Open Forum Infectious Diseases

MAJOR ARTICLE

Association Between Inflammation and Coagulation Biomarkers and Carotid Atherosclerosis Among Treated People With Human Immunodeficiency Virus

Bowen Zhu,[1,2,3] Xiaoxiao Chen,[4] Weiwei Shen,[4] Yingying Ding,[1,2,3] Haijiang Lin,[1,4] and Na He[1,2,3]

[1]School of Public Health, Fudan University, Shanghai, China, [2]Key Laboratory of Public Health Safety of Ministry of Education, Fudan University, Shanghai, China, [3]Yiwu Research Institute of Fudan University, Shanghai, China, and [4]Taizhou City Center for Disease Control and Prevention, Zhejiang Province, China

Background. Atherosclerotic cardiovascular disease (CVD) is a common cause of morbidity among people with human immunodeficiency virus (PWH) who initiate antiretroviral therapy (ART). Little is known about the roles of inflammation in atherosclerotic CVD among PWH.

Methods. This cross-sectional evaluation included 178 PWH between 40 and 70 years on stable (>3 months) ART who were derived from the ongoing, prospective cohort for Comparative HIV and Aging Research in Taizhou (CHART), China, from February 2017 to August 2018. Carotid intima-media thickness (cIMT) ≥1 mm was considered as cIMT thickening indicative of atherosclerotic CVD. Plasma inflammation and coagulation biomarkers were quantified by a multiplex bead cytokine assay for 27 cytokines and enzyme-linked immunosorbent assay (ELISA) for soluble CD14 and D-dimer, respectively. We performed a series of multiparametric analyses of biomarkers and developed a composite score for atherosclerotic CVD assessment among PWH.

Results. Of 178 PWH, 53 (30.9%) had cIMT thickening. In multivariable logistic analysis adjusting for CVD and human immunodeficiency virus-specific risk factors, interleukin (IL)-4 (odds ratio [OR] = 19.0; 95% confidence interval [CI], 1.6–226.5), IL-7 (OR = 16.7; 95% CI, 1.8–151.7), IL-10 (OR = 11.9; 95% CI, 2.0–72.1), and D-dimer (OR = 3.1; 95% CI, 1.0–10.1) were significantly associated with cIMT thickening. We also developed a composite score incorporating markers (IL-7, IL-10, D-dimer, and hypertension) that accurately evaluated atherosclerotic CVD.

Conclusions. The associations of IL-4, IL-7, IL-10, and D-dimer with atherosclerosis underscores research needs to further understand the inflammatory mechanisms in the pathogenesis of atherosclerosis CVD among treated PWH. The composite score for atherosclerotic CVD assessment could be useful for risk stratification in PWH.

Keywords. carotid atherosclerosis; coagulation; HIV; inflammation.

Combination antiretroviral therapy (cART) has dramatically reduced human immunodeficiency virus (HIV)-related mortality and morbidity among people with HIV (PWH), leading to prolonged life expectancy approaching that of the general population [1]. Growing evidence indicates that atherosclerotic cardiovascular disease (CVD) is a major cause of premature death in PWH [2]. Of note, there is an increase in incidental carotid plaque among PWH, and the presence and characteristics of carotid plaque are associated with subsequent vascular events [3]. Atherosclerotic CVD (ASCVD) in PWH may be associated with traditional CVD risk factors [4, 5], but those factors alone could not explain the excessive CVD risks in PWH.

Human immunodeficiency virus infection even under viral suppression has been characterized by chronic inflammation and immune activation [4, 6], which may play a vital role in the process of atherosclerosis in PWH on cART [7]. Elevated levels of inflammatory cytokines such as interleukin (IL)-6, C-reactive protein (CRP), D-dimer, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF)-α, soluble (s) CD163, inducible protein-10 (IP10), and sCD14 are significantly associated with atherosclerosis in some but not all studies with PWH [8–11]. The inconsistent findings across studies may be due to differences in sample size, participant characteristics, and adjustment for potential confounders as well as the limited number of examined cytokines.

The number of aging PWH has been growing rapidly over the past decade in China, and noncommunicable chronic diseases are increasingly the main causes of death in HIV-infected elders [12]. Our earlier study in Eastern China

Received 15 February 2022; editorial decision 11 April 2022; accepted 14 April 2022; published online 17 April 2022

Correspondence: Na He, MD, PhD, Professor of Epidemiology, School of Public Health, Fudan University, P.O. Box 289, 138 Yi Xue Yuan Road, Shanghai 200032, China (nhe@fudan.edu.cn).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofac208

also reported that despite lower exposure to traditional risk factors of CVD, PWH on suppressive cART had a significantly higher prevalence of subclinical atherosclerosis than their HIV-negative counterparts [13]. They also presented characteristic electrocardiographic abnormalities, especially sinus tachycardia and ST/T alterations [14]. Nonetheless, the underlying mechanisms, especially the role of inflammation in the link between HIV infection and ASCVD, have not been well addressed. This is particularly relevant in the current era of the "Treat for All" HIV control strategy in China. Therefore, we proposed a study to examine the association between a confluence of inflammation and coagulation biomarkers and atherosclerosis among PWH on cART for at least 3 months.

METHODS

Study Design and Participants

Data for the present study were derived from the baseline survey of Comparative HIV and Aging Research in Taizhou (CHART) study, China, which is an ongoing prospective cohort study of HIV and aging-related comorbidities among PWH and HIV-negative individuals since February 2017. Details about the CHART cohort have been described elsewhere [13]. In brief, PWH in Taizhou prefecture of Zhejiang province in Eastern China was recruited when they came to local Centers for Disease Prevention and Control to receive routine follow-up clinical care. Community-based age- and gender-matched HIV-negative controls were recruited from the same areas. Enrolled participants received a comprehensive baseline assessment including standardized questionnaire interviews, physical examinations, and biochemical tests using venous blood. For the current analysis (using Proc SurveySelect in SAS software), 178 PWH aged 40–70 years who were on stable cART (>3 months) were included. In addition, 121 HIV-negative individuals during the present study period from February 2017 to August 2018 were randomly selected from the whole sample with available cIMT measurements. The study was reviewed and approved by the Institutional Review Board of Fudan University, Shanghai, China. The patient's written consent was obtained by all study participants.

