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ORIGINAL RESEARCH

Soluble CD14, Ischemic Stroke, and Coronary Heart Disease Risk in a Prospective Study: The REGARDS Cohort

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BACKGROUND: Soluble CD14 (sCD14), a circulating pattern recognition receptor, has been suggested as a cardiovascular disease risk factor. Prospective studies evaluating sCD14 with incident cardiovascular disease events are limited, particularly among racially diverse populations.

METHODS AND RESULTS: Between 2003 and 2007, the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study recruited 30 239 black and white participants across the United States. In a nested case–cohort study, sCD14 was measured in baseline serum from 548 cases of incident ischemic stroke, 612 cases of incident coronary heart disease (CHD), and a cohort random sample (n=1039). Cox models estimated hazards ratios (HR) of incident ischemic stroke or CHD per 1 SD higher sCD14, adjusting for cardiovascular disease risk factors. There was a differential association of sCD14 with ischemic stroke and CHD risk by race. Among blacks, the adjusted HR of stroke per SD increment of sCD14 was 1.42 (95% CI: 1.12, 1.80), with no association among whites (HR 1.02 [95% CI: 0.82, 1.27]). Higher sCD14 was associated with increased CHD risk in blacks but not whites, and relationships between sCD14 and CHD were stronger at younger ages. Adjusted for risk factors, the HR of CHD per SD higher sCD14 among blacks at age 45 years was 2.30 (95% CI: 1.45, 3.65) compared with 1.56 (95% CI: 0.94, 2.57) among whites. At age 65 years, the CHD HR was 1.51 (95% CI: 1.20, 1.91) among blacks and 1.02 (95% CI: 0.80, 1.31) among whites.

CONCLUSIONS: sCD14 may be a race-specific stroke and CHD risk marker.

Key Words: biomarker ■ cardiovascular disease risk factors ■ epidemiology ■ inflammation ■ race

CD14 is a pattern recognition receptor that plays an important role in the innate immune response by pathogen surveillance and responding to bacterial lipopolysaccharide (endotoxin). Upon stimulation, CD14 contributes to the activation of several pro-inflammatory signaling pathways and may influence metabolism and obesity.[1–3]

CD14 is present as membrane-bound and soluble forms. The membrane-bound form (mCD14) contains a glycosylphosphatidylinositol anchor and is expressed on the surface of monocytes, macrophages, neutrophils, dendritic cells, myeloid-derived suppressor cells, and some nonmyeloid cells.[3,4] The soluble form of CD14 (sCD14) is secreted by hepatocytes in response to interleukin-6 (IL-6) and is considered an acute-phase reactant.[5] Circulating sCD14 is also generated by cell surface cleavage of mCD14[6,7] and the release of intracellular vesicles,[8] processes that occur during cell activation or apoptosis.

CD14 is required for endotoxemia and septic shock induced by lipopolysaccharide or *Escherichia coli*. Mice with genetic deficiency in CD14 are resistant to lethal doses of these pathogens.[9] Although the

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CLINICAL PERSPECTIVE

What Is New?

- In a population-based study of adult men and women sampled from across the contiguous United States, higher blood levels of soluble CD14 were associated with increased risks of future ischemic stroke and future coronary events among black participants but not among white participants.
- Relationships between higher soluble CD14 and increased coronary risk among blacks were stronger at younger ages.

What Are the Clinical Implications?

- Soluble CD14 may be a race-specific cardiovascular risk marker among black adults, with implications for understanding racial disparities in the risk of stroke.

Nonstandard Abbreviations and Acronyms

CHS	Cardiovascular Health Study
CRP	C-reactive protein
HR	hazard ratio
LPS	lipopolysaccharide
LVH	left ventricular hypertrophy
mCD14	membrane-bound CD14
sCD14	soluble CD14
REGARDS	Reasons for Geographic and Racial Differences in Stroke study

importance of infectious pathogens in the progression of atherosclerosis remains unclear, they may play roles by promoting inflammation. Activation of monocytes and macrophages through mCD14 signaling leads to secretion of the pro-inflammatory cytokines tumor necrosis factor-α, IL-1β, and IL-6.[10] Activation of endothelial cells through sCD14 can increase cell adhesion molecule expression and procoagulant activity,[11] which could serve as a potential triggering event for clinical cardiovascular disease (CVD).

