Immunologic predictors of coronary artery calcium progression in a contemporary HIV cohort

Jason V. Baker[a,b], Katherine Huppler Hullsiek[c], Amrit Singh[d], Eleanor Wilson[d], Keith Henry[a,b], Ken Lichtenstein[e], Nur Onen[f], Erna Kojic[g], Pragna Patel[h], John T. Brooks[h], Howard N. Hodis[i], Matt Budoff[j], Irini Sereti[d], for the CDC SUN Study Investigators

Background: Identifying immunologic mechanisms that contribute to premature cardiovascular disease (CVD) among HIV-positive patients will inform prevention strategies.

Methods: Coronary artery calcium (CAC) progression was studied in an HIV cohort. Immunophenotypes were measured on baseline cryopreserved peripheral blood mononuclear cells using multicolor flow cytometry. Logistic regression identified predictors of CAC progression after adjusting for traditional and HIV-related risk factors.

Results: Baseline characteristics for the analysis cohort ($n = 436$) were median age 42 years, median $CD4^+$ cell count 481 cells/μl, and 78% receiving antiretroviral therapy. Higher frequencies of $CD16^+$ monocytes were associated with greater likelihood of CAC progression, after adjusting for traditional and HIV risk factors [odds ratio per doubling was 1.66 for $CD14^+/CD16^+$ ($P = 0.02$), 1.36 for $CD14^{dim}/CD16^+$ ($P = 0.06$), and 1.69 for $CD14^{var}/CD16^+$ ($P = 0.01$)]. Associations for $CD16^+$ monocytes persisted when restricted to participants with viral suppression. We found no significant associations for CAC progression with other cellular phenotypes, including T-cell activation and senescence markers.

Conclusion: Circulating $CD16^+$ monocytes, potentially reflecting a more pro-atherogenic subpopulation, independently predicted greater CAC progression among HIV-infected persons at low risk for AIDS. In contrast to T-cell abnormalities classically associated with AIDS-related disease progression, these data highlight the potential role of monocyte activation in HIV-related CVD risk.

AIDS 2014, **28**:000–000

Keywords: cardiovascular disease, coronary artery calcium, HIV, immune activation, inflammation, monocyte activation

Introduction

HIV-positive persons are at increased risk for premature atherosclerotic cardiovascular disease (CVD), which is now a leading cause of morbidity and mortality among contemporary patients with access to effective combination antiretroviral therapy (ART) [1,2]. Pro-atherogenic factors among HIV-positive patients include a greater prevalence of traditional risk factors (e.g., smoking), consequences of HIV replication, and

[a]Department of Medicine, University of Minnesota, [b]Division of Infectious Diseases, Hennepin County Medical Center, [c]Division of Biostatistics, University of Minnesota, Minneapolis, Minnesota, [d]National Institute of Allergy and Infectious Disease (NIAID), NIH, Bethesda, Maryland, [e]National Jewish Medical and Research Center, Denver, Colorado, [f]Washington University School of Medicine, St Louis, Missouri, [g]Miriam Hospital, Providence, Rhode Island, [h]Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, [i]Atherosclerosis Research Unit, University of Southern California, and [j]Los Angeles Biomedical Research Institute at Harbor-UCLA, Los Angeles, California, USA.

Correspondence to Jason Baker, MD, MS, 701 Park Avenue (MC G5), Minneapolis, MN 55415, USA.

Tel: +1 612 873 2705; e-mail: baker459@umn.edu

Received: 22 September 2013; revised: 08 November 2013; accepted: 08 November 2013.

DOI:10.1097/QAD.0000000000000145

ISSN 0269-9370 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

exposure to certain antiretroviral medications [3–5]. We have shown that both HIV replication and exposure to protease inhibitors are associated with greater progression of subclinical atherosclerotic disease among participants in the SUN study (Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy) [6].

Recent data suggest that chronic inflammation may partly account the excess CVD risk attributable to HIV infection [7,8]. However, few data exist on the immunologic mechanisms underlying inflammation-CVD risk associations among HIV-positive patients. Cardiac computed tomography (CT) estimates of coronary artery calcified plaque (coronary artery calcium, CAC) provide a noninvasive assessment of subclinical atherosclerosis that also correlates with the extent of histologically confirmed noncalcified plaque [9,10]. In addition, CAC progression is independently associated with future risk for atherosclerotic CVD events and all-cause mortality, and adding CAC assessments to traditional risk factor prediction significantly improves risk classification for coronary heart disease (CHD) or stroke [11–14].

We conducted a longitudinal analysis of participants in the SUN study, and hypothesized that pro-inflammatory cellular phenotypes would predict CAC progression independent of traditional and HIV infection-related clinical factors. The most widely studied measure of immune activation in HIV-positive persons is the frequency of $CD4^+$ or $CD8^+$ T-cell expressing activation markers (e.g., CD38/HLA-DR), which independently predicts risk for AIDS progression and has more recently been associated with subclinical CVD [15,16]. Abnormalities in innate, or nonspecific, immunity have also been described among HIV-positive patients, such as a higher prevalence of circulating monocytes that express $CD16^+$ cell [17,18]. Three distinct monocyte subpopulations have been defined, based on expression of CD14 and CD16: classical ($CD14^+/CD16^-$), intermediate ($CD14^+/CD16^+$), and nonclassical ($CD14^{dim}/CD16^+$) phenotypes [19]. Intermediate and nonclassical monocyte phenotypes may reflect a more activated immunologic state, demonstrating greater release of pro-inflammatory cytokines and an affinity for attaching to vascular surfaces, respectively [20–23]. These data, along with the well accepted role of monocytes in CVD pathogenesis [24], motivated our decision to study monocytes subpopulations along with more traditional T-cell phenotypes associated with HIV disease risk.

