

Proprotein Convertase Subtilisin/Kexin 9 Levels in Relation to Systemic Immune Activation and Subclinical Coronary Plaque in HIV

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Background. Proprotein convertase subtilisin/kexin 9 (PCSK9) is known to mediate homeostasis of low-density lipoprotein cholesterol (LDL-c), but it may also participate in immune reactivity and atherogenesis.

Methods. We compared circulating PCSK9 levels among asymptomatic individuals with and without HIV. Further, within each group, we assessed the relationship between PCSK9 levels, traditional cardiovascular disease risk factors, immune activation, and subclinical coronary atherosclerotic plaque.

Results. PCSK9 levels were higher among HIV-infected ($n = 149$) vs matched non-HIV-infected subjects ($n = 69$; 332 [272, 412] ng/mL vs 304 [257, 375] ng/mL; $P = .047$). Among HIV-infected subjects, significant albeit modest positive associations were noted between PCSK9 levels and markers of systemic monocyte activation including sCD14 ($\rho = 0.22$; $P = .009$) and sCD163 ($\rho = 0.23$; $P = .006$). In this group, PCSK9 levels related weakly to LDL-c ($\rho = 0.16$; $P = .05$) and also to Framingham Point Score but did not relate to subclinical coronary atherosclerotic plaque parameters.

Conclusions. Among HIV-infected individuals, circulating PCSK9 levels are elevated and related to systemic markers of monocyte activation but not to coronary plaque parameters. Additional studies are needed to determine the effects of PCSK9 on immune activation and atherogenesis in HIV and to assess whether PCSK9 inhibition reduces immune activation and coronary atherosclerotic plaque burden.

Clinical Trial Registration. NCT00455793.

Keywords. cardiovascular disease risk; HIV; inflammation; lipids; plaque; PCSK9.

A series of elegant discoveries in the 2000s increased our understanding of the critical role proprotein convertase subtilisin/kexin 9 (PCSK9) plays in low-density lipoprotein cholesterol (LDL-c) homeostasis. In 2003, PCSK9 gain-of-function mutations were identified as causing autosomal dominant hypercholesterolemia, characterized by markedly elevated LDL-c levels [1]. Next, PCSK9 loss-of-function, nonsense mutations were shown to be associated with life-long low LDL-c levels and a dramatic reduction in the risk of coronary heart disease [2]. Protein structure/function analyses revealed that PCSK9, highly expressed by hepatocytes and intestinal cells, promotes degradation of the cell surface LDL

receptor (LDLR), impeding cellular uptake of bloodstream LDL-c [3]. Monoclonal antibodies inhibiting PCSK9 were developed, and these therapies, by preventing degradation of the cell surface LDLR, elicited striking 40%–70% reductions in circulating LDL-c among patients at high risk for cardiovascular disease (CVD) [4].

Intriguingly, a pro-inflammatory milieu stimulates diverse cell types to release PCSK9 into the circulation [5]. Indeed, patients with bacterial infections [6] and viral infections [7] have been shown to have elevated PCSK9 levels, which, notably, do not track with levels of LDL-c. Conversely, PCSK9 may elicit pro-inflammatory responses in monocytes/macrophages, with relevance to systemic immune activation, arterial inflammation, and atherogenesis/atherothrombosis [8]. In select general population studies, PCSK9 levels related to major adverse cardiovascular events, even after controlling for levels of LDL-c [9, 10].

In the present study, we compared circulating levels of PCSK9 among asymptomatic individuals with and without HIV, considering the potential influence of concomitant hepatitis C virus (HCV) infection. As individuals with HIV are known to have an increased prevalence of subclinical coronary atherosclerotic plaque and high-level systemic immune activation [11–13], we

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therefore assessed, within the HIV-infected group and separately within the non-HIV-infected group, whether PCSK9 levels related to levels of systemic monocyte activation markers and/or to subclinical coronary atherosclerotic plaque parameters. Our primary hypothesis was that PCSK9 levels would be elevated among individuals with HIV (vs age/sex-matched, non-HIV-infected control subjects). Further, we hypothesized that among individuals with HIV, PCSK9 levels would relate to levels of systemic monocyte activation markers and/or measures of coronary atherosclerotic plaque burden.