Data Collection

All CHART study data were collected by trained study staff. Baseline information on demographic characteristics, lifestyle risk factors, and use of medications were obtained by a standardized structured questionnaire. Physical examinations of waist circumference, hip circumference, height, weight, and blood pressure (BP) were carried out after the questionnaire had been completed. Seated BP measurements using an aneroid sphygmomanometer after at least 5 minutes of quiet rest after physical examinations were obtained by trained and certified staff who followed a standard protocol, with the averages of 2 measurements. Levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglycerides (TG) were measured using standard laboratory methods. Regular exercise, smoking, and alcohol use habit were self-reported and used as covariable in the analysis. Regular exercise was 3 or more times exercises every week. Heavy alcohol use was defined as ≥4 drinks on any day or 14 per week for men or ≥3 drinks on any day or 7 per week for women. Body mass index ([BMI] calculated as weight in kilograms divided by height in meters squared [kg/m^2]) was categorized as <18.5 (underweight), 18.5–23.9 (normal weight), and ≥24 (overweight/obese). Waist/hip circumference and BP were the means of the 2 routine measurements. The cutoff of the waist-to-hip ratio (WHR) was 0.9 for men and 0.85 for women. We defined hypertension as systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg, or prior clinical diagnosis of hypertension [15]. Diabetes was defined as a prior clinical diagnosis of diabetes or hemoglobin A_{1c} ≥6.5%. Dyslipidemia was defined as TC ≥5.2 mmol/L or LDL cholesterol ≥3.4 mmol/L, or TG ≥1.7 mmol/L [16]. Framingham Risk Score (FRS) was calculated using the formula from the Framingham Heart Study [17]. We categorized individuals based on 3 levels of CVD risk: low (<10%), moderate (10%–20%), and high (>20%). Human immunodeficiency virus-related variables were extracted from the HIV/AIDS Comprehensive Response Information Management System, including time of HIV diagnosis, baseline, time-update CD4 cell counts, ART regimes, and time-update HIV ribonucleic acid (RNA). The time-update CD4 cell and HIV ribonucleic acid were obtained within 3 months of carotid intima-media thickness (cIMT) measurement.

Measurement of Carotid Intima-Media Thickness

Intima-media thickness of the left common carotid artery was measured by trained sonographers using a high-resolution B-mode ultrasound imager (LOGIQ P5 pro; GE, Indianapolis, IN), by standard procedures. In briefly, an IMT image was obtained on approximately 10 mm of the longitudinal carotid length, which is free of plaque with an identified double-line pattern. According to whether the cIMT was ≥1 mm or not, these individuals were divided into the cIMT thickening group and the cIMT nonthickening group.

Measurement of Inflammation and Coagulation Markers

In subsets of participants who also participated in the CHART study, venous blood was collected by KEDTA-anticoagulant tube and centrifuged at 3000 rpm for 15 minutes, and the upper plasma sample was stored at −80°C for detection of biomarkers. Inflammation biomarkers were measured in plasma samples using a multiplex assay. Multiplex bead array cytokine assays (Bio-Plex Protrademark Human Cytokine 27-plex Assay), obtained from Bio-Rad Laboratories (Veenendaal, Netherlands), were used to quantify the following plasma

biomarkers levels on the Luminex instrument: interleukin family such as IL-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-15, IL-17; interferon-γ (IFN-γ); TNF-α; colony-stimulating factors (CSFs) such as granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF); growth factors such as fibroblast growth factor (basic FGF), platelet-derived growth factor two B subunits (PDGF-bb), and vascular endothelial growth factor (VEGF); chemokines such as MCP-1 (or CCL2), macrophage inflammatory protein-1α ([MIP-1α] or CCL3), and MIP-1β (or CCL4), regulated upon activation normal T-cell expressed, and presumably secreted (RANTES or CCL5); IFN-γ IP10 (or CXCL10); and IL-8. Plasma sCD14 and D-dimer were detected using enzyme-linked immunosorbent assay (ELISA). Soluble CD14 was measured by sCD14 ELISA kit (Hycult Biotech, Wayne, PA). The coagulation marker measured as D-dimer was measured by the human D-dimer ELISA kit (ab196269; Abcam, Cambridge, MA). The assays were performed according to the manufacturer's guidelines. The raw data were analyzed using the SpectraMax i3x plate reader (Molecular Devices, San Jose, CA).