Methods

Study design

The SUN study is a Centers for Disease Control and Prevention (CDC)-funded prospective observational cohort study of HIV-infected participants enrolled at seven clinics in four US cities (Denver, Minneapolis, Providence, and St Louis) between March 2004 and June 2006 [25]. The protocol was approved by ethics committees at the CDC and each clinical site. Participants provided written informed consent. The SUN study design and cohort have been previously described [25]. Participant visits occurred at baseline and every 6 months thereafter, with a CAC measurement obtained at the baseline and year 2 visit. Participants enrolled in the SUN study were either naive to ART or their prior antiretroviral exposure had consisted solely of combination ART [$\geq$three nucleoside reverse transcriptase inhibitors (NRTIs) or $\geq$ three antiretroviral drugs from at least two different classes], and were expected to survive at least 2 years. Participants were included in this analysis if they had CAC measurements at both the baseline and 2-year visit.

Clinical data, including all medications and diagnoses, were abstracted and entered into a database (Clinical Practice Analyst; Cerner Corporation, Vienna, Virginia, USA), with additional data provided by a study-specific physical examination, laboratory testing, and an audio computer-assisted self-interview (ACASI). The presence of hypertension was defined as a blood pressure (BP) more than 140/90 mmHg, prescription of antihypertensives, or a diagnosis of hypertension. We estimated glomerular filtration rate (GFR) using the Cockcroft–Gault equation and Framingham 10-year CHD risk score (FRS) using published score sheets [26].

Laboratory measurements

Following each visit, fasting whole blood and plasma specimens were shipped overnight to the CDC. Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved in liquid nitrogen centrally at a CDC laboratory within 30 h of blood draw. Clinical site laboratory testing included measurement of fasting serum lipids, plasma HIV RNA viral load, and $CD4^+$ T-cell counts.

Immunophenotyping was performed on cryopreserved PBMCs using multicolor flow cytometry. Panels of fluorochrome-conjugated antibodies for cell surface markers not affected by cryopreservation (and viability dye to exclude nonviable cells) have been validated [27]. The fluorochrome-conjugated antibodies used to characterize cellular phenotypes were as follows: anti-CD38 PE-Cy7(Clone:HIT2), anti-CX3CR1 PE(Clone:2A9–1), anti-CD28 PE-Cy7(Clone:CD28.2), anti-CD4 efluor605(Clone:OKT4), and anti-HLADR efluor605 (Clone:LN3) from eBioscience (San Diego, California, USA), anti-CD57 APC(Clone:HCD57), anti-CD27 Ax700(Clone:O323), anti-CX3CR1 APC(Clone:2A9-1), anti-CD16 PE-Cy7(Clone:3G8), and anti-CCR2 PerCp-Cy5.5(Clone:TG5) from BioLegend (San Diego, California, USA), anti-HLADR PE(Clone:G46-6), anti-CD3 APC-Cy7(Clone:SK7), anti-CD8 PB(Clone:

RPA-T8), anti-CD14 PE(Clone:M5E2), anti-CCR5 APC-Cy7(Clone:2D7), and anti-CD56 PB(Clone:B159) from BD Biosciences (San Jose, California, USA), anti-TF FITC(Clone:VIC7) from American Diagnostica (Stamford, Connecticut, USA), and anti-CD45RO ECD(Clone:UCHL1) from Beckman Coulter (Brea, California, USA), and Live/Dead Fixable Blue Dead Cell Stain Kit with UV excitation from Invitrogen (Grand Island, New York, USA). Samples were acquired on an LSR-II flow cytometer (BD Diagnostic Systems; Franklin Lakes, New Jersey, USA) and data were analyzed using FlowJo software version 9.5.3 (Treestar Inc., Ashland, Oregon, USA).

The following monocyte cell phenotypes were characterized and expressed as proportional percentages: classical phenotype ($CD14^{+}/CD16^{-}$), intermediate phenotype ($CD14^{+}/CD16^{+}$), nonclassical phenotype ($CD14^{dim}/CD16^{+}$), $CD14^{var}/CD16^{+}$ (intermediate and nonclassical combined, or $CD16^{+}$), tissue factor expression (TF^{+}), and expression of tissue migration markers ($CCR2^{+}$, $CCR5^{+}$, $CX3CR1^{+}$). $CD4^{+}$ and $CD8^{+}$ T cells phenotypes included the following: CX3CR1 expression, activated phenotype ($HLA\text{-}DR^{+}/CD38^{+}$), and senescence phenotype ($CD57^{+}/CD38^{+}$). Representative flow cytometry plots are shown in Fig. 1. Only gated live cells were included in analyses; all cryopreserved samples had more than 75% viability.