METHODS

Study Design and Subjects

Asymptomatic individuals with and without HIV age 18–60 years were prospectively recruited into a cross-sectional study assessing subclinical atherosclerosis in relation to immune and metabolic parameters [11–13]. HIV-infected subjects were recruited primarily from HIV clinics and community health centers in the Boston area. Non-HIV-infected individuals were recruited as control subjects from the same communities. Subjects in both groups were required to have no current or prior history of cardiovascular disease. All participants provided informed consent before initiating any study procedures. The study was approved by the Institutional Review Board of the Massachusetts General Hospital and the Massachusetts Institute of Technology (MIT) and is registered on clinicaltrials.gov (NCT0045579) [11, 13]. In the current analysis, 218 out of 234 originally recruited participants were included. Subjects with insufficient blood samples for PCSK9 testing ($n = 16$) were excluded.

Demographic and Clinical Characteristics

Medical history was elicited by interview during the screening and entry study visits. Framingham Point Score was calculated using standard methods. Duration of HIV infection, nadir CD4⁺ T cell count, and antiretroviral therapy (ART) history are per subject report.

Metabolic and Immune Parameters

Fasting total cholesterol, high-density lipoprotein cholesterol (HDL-c), LDL-c, triglycerides, creatinine, and glucose were assessed using standard techniques. Circulating PCSK9 levels were measured from serum samples with enzyme-linked immunosorbent assay (ELISA; R & D Systems; Minneapolis, MN; sensitivity 0.219 ng/mL; observed mean coefficient of variability 3.8% [0.2% SEM] between replicates). Immune and inflammatory biomarkers—soluble CD14 (sCD14), soluble CD163 (sCD163), monocyte chemoattractant protein-1 (MCP-1), lipoprotein-associated phospholipase A₂ (Lp-PLA₂), oxidized LDL (ox LDL), C-reactive protein (CRP), and high-sensitivity interleukin-6 (hsIL-6)—were assessed by ELISA as previously described [11–14]. For non-HIV-infected subjects, HIV testing was performed with chemiluminometric immunoassay. For HIV-infected subjects, CD4⁺ T-cell counts were determined with flow cytometry

and HIV viral load was assessed with ultrasensitive reverse-transcription polymerase chain reaction (Roche COBAS Amplicor).

Cardiac Computed Tomography Angiography

Cardiac computed tomography (CT) imaging was performed with a 64-slice CT scanner or 64-slice dual-source CT scanner (Siemens Medical Solutions), as previously described [11, 13]. Parameters including number of subclinical coronary artery plaque segments and number of noncalcified coronary plaque segments were assessed using previously described analytic techniques [11, 13].

Statistical Analysis

The primary end point for this analysis was the between-group difference in circulating PCSK9 levels among HIV-infected subjects and non-HIV-infected subjects. Normality of all variables was assessed with the Shapiro-Wilk test. Normally distributed variables are presented as mean \pm standard deviation. Non-normally distributed variables are presented as medians and interquartile ranges (IQRs). Categorical variables are displayed as percentages. For between-group comparisons of circulating PCSK9 levels, as well as previously assessed baseline characteristics, the following tests were employed: the Student's *t* test for normally distributed continuous variables; the Wilcoxon rank sum test for non-normally distributed continuous variables; and the χ^2 test for categorical variables. Multivariate regression modeling was performed to adjust for current statin use. A sensitivity analysis was also performed in which statin users ($n = 23$) were excluded, and circulating PCSK9 levels were compared between groups using the Wilcoxon rank sum test. The Kruskal-Wallis test was used to compare circulating PCSK9 levels across 4 groups: non-HIV-infected subjects (with and without HCV) and HIV-infected subjects (with and without HCV). As significant across-group differences were noted by the Kruskal-Wallis test, the Wilcoxon rank sum test was subsequently employed to identify significant between-group differences.