Statistical Analysis

Data were presented as mean ± standard deviation (SD) and median with interquartile range for continuous variables or N (%) for categorical variables. Natural logarithmic (ln) transformation was used to normalize the values of concentration of plasma biomarkers. If the plasma biomarkers level was below the level of detection, the midpoint between zero and the lowest level of reliable detection was imputed (Supplementary Table 1). Mean differences in concentration of inflammation and coagulation makers stratified by cIMT thickening were evaluated using a *t* test. Univariable and multivariable logistic regression was used to evaluate the associations of inflammation and coagulation makers with cIMT thickening. Multivariable logistic models were sequentially adjusted for the following: age (as continuous), gender, BMI, WHR, hypertension, diabetes, smoking status, drinking status (Model 2); and baseline CD4 cell count, duration of HIV diagnosis (Model 3). Profiles of correlations between biomarkers were examined using network analysis of the Spearman correlation matrices. To account for multiple comparisons, an alpha of $0.05/[29 \times (29-1)/2+1]$, which was 0.0001, was used as significant. In such analyses, biomarkers that exhibited high correlation profiles (correlation coefficient >0.6) were marked. A composite score was created using the variables including IL-7, IL-10, D-dimer, and hypertension, which were statistically different between cIMT thickening and non-cIMT thickening after *P* value adjustment (threshold, .05) (Table 3 and Supplementary Table 2). A score of 1 (+1) was attributed whenever IL-7, IL-10, or D-dimer values were above the 80th percentile and could be diagnosed as hypertension. This composite score could then range between 0 and 4. The score obtained were compared using univariable and multivariable logistic regression. Receiver operator characteristics (ROC) curves were used to test the performance of the composite score to distinguish cIMT thickening cases from nonthickening individuals. To confirm the specific biomarkers in PWH, multivariable Pearson correlation analyses were used to evaluate the associations of inflammation and coagulation makers with continuous cIMT in both PWH and HIV-negative individuals (Supplementary Table 3). The differences in demographics, comorbidity, health-related behaviors, and biomarkers were described in Supplementary Tables 4 and 5. Sensitivity analysis was done to assess the robustness of the results by defining the cIMT cutoff point to more than 0.9 mm (Supplementary Tables 6 and 7) and treating cIMT as a continuous variable (Supplementary Table 8), respectively. Prespecified subgroup analyses were conducted based on gender, current CD4 count (cutoff value is 350 cells/μL), and HIV viral load (cutoff value is 200 copies/mL) in secondary analyses. SAS 9.4 software (SAS Institute, Cary, NC) was used for statistical analyses. The ROC curves were used to test the performance of the FRS and integration of each single biomarker to distinguish cIMT thickening cases from nonthickening individuals in PWH. A 2-sided $P < .05$ was used as the threshold for statistical significance.

RESULTS

Participant Characteristics

In total, 178 PWH were included in the analysis. Among them, 82.6% were male, 29.8% of whom had cIMT thickening (cIMT ≥1 mm). For HIV-specific characteristics, 81.8% of PWH had current HIV viral suppression, 42.7% had baseline CD4 count ≤200 cells/μL, 13.1% had current CD4 count ≤200 cells/μL, 57.9% were diagnosed with HIV infection for more than 3 years, and 51.1% had received cART for at least 3 years. People with HIV with cIMT thickening were more likely to be older, with higher proportions of hypertension and CVD risks than those without cIMT thickening (Table 1).

Inflammation and Coagulation Biomarkers by Carotid Intima-Media Thickness Thickening

As shown in Table 2, compared with PWH without cIMT thickening, those with cIMT thickening had significantly higher median levels of IL-4 (mean ± SD: 0.74 ± 0.18 vs 0.67 ± 0.17, ln pg/mL; $P = .017$), IL-7 (mean ± SD: 1.49 ± 0.21 vs 1.41 ± 0.17, ln pg/mL; $P = .004$), IL-10 (mean ± SD: 1.03 ± 0.25 vs 0.93 ± 0.24, ln pg/mL; $P = .014$), and D-dimer (mean ± SD: 2.20 ± 0.38 vs 2.05 ± 0.35, ln pg/mL; $P = .032$).

Associations of Inflammation and Coagulation Maker Levels With Carotid Intima-Media Thickness Thickening

In unadjusted logistic regression analysis, higher levels of plasma IL-4 (odds ratio [OR] = 11.5; 95% confidence interval [CI], 1.2–114.2), IL-7 (OR = 20.2; 95% CI, 2.4–168.9), IL-10

Table 1. Characteristics of PWH

Characteristics	cIMT Thickening (*N* = 53)	Non-cIMT Thickening (*N* = 125)	*P* Value*
Demographics			
Male sex	48 (90.6)	99 (79.2)	.068
Age, years			<.001
40–49	6 (11.3)	70 (56.0)	
50–59	22 (41.5)	35 (28.0)	
60–70	25 (47.2)	20 (16.0)	
Mean (SD)	59.0 (7.4)	50.8 (7.7)	<.001
Traditional CVD Risk Factors			
Current smoker	13 (24.5)	40 (32.0)	.319
Heavy alcohol use	7 (13.2)	15 (12.0)	.823
Regular exercise	18 (34.0)	41 (32.8)	.880
BMI Category, kg/m^2			.688
<18.5	3 (5.7)	7 (5.6)	
18.5 to <24	40 (75.5)	87 (69.6)	
≥24	10 (18.9)	31 (24.8)	
Mean (SD)	22.4 (2.6)	22.2 (3.2)	.996
Diabetes	7 (13.2)	15 (12.0)	.823
Hypertension	24 (45.3)	28 (22.4)	.002
Obese WHR	32 (60.4)	56 (44.8)	.057
Dyslipidemia	42 (79.3)	82 (65.6)	.070
Framingham Risk Score, %			<.001
<10	9 (17.0)	57 (45.6)	
10–20	22 (41.5)	44 (35.2)	
>20	22 (41.5)	24 (19.2)	
Median (IQR)	18.3 (14.0–24.6)	11.1 (5.5–18.4)	.004
HIV-Specific Characteristics			
Time since HIV diagnosis ≥3 years	32 (60.4)	71 (56.8)	.659
Baseline CD4 count <200, cells/μL	25 (47.2)	51 (40.8)	.432
Current CD4 count <200, cells/μL	9 (17.3)	14 (11.4)	.289
Current HIV RNA, copies/mL[a]			.196
≤50	34 (75.6)	92 (84.4)	
>50	11 (24.4)	17 (15.6)	
On cART ≥3 years	29 (54.7)	62 (49.6)	.532
ART Regimen			
Ever AZT use	35 (66)	85 (68)	.798
Ever D4T use	4 (7.6)	14 (11.2)	.460

Abbreviations: ART, antiretroviral therapy; AZT, zidovudine; BMI, body mass index; cART, combination ART; cIMT, carotid intima-media thickness; CVD, cardiovascular disease; D4T, stavudine; HIV, human immunodeficiency virus; IQR, interquartile range; PWH, people with HIV; RNA, ribonucleic acid; SD, standard deviation; WHR, waist-to-hip circumference ratio.