The following soluble biomarkers were measured from stored plasma at the Diabetes Research and Training Center Radioimmunoassay Core Laboratory (Washington University School of Medicine, St Louis, Missouri, USA): high-sensitivity C-reactive protein (hsCRP; Kamiya Biomedical Company, Seattle, Washington, USA) and D-dimer (Roche Diagnostics, Indianapolis, Indiana, USA) using immunoturbidometric assays on a Hitachi 917 analyzer; interleukin-6 (IL-6) using electrochemiluminescence assay on Immulite system (Siemens, Erlangen, Germany), and soluble CD14 by ELISA (R&D Systems, Minneapolis, Minnesota, USA).

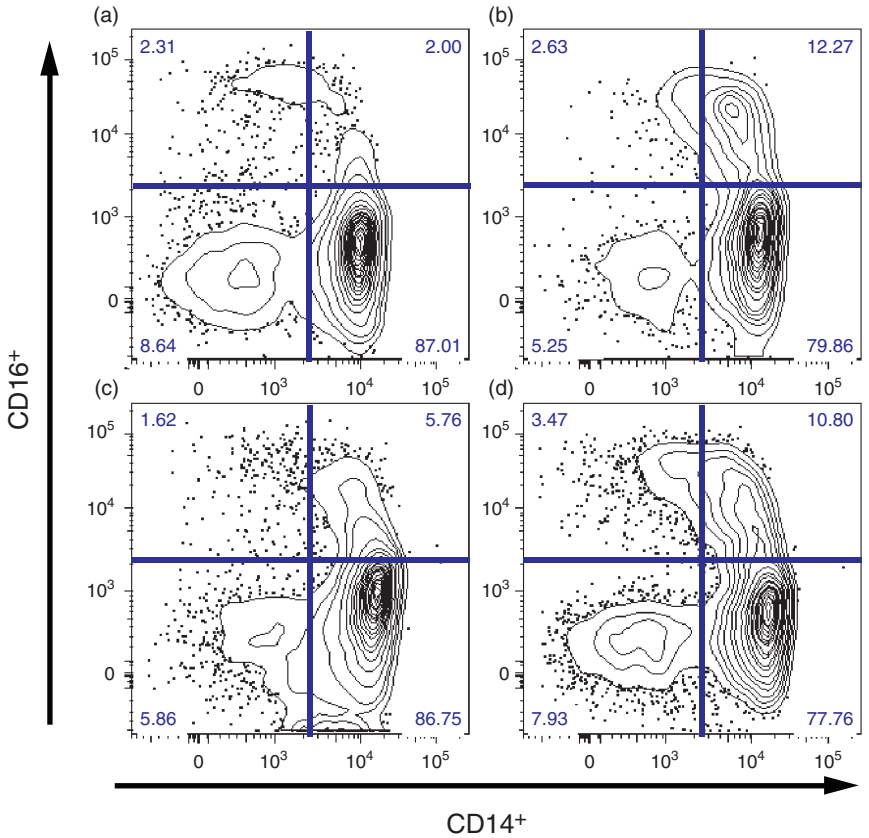

Fig. 1. Monocyte phenotype by coronary artery calcium outcome. After gating on live cells that express HLA-DR and lack expression of markers of T, B, and natural killer (NK) cells (CD2, CD3, CD19, CD20, and CD56), the proportions of monocytes expressing CD14 (horizontal axis) and CD16 (vertical axis) are shown for representative patients with: (a) no coronary artery calcium (CAC): absent at baseline and at year 2 follow-up, (b) incident CAC: absent at baseline, but detectable at year 2 follow-up, (c) stable CAC: detectable at baseline, but without significant increase over 2 years of follow-up, and (d) increased CAC: detectable at baseline with a significant increase over 2 years of follow-up. The percentage of cells for each quadrant are reported in the outer most corner. Greater frequencies of $CD14^{+}/CD16^{+}$ (upper right quadrant) and $CD14^{var}/CD16^{+}$ (both upper quadrants combined) are seen with subsequent CAC progression ('b' and 'd').

Coronary artery calcium measures

CAC was measured from multislice CT scans performed at the baseline and year 2 SUN study visits. Thirty to forty contiguous tomographic slices were obtained at 3-mm intervals beginning 1 cm below the carina and progressing caudally to include the entire coronary tree. All scans were analyzed with a commercially available software package (Neo Imagery Technologies, City of Industry, California, USA). An attenuation threshold of 130 Hounsfield units (HU) and a minimum of three contiguous pixels were utilized to identify a calcific lesion. Each focus was scored using the algorithm developed by Agatston *et al.* [28] and the total CAC score was determined by summing individual lesion scores from each of four anatomical sites: the left main, left anterior descending, left circumflex, and right coronary arteries [9]. An expert reader, blinded to all clinical and demographic information, read each case for total and per-vessel CAC score.

Statistical methods

We analyzed baseline predictors of progression in CAC over 2 years. Significant CAC progression was defined as incident CAC (score of 0 at baseline and >0 at year 2), or increase in CAC (score >0 at baseline with a positive change at year 2 greater than the measurement error). Previously established methods were applied that defined a threshold for CAC increase beyond measurement error [12,29]. For this approach, data were square-root transformed and the difference between the CAC values at year 2 and baseline was then calculated ('SQRT method'). A threshold of at least 2.5 mm from the SQRT method corresponded to more than 99th percentile for interscan variability. Given that the majority of the cohort had no detectable CAC, additional methods for studying changes in CAC on a continuous scale could not be applied.