To assess for differences in baseline demographic parameters across the 4 groups delineated above, analysis of variance or Kruskal-Wallis tests were used, as appropriate. Within the HIV-infected group and separately within the non-HIV-infected group, Spearman's *rho* was employed to assess associations between circulating PCSK9 levels and cardiometabolic risk parameters. Statistical significance was defined as $P \leq .05$. All analyses were performed using JMP software (version 12.0; SAS Institute).

RESULTS

Baseline Characteristics of HIV-Infected and Non-HIV-Infected Individuals

Demographic and clinical characteristics of the subjects are displayed in Table 1. Age, sex, race, body mass index (BMI), hypertension, diabetes, and smoking did not differ significantly between groups.

Table 1. Baseline Characteristics Among HIV-Infected and Non-HIV-Infected Subjects^a

Parameter	Non-HIV-Infected (n = 69)	HIV-Infected (n = 149)	P Value	P Value Adjusted ^b
Demographics and traditional CVD risk parameters				
Race/ethnicity			.47	—
Asian	4% (3/69)	1% (1/149)		
White	49% (34/69)	52% (77/149)		
Black/African American	35% (24/69)	34% (51/149)		
American Indian/Alaskan Native	3% (2/69)	3% (4/149)		
More than 1 race	3% (2/69)	1% (2/149)		
Hispanic	6% (4/69)	9% (14/149)		
Male sex	59% (41/69)	65% (97/149)	.42	.52
Age, y	47 (43, 51)	48 (43, 52)	.33	.23
Current statin use	4% (3/67)	14% (20/146)	.03	—
Current HTN	17% (12/69)	22% (33/147)	.39	.53
Current DM	7% (5/69)	9% (14/148)	.59	.67
Current smoking	38% (26/69)	42% (62/148)	.56	.46
Total cholesterol, mg/dL	178 (159, 208)	178 (156, 205)	.98	.78
LDL-c, mg/dL	107 ± 31	103 ± 33	.36	.31
HDL-c, mg/dL	49 (42, 63)	49 (40, 60)	.64	.93
Triglycerides, mg/dL	84 (63, 126)	96 (77, 174)	.002	.03
BMI, kg/m ²	26.9 (23.9, 30.2)	26.4 (23.5, 29.8)	.27	.16
WHR	0.9 ± 0.1	0.9 ± 0.1	.31	.41
HCV infection	9% (6/69)	25% (37/149)	.003	.004
Total Framingham Point Score	9 (5, 11)	9 (7, 12)	.20	.26
HIV-specific parameters				
Duration since HIV diagnosis, y	—	14 (10, 19)	—	—
Currently on ART	—	99% (148/149)	—	—
Currently on NRTIs	—	95% (141/149)		
Currently on NNRTIs	—	38% (57/149)		
Currently on PIs	—	56% (83/149)		
Currently on integrase inhibitors	—	14% (20/146)		
Total duration of ART, y	—	8 (4, 11)	—	—
CD4 count, cells/mm ³	—	484 (347, 741)	—	—
Nadir CD4, cells/mm ³	—	180 (53, 263)	—	—
VL, copies/mL	—	<50 (<50, <50)	—	—
Immune parameters				
sCD14, ng/mL	326.5 (163.9, 1316.6)	431.1 (223.3, 1755.7)	.047	.08
sCD163, ng/mL	831.5 (607.9, 1134.3)	1224.9 (806.4, 1787.0)	<.0001	.001
MCP-1, pg/mL	223.5 (170.8, 273.0)	252.0 (179.0, 358.0)	.03	.045
Lp-PLA ₂ , nmol/min/mL	174.2 (147.0, 218.7)	188.9 (155.3, 234.2)	.10	.10
Oxidized LDL, U/L	44.4 (36.3, 56.5)	43.9 (35.2, 53.5)	.52	.51
CRP, mg/L	1.3 (0.5, 3.6)	1.4 (0.5, 4.1)	.60	.13
hsIL-6, pg/mL	1.0 (0.6, 1.8)	1.1 (0.7, 1.9)	.31	.77
Cardiac CT parameters^c				
Total No. of plaque segments	0.0 (0.0, 3.0)	1.0 (0.0, 3.0)	.09	.38
No. of noncalcified plaque segments	0.0 (0.0, 0.0)	0.0 (0.0, 1.8)	.003	.03
Miscellaneous				
PCSK9, ng/mL	304 (257, 375)	332 (272, 412)	.047	.02

