11 (100%)	
Body mass index	116	25.6 (22.2, 29.8)	11	22.7 (22.0,23.1)	
White Blood Cell ($\times 10^9$/L)	117	9.2 (7.8, 11.4)	11	6.8 (4.4,9.3)	0.0104
Hemoglobin (g/dL)	117	8.8 (7.6, 10.1)	11	13.4 (12.2,13.9)	<0.0001
Platelet Count ($\times 10^9$/L)	117	410 (314, 498)	11	222 (201,291)	<0.0001
Reticulocyte Count (%)	116	6.6 (4.5, 9.4)	11	1.7 (1.3,2.2)	<0.0001
Hemoglobin F (%)	116	6.0 (3.2, 10.9)	11	0.5 (0.3, 2.0)	<0.0001
Absolute Neutrophil Count ($\times 10^9$/L)	117	4.8 (3.9, 6.2)	11	3.8 (2.1, 4.5)	0.0068
Absolute Monocyte Count ($\times 10^9$/L)	117	0.5 (0.3, 0.7)	11	0.3 (0.2,0.4)	0.0026
Lactate Dehydrogenase (U/L)	115	866.0 (65.02, 1214.0)	11	481 (380,537)	<0.0001
Total Bilirubin (mg/dL)	117	1.9 (1.0, 3.0)	11	0.5 (0.3,0.6)	<0.0001
Direct Bilirubin (mg/dL)	115	0.1 (0.09, 0.1)	11	0.09 (0.09,0.1)	0.0193
Indirect Biliribin (mg/dL)	115	1.7 (0.9, 2.8)	11	0.36 (0.2,0.51)	<0.0001
Creatinine (mg/dL)	117	0.7 (0.6, 1)	11	1.0 (0.7,1.1)	0.1589

Table 2

Association of Lipid Variables with Clinical Complications in Patients with Sickle Cell Disease

Lipid Variable	Clinical Variable	N	Yes (Median, IQR)	N	No (Median, IQR)	p value
Total Cholesterol	History of Stroke	14	99.6 [83.0, 108.5]	103	103.0 [86.5, 123.3]	0.54
	Avascular necrosis	48	103.3 [90.9, 121.7]	69	99.8 [85.0, 122.5]	0.35
	History of leg ulcer	31	93.6 [83.8, 109.5]	86	103.1 [90.9, 123.8]	0.084
	Use of hydroxyurea	65	102.4 [86.5, 120.1]	51	102.5 [86.8, 122.8]	0.85
	History of retinopathy	37	96.7 [85.5, 117.2]	80	103.1 [88.3, 123.9]	0.22
	Pain crisis ≥ 3 in previous year	66	102.4 [83.9, 117.9]	51	103.3 [89.6, 130.2]	0.38
	History of acute chest syndrome	101	102.5 [86.4, 123.3]	16	102.6 [92.3, 112.0]	0.96
	History of priapism	18	86.3 [80.1, 108.5]	67	102.8 [86.5, 120.3]	0.063
	Suspected pulmonary hypertension	33	95.9 [80.1, 109.5]	84	104.9 [90.2, 123.9]	0.011
HDL-Cholesterol	History of stroke	14	48.5 [32.0, 70.8]	103	42.0 [34.4, 50.6]	0.32
	Avascular necrosis	48	43.5 [32.0, 56.3]	69	42.0 [35.0, 50.9]	0.71
	History of leg ulcer	31	42.6 [33.0, 53.8]	86	42.0 [35.3, 52.9]	0.65
	Use of hydroxyurea	65	42.6 [32.4, 52.0]	51	42.0 [35.0, 55.4]	0.71
	History of retinopathy	37	40.0 [34.4, 48.2]	80	44.1 [34.0, 55.1]	0.39
	Pain crisis ≥ 3 in previous year	66	42.9 [32.4, 53.8]	51	42.0 [35.0, 52.9]	0.80
	History of acute chest syndrome	101	42.6 [34.0, 52.9]	16	41.4 [37.0, 52.9]	0.78
	History of priapism	18	38.0 [33.0, 45.7]	67	48.2 [37.4, 55.6]	0.015
	Suspected pulmonary hypertension	33	41.2 [34.4, 55.0]	84	42.8 [34.0, 52.4]	0.97
Pro-inflammatory HDL-Cholesterol	History of stroke	13	3.4 [2.6, 4.1]	100	3.1 [2.1, 4.2]	0.41
	Avascular necrosis	46	3.2 [2.4, 4.5]	67	3.0 [2.1, 4.0]	0.47
	History of leg ulcer	31	3.8 [2.1, 4.6]	82	2.9 [2.2, 3.7]	0.094
	Use of hydroxyurea	65	2.9 [2.1, 4.1]	47	3.3 [2.3, 4.3]	0.29
	History of retinopathy	35	3.0 [2.2, 4.0]	78	3.2 [2.1, 4.3]	0.60
	Pain crisis ≥ 3 in previous year	66	3.2 [2.2, 4.3]	47	3.1 [2.1, 4.0]	0.98
	History of acute chest syndrome	99	3.2 [2.1, 4.2]	14	3.0 [2.5, 4.0]	0.95
	History of priapism	18	2.7 [2.0, 4.0]	63	3.7 [2.7, 4.6]	0.035
	Suspected pulmonary hypertension	30	3.6 [2.7, 5.0]	83	2.9 [2.0, 4.0]	0.0099