Descriptive statistics of characteristics at baseline and year 2 are presented as medians with interquartile range (IQR) or frequency (number and percentage). Logistic regression models were used to explore the predictors of CAC progression. Univariate models examined associations with baseline characteristics and HIV-related parameters. The frequencies of monocyte and T-cell phenotypes were then studied as predictors of CAC progression (versus no progression) after adjustment for traditional and HIV-related risk factors. Unless otherwise stated, fully adjusted models included the following covariates: age, gender, race/ethnicity, tobacco smoker, diabetes, hepatitis B or C coinfection, treatment with BP or lipid-lowering therapy, baseline $CD4^+$ cell count, and plasma HIV RNA level (undetectable versus not). Analyses were performed using SAS 9.3 (SAS Institute, Cary, North Carolina, USA); plots were generated with R statistical software 2.10.1 (http://www.R-project.org).

Results

Study population

Of the 691 SUN study participants, baseline or year-2 CAC data were not available for 255 participants, resulting in 436 SUN study participants with paired baseline and 2-year follow-up CAC measurements. Of those participants excluded from the analysis ($n = 255$), the most common reasons for missing data were as follows: missed CAC measure at either baseline or year 2, but continued in the study ($n = 72$), withdrew from study ($n = 59$), moved ($n = 36$), lost to follow-up ($n = 27$), and death ($n = 11$). Compared with any SUN study participant excluded from this analysis, the analysis cohort was slightly older (mean age 42 versus 40 years; $P = 0.01$), had fewer baseline tobacco smokers (41 versus 48%; $P = 0.10$), and had a lower prevalence of prior injection drug use (12 versus 17%; $P = 0.07$), respectively (characteristics for those excluded from analyses presented along with the analysis cohort in Appendix Table A, http://links.lww.com/QAD/A453).

Baseline characteristics for analysis cohort are presented in Table 1. Median age was 42 years, the majority were men, over half were non-Hispanic whites, and 41% of participants smoked cigarettes at baseline. Median total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were within desired ranges for persons without known CVD (67% with TC <200 mg/dl and 73% with LDL-C <130 mg/dl) [30], and FRSs were consistent with a low CHD risk as estimated by traditional risk factors.

At baseline, 78% of participants were prescribed ART. Of these, 88% had suppressed plasma HIV RNA viral load at baseline and 65% maintained a suppressed HIV RNA viral load at baseline and year 2. The proportion of participants prescribed nonnucleoside reverse transcriptase inhibitor (NNRTI)-based or protease inhibitor-based ART were similar at baseline, and among those prescribed a protease inhibitor atazanavir (35%) and lopinavir (46%) were the most common. Sixty percentage of participants not prescribed ART at baseline started ART after baseline.

Baseline median (IQR) frequencies for monocyte and T-cell phenotypes, and levels of soluble plasma biomarkers, for all SUN study participants are shown in Appendix Table B, http://links.lww.com/QAD/A453. When compared with participants excluded from analyses, the analysis cohort did not differ significantly with respect to the frequency of any of the cellular immunophenotypes studied.

Two-year coronary artery calcium progression

Most participants had no detectable CAC at baseline (82%), and similarly at year 2 (75%). Median (IQR) detectable CAC score was 39 (8–136) Agatston units at

Table 1. Characteristics of SUN participants with paired coronary artery calcium assessments ($N = 436$).

	Baseline visit	2-year visit
Demographics		
Median age, years (IQR)	42 (36–48)	44 (38–50)
Male sex, *n* (%)	339 (78)	–
Race/ethnicity, *n* (%)		
White, non-Hispanic	258 (59)	–
Black, non-Hispanic	119 (27)	–
Hispanic	48 (11)	–
Other	11 (3)	–
Clinical characteristics		
Median BMI (kg/m^2) (IQR)	26 (23–29)	26 (23–30)
Hepatitis B or C coinfection, *n* (%)	70 (16)	79 (18)
Tobacco smoking, *n* (%)	175 (41)	176 (40)
Injection drug use (ever), *n* (%)	52 (12)	–
Diabetes (diagnosis or treatment), *n* (%)	40 (9)	38 (9)
Prescribed antihypertensive therapy, *n* (%)	89 (20)	89 (20)
Prescribed lipid-lowering therapy, *n* (%)	48 (11)	77 (18)
10-year Framingham Risk Score, median (IQR)	4.5 (2.0–8.2)	5.4 (2.2–8.7)
Clinical laboratories		
Total cholesterol, median (mg/dl) (IQR)	180 (155–209)	183 (158–210)
Triglycerides, median (mg/dl) (IQR)	141 (97–213)	136 (92–211)
LDL-C, median (mg/dl) (IQR)	104 (84–130)	104 (86–131)
HDL-C, median (mg/dl) (IQR)	41 (34–50)	43 (36–52)
GFR, median (ml/min per 1.73m^2) (IQR)	100 (86–111)	99 (84–111)
HIV parameters		
Median years with HIV diagnosis (IQR)	4.7 (2.2–7.9)	6.7 (4.2–10.0)
Prior AIDS-defining event, *n* (%)	106 (24)	113 (26)
Median baseline CD4$^+$ cell count (cells/μl) (IQR)	481 (339–685)	516 (369–695)
Median nadir CD4$^+$ cell count (cells/μl) (IQR)	205 (77–312)	188 (74–296)
HIV RNA viral load <400 copies/ml, *n* (%)	314 (72)	358 (84)
Median years of ART exposure (IQR)	2.8 (1.1–5.4)	4.3 (2.6–6.8)
Baseline ART exposure, *n* (%)	339 (78)	366 (84)
Abacavir use, *n* (%)	100 (29)	97 (27)
Tenofovir use, *n* (%)	161 (47)	219 (60)
NNRTI use, *n* (%)	165 (49)	181 (49)
Protease inhibitor use, *n* (%)	152 (45)	175 (48)