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CRP, C-reactive protein; CT, computed tomography; DM, diabetes mellitus; HCV, hepatitis C virus; HDLc, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; hsIL-6, high-sensitivity interleukin-6; HTN, hypertension; LDLc, low-density lipoprotein cholesterol; Lp-PLA₂, lipoprotein-associated phospholipase A₂; MCP-1, monocyte chemoattractant protein-1; NNRTIs, Non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PCSK9, proprotein convertase subtilisin/kexin 9; PIs, protease inhibitors; sCD14, soluble CD14; sCD163, soluble CD163; VL, viral load; WHR, waist-to-hip ratio.

^aNormally distributed variables are presented as mean ± standard deviation; non-normally distributed data are presented as median (interquartile range).

^bP values are adjusted for current statin use.

^cBased on data from 206 participants who completed CT procedures.

The median Framingham Point Score was 9 in both groups. Total cholesterol, LDL-c, and HDL-c did not differ significantly between groups, but triglycerides were higher among

the HIV-infected subjects vs the non-HIV-infected subjects. Compared with non-HIV-infected subjects, HIV-infected subjects were noted to have a higher prevalence of HCV (25% vs 9%;

$P = .003$) and a higher prevalence of statin use (14% vs 4%; $P = .03$). For HIV-infected subjects, the median duration since diagnosis was 14 years (14 [10, 19] years); 99% of HIV-infected subjects reported current ART use, with a median duration of ART use of 8 years. The median CD4⁺ T cell count was 484 cells/mm³, and the median viral load was <50 copies/mL. Compared with non-HIV-infected subjects, HIV-infected subjects exhibited significantly higher levels of select markers of systemic immune activation, as well as a larger number of noncalcified coronary atherosclerotic plaque segments, in line with previously reported findings [11, 13]. After adjustment for current statin use, between-group differences in sCD163, MCP-1, and a number of noncalcified plaque segments remained significant. Supplementary Table 1 delineates demographic and clinical characteristics of subjects stratified by both HIV and HCV status.

Circulating PCSK9 Level by HIV Status and the Effect of HCV Status

PCSK9 levels were higher among HIV-infected vs non-HIV-infected subjects (332 [272, 412] ng/mL vs 304 [257, 375] ng/mL; $P = .047$) (Figure 1A). In a multivariate analysis controlling for current statin use, this relationship remained significant (adjusted $P = .02$). Furthermore, the between-group difference in PCSK9 levels remained significant in a sensitivity analysis performed after the exclusion of current statin users ($P = .05$). When subjects were stratified by both HIV status and HCV status, an overall significant difference in PCSK9 levels between groups was observed by Kruskal-Wallis test ($P = .0003$) (Figure 1B). Between-group comparisons revealed significant effects of the following on PCSK9 levels: (1) HCV alone, as demonstrated by the difference between non-HIV-infected, non-HCV-infected and non-HIV-infected, HCV-infected subjects (294 [244, 360] ng/mL vs 441 [408, 499] ng/mL; $P = .0009$); (2) HIV alone, in the absence of HCV, as demonstrated by the difference between non-HIV-infected, non-HCV-infected and HIV-infected, non-HCV-infected subjects (294 [244, 360] ng/mL vs 318 [262, 404] ng/mL; $P = .046$); and (3) HCV and HIV combined, as demonstrated by the difference between non-HIV-infected, non-HCV-infected and HIV-infected, HCV-infected subjects (294 [244, 360] ng/mL vs 366 [301, 427] ng/mL; $P = .0006$). As noted above, in the non-HIV-infected group, HCV-infected subjects demonstrated higher PCSK9 levels than non-HCV infected subjects. By contrast, in the HIV-infected group, HCV-co-infected subjects did not demonstrate significantly higher PCSK9 levels than non-HCV-co-infected subjects.