As a biomarker of inflammation, immune cell activation, and bacterial translocation, sCD14 has gained interest as a potential CVD risk factor, but prospective studies of this question are limited. Higher sCD14 was associated with progression of subclinical atherosclerosis among men and women with HIV infection.[12,13] In patients with chronic kidney disease, higher sCD14 was associated with incident CVD events independent of other risk factors.[14] Higher sCD14 was associated with increased risk of incident coronary heart disease (CHD), stroke, and heart failure after adjustment for CVD risk factors in cohorts of middle-age and older American adults.[15,16] In contrast, sCD14 levels were not related to incident CHD in a cohort of European men.[17]

As sCD14 levels vary by race,[15] and because there are racial differences in systemic inflammation, stroke, and CHD mortality,[18,19] we sought to determine whether there were differences in the relationships between sCD14 and CVD risk by race. In black and white participants of the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study, we evaluated associations of sCD14 with risk of incident ischemic stroke and CHD and tested whether relationships were different by race, age, or sex.

METHODS

Data and Resource Availability

The data underlying the findings include potentially identifying participant information, and cannot be made publicly available because of ethical/legal restrictions. However, data including statistical code from this article are available to researchers who meet the criteria for access to confidential data. Data can be obtained upon request through the University of Alabama at Birmingham at regardsadmin@uab.edu.

Cohort

REGARDS recruited 30 239 participants from the contiguous United States between 2003 and 2007. The study aimed to recruit a cohort that was half women, half black, and half residents of the "stroke belt" or "stroke buckle" regions of the Southeastern United States (Stroke Belt: North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Arkansas, and Louisiana; Stroke Buckle: Coastal Plains of North Carolina, South Carolina, and Georgia).[20]

Exclusion criteria included self-reported race other than black or white, Hispanic ethnicity, non-English speaker, active cancer or cancer treatment within the past year, nursing home residence or waiting-list, or a medical condition preventing long-term study participation. The final cohort was 55% women, 41% black, and 56% residents of the Southeast. Details about participant computer-assisted telephone interviews and in-home visits are published.[20]

The current study is a nested case–cohort study performed within REGARDS to study associations of biomarkers with vascular disease outcomes.[21–23] The case–cohort sample included cases of stroke and CHD, and a cohort random sample. The cohort random sample was selected using stratified sampling to ensure representation across age, sex, and, race. Participants in the cohort random sample were randomly selected to fulfill the desired distributions: 20%

age 45 to 54, 20% age 55 to 64, 25% age 65 to 74, 25% age 75 to 84, and 10% age ≥85 years, 50% female, and 50% black.[22] The present study included 2199 case–cohort participants with sCD14 measurements. The study's methods were reviewed and approved by the Institutional Review Boards at the participating institutions[20] and all study participants provided written informed consent.

Stroke and CHD Ascertainment

Event ascertainment methods in REGARDS are published.[24,25] Participants (or their proxies) were contacted at 6-month intervals to ascertain potential stroke or CHD events. Deaths, and causes of death, were ascertained. Medical records were obtained for suspected stroke or coronary events and reviewed by separate adjudication committees. Stroke events were defined by the World Health Organization's definition as "rapidly developing clinical signs of focal, at times global, disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin"[26] or by review of final neuroimaging reports consistent with acute ischemia. Strokes were classified as ischemic or hemorrhagic. For this analysis, strokes identified through September 1, 2011 were included.

CHD events were adjudicated from medical records by a team of experts as described previously.[25] CHD was defined as definite or probable myocardial infarction or definite or probable CHD-related death. Definite MIs were defined as those with diagnostic cardiac enzymes (cardiac troponin or creatine phosphokinase-MB) or diagnostic electrocardiogram. Probable myocardial infarctions were defined as those with elevated, but not diagnostic, cardiac enzymes with a positive, but not diagnostic, ECG. If enzyme information was missing, probable MIs included those with a positive ECG in the presence of signs or symptoms of ischemia. MIs included those adjudicated to have been caused by an invasive medical procedure. Ascertainment of CHD events was conducted in a separate ancillary study within REGARDS and follow-up was complete through December 31, 2009.

Definitions

Race was by participant self-identification. CVD risk factors and clinical events were assessed at the baseline visit. CVD was defined as ECG evidence of myocardial infarction or by participant self-report of CHD, stroke, or peripheral artery disease. Atrial fibrillation was defined by participant self-report of a physician diagnosis or by ECG obtained during the in-home visit. Left ventricular hypertrophy was defined by ECG criteria. Diabetes mellitus was defined as a fasting glucose level ≥126 mg/dL, a nonfasting glucose level ≥200 mg/dL, or by participant self-report of diabetes mellitus medication use. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or self-reported use of antihypertensive medications. Smoking status was by self-report.