Hypertension: clinical diagnosis; prescribed BP-lowering medication, or BP more than 140/90 mmHg. Hepatitis C: IgG antibody-positive. ART, combination antiretroviral therapy; BP, blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SUN, Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy.

baseline, and 39 (7–114) Agatston units at year 2. Fifty five participants (12.6%) fulfilled criteria for CAC progression (6.4% with incident CAC and 6.2% with an increase in detectable CAC). The frequency of detectable CAC at baseline and CAC progression over 2 years are presented for subgroups in Table 2. From unadjusted models, traditional CVD risk factors associated with significant CAC progression included older age, male sex, prescription of BP-lowering therapy, prescription of lipid-lowering therapy, and higher FRS (Table 3). The duration of HIV diagnosis and duration of ART exposure were each associated with greater frequency of CAC progression (Table 3). Protease inhibitor-based ART was associated with greater CAC progression, but having a detectable HIV viral load (versus <400 copies/ml) was not.

In the fully adjusted model, a higher CD4$^+$ cell count at baseline (odds ratio, OR 1.12 per 100 cells/μl increase; 95% confidence interval, CI 1.00–1.24; $P = 0.04$) was, unexpectedly, associated with greater CAC progression. As a result, we further explored the relationship between baseline CD4$^+$ cell count and duration of HIV diagnosis. Mean (SD) duration of HIV diagnosis at baseline was 5.03 (4.93) years for participants with a CD4$^+$ cell count less than 500 cells/μl ($n = 235$), and 6.75 (4.38) years for those with a CD4$^+$ cell count at least 500 cells/μl ($n = 195$; $P = 0.002$ for difference). When duration of HIV diagnosis was added to the fully adjusted model, baseline CD4$^+$ cell count was no longer predictive of CAC progression (OR 1.08 per 100 cells/μl higher; 95% CI 0.96–1.20; $P = 0.21$), but duration of HIV diagnosis remained significant (OR 1.08 per year; 95% CI 1.02–1.15; $P = 0.008$).

Immunologic predictors of coronary artery calcium progression

Figure 1 presents flow cytometry plots used to quantify monocyte subsets for representative participants based on presence or absence of CAC progression. After adjusting for traditional and HIV-related risk factors, greater frequencies of CD14$^+$/CD16$^+$ and of CD14^{var}/CD16$^+$ monocytes at baseline were independently associated with greater likelihood of CAC progression;

Table 2. Presence of coronary artery calcium at baseline and 2-year progression by subgroups ($n = 436$).

Baseline variable	CAC at baseline		CAC progression[a]	
	Yes	No	Yes	No
Totals for analysis cohort, # (%)	80 (18.3%)	356 (81.7%)	55 (12.6%)	381 (87.4%)
Age (years)				
<40	6 (7.5%)	164 (46.1%)	14 (25.5%)	156 (40.9%)
40–49	38 (47.5%)	150 (42.1%)	21 (38.2%)	167 (43.8%)
>50	36 (45.0%)	42 (11.8%)	20 (36.4%)	58 (15.2%)
Duration of HIV diagnosis (years)				
0.1–2.2 (1st quartile)	11 (13.8%)	97 (27.6%)	7 (13.0%)	101 (26.7%)
2.2–4.7 (2nd quartile)	21 (26.3%)	87 (24.7%)	8 (14.8%)	100 (26.5%)
4.7–7.9 (3rd quartile)	28 (35.0%)	81 (23.0%)	17 (31.5%)	92 (24.3%)
7.9–21.6 (4th quartile)	20 (25.0%)	87 (24.7%)	22 (40.7%)	85 (22.5%)
$CD4^+$ cell count				
<350 cells/μl	20 (25.0%)	99 (28.0%)	13 (23.6%)	106 (28.0%)
350–499 cells/μl	20 (25.0%)	96 (27.2%)	12 (21.8%)	104 (27.5%)
≥500 cells/μl	40 (50.0%)	158 (44.8%)	30 (54.5%)	168 (44.4%)
HIV viral load				
<400 copies/ml	65 (81.3%)	249 (70.3%)	41 (74.5%)	273 (72.0%)
≥400 copies/ml	15 (18.8%)	105 (29.7%)	14 (25.5%)	106 (28.0%)
Lipid-lowering therapy				
Yes	20 (25.0%)	28 (7.9%)	12 (21.8%)	36 (9.4%)
No	60 (75.0%)	328 (92.1%)	43 (78.2%)	345 (90.6%)
Blood pressure-lowering therapy				
Yes	26 (32.5%)	63 (17.7%)	17 (30.9%)	72 (18.9%)
No	54 (67.5%)	293 (82.3%)	38 (69.1%)	309 (81.1%)

[a]Coronary artery calcium (CAC) progression defined as either incident (change from 0 to detectable) or significant increase from a detectable measure at baseline (difference ≥2.5 on square root scale).

the association with $CD14^{dim}/CD16^+$ approached significance (Table 4 column A and Appendix Figure A, http://links.lww.com/QAD/A453). Associations with monocyte phenotypes appeared to be driven largely by the subset of participants with a significant increase in CAC (Table 4 column C). When restricted to participants with an undetectable HIV viral load at baseline (Table 4 column B), ORs for CAC progression became more

Table 3. Univariate associations for baseline traditional and HIV infection-related risk factors with coronary artery calcium progression.