Table 3

Correlation of Lipid Variables with Laboratory Measures of Hemolysis and Inflammation in Patients with Sickle Cell Disease

Lipid Variable	Laboratory Variable	Number of Patients	r value	(95%) Confidence Interval	p value
Total Cholesterol	White blood count	117	−0.031	−0.212 – 0.151	0.74
	Absolute neutrophil count	117	0.106	−0.077 – 0.283	0.25
	Absolute monocyte count	117	0.022	−0.160 – 0.203	0.81
	Hemoglobin	117	0.159	−0.023 – 0.331	0.085
	Platelet count	117	−0.014	−0.195 – 0.168	0.88
	Reticulocyte count	116	−0.096	−0.273 – 0.088	0.31
	Hemoglobin F	116	0.186	0.004 – 0.356	0.045
	Lactate dehydrogenase	115	−0.20	−0.370 - −0.018	0.031
	Total bilirubin	117	−0.215	−0.382 - −0.035	0.019
	Direct bilirubin	115	0.020	−0.164 – 0.202	0.84
	Indirect bilirubin	115	−0.197	−0.367 - −0.015	0.034
	NT-proBNP	114	−0.048	−0.230 – 0.137	0.61
	Soluble VCAM-1	117	−0.336	−0.488 - −0.164	0.0002
	Thrombin-antithrombin complexes	109	0.006	−0.183 – 0.193	0.95
	D-dimer	105	−0.082	−0.269 – 0.111	0.40
	Prothrombin 1+2	69	−0.097	−0.326 – 0.143	0.43
HDL-Cholesterol	White blood count	117	−0.020	−0.200 – 0.162	0.8333
	Absolute neutrophil count	117	−0.126	−0.300 – 0.057	0.1758
	Absolute monocyte count	117	−0.068	−0.247 – 0.115	0.4646
	Hemoglobin	117	0.002	−0.180 – 0.183	0.9865
	Platelet count	117	−0.206	−0.374 - −0.026	0.0247
	Reticulocyte count	116	0.027	−0.156 – 0.208	0.7748
	Hemoglobin F	116	−0.088	−0.266 – 0.095	0.3438
	Lactate dehydrogenase	115	0.055	−0.130 – 0.235	0.56
	Total bilirubin	117	0.103	−0.080 – 0.280	0.27
	Direct bilirubin	115	0.389	0.220 – 0.533	<0.0001
	Indirect bilirubin	115	0.086	−0.099 – 0.265	0.36
	NT-proBNP	114	−0.007	−.0.177 – 0.191	0.94
	Soluble VCAM-1	117	−0.012	−0.0193 – 0.170	0.90
	Thrombin-antithrombin complexes	109	0.119	−0.072 – 0.299	0.22
	D-dimer	105	0.154	−0.039 – 0.336	0.12
	Prothrombin 1+2	69	0.171	−0.068 – 0.392	0.16
Pro-inflammatory HDL-Cholesterol	White blood count	113	−0.037	−0.220 – 0.148	0.69
	Absolute neutrophil count	113	−0.094	−0.274 – 0.092	0.32
	Absolute monocyte count	113	−0.001	−0.186 – 0.184	0.99
	Hemoglobin	113	−0.140	−0.317 – 0.046	0.14

Lipid Variable	Laboratory Variable	Number of Patients	r value	(95%) Confidence Interval	p value
	Platelet count	113	−0.108	−0.287 – 0.079	0.26
	Reticulocyte count	112	0.122	−0.065 – 0.301	0.20
	Hemoglobin F	112	−0.147	−0.324 – 0.039	0.12
	Lactate dehydrogenase	111	0.310	0.131 – 0.469	0.0008
	Total bilirubin	113	0.233	0.050 – 0.400	0.013
	Direct bilirubin	111	0.481	0.322 – 0.611	<0.0001
	Indirect bilirubin	111	0.208	0.022 – 0.380	0.028
	NT-proBNP	110	0.132	−0.057 – 0.311	0.17
	Soluble VCAM-1	113	0.118	−0.068 – 0.296	0.21
	Thrombin-antithrombin complexes	105	0.221	0.030 – 0.395	0.023
	D-dimer	101	0.274	0.082 – 0.445	0.0053
	Prothrombin 1+2	65	0.332	0.096 – 0.533	0.0062

NT-proBNP – N-terminal pro-brain natriuretic peptide

Soluble VCAM-1 – Soluble vascular cell adhesion molecule-1

Table 4

Multivariable Analysis

Dependent Variable	Covariate	Number of patients	Estimate	Standard Error	95% Confidence Interval	p value
Total Cholesterol	Intercept	117	122.05	5.78	110.71, 133.39	<0.0001
	Soluble VCAM-1		−0.015	0.005	−0.026, −0.005	0.003
HDL Cholesterol	Intercept	109	43.26	1.52	40.28, 46.23	<0.0001
	Thrombin anti-thrombin complex		0.068	0.033	0.0035, 0.13	0.039
Pro-inflammatory HDL Cholesterol	Intercept	110	1.91	0.31	1.29, 2.52	<0.0001
	Direct bilirubin		1.4	0.71	0.02, 2.78	0.047
	Lactate dehydrogenase		0.0011	0.00029	0.0005, 0.0016	0.00024

Soluble VCAM-1 – Soluble vascular cell adhesion molecule – 1

HDL cholesterol – High density lipoprotein cholesterol

LDL cholesterol – High density lipoprotein cholesterol