NOTE: Data are presented as No. (%) or median (IQR).

[a]Twenty-four PWH were not measured for HIV RNA. **P* values were calculated by using *t* test or wilcoxon test for continuous variables and $\chi 2$ test for categorical variables.

Table 2. Inflammation and Coagulation Biomarkers by cIMT Thickening Among PWH[a]

Biomarkers	cIMT Thickening (*N* = 53)	Non-cIMT Thickening (*N* = 125)	*P* Value*
Inflammation			
IL-1β	0.42 (0.39)	0.41 (0.40)	.969
IL-1rα	2.56 (0.39)	2.64 (0.47)	.293
IL-2	0.93 (0.21)	0.88 (0.20)	.148
IL-4	0.74 (0.18)	0.67 (0.17)	.017
IL-5	1.43 (0.25)	1.38 (0.25)	.212
IL-6	0.42 (0.29)	0.37 (0.35)	.438
IL-7	1.49 (0.21)	1.41 (0.17)	.004
IL-9	2.02 (0.18)	2.01 (0.12)	.919
IL-10	1.03 (0.25)	0.93 (0.24)	.014
IL-12	0.88 (0.35)	0.78 (0.38)	.114
IL-13	0.97 (0.34)	0.87 (0.32)	.078
IL-15	2.23 (0.21)	2.2 (0.24)	.345
IL-17	1.34 (0.19)	1.28 (0.18)	.066
IFN-γ	0.95 (0.23)	0.88 (0.25)	.081
TNF-α	1.56 (0.14)	1.53 (0.15)	.148
G-CSF	2.70 (0.30)	2.65 (0.30)	.157
GM-CSF3	0.69 (0.33)	0.61 (0.33)	.323
Chemoattractant			
MCP-1	1.57 (0.26)	1.51 (0.26)	.166
MIP-1α	0.45 (0.18)	0.44 (0.15)	.570
MIP-1β	2.02 (0.16)	2.05 (0.14)	.226
RANTES	6.84 (2.78)	7.02 (2.94)	.703
IP10	3.11 (0.24)	3.10 (0.22)	.674
Eotaxin	2.11 (0.16)	2.09 (0.14)	.252
IL-8	1.28 (0.37)	1.33 (0.51)	.441
Growth Factor			
FGF-basic	1.55 (0.15)	1.55 (0.13)	.964
PDGF-bb	3.28 (0.50)	3.31 (0.52)	.662
VEGF	2.45 (0.19)	2.42 (0.24)	.518
Others			
sCD14	0.46 (0.23)	0.44 (0.25)	.670
Coagulation			
D-dimer	2.20 (0.38)	2.05 (0.35)	.009

Abbreviations: cIMT, carotid intima-media thickness; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IMT, intima-media thickness; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF-bb, platelet-derived growth factor, two B subunits; PWH, people with HIV; sCD14, soluble CD14; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

[a]The concentration of markers were natural logarithmic transformation; unit of sCD14, μg/mL; units of other markers, pg/mL. **P* values were calculated by using *t* test.

(OR = 9.0; 95% CI, 1.7–47.4), IFN-γ (OR = 6.1; 95% CI, 1.3–28.1), and GM-CSF3 (OR = 3.3; 95% CI, 1.1–10.5) were significantly associated with cIMT thickening. After further adjusting for age, sex, BMI, WHR, hypertension, diabetes, smoking status, drinking status, baseline CD4$^+$ T-cell count, duration of HIV diagnosis (Model 2 and 3, Table 3), the associations of IL-4, IL-7, and IL-10 with cIMT thickening remained significant. Furthermore, D-dimer appeared positively associated with cIMT thickening in multivariate models (Model 2 and 3, Table 3). In sensitivity analysis defining the cIMT cutoff point of 0.9 mm for subclinical atherosclerosis or treating cIMT as a continuous variable, similar findings were observed (Supplementary Tables 6–8).

Composite Score of Inflammatory Markers to Evaluate Carotid Intima-Media Thickness Thickening

An exploratory analysis using networks from correlation matrices highlighted strongly positive interrelationships among IL-4, IL-7, and IL-10 (Figure 1A) . We then constructed an

Table 3. Univariate and Multivariate Logistic Regression Analyses of Inflammation and Coagulation Biomarkers With cIMT Thickening Among PWH[a]