Clinical characteristics and laboratories	OR[a] (95% CI)	*P* value
Age (per 10 years older)	1.79 (1.28–2.50)	0.001
Male (versus female)	2.56 (1.06–6.18)	0.04
Race/ethnicity (white versus other)	1.80 (0.97–3.34)	0.06
Smoking (current versus not)	1.33 (0.75–2.38)	0.33
Injection drug use (ever versus never)	0.72 (0.27–1.91)	0.52
Hepatitis B or C coinfection (versus not)	1.33 (0.65–2.73)	0.43
BMI (per kg/m^2 higher)	0.62 (0.21–1.79)	0.37
Diabetes (versus not)	1.86 (0.81–4.27)	0.15
Blood pressure-lowering therapy (versus not)	1.92 (1.03–3.59)	0.04
Lipid-lowering therapy (versus not)	2.68 (1.29–5.53)	0.01
Framingham risk score (per unit higher)	1.29 (1.05–1.59)	0.02
LDL-C (per mg/dl higher)	0.62 (0.35–1.09)	0.10
Total-to-HDL-C ratio (per unit higher)	0.99 (0.53–1.84)	0.97
HIV infection-related parameters at baseline		
Duration of HIV diagnosis (per year)	1.10 (1.04–1.16)	<0.001
Prior AIDS-defining event (versus not)	1.33 (0.71–2.49)	0.38
Baseline $CD4^+$ cell count (per 100 cells/μl higher)	1.40 (0.95–2.07)	0.09
Nadir $CD4^+$ cell count (per 100 cells/μl higher)	1.01 (0.86–1.19)	0.90
HIV RNA viral load <400 copies/ml (versus not)	1.14 (0.60–2.17)	0.70
Duration of ART exposure (per year)	1.19 (1.07–1.33)	0.002
Baseline ART use (versus not)	1.79 (0.82–3.93)	0.15
Abacavir use (versus not)	0.90 (0.45–1.79)	0.77
Protease inhibitor use (versus not)	0.47 (0.24–0.92)	0.03

ART, combination antiretroviral therapy; CAC, coronary artery calcium; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse transcriptase inhibitor; OR, odds ratio.
[a]OR represents likelihood of a significant 2-year change in CAC, defined as incident or significant progression in CAC.

extreme for all the $CD16^+$ monocyte populations. Finally, adding duration of HIV diagnosis or use of protease inhibitor-based ART to multivariate models did not attenuate associations between monocyte phenotypes and CAC progression (data not shown).

TF expression on monocytes was not associated with CAC progression, nor was expression of the monocyte migration markers CCR2, CCR5, or CX3CR1 in fully adjusted models. Similarly, none of the $CD8^+$ T-cell phenotypes studied in Table 4 demonstrated independent associations. Finally, none of the inflammatory and coagulation plasma biomarkers were associated with CAC progression.

Discussion

In this cohort of HIV-infected patients with relatively restored immune function and at very low risk for AIDS complications, we describe a novel finding that higher frequencies of $CD16^+$ monocytes (intermediate and nonclassical phenotypes) predicted greater CAC progression, independent of traditional and HIV-related risk factors. We found no significant associations for other cellular phenotypes, including those reflecting T-cell activation, with CAC progression. These data suggest a potentially important role for monocyte-related cellular activation in coronary atherosclerotic progression among HIV-positive patients, and suggest that the immunologic abnormalities that contribute to risk for non-AIDS-defining comorbidities like CVD among contemporary patients may not predominantly involve T-cell abnormalities that are classically associated with risk for AIDS-defining complications.

The hallmark immunologic abnormalities associated with HIV infection include immune depletion (i.e., low $CD4^+$ T-cell counts) as well as chronic immune activation, which is most pronounced in untreated disease, but remains present in persons effectively treated with ART [31,32]. At present, epidemiologic data have been inconsistent with respect to whether CVD risk is associated with immune depletion when measured by absolute $CD4^+$ cell count [6,33–36]. The classic indicator of HIV-related T-cell immune activation – the frequency of $CD4^+$ and $CD8^+$ T cells with a $CD38^+$/$HLA\text{-}DR^+$ phenotype – independently predicts greater risk for AIDS disease progression [15]. In cross-sectional studies, T-cell activation was associated with an increased prevalence of carotid artery atherosclerotic lesions [16,37] and greater carotid artery stiffness [38]. In contrast, we did not find that $CD4^+$ or $CD8^+$ T-cell phenotypes were potential predictors for greater CAC progression, and the potential association with absolute $CD4^+$ cell count was accounted for by the duration of HIV diagnosis in our analyses.

Table 4. Multivariate models for coronary artery calcium progression by immunologic predictors.