Relationships Between PCSK9 Levels and Demographic, Systemic Immune, and Coronary Plaque Parameters

Relationships between PCSK9 levels and demographic, systemic immune, and coronary plaque parameters are presented in Table 2. Among non-HIV-infected subjects, PCSK9 levels related significantly to age ($\rho = 0.35$; $P = .003$) and to Framingham Point Score ($\rho = 0.51$; $P < .0001$). Among

HIV-infected subjects, PCSK9 levels related to select traditional CVD risk parameters including Framingham Point Score ($\rho = 0.33$; $P < .0001$), total cholesterol ($\rho = 0.28$; $P = .0005$), and LDL-c ($\rho = 0.16$; $P = .05$). Further, among HIV-infected subjects, significant positive associations were noted between PCSK9 levels and levels of systemic monocyte activation markers including sCD14 ($\rho = 0.22$; $P = .009$) and sCD163 ($\rho = 0.23$; $P = .006$). Among HIV-infected subjects, PCSK9 levels did not relate to subclinical coronary atherosclerotic plaque parameters—total number of plaque segments and number of noncalcified plaque segments. Significant relationships between PCSK9 levels and coronary plaque parameters were also not observed among non-HIV-infected subjects.

DISCUSSION

Asymptomatic HIV-infected individuals in our cohort had higher circulating PCSK9 levels than non-HIV-infected control subjects well matched on age, sex, and race. The relationship between HIV status and PCSK9 levels remained significant even after adjusting for use of statin medications, which are known to elicit increases in circulating PCSK9 levels [15]. In sensitivity analyses performed after excluding current statin users, PCSK9 levels were again noted to be higher among individuals with vs without HIV. A previously published study by Kohli et al. revealed that HIV/HCV-co-infected subjects had higher PCSK9 levels than HIV-mono-infected subjects and non-HIV-infected control subjects but that HIV-mono-infected subjects did not have higher PCSK9 levels than control subjects [7]. In our cohort, HCV infection was more common among HIV-infected subjects than non-HIV-infected subjects. In light of the findings by Kohli et al., we sought to disentangle the effects of HIV and HCV on PCSK9 levels. We noted that when all subjects with HCV were excluded from the analysis, PCSK9 levels were still higher among individuals with (vs without) HIV. This finding suggests for the first time a clear influence of HIV status on PCSK9 levels, independent of HCV. However, we also noted that HCV status positively influences PCSK9 levels. Considering non-HIV-infected individuals, those with HCV had higher PCSK9 levels than those without HCV. Of interest, PCSK9 levels among individuals co-infected with HIV and HCV were not significantly higher than levels among either those mono-infected with HCV or those mono-infected with HIV. That is, *either* HCV infection *or* HIV infection markedly increased PCSK9 levels, but not in an additive or synergistic fashion.

That our findings differ from those of Kohli et al. may relate to underlying differences in the cohorts studied. Subjects in the study by Kohli et al. were originally recruited into the SCOPE study across 5 categories—HIV-infected, ART-untreated; HIV-infected ART-treated/virally suppressed; HIV-infected ART-treated/virally non-suppressed; HIV-infected ART-untreated/virally suppressed (elite controllers); and non-HIV infected [7]. In the cohort of HIV-infected subjects we assessed, 99% were