Laboratory Methods

The overall laboratory design for REGARDS is published.[27] sCD14 was measured by ELISA in the REGARDS case–cohort sample (R&D Systems, Minneapolis, MN) using fasting serum collected at baseline and stored at −80°C. The interassay coefficients of variation were 7.9% to 9.0%. High-sensitivity CRP (C-reactive protein) was measured on a BNII nephelometer using particle-enhanced immunonephelometry (Dade Behring, Deerfield, IL) (coefficients of variation 2.1–5.7%). D-dimer was measured using an immuno-turbidometric assay on the STA-R automated coagulation analyzer (Diagnostica Stago, Parsippany, NJ) (coefficients of variation 3.2–26.8%).

Statistical Analyses

In the cohort random sample, cross-sectional associations of demographic variables, CVD risk factors, and inflammation biomarkers with sCD14 were assessed by linear regression models adjusted for age, sex, and race.

Relationships between baseline sCD14 and risk of incident ischemic stroke or CHD were evaluated using Cox proportional hazards models. Analyses used sample weighting so the stratified cohort sample would reflect the entire REGARDS cohort. 95% CIs were calculated using robust sandwich estimators.[28] sCD14 was analyzed per 1 SD increment and by age-, sex-, and race-specific quartiles of the study population's sCD14 distribution. Cox models were sequentially adjusted for demographic variables, Framingham stroke or CHD risk factors,[29,30] and the inflammation and thrombosis biomarkers, CRP and D-dimer.

We tested for sCD14-by-age, -sex, and -race interactions individually in the ischemic stroke and CHD models by including interaction terms (eg, sCD14×race). We chose a type I error rate of 0.10 for our tests of interactions a priori.[31] We hypothesized a priori that there were racial differences in the relationships between sCD14 with ischemic stroke and CHD. Testing for sCD14-by-age and sCD14-by-sex interactions occurred post hoc, guided by the significant correlations observed between sCD14 and age and sex in linear regression models. Since each of the 6 interaction terms were statistically significant ($P<0.10$), we used backwards stepwise elimination models to assess all possible 2- (eg, sCD14×age), 3- (eg, sCD14×age×race) and 4-way (ie,

sCD14×age×race×sex) interaction terms simultaneously in separate Cox models of stroke and CHD (for a total of 11 interaction terms entered as candidates in the starting models). Nonsignificant 4-way interaction terms were removed from models first, followed by removal of the least significant 3-way terms, with the least significant 2-way interaction terms removed last. Only statistically significant interaction terms were retained in the final models. In the final model for stroke, 4 interaction terms were retained (sCD14×race, age×race, age×sex, and race×sex). In the final model for CHD, 2 interactions terms were retained (sCD14×race and sCD14×age).

After exclusion of participants with missing values for sCD14, baseline stroke, and incident hemorrhagic stroke, 548 ischemic stroke cases, and 964 cohort random sample participants were included in the final ischemic stroke analysis. Demographic-adjusted Cox models included adjustment for age, sex, race, region, and an age×race interaction term (because of the known age-by-race interaction for stroke[32]). Risk factor models included adjustment for systolic blood pressure, use of antihypertensive medications, diabetes mellitus, current smoking, and baseline CVD, atrial fibrillation, and left ventricular hypertrophy.

Following exclusion of participants with missing values for sCD14 and those with CHD at the baseline examination, 612 CHD cases and 856 participants from the cohort random sample were included in the final CHD analysis. Demographic-adjusted CHD models included age, sex, race, and region. Risk factor–adjusted models included demographics plus systolic blood pressure, antihypertensive medication use, diabetes mellitus, current smoking, total-cholesterol, high-density lipoprotein cholesterol, and use of cholesterol-lowering medications. For stroke and CHD analyses, a third model included adjustment for CRP and D-dimer in addition to CVD risk factors. In a sensitivity analysis, we included additional adjustment for body mass index in all models of stroke and CHD.

Statistical analyses were performed using SAS 9.4. All authors had access to the data and contributed to the interpretation of the results.