Monocytes (%)	(A) Multivariate model ($n=436$) OR* (95% CI)	(B) Restrict cohort to suppressed viral load ($n=314$) OR* (95% CI)	(C) Restrict outcome to CAC increase ($n=436$) OR* (95% CI)	(D) Restrict outcome to CAC incidence ($n=436$) OR* (95% CI)
$CD14^+/CD16^-$	0.64 (0.32–1.25)	0.65 (0.32–1.31)	0.04 (0.00–0.79)	1.53 (0.31–7.42)
$CD14^+/CD16^+$	1.66 (1.09–2.55)	2.02 (1.21–3.38)	2.87 (1.21–6.77)	1.13 (0.67–1.89)
$CD14^{dim}/CD16^+$	1.36 (0.98–1.88)	1.48 (1.01–2.17)	1.81 (1.01–3.25)	1.10 (0.73–1.67)
$CD14^{var}/CD16^+$	1.69 (1.13–2.55)	1.96 (1.21–3.18)	3.13 (1.35–7.28)	1.16 (0.71–1.89)
TF^+	1.13 (0.86–1.48)	1.08 (0.79–1.48)	1.28 (0.80–2.05)	0.96 (0.65–1.42)
$CCR2^+$	1.04 (0.10–11.34)	0.35 (0.03–4.53)	0.47 (0.01–16.48)	3.78 (0.13–112.2)
$CCR5^+$	1.01 (0.99–1.03)	1.01 (1.00–1.03)	1.02 (0.99–1.06)	0.99 (0.95–1.02)
$CX3CR1^+$	0.95 (0.72–1.26)	1.10 (0.78–1.55)	0.84 (0.53–1.32)	1.08 (0.74–1.57)
T cells (%)				
$CD4^+/HLADR^+/CD38^+$	1.17 (0.87–1.58)	1.10 (0.78–1.55)	1.16 (0.58–2.31)	1.10 (0.74–1.63)
$CD4^+/CD57^+/CD38^+$	1.09 (0.83–1.41)	1.26 (0.93–1.70)	1.60 (0.98–2.60)	0.83 (0.58–1.19)
$CD4^+/CD57^+$	1.03 (0.80–1.31)	1.02 (0.76–1.35)	1.43 (0.93–2.21)	0.84 (0.61–1.14)
$CD4^+/CX3CR1^+$	1.01 (0.80–1.29)	1.08 (0.82–1.43)	1.49 (0.96–2.32)	0.88 (0.65–1.20)
$CD8^+/HLADR^+/CD38^+$	0.94 (0.69–1.28)	0.85 (0.60–1.22)	0.74 (0.39–1.42)	1.05 (0.70–1.58)
$CD8^+/CD57^+/CD38^+$	0.95 (0.68–1.33)	0.93 (0.65–1.32)	0.98 (0.47–2.05)	0.96 (0.63–1.47)
$CD8^+/CD57^+$	0.76 (0.47–1.23)	0.63 (0.36–1.09)	1.01 (0.42–2.41)	0.84 (0.45–1.56)
$CD8^+/CX3CR1^+$	0.94 (0.72–1.22)	1.00 (0.73–1.35)	1.30 (0.78–2.16)	0.99 (0.70–1.38)
Plasma biomarkers				
hsCRP (mg/dl)	0.83 (0.61–1.12)	0.82 (0.58–1.15)	0.74 (0.39–1.40)	0.97 (0.67–1.41)
IL-6 (pg/ml)	0.95 (0.56–1.62)	0.96 (0.53–1.73)	1.09 (0.38–3.14)	0.96 (0.48–1.92)
sCD14 (μg/ml)	0.76 (0.33–1.76)	1.01 (0.38–2.68)	0.58 (0.11–3.07)	1.10 (0.37–3.28)
sCD163 (ng/ml)	0.92 (0.64–1.33)	0.91 (0.61–1.38)	0.89 (0.43–1.82)	0.92 (0.56–1.52)
D-dimer (μg/ml)	1.24 (0.41–3.77)	0.81 (0.19–3.48)	3.11 (0.35–27.80)	0.93 (0.17–5.07)

*Odds ratios (ORs) are per log-2 unit higher, which corresponds to a doubling in frequency of given monocyte phenotype, adjusted for age, gender, race/ethnicity, tobacco smoking, diabetes, hepatitis B or C coinfection, treatment for hypertension, treatment for hyperlipidemia, baseline $CD4^+$ cell count, and undetectable plasma HIV RNA level – except for model restricted to participants with suppressed viral load (<400 copies/ml).

HIV infection, including treated disease, is also characterized by elevations in inflammatory markers and by abnormalities in innate, or nonspecific, immunity, such as a higher prevalence of circulating monocytes that express $CD16^+$ cell [17,18,31,39,40]. Although the functional characteristics of intermediate and nonclassical monocyte phenotypes (combined as CD14var/$CD16^+$, or $CD16^+$, in these analyses) remain controversial, these subsets may be permissive to infection by HIV [41] and exhibit properties that promote atherogenesis [20–23]. Specifically, the nonclassical phenotype may act as a 'patrolling' subset that has greater affinity for vascular surfaces and preferentially migrate into atherosclerotic lesions [20,21]. The intermediate monocyte phenotype appears to be functionally more pro-inflammatory, with greater cytokine release after stimulation [20,22,23]. Consistent with $CD16^+$ monocytes being precursors for tissue macrophages, this population has been shown to localize to tissue sites of inflammation and fibrosis, with transendothelial migration facilitated by CX3CL1 [42]. Epidemiologic data from HIV-uninfected participants at risk for CVD ($n = 951$) also demonstrate that the intermediate monocyte phenotype independently predicts higher risk for subsequent CVD events (i.e. myocardial infarction, stroke, or CVD death) [43]. Our findings are consistent with these data and suggest that nonclassical and intermediate monocyte phenotypes, reflecting greater activation and potential for transendothelial migration, may contribute to excess coronary atherosclerosis in the context of HIV infection. The relative importance of these associations in the context of other known risk factors requires further clarification. While a doubling in the frequency of $CD16^+$ monocytes conferred risk for CAC progression similar to being 10 years older, this degree of difference in $CD16^+$ monocytes is substantial (e.g., corresponds to a change from the 25th to 75th percentile).