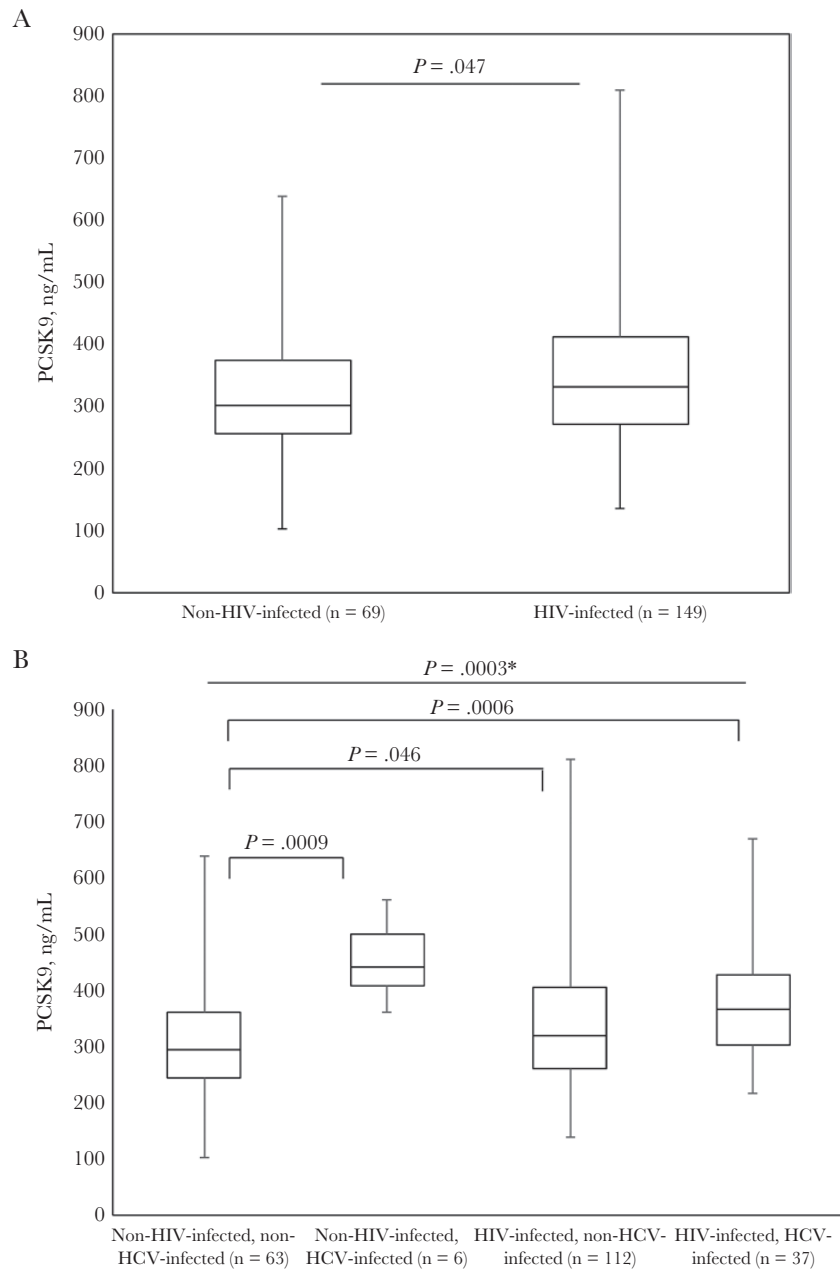


Figure 1. (A) PCSK9 levels among HIV-infected and non-HIV-infected subjects. PCSK9 was compared among HIV-infected vs non-HIV infected subjects. HIV-infected subjects (n = 149) exhibited higher PCSK9 levels relative to non-HIV-infected subjects (n = 69); 332 [272, 412] ng/mL vs 304 [257, 375] ng/mL; $P = .047$. Results are displayed as medians (horizontal lines) and interquartile ranges (upper and lower edges of boxes), and the error bars stretch from the quartiles to the maximum and minimum. (B) PCSK9 Levels by HIV and HCV Status. PCSK9 levels were compared among HIV-infected and non-HIV-infected subjects secondarily stratified by hepatitis C virus (HCV) infection. An overall Kruskal-Wallis test revealed a significant difference in PCSK9 levels across groups (overall P value by Kruskal-Wallis test). Between-group comparisons were then made using the Wilcoxon rank sum test. Significant between-group differences in PCSK9 levels were noted when the following comparisons were made: (1) non-HIV-infected, non-HCV-infected vs non-HIV-infected, HCV-infected subjects [294 [244, 360] ng/mL vs 441 [408, 499] ng/mL; $P = .0009$]; (2) non-HIV-infected, non-HCV-infected vs HIV-infected, non-HCV-infected subjects [294 [244, 360] ng/mL vs 318 [262, 404] ng/mL; $P = .046$]; and (3) non-HIV-infected, non-HCV-infected vs HIV-infected, HCV-infected subjects [294 [244, 360] ng/mL vs 366 [301, 427] ng/mL; $P = .0006$]. Results are displayed as medians (horizontal lines) and interquartile ranges (upper and lower edges of boxes), and the error bars stretch from the quartiles to the maximum and minimum.