Biomarkers	Model 1 aOR (95% CI)	Model 2 aOR (95% CI)	Model 3 aOR (95% CI)
Inflammation			
IL-1β	1.0 (.4–2.6)	0.6 (.2–1.7)	0.6 (.2–1.9)
IL-1rα	0.8 (.4–1.9)	0.7 (.3–1.8)	0.7 (.3–1.7)
IL-2	5.1 (.8–34.9)	4.3 (.6–32.3)	4.1 (.5–31.8)
IL-4	11.5 (1.2–114.2)*	19.3 (1.6–233.1)*	19.0 (1.6–226.5)*
IL-5	5.1 (1.0–25.8)*	5.5 (1.0–30.9)	5.4 (.9–30.8)
IL-6	2.3 (.7–7.2)	2.0 (.6–7.2)	2.1 (.6–7.6)
IL-7	20.2 (2.4–168.9)**	17.8 (2.0–161.4)**	16.7 (1.8–151.7)**
IL-9	4.4 (.3–57.4)	2.0 (.1–33.8)	1.9 (.1–31.1)
IL-10	9.0 (1.7–47.4)**	11.9 (2.0–69.4)**	11.9 (2.0–72.1)**
IL-12	2.8 (.9–8.4)	2.4 (.7–8.2)	2.6 (.8–9.0)
IL-13	2.5 (.8–7.7)	2.0 (.6–6.7)	2.1 (.6–7.4)
IL-15	3.9 (.7–21.2)	3.3 (.5–20.8)	3.3 (.5–21.2)
IL-17	7.2 (.9–55.7)	7.2 (.9–59)	7.4 (.9–62)
IFN-γ	6.1 (1.3–28.1)*	4.8 (1.0–24.6)	5.2 (1.0–27.2)
TNF-α	6.8 (.4–106.7)	6.2 (.4–108.8)	6.8 (.4–123.6)
G-CSF	2.4 (.6–9.1)	2.6 (.6–10.7)	2.7 (.7–11.1)
GM-CSF3	3.3 (1.1–10.5)*	2.1 (.6–7.8)	2.2 (.6–8.0)
Chemoattractant			
MCP-1	2.1 (.5–8.5)	3.6 (.7–17.4)	3.5 (.7–17.2)
MIP-1α	0.5 (.1–5.6)	0.5 (.1–5.2)	0.4 (.1–4.3)
MIP-1β	0.8 (.1–8.9)	0.4 (.1–6.0)	0.4 (.1–5.5)
RANTES	0.9 (.8–1.1)	0.9 (.8–1.1)	0.9 (.8–1.1)
IP10	1.1 (.2–5.6)	1.6 (.3–10)	1.3 (.2–8.4)
Eotaxin	1.4 (.1–17.5)	2.1 (.1–33.3)	2.2 (.1–36.3)
IL-8	0.8 (.4–1.9)	0.7 (.3–1.7)	0.7 (.3–1.6)
Growth Factor			
FGF-basic	1.8 (.1–28.0)	1.2 (.1–23.2)	1.4 (.1–28.8)
PDGF-bb	1.1 (.5–2.4)	1.0 (.5–2.4)	1.0 (.4–2.3)
VEGF	3.6 (.6–21.5)	3.6 (.5–25.2)	4.0 (.5–29.3)
Others			
sCD14	1.2 (.3–5.6)	0.8 (.2–4.4)	0.9 (.2–4.7)
Coagulation			
D-dimer	2.0 (.7–5.6)	3.2 (1.0–10.1)*	3.1 (1.0–10.1)*

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IMT, intima-media thickness; IP10, inducible protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; OR, odds ratio; PDGF-bb, platelet-derived growth factor, two B subunits; PWH, people with HIV; sCD14, soluble CD14; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

[a]The concentration of biomarkers was natural logarithmic transformation; unit of sCD14, μg/mL; units of other markers, pg/mL. Model 1, OR was adjusted for age and gender; Model 2, OR was adjusted for body mass index, waist-to-hip circumference ratio, hypertension, diabetes, smoking status, drinking status, and other variables listed in Model 1; Model 3, OR was adjusted for baseline $CD4^+$ T-cell count, duration of HIV diagnosis, and other variables listed in Model 2.

*$P < .05$.

**$P < .01$.

inflammatory score composed of clinical and laboratory meters for the prediction of cIMT thickening. We included variables significantly associated with cIMT thickening after adjustment for multiple variables (Table 3 and Supplementary Table 2), including IL-7, IL-10, D-dimer, and hypertension (Figure 1B). The ROC curves confirmed that the composite score plus age had the potential to identify cIMT thickening cases in this study (area under the curve [AUC], 0.833; 95% CI, 0.768–0.899) (Figure 1C). When scored into low (score = 0), moderate (score = 1), and high-risk (score, 2–4) groups, the rate of cIMT thickening was 12.2%, 34.3%, and 56.8%, respectively. Compared with the low-risk group as a reference, the OR (95% CI) for cIMT thickening was 4.8 (95% CI, 1.7–13.7) for those in the moderate-risk group and 11.3 (95% CI, 3.5–36.5) for those in the high-risk group (Figure 1D, Model 3).

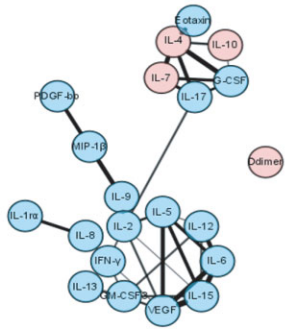
Sensitivity and Explanatory Analyses

In the Supplementary Analysis, inflammation and coagulation biomarkers were categorized into quartiles: the first quartile of biomarkers was used as a reference. Multivariable logistic regression was adjusted for age, gender, BMI, WHR, hypertension, diabetes, smoking status, drinking status, baseline $CD4^+$ T-cell count, and duration of HIV diagnosis to determine whether each level of biomarkers quartiles was independently associated with cIMT thickening. High levels of IL-7, IL-10, G-CSF, MCP-1, and VEGF were associated with cIMT thickening among PWH (Supplementary Table 9). In addition, high levels of IL-4, IL-7, MCP-1, and D-dimer were associated with continuous cIMT in multivariable Pearson correlation analysis. Moreover, specified subgroup analyses between biomarkers and cIMT thickening were conducted based on gender, current CD4 count (cutoff value is 350 cells/μL), and HIV viral load (cutoff value is 200 copies/mL). In males, high levels of IL-4, IL-7, and IL-10 were significantly associated with cIMT thickening after adjusting for age, BMI, WHR, hypertension, diabetes, smoking status, drinking status, baseline $CD4^+$ T-cell count, and duration of HIV diagnosis. Most parameters were not available due to the relatively small number of females in the study (Supplementary Table 10). In PWH with CD4 >350 cells/μL, a high level of IL-7 was significantly associated with cIMT thickening in multivariate logistic regression analyses. In PWH with CD4 ≤350 cells/μL, no statistical correlation was observed between biomarkers and cIMT thickening (Supplementary Table 11). Among individuals with and without current HIV viral load <200 copies/mL, high levels of IL-4, IL-7, and IL-10 were significantly associated with cIMT thickening in multivariate regression analyses (Supplementary Table 12). In the ROC curves used to test the performance of the FRS and integration of each single biomarker, all AUC values of the models were lower than 0.75, which indicated that the composite score had better evaluation ability for atherosclerotic CVD in PWH compared with the traditional CVD risk model (Supplementary Table 13, Supplementary Figure 1).