Table 1. Baseline Characteristics of the REGARDS Case–Cohort Study Population

	Cohort Random Sample (n=1039)	Ischemic Stroke Cases (n=548)	CHD Cases (n=612)
Age (y), mean (SD)	68 (12)	70 (8)	68 (9)
Female sex, n (%)	518 (49.9)	266 (48.5)	224 (36.6)
Black race, n (%)	518 (49.9)	227 (41.4)	266 (43.5)
Region			
Stroke belt, n (%)	359 (34.6)	193 (35.2)	248 (40.5)
Stroke buckle, n (%)	187 (18.0)	111 (20.3)	100 (16.3)
Antihypertensive medications, n (%)	539 (51.9)	359 (65.5)	350 (57.2)
Systolic BP (mm Hg), mean (SD)	128 (18)	133 (17)	134 (19)
Total-cholesterol (mg/dL), mean (SD)	188 (39)	190 (43)	193 (42)
HDL-cholesterol (mg/dL), mean (SD)	52 (17)	50 (17)	48 (14)
Lipid-lowering medications, n (%)	331 (31.9)	193 (35.2)	170 (27.8)
Current smoker, n (%)	148 (14.2)	101 (18.4)	122 (19.9)
Former smoker, n (%)	393 (37.8)	222 (40.5)	264 (43.1)
BMI, mean (SD)	28.7 (5.8)	28.7 (5.8)	29.3 (6.2)
Diabetes mellitus, n (%)	214 (20.6)	150 (27.4)	193 (31.5)
CVD, n (%)	245 (23.6)	162 (29.6)	83 (13.6)
Atrial fibrillation, n (%)	93 (9.0)	78 (14.2)	73 (11.9)
LVH, n (%)	96 (9.2)	82 (15.0)	70 (11.4)
CRP (mg/L), median (25th, 75th)	2.1 (0.9, 4.9)	2.9 (1.2, 6.3)	3.0 (1.2, 5.8)
D-dimer (μg/mL), median (25th, 75th)	0.48 (0.26, 0.90)	0.55 (0.33, 0.92)	0.55 (0.33, 0.92)
sCD14 (pg/mL), mean (SD)	1911 (442)	1996 (450)	1963 (448)

The cohort random sample was selected with stratification by age, sex, and race, as described in the Methods. BMI indicates body mass index; BP, blood pressure; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease (defined as ECG evidence of myocardial infarction or participant self-report of CHD, stroke, or peripheral artery disease at the baseline examination); LVH, left ventricular hypertrophy; and REGARDS, Reasons for Geographic and Racial Differences in Stroke.

RESULTS

There were 548 incident ischemic stroke cases over a median 5.4 years of follow-up (interquartile range: 4.1–7.0 years) and 612 incident CHD events over a median 4.2 years of follow-up (interquartile range: 3.1–5.5 years). Table 1 shows the baseline characteristics of the cohort random sample (n=1039), ischemic stroke, and CHD cases. sCD14 levels were normally distributed with a mean (SD) of 1911 pg/mL (442 pg/mL) in the cohort random sample, 1996 pg/mL (450 pg/mL) in ischemic stroke cases, and 1963 pg/mL (448 pg/mL) among incident CHD cases.

As shown in Table 2, sCD14 levels were higher with older age, in women, and lower among blacks (all $P<0.0001$). Smoking, diabetes mellitus, CVD, and higher CRP and D-dimer levels were associated with higher sCD14 (in models adjusted for age, sex, and race).

In testing for interactions, for both stroke and CHD, the interaction terms for sCD14 and age, sex, and race were all statistically significant (all P-interaction <0.10). The backwards stepwise elimination models that simultaneously included all possible 2-, 3- and 4-way interaction terms showed that interactions of sCD14 with race for stroke (P-interaction=0.05), and with age and race for CHD (P-interaction=0.08 and 0.04, respectively), remained significant. These interaction terms were included in final models.

Table 3 shows associations of sCD14 with ischemic stroke risk. As there was a statistically significant interaction for sCD14-by-race, analyses were stratified by race. Adjusted for Framingham stroke risk factors, each SD higher sCD14 was associated with a HR of 1.42 for ischemic stroke among black participants (95% CI: 1.12, 1.80) and there was no association among whites (HR: 1.02; 95% CI: 0.82, 1.27). The association of sCD14 with stroke in blacks was minimally attenuated by added adjustment for CRP and D-dimer (HR: 1.36; 95% CI: 1.06, 1.74) (Table 3). Interpretations were similar considering sCD14 in age-, sex-, and race-specific quartiles; the HRs of stroke for sCD14 in the fourth versus first quartile were 1.90 (95% CI: 1.04, 3.45) among black participants and 0.91 (95% CI: 0.53, 1.57) among whites (Table S1). Interpretation of results was the