on ART and the median VL was suppressed at <50 copies/mL. Thus, this cohort is comprised of a highly clinically relevant population, similar to the majority of those under treatment in the current era of ART. Of note, our cohort featured few

subjects with HCV mono-infection, limiting the power to analyze isolated HCV effects on PCSK9 levels.

In our cohort, among asymptomatic HIV-infected subjects, we noted a significant relationship between circulating PCSK9

Table 2. Associations Between PCSK9 Levels and Cardiometabolic Risk Parameters Among Individuals With and Without HIV

	Non-HIV-Infected		HIV-Infected	
	Spearman's <i>rho</i>	<i>P</i> Value	Spearman's <i>rho</i>	<i>P</i> Value
Demographics and traditional CVD risk parameters				
Age, y	0.35	.003	0.002	.98
Total cholesterol, mg/dL	0.13	.31	0.28	.0005
LDLc, mg/dL	0.07	.58	0.16	.05
HDLc, mg/dL	0.14	.26	0.16	.04
Triglycerides, mg/dL	0.22	.07	0.02	.77
BMI, kg/m ²	0.15	.21	0.02	.77
WHR	0.05	.69	-0.13	.14
Total Framingham Point Score	0.51	<.0001	0.33	<.0001
HIV-specific parameters				
Duration since HIV diagnosis, y	—	—	0.007	.93
Total duration of ART, y	—	—	-0.07	.50
CD4 count, cells/mm ³	—	—	0.02	.80
Nadir CD4, cells/mm ³	—	—	0.10	.30
LogVL	—	—	-0.12	.19
Immune parameters				
sCD14, ng/dL	0.02	.90	0.22	.009
sCD163, ng/mL	-0.05	.67	0.23	.006
MCP-1, pg/mL	0.17	.18	0.02	.78
Lp-PLA ₂ , U/L	-0.02	.89	0.01	.86
Oxidized LDL, U/L	0.14	.25	0.10	.24
CRP, mg/L	-0.21	.09	0.04	.64
hsIL-6, pg/mL	-0.05	.72	0.16	.07
Cardiac CT parameters				
Total No. of plaque segments	-0.11	.37	-0.009	.92
No. of noncalcified plaque segments	-0.11	.37	0.001	.99

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CRP, C-reactive protein; CT, computed tomography; CVD, cardiovascular disease; HDLc, high density lipoprotein cholesterol; HIV, human immunodeficiency virus; hsIL-6, high sensitivity interleukin-6; LDLc, low density lipoprotein cholesterol; Lp-PLA₂, lipoprotein-associated phospholipase A₂; MCP-1, monocyte chemoattractant protein-1; PCSK9, proprotein convertase subtilisin/kexin 9; sCD14, soluble CD14; sCD163, soluble CD163; VL, viral load; WHR, waist-to-hip ratio.

levels and levels of markers of systemic monocyte activation—sCD163 and sCD14. By contrast, among non-HIV-infected subjects, PCSK9 levels related only to traditional CVD risk factors—age and Framingham Point Score. Individuals with HIV are known to have heightened systemic immune activation, even when HIV viral load is well controlled by ART [16]. Ex vivo cell-based studies have suggested that inflammatory mediators may trigger diverse cell types to release PCSK9 into the circulation. For example, LPS, TNF- α , and CRP have all been shown to stimulate PCSK9 expression [17–19]. Conversely, cell-based studies, murine model studies, and human physiology studies implicate PCSK9 as a mediator of monocyte/macrophage activation [8, 20]. To our knowledge, ours is the first study relating circulating PCSK9 levels to markers of monocyte activation in HIV. Additional studies are needed to determine the causal directionality of the observed relationship in HIV. Moreover, dedicated study will be required to better understand how PCSK9 levels are influenced by HCV—a driver of systemic and in situ hepatic inflammation [21]. Of interest, HCV virions may use the LDLR to infect hepatocytes [22]. Thus, elevated PCSK9 levels, by increasing LDLR degradation, may theoretically confer a measure of antiviral protection by reducing HCV infectivity

[23]. Genotype-specific effects of HCV on PCSK9 and LDLR have also been suggested [24].