DISCUSSION

In this study, we assessed the relationship between inflammation and coagulation biomarkers and carotid atherosclerosis

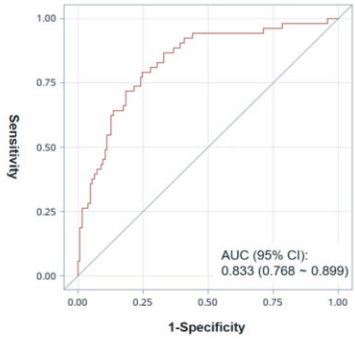
A Correlation of biomakers



B Composite score

Mark	Criterion	Points
IL-7	above 80th	1
IL-10	above 80th	1
D-dimer	above 80th	1
Hypertension	Yes	1
Min score value: 0		
Max score value: 4		

C ROC curves



D Composite marker Risk Score for association of cIMT thickening among HIV-positive individuals

Risk group	Score	Rate	Model 1	Model 2	Model 3
			OR (95% CI)	OR (95% CI)	OR (95% CI)
Low	0	12.2 (9/74)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Moderate	1	34.3 (23/67)	3.8 (1.6 ~ 8.9)**	4.3 (1.6 ~ 11.8)**	4.8 (1.7 ~ 13.7)**
High	2 ~ 4	56.8 (21/37)	9.5 (3.7 ~ 24.6)***	10.5 (3.3 ~ 33.5)***	11.3 (3.5 ~ 36.5)***

Model 1: OR were adjusted for age and gender; Model 2: OR were adjusted for BMI, WHR, diabetes, smoking status, drinking status and other variables listed in model 1; Model 3: OR were adjusted for baseline CD4+ T-cell count, duration of HIV diagnosis and other variables listed in Model 2: *$P < .05$; ** $P < .01$; *** $P < .001$

Figure 1. A composite score of markers to predict carotid intima-media thickness (cIMT) thickening. (*A*) Pearson correlation of inflammation and coagulation biomarkers among people with human immunodeficiency virus (PWH). Blackline denotes correlation coefficient >0.6 and $P < .0001$. (*B*) A composite score was created using the variables shown to be statistically different in the cIMT thickening group compared with the other groups (interleukin [IL]-7, IL-10, D-dimer, and hypertension). (*C*) Receiver operator characteristic (ROC) curves were used to test the performance of the composite score and age to distinguish the cIMT thickening group from the other group. (*D*) Composite marker risk score for the association of cIMT thickening among PWH (define low risk group [score = 0] as a reference,). AUC, area under the curve; BMI, body mass index; CI, confidence interval; GM-CSF, granulocyte macrophage colony-stimulating factor; HIV, human immunodeficiency virus; IFN, interferon; max, maximum; min, minimum; OR, odds ratio; MIP, macrophage inflammatory protein; PDGF-bb, platelet-derived growth factor, two B subunits; VEGF, vascular endothelial growth factor; WHR, waist-to-hip circumference ratio.

among PWH on cART. Our primary results showed that elevated levels of IL-4, IL-7, IL-10, and D-dimer were significantly associated with the presence of cIMT thickening even after adjusting for traditional CVD risk factors. In addition, we have reported that a combination of markers, including IL-7, IL-10, D-dimer, hypertension, and age, could accurately evaluate atherosclerosis among treated PWH.

We also investigated the inflammation and coagulation profile of PWH and found statistically significant differences in biomarker concentrations compared with those who were not infected with HIV (Supplementary Table 3). We hypothesize that the HIV triggered immune responses, which resulted in a strong T helper (Th)1/Th2 disturbance that would lead to a distinctive biomarker signature and may have different pathways that promote immune activation [18–20].

It is notable that we found a significantly higher IL-7 level in PWH with cIMT thickening. Such association was first reported in PWH but has been observed in the general population [21]. Bullenkamp et al's [22] in vitro studies from patients with acute coronary syndromes have provided compelling evidence supporting the role of IL-7 in the expansion and dysregulation of cytotoxic $CD4^{+}CD28^{null}$ T cells in acute coronary disease. Previous studies reported that the activation of the persistence of IL-7 contributes to the adhesion/migration of chemoattractant and aggregation of monocyte/macrophage, thus promoting the expression of VEGF level via inflammatory reaction [23, 24]. Our results also first demonstrated a significant association between IL-4 and cIMT thickening. There was a strong correlation between IL-7 and IL-4 ($r = 0.77$, $P < .0001$). Among treated PWH, host factors such as IL-7, IL-4, and immune activation enhanced CDl27 expression [25, 26], the role and regulatory mechanism of which in HIV-related atherosclerosis remain to be further elucidated.

Studies in treated HIV infection have reported that circulating IL-7 is strongly associated with CD4 cell depletion [27, 28]. However, we did not observe a significant difference in IL-7 plasma levels between PWH with and without baseline or current CD4 cell depletion (data not shown), which was possibly due to the small sample size of CD4 cell depletion. Some research highlights the potential value of IL-7 and its receptor for intervention and immunization reconstruction in HIV infection by promoting the proliferation of T cells such as CD4

and CD8 cells [29, 30]. Considering the positive correlation between IL-7 and atherosclerosis in our study, whether IL-7 could be used as a clinical adjuvant remains to be explored.