Table 2. Associations of Cardiovascular Disease Risk Factors With sCD14 in the REGARDS Cohort Random Sample

	sCD14 (pg/mL)	
	Difference in sCD14 (95% CI)	*P* Value
Age (per 12.2 y)	84 (58, 109)	<0.0001
Female sex	129 (77, 180)	<0.0001
Black race	−160 (−212, −109)	<0.0001
Region		
Nonstroke belt region	0.0 (Reference)	
Stroke belt	6.3 (−52, 64)	0.83
Stroke buckle	17.6 (−55, 89)	0.63
Antihypertensive medications use (yes vs no)	48 (−6.0, 103)	0.08
Systolic BP (per 17 mm Hg)	10.7 (−16.1, 37.5)	0.44
Total cholesterol (per 39 mg/dL)	11.4 (−14.9, 37.6)	0.40
HDL cholesterol (per 17 mg/dL)	−20.9 (−49.1, 7.3)	0.15
Lipid-lowering medications (yes vs no)	4.1 (−52, 60)	0.89
Smoking status		
Never smoker	0.0 (Reference)	
Former smoker	37 (−20, 93)	0.21
Current smoker	152 (72, 232)	0.0002
BMI (per 5.8 kg/m^2)	−37 (−64, −10.3)	0.007
Diabetes mellitus	131 (67, 194)	<0.0001
Baseline CVD	110 (48, 173)	0.0005
Atrial fibrillation	85 (−5.6, 176)	0.07
LVH	17.2 (−72, 107)	0.71
CRP (per 1.20 mg/L)	89 (63, 115)	<0.0001
D-dimer (per 0.89 µg/mL)	52 (23, 84)	0.0006

Differences in sCD14 levels were estimated by linear regression model beta coefficients per SD higher exposure variable values (shown in parentheses), with adjustment for age, sex, and race (age, sex, and race estimates were only adjusted for the 2 remaining variables). The SD (standard deviation) of sCD14 was 442 pg/mL. CRP and D-dimer were natural log-transformed. BMI indicates body mass index; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; LVH, left ventricular hypertrophy; and REGARDS, Reasons for Geographic and Racial Differences in Stroke.

Table 3. Associations of 1-SD Higher sCD14 With Incident Ischemic Stroke Stratified by Race

	HR of Ischemic Stroke (95% CI) Per SD Increment sCD14
Model 1	
Black	1.41 (1.16, 1.71)
White	1.07 (0.90, 1.29)
Model 2	
Black	1.42 (1.12, 1.80)
White	1.02 (0.82, 1.27)
Model 3	
Black	1.36 (1.06, 1.74)
White	0.99 (0.80, 1.24)

Model 1 adjusted for age, sex, region, and age×sex. Race×sCD14 P value=0.05. Model 2 adjusted for variables in Model 1 plus baseline systolic blood pressure, antihypertensive medication use, smoking, diabetes mellitus, cardiovascular disease, atrial fibrillation, and left ventricular hypertrophy. Race×sCD14 P value=0.04. Model 3 adjusted for variables in Model 2 plus C-reactive protein and D-dimer. Race×sCD14 P value=0.06. HR indicates hazard ratio.

Table 4. Associations of 1 SD Higher sCD14 With Incident Coronary Heart Disease at Different Ages Stratified by Race

	HR of CHD (95% CI) Per SD Increment sCD14	
	White	Black
Model 1		
Point estimate at age 45 y	1.51 (0.95, 2.41)	2.04 (1.30, 3.22)
Point estimate at age 55 y	1.31 (0.95, 1.82)	1.78 (1.28, 2.46)
Point estimate at age 65 y	1.14 (0.92, 1.42)	1.54 (1.22, 1.95)
Point estimate at age 75 y	0.99 (0.81, 1.21)	1.34 (1.06, 1.69)
Point estimate at age 85 y	0.86 (0.65, 1.14)	1.16 (0.85, 1.60)
Model 2		
Point estimate at age 45 y	1.56 (0.94, 2.57)	2.30 (1.45, 3.65)
Point estimate at age 55 y	1.26 (0.88, 1.80)	1.87 (1.35, 2.58)
Point estimate at age 65 y	1.02 (0.80, 1.31)	1.51 (1.20, 1.91)
Point estimate at age 75 y	0.73 (0.66, 1.04)	1.23 (0.95, 1.58)
Point estimate at age 85 y	0.67 (0.49, 0.93)	0.99 (0.69, 1.43)
Model 3		
Point estimate at age 45 y	1.58 (0.83, 2.68)	2.51 (1.53, 4.12)
Point estimate at age 55 y	1.25 (0.86, 1.81)	1.98 (1.40, 2.81)
Point estimate at age 65 y	0.99 (0.77, 1.27)	1.57 (1.22, 2.02)
Point estimate at age 75 y	0.78 (0.62, 0.99)	1.24 (0.95, 1.62)
Point estimate at age 85 y	0.62 (0.44, 0.87)	0.98 (0.67, 1.44)