Contrary to our initial hypothesis, circulating PCSK9 levels did not relate to subclinical coronary atherosclerotic plaque parameters among asymptomatic HIV-infected subjects. Moreover, PCSK9 levels related only modestly to LDL-c in this group. A fascinating literature has emerged suggesting that PCSK9 secreted by arterial wall endothelial and smooth muscle cells [25] may promote inflammation and atherogenesis in situ through the following mechanisms: (1) upregulating endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), thus facilitating monocytes-to-endothelial adhesion [25]; (2) promoting a pro-inflammatory response by local macrophages [8, 26, 27]; and (3) inhibiting macrophage expression of ATP-binding cassette subfamily A member 1 (ABCA1), thus impeding reverse cholesterol transport and engendering foam cell formation [28]. Indeed, in murine models, PCSK9 has been shown to accumulate in the arterial wall, influencing atheroma size and composition, independent of LDL-c levels [29]. In pathologic studies, PCSK9 has been detected in human atherosclerotic plaques [30]. Further, general population clinical studies have shown that PCSK9 levels relate to carotid and

coronary plaque burden even after controlling for LDL-c and/or additional CVD risk factors [31–34]. Of interest, in a large intravascular ultrasound virtual histology study conducted among individuals with acute coronary syndrome or stable angina (who were not known to be HIV infected), PCSK9 levels did not relate to total plaque burden but rather to the fraction of plaque consisting of necrotic core tissue and to the absolute volume of necrotic core (after adjusting for LDL-c) [35]. Future studies will be needed to determine whether PCSK9 contributes to the development of thin-capped fibroatheroma (thin cap, thick necrotic core) in HIV.

A strength of the current study includes a focus on a contemporary cohort of HIV-infected individuals without prior CVD, with suppressed viremia on ART. Moreover, the matching of our non-HIV-infected control subjects to the HIV-infected subjects on the criteria of age, sex, and race represents a strength. Finally, the wealth of available phenotypic data in this cohort—including detailed immunologic and coronary plaque parameters—facilitated novel analyses on relationships between PCSK9 levels and both traditional and nontraditional cardiometabolic risk parameters among asymptomatic individuals with and without HIV. Study limitations include (1) the cross-sectional design, which precludes definitive inferences on the causality of observed relationships; (2) the small number of participants who were non-HIV-infected and HCV-infected, limiting the strength of inferences about the effects of HCV mono-infection on PCSK levels; (3) the absence of subclinical atherosclerotic plaque in a sizable fraction of the studied subjects (who were selected by study design to be asymptomatic, with no known cardiovascular disease), limiting power to detect relationships between PCSK9 levels and plaque parameters; and (4) the uncertain clinical significance of modest differences in PCSK9 levels among studied individuals with and without HIV. Additional research is needed to determine the relationship between PCSK9 and inflammation and to determine whether circulating PCSK9 levels influence atherogenesis in HIV. A recent study in the general population showed that PCSK9 levels do not predict LDL-c response to PCSK9 inhibitors [36], suggesting that PCSK9 levels should not be used to influence PCSK9 inhibitor treatment decisions. Studies in the general population have also shown that PCSK9 inhibition reduces plaque burden [37] and major adverse cardiovascular events [38] among high-risk patients on statins; however, it is unclear the extent to which these effects are driven by LDL lowering. Of note, in a large, general population study, PCSK9 inhibition did not result in a reduction in circulating levels of select pro-inflammatory cytokines [39]. It remains to be determined whether PCSK9 inhibition in HIV influences cytokine levels or plaque and whether baseline PCSK9 levels predict the magnitude of immune-modulatory or plaque response.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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