Higher IL-10 level was positively associated with cIMT thickening, which was observed at a higher level in PWH compared with those among HIV-negative individuals (Supplement Table 3). In their previous study [31], Elizabeth Gori et al indicated that the presence of secondary or opportunistic infections contributes to higher levels of IL-10. However, the major roles of IL-10 in the pathogenesis of atherosclerosis include inhibition of macrophage activation, inhibition of matrix metalloproteinase, proinflammatory cytokines, and cyclooxygenase-2 expression in lipid-loaded and activated macrophage foam cells [32]. Nonetheless, there was no existing literature reporting the association of IL-10 with cIMT in PWH. Moraitis et al [33] reported elevated levels of IL-10 as a cause of dyslipidemia and high-density lipoprotein cholesterol deficiency in HIV-infected individuals, which may contribute to the pathogenesis of ASCVD. More importantly, IL-10 was positively associated with ASCVD in PWH, irrespective of the negative relationship between IL-10 and ASCVD among HIV-negative individuals. The conflicting results in PWH and HIV-negative individuals are also likely due to the release of immunosuppressive anti-inflammatory cytokines resulting from the activation of the innate and acquired immune system [34].

It is well known, among HIV-negative individuals, that ASCVD is orchestrated through the induction of a Th1 cytokine profile such as IL-17 or TNF-α [35]. Because HIV infection is characterized by the depletion of $CD4^+$ T cells and several changes in the humoral immune response, the immune regulation mechanism on ASCVD in PWH might be distinct from that of HIV-negative individuals. It is noteworthy that the different phenomenon between PWH and HIV-negative individuals was also confirmed in our study, which showed that IL-4 and IL-10 were positively associated with cIMT, whereas in the HIV-negative population, these biomarkers were negatively associated with cIMT (Supplementary Table 4), further supporting the hypothesis that disease progression is mainly orchestrated through the balance between Th1 and Th2 subtypes or a mixed cytokine profile in PWH [36].

Consistent with the SMART study [37], our data reinforced that the increased concentration of D-dimer was associated with atherosclerosis in HIV infection [38, 39]. Tissue factor activation is another mechanism to alter coagulation pathways in HIV infection [40]. Elevation of D-dimer may reflect a prothrombotic state that increases susceptibility to a major thrombotic event when there are additional underlying risk factors [41]. There is also evidence that high D-dimer levels reflect an inflammatory state in the LIPID study: D-dimer correlated with levels of high-sensitivity (hs)CRP and remained a highly significant predictor even after adjustment for hsCRP [42]. Its involvement in HIV-related inflammation associated with atherosclerosis requires further clarification.

More importantly, we proposed a score composed of 3 blood biomarkers and 1 clinical parameter plus age, which showed great power for the evaluation of atherosclerosis CVD. Thus, this composite score could be useful for risk stratification of atherosclerosis CVD in treated PWH. Furthermore, the composite score had better evaluation ability for atherosclerotic CVD in PWH compared with the traditional CVD risk model (Supplementary Table 13, Supplementary Figure 1). Of note, TNF-α, IL-1β, and IL-6, which are classically implicated in the pathophysiology of atherosclerosis, were not included in the composite score due to a lack of significant associations with cIMT thickening in the present study. Whether TNF-α, IL-1β, and IL-6 are implicated in the pathophysiology of atherosclerosis in PWH still needs to be confirmed by randomized controlled trials.

Several limitations should be noted. First, the time-varying changes in biomarkers and cIMT were not considered in this study. This was limited by the cross-sectional structure of our data. In addition, the constructed model was not tested using a validation cohort, which needs to be addressed in future longitudinal studies. Second, the sample size for the present study was relatively small while a total of 29 plasma biomarkers were examined simultaneously. Given the complexity of cytokine networks during HIV infection, studies with larger sample sizes are warranted.

CONCLUSIONS

In summary, plasma levels of IL-4, IL-7, IL-10, and D-dimer are indicative of atherosclerosis among treated PWH. The composite score with the integration of certain plasma inflammatory biomarkers such as IL-7, IL-10, D-dimer, and hypertension could accurately differentiate treated PWH with or without atherosclerosis CVD, which is useful for HIV care and management.

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.

Acknowledgments

Authors contributions. Y. D. and N. H. contributed to the conception or design of the work. B. Z. contributed to data analysis and drafted the manuscript. Y. D. and N. H. critically revised the manuscript. B. Z., X. C., W. S., Y. D., H. L., and N. H. contributed to the acquisition, analysis, or interpretation of the work. All authors gave final approval and agree to be accountable for all aspects of work and ensure integrity and accuracy.

Financial support. This work was funded by the National Natural Science Foundation of China (Grant Number 81872671; to Y. D.), Yiwu Research Institute of Fudan University (Grant Number KCF201512; to