Age was included in the models as a continuous variable and results present point estimates for CHD risk at specific ages. Model 1 adjusted for age, sex, region, and age×sCD14. Age×sCD14 *P* value=0.08; Race×sCD14 *P* value=0.04. Model 2 adjusted for variables in Model 1 plus baseline hypertension medication use, systolic blood pressure, smoking, total cholesterol, HDL cholesterol, lipid-lowering medication, and diabetes mellitus. Age×sCD14 *P* value=0.02; Race×sCD14 *P* value=0.02. Model 3 adjusted for variables in Model 2 plus C-reactive protein and D-dimer. Age×sCD14 *P* value=0.01; Race×sCD14 *P* value=0.006. CHD indicates coronary heart disease; HDL, high-density lipoprotein; and HR, hazard ratio.

same in sensitivity analyses that added adjustment for body mass index. For example, in a model adjusted for stroke risk factors (Model 2) plus body mass index, the HR (95% CI) for ischemic stroke among blacks was 1.41 (1.11, 1.79) and among whites was 1.01 (0.81, 1.26).

Table 4 shows associations of sCD14 with CHD risk. As interaction terms for sCD14-by-race and sCD14-by-age were statistically significant, analyses were stratified by race and point estimates presented for different ages. Each 1-SD higher sCD14 was associated with an increased CHD risk in blacks, and the relationship was stronger in younger compared with older participants. For example, adjusted for risk factors, the HR of CHD per SD higher sCD14 among blacks at age 45 years was 2.30 (95% CI: 1.45, 3.65) compared with 1.51 (95% CI: 1.20, 1.91) at age 65 years and 1.23 (0.95, 1.58) at age 75 years. This pattern was similar in whites (*P*-interaction of sCD14×age=0.03 among whites in model 2), but the association of sCD14 with incident CHD was nominally smaller in magnitude and not statistically significant. For example, the HR in whites at age 45 years was 1.56 (95% CI: 0.94, 2.57), while there was an inverse association at age 85 years. Among blacks, addition of CRP and D-dimer to the model modestly accentuated the HRs but did not impact them in whites (Table 4). Analyzed as age-, sex-, and race-specific sCD14 quartiles, the CHD HR was 2.03 (95% CI: 1.11, 3.72) among blacks in the fourth versus first sCD14 quartile (Table S2). Among whites, the CHD HR was 0.61 (95% CI: 0.32, 1.14) comparing the fourth with the first quartile (Table S2). In sensitivity analyses that included body mass index as a covariate, interpretation of the results did not change.

DISCUSSION

In a prospective population-based study of black and white men and women living across the contiguous United States, higher circulating sCD14 was associated with risk of incident ischemic stroke and CHD among blacks but not whites. The association with CHD was greater at younger ages. These findings suggest sCD14 may be a race-specific risk marker for ischemic stroke and CHD.

Ours is 1 of only a few studies to evaluate prospective relationships of sCD14 with incident stroke or CHD.[15–17] CD14 is a pattern recognition receptor that contributes to pro-inflammatory responses,[3] and circulating sCD14 concentrations increase during inflammatory conditions.[5] The observed relationships here between higher sCD14 and increased risk of stroke and CHD among blacks are consistent with a role of innate immunity and chronic inflammation in the progression of CVD.

To our knowledge, race and age differences in the associations of sCD14 with risk of stroke or CHD have not been reported. Higher sCD14 was associated with increased risk of stroke and CHD in the CHS (Cardiovascular Health Study), a cohort of black and white adults ≥65 years, but race-stratified associations were not reported and the number of black participants was relatively low.[15] Results here showing that higher sCD14 was related to ischemic stroke

and CHD risk in blacks, but not whites, may suggest that findings from the CHS were driven by the African-American participants. Consistent with results in REGARDS, CHS reported statistically significant differences in sCD14 levels by age, sex, and race. The lack of association of sCD14 with CHD risk among whites in the current study is consistent with findings from the PRIME Study showing sCD14 was not related to incident CHD among a cohort of European men aged 50 to 59 years,[17] and the Framingham Heart Study (comprising white men and women, mean age 62 years) reporting that sCD14 was not associated with incident atherosclerotic CVD (defined as a composite end point of nonfatal myocardial infarction, revascularization, atherothrombotic stroke, and CHD death).[16]

Despite black participants having higher levels of other inflammation biomarkers compared with whites in REGARDS,[33] sCD14 levels were lower in blacks than whites. This is consistent with results from the CHS.[15] These differences may be explained in part by interpopulation heterogeneity in CD14 variant allele frequencies. The minor allele frequency of a polymorphism in the *CD14* promoter, rs2569190 (also referred to as C-159T or C-260T), associated with higher sCD14 levels, is more common among those of European than African ancestry.[34] A sCD14-lowering haplotype identified in the CHS was present only among African-Americans (tagged by rs5744451; minor allele frequency: 9%).[15]

Lower basal-state concentrations of sCD14 among blacks may suggest that, compared with whites, a lower sCD14 threshold is required to reflect a risk of stroke or CHD. sCD14 is generated by a number of different mechanisms, including hepatic secretion during an acute-phase response, cell surface cleavage of membrane-bound CD14, and release of CD14-bearing microvesicles.[5–7,35] As such, racial differences in stroke or CHD risk in relation to sCD14 may also reflect the importance of distinct sources of sCD14. For example, African-Americans have lower neutrophil counts (which express CD14) compared with European-Americans.[36] A greater proportion of sCD14 generated by hepatocytes in blacks compared with whites would be consistent with a greater burden of chronic inflammation. If a greater proportion of sCD14 among blacks was generated from cells (such as monocytes) or cellular processes (such as microvesicle release) implicated in CVD risk,[35,37] this could also potentially account for a stronger association with CVD events. Results may also be explained by interrelationships of sCD14 with other CVD-related factors that differ by race.

In addition to an interaction with race, we identified a significant sCD14-by-age interaction on the risk of CHD. This suggests sCD14 may be a stronger risk marker for CHD risk among adults at younger ages, particularly among blacks. Although sCD14 was not statistically significantly associated with CHD risk among whites, there was a similar trend for stronger relationships at younger ages. While reasons for potential differences between sCD14 and CHD risk by age are unknown, weaker associations at older ages may reflect the increased importance of other risk factors, or their interrelationships, that track with advancing age. This hypothesis is generally consistent with previous studies demonstrating that relationships between causal risk factors such as cholesterol and hypertension and CHD risk are diminished in the elderly.[38]

The implications of these findings to clinical practice are unknown. Currently, there is no evidence that sCD14 improves CVD risk prediction. Genetic studies do not support a causal role of sCD14 towards CVD risk because a common polymorphism in the *CD14* promoter (C-260T) was not associated with incident CHD or cerebrovascular disease in meta-analyses comprising predominately European-ancestry populations.[39,40] The observed associations of sCD14 with stroke and CHD in the present study, however, were independent of the acute-phase protein CRP, a risk marker for stroke and CHD. This suggests that sCD14 represents different aspects of inflammation than CRP. Further study of the potential utility of CRP and sCD14 in risk assessment would be informative. Given the observed race-specific associations of sCD14 with stroke and CHD, evaluation of sCD14 may be especially useful among African-Americans. Our findings also raise hypotheses into potential genetic and/or environmental factors between sCD14 and CVD risk that differ by race.

Limitations of this study warrant consideration. Identification of significant sCD14-by-race, -age, and -sex interactions introduced complexity into our statistical analyses and the interpretation of our results. While we had hypothesized sCD14-by-race interactions a priori, the interactions with age and sex were post hoc. We cannot rule out the possibility of residual confounding or chance findings. Given that there are only limited data investigating sCD14 and CVD risk by racial group, replication of our findings, as well as genetic association studies of sCD14 in cohorts of African-Americans and other race/ethnic groups, are important. REGARDS does not have genetic data available for the entire case–cohort study sample and this is a limitation. Strengths of the study also merit discussion. REGARDS is a contemporary prospective cohort study recruited during the statin era, with low rates of loss to follow-up and detailed phenotyping of participants based on in-person baseline visits and frequent telephone contact. Measurement of risk factors, including sCD14, prior to stroke or CHD events minimizes the likelihood of reverse causality for associations that would be possible in a retrospective study.

In summary, higher sCD14 was associated with risk of ischemic stroke in blacks but not whites, and with increased CHD risk in blacks, with a greater CHD risk at younger ages. These findings suggest sCD14 may be a race-specific CVD risk marker among black adults and could have implications for understanding racial differences in other chronic inflammatory diseases. Further research on sCD14 and CVD, and other health risks, in blacks is important.

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Disclosures

None.

Supplementary Material

Tables S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Race-specific hazards ratios for incident ischemic stroke across quartiles of sCD14.