N. H.), and Shanghai Municipal Health and Family Planning Commission (Grant Number GWV-10.1-XK16; to N. H.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Sabin CA. Do people with HIV infection have a normal life expectancy in the era of combination antiretroviral therapy? BMC Med **2013**; 11:251.
2. Paisible AL, Chang CC, Soarmah KA, et al. HIV infection, cardiovascular disease risk factor profile, and risk for acute myocardial infarction. J Acquir Immune Defic Syndr **2015**; 68:209–16.
3. Janjua SA, Staziaki PV, Szilveszter B, et al. Presence, characteristics, and prognostic associations of carotid plaque among people living with HIV. Circ Cardiovasc Imaging **2017**; 10:e005777.
4. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med **2013**; 173:614–22.
5. Armah KA, Chang CC, Baker JV, et al. Prehypertension, hypertension, and the risk of acute myocardial infarction in HIV-infected and uninfected veterans. Clin Infect Dis **2014**; 58:121–9.
6. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. J Leukoc Biol **2010**; 87:589–98.
7. Shaked I, Hanna DB, Gleißner C, et al. Macrophage inflammatory markers are associated with subclinical carotid artery disease in women with human immunodeficiency virus or hepatitis C virus infection. Arterioscler Thromb Vasc Biol **2014**; 34:1085–92.
8. Grunfeld C, Delaney JA, Wanke C, et al. Pre-clinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. AIDS **2009**; 23:1841–9.
9. Hsue PY, Lo JC, Franklin A, et al. Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection. Circulation **2004**; 109:1603–8.
10. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. J Leukoc Biol **2010**; 87:589–98.
11. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis **2012**; 206:1558–67.
12. He N, Ding Y, Li J, et al. HIV and aging in mainland China: implications for control and prevention research. Curr HIV/AIDS Rep **2019**; 16:439–47.
13. Lin H, Ding Y, Ning C, et al. Age-specific associations between HIV infection and carotid artery intima-media thickness in China: a cross-sectional evaluation of baseline data from the CHART cohort. Lancet HIV **2019**; 6:e860–8.
14. Ding Y, Zhu B, Lin H, et al. HIV infection and electrocardiogram abnormalities: baseline assessment from the CHART cohort. Clin Microbiol Infect **2020**; 26: 1689.e1–7.
15. Writing Group of Chinese Guidelines for the Management of Hypertension. The 2018 Chinese guidelines for the management of hypertension. Chin J Cardiovasc Med **2019**; 24:25.
16. Joint Committee of guidelines for the prevention and treatment of dyslipidemia in Chinese adults. Guidelines for prevention and treatment of dyslipidemia in Chinese adults. Chin J Cardiovasc Dis **2007**; 19:4–14.
17. D'Agostino RB, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham heart study. Circulation **2008**; 117:743.
18. Pina AF, Matos VTG, Bonin CM, Dal Fabbro MMFJ, Tozetti IA. Non-polarized cytokine profile of a long-term non-progressor HIV infected patient. Braz J Infect Dis **2018**; 22:142–5.
19. Wen J, Li X, Zhao QX, et al. Pharmacological suppression of glycogen synthase kinase-3 reactivates HIV-1 from latency via activating Wnt/β-catenin/TCF1 axis in CD4+ T cells. Emerg Microbes Infect **2022**; 11:391–405.
20. Romagnani S, Del Prete G, Manetti R, et al. Role of TH1/TH2 cytokines in HIV infection. Immunol Rev **1994**; 140:73–92.
21. Vanloan N, Andrew M, Larrick JW. Interleukin-7 and immunosenescence. J Immunol Res **2017**; 2017:4807853.
22. Bullenkamp J, Mengoni V, Kaur S, et al. Interleukin-7 and interleukin-15 drive $CD4^{+}CD28^{null}$ T lymphocyte expansion and function in patients with acute coronary syndrome. Cardiovasc Res **2021**; 117:1935–48.
23. Yang J, Dong T, Wang J, Zhang Y, Mao P. Correlation between interleukin-7 and vascular endothelial growth factor in essential hypertension. J Cardiovasc Rehabil Med **2008**; 17:538–41.
24. Crawley AM, Angel JB. The influence of HIV on CD127 expression and its potential implications for IL-7 therapy. Semin Immunol **2012**; 24:231–40.
25. Seddiki N, Sasson SC, Santner-Nanan B, et al. Proliferation of weakly suppressive regulatory CD4+ T cells is associated with over-active CD4+ T-cell responses in HIV-positive patients with mycobacterial immune restoration disease. Eur J Immunol **2009**; 39:391–403.
26. Kerzerho J, Mcilvaine EJ, Anthony P, et al. Impact of hepatitis C virus on the circulating levels of IL-7 in HIV-1 coinfected women. J Acquir Immune Defic Syndr **2016**; 71:172–80.
27. Shive CL, Clagett B, McCausland MR, et al. Inflammation perturbs the IL-7 axis, promoting senescence and exhaustion that broadly characterize immune failure in treated HIV infection. J Acquir Immune Defic Syndr **2016**; 71:483–92.
28. Leen AM, Sukumaran S, Watanabe N, et al. Reversal of tumor immune inhibition using a chimeric cytokine receptor. Mol Ther **2014**; 22:1211–20.
29. Gavegnano C, Brehm JH, Dupuy FP, et al. Novel mechanisms to inhibit HIV reservoir seeding using Jak inhibitors. PLoS Pathog **2017**; 13:e1006740.
30. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med **2008**; 5:e203.
31. Gori E, Mduluza T, Nyagura M, et al. Inflammation-modulating cytokine profile and lipid interaction in HIV-related risk factors for cardiovascular diseases. Ther Clin Risk Manag **2016**; 12:1659–66.
32. Han X, Boisvert WA. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. Thromb Haemost **2015**; 113: 505–12.
33. Moraitis AG, Freeman LA, Shamburek RD, et al. Elevated interleukin-10: a new cause of dyslipidemia leading to severe HDL-c deficiency. J Clin Lipidol **2015**; 9: 81–90.
34. Sabat R, Grutz G, Warszawska K, et al. Biology of interleukin-10. Cytokine Growth Factor Rev **2010**; 21:331–44.
35. Nevers T, Salvador AM, Velazquez F, et al. Th1 effector T cells selectively orchestrate cardiac fibrosis in nonischemic heart failure. J Exp Med **2017**; 214:3311–29.
36. Klein SA, Dobmeyer JM, Dobmeyer TS, et al. Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. AIDS **1997**; 11:1111–8.
37. Kaplan RC, Landay AL, Hodis HN, et al. Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment. J Acquir Immune Defic Syndr **2012**; 60:359–68.
38. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis **2014**; 210:1248–59.
39. Ford ES, Greenwald JH, Richterman AG, et al. Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. AIDS **2010**; 24:1509–17.
40. Eugenin EA, Morgello S, Klotman ME, et al. Human immunodeficiency virus (HIV) infects human arterial smooth muscle cells in vivo and in vitro: implications for the pathogenesis of HIV mediated vascular disease. Am J Pathol **2008**; 172:1100–11.
41. Aisabokhale FA, Akingbola TS, Bamidele K. D-dimer as a predictor of altered coagulation in HIV patients in Nigeria. Niger J Physiol Sci **2019**; 34:99–105.
42. Simes J, Robledo KP, White HD, et al. D-dimer predicts long-term cause-specific mortality, cardiovascular events, and cancer in patients with stable coronary heart disease: LIPID study. Circulation **2018**; 138:712–23.