Ischemic Stroke	Q1	Q2	Q3	Q4
n Stroke / N at Risk: Black	54 / 236	49 / 230	65 / 224	59 / 219
n Stroke / N at Risk: White	73 / 285	78 / 291	85 / 277	85 / 276
Model 1 (HR 95% CI)				
Black	1.00 (ref)	1.28 (0.75, 2.21)	1.60 (0.96, 2.65)	2.48 (1.45, 4.23)
White	1.00 (ref)	0.81 (0.50, 1.30)	1.25 (0.76, 2.05)	1.07 (0.66, 1.74)
Model 2 (HR 95% CI)				
Black	1.00 (ref)	1.18 (0.66, 2.11)	1.42 (0.84, 2.41)	1.90 (1.04, 3.45)
White	1.00 (ref)	0.77 (0.47, 1.28)	1.10 (0.64, 1.89)	0.91 (0.53, 1.57)
Model 3 (HR 95% CI)				
Black	1.00 (ref)	1.23 (0.68, 2.22)	1.32 (0.76, 2.28)	1.69 (0.90, 3.17)
White	1.00 (ref)	0.73 (0.44, 1.23)	1.13 (0.65, 1.96)	0.84 (0.48, 1.47)

Model 1 adjusted for age, sex, region, and age*sex.

Model 2 adjusted for variables in Model 1 plus baseline systolic blood pressure, antihypertensive medication use, smoking, diabetes, cardiovascular disease, atrial fibrillation, and left ventricular hypertrophy.

Model 3 adjusted for variables in Model 2 plus C-reactive protein and D-dimer.

sCD14 was evaluated as age-, sex-, and race-specific quartiles of the sCD14 distribution:

			sCD14 [pg/mL]			
Age	**Sex**	**Race**	**Q1**	**Q2**	**Q3**	**Q4**
>68	F	B	1048-1679	1680-1924	1925-2219	2220+
>68	F	W	1174-1830	1831-2110	2111-2408	2409+
>68	M	B	859-1509	1510-1808	1809-2103	2104+
>68	M	W	922-1698	1699-1906	1907-2206	2207+
≤68	F	B	865-1540	1541-1794	1795-2180	2181+
≤68	F	W	1189-1778	1779-1992	1993-2304	2305+
≤68	M	B	802-1460	1461-1716	1717-2007	2008+
≤68	M	W	1055-1617	1618-1832	1833-2104	2105+

Table S2. Race-specific hazards ratios for incident coronary heart disease (CHD) across sCD14 quartiles.

CHD	Q1	Q2	Q3	Q4
n Stroke / N at Risk: Black	60 / 223	67 / 221	64 / 219	75 / 199
n Stroke / N at Risk: White	89 / 254	86 / 257	87 / 241	84 / 231
Model 1 (HR 95% CI)				
Black	1.00 (ref)	1.54 (0.93, 2.57)	1.28 (0.78, 2.11)	2.47 (1.47, 4.13)
White	1.00 (ref)	0.79 (0.48, 1.31)	1.13 (0.66, 1.93)	0.72 (0.55, 1.54)
Model 2 (HR 95% CI)				
Black	1.00 (ref)	1.66 (0.97, 2.84)	1.14 (0.66, 1.97)	1.98 (1.11, 3.53)
White	1.00 (ref)	0.80 (0.47, 1.37)	1.20 (0.68, 2.11)	0.66 (0.36, 1.20)
Model 3 (HR 95% CI)				
Black	1.00 (ref)	1.68 (0.95, 2.96)	1.09 (0.61, 1.98)	2.03 (1.11, 3.72)
White	1.00 (ref)	0.76 (0.40, 1.34)	1.16 (0.63, 2.11)	0.61 (0.32, 1.14)

Model 1 adjusted for age, sex, and region. Race*sCD14 quartile p-value=0.05; Age*sCD14 quartile p-value=0.55.

Model 2 adjusted for variables in Model 1 plus baseline hypertension medication use, systolic blood pressure, smoking, total-cholesterol, HDL-cholesterol, lipid-lowering medication, and diabetes. Race*sCD14 quartile p-value=0.03; Age*sCD14 quartile p-value=0.23.

Model 3 adjusted for variables in Model 2 plus C-reactive protein and D-dimer. Race*sCD14 quartile p-value=0.02; Age*sCD14 quartile p-value=0.35.

sCD14 was evaluated as age-, sex-, and race-specific quartiles of the sCD14 distribution as presented in the footnote in Supplemental Table 1.