Recent data support the hypothesis that monocyte-related inflammation has clinical consequences for HIV-positive patients [18,44–47]. Soluble CD14 and CD163 levels, both reflecting monocyte activation, have been associated with greater subclinical atherosclerosis among HIV-positive patients and all-cause mortality [45–47]. Data from the AIDS Clinical Trials Group report further demonstrate that the degree of common carotid intima–media thickness progression was also positively associated with plasma levels of lipopolysaccharide (LPS), potentially reflecting translocation of microbial products across damaged mucosal surfaces – a mechanism hypothesized to be an important driver of persistent immune activation among HIV-positive patients [45,48]. Furthermore, Funderburg *et al.* [18] have shown that LPS levels correlated with the frequency of the intermediate monocyte phenotype ($CD14^+/CD16^+$) among virally suppressed HIV-positive patients.

An important observation is that the rate of significant CAC progression (12.4% over 2 years) was low among this HIV positive cohort, who were at low risk for CVD (by traditional risk factors) and had high utilization of antiretrovirals that have not been associated with CVD event risk (i.e., tenofovir, atazanavir, NNRTIs) [49,50]. Specifically, the CAC incident rate was within the expected range for HIV-uninfected participants less than 50 years old reported in the general population cohort MESA (Multi-Ethnic Study of Atherosclerosis) [51]. However, the vast majority of SUN study participants with a detectable CAC score at baseline would still be considered to be at high or very high age-adjusted risk for CHD event (i.e. CAC >75th percentile for age), and would prompt some guidelines to recommend lipid-lowering prevention therapy with LDL-C goal of less than 100 or less than 70 mg/dl, respectively [52]. Only 25% of the SUN study participants with detectable CAC at baseline were prescribed lipid-lowering therapy, of which 45% had an LDL-C less than 100 mg/dl. Furthermore, our subgroup analyses demonstrated that the association between CAC progression and the frequency of $CD16^+$ monocytes was driven by participants who had a significant increase in detectable CAC (versus those with de-novo incident CAC). These data emphasize the need for aggressive risk factor modification among HIV-positive patients with evidence of CHD by noninvasive imaging, and suggest this target population in particular may benefit from adjunct anti-inflammatory treatment strategies.

Ultimately, the low rate of CAC progression limited our ability to detect modest associations with potentially important predictors (e.g. TF expression on monocytes and D-dimer levels). However, the lack of association between T-cell phenotypes and CAC progression specifically did not appear to be due to inadequate statistical power, as the OR estimates were very close to one. Additional limitations of our analyses included the lack of an HIV-uninfected comparison population, the inability to adjust for cytomegalovirus serostatus or viremia, the uncertain clinical implications for the degree of CAC change observed, and differences in methodology precluding direct comparison of monocyte subset frequencies to other HIV-uninfected populations. As with any cohort study, causation cannot be established and channeling bias and other unmeasured confounding may be present. The degree to which circulating monocytes reflect pathologic changes in tissue macrophages (e.g. those within atherosclerotic plaques) is also unclear, and our methods did not assess for earlier atherosclerotic lesions such as noncalcified plaque.

In summary, we report the novel finding that higher frequencies of circulating $CD16^+$ monocytes, reflecting an activated, pro-inflammatory and pro-atherogenic state, predicted greater short-term progression of subclinical coronary atherosclerosis as estimated by CAC. These findings, combined with the lack of associations for activated T cells or immune depletion *per se*, suggest

reducing innate immune activation is a potential CVD prevention strategy for HIV-positive patients.

Acknowledgements

The authors would like to sincerely thank all of the SUN study participants, as well as all the clinical site investigators and staff.

The investigation followed the guidelines of the US Department of Health and Human Services regarding protection of human participants. The study protocol was approved and renewed annually by each participating institutions' ethical review board. All study participants provided written, informed consent.

The study received financial support from Centers for Disease Control and Prevention contract numbers 200-2002-00610, 200-2002−00611, 200−2002−00612, 200−2002−00613, 200-2007-23633, 200-2007-23634, 200-2007-23635, and 200-2007-23636. Additional support was provided by the NIH 1KL2RR033182-01. The work of E.W., A.S., and I.S. was supported by the intramural program of NIAID.

The findings and conclusions from this review are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Conflicts of interest

No conflicts of interest related to collection and presentation of these data are reported.

Conference on Retroviruses and Opportunistic Infections, Atlanta, GA, 3−6 March 2013, Abstract #66LB

References

1. Mocroft A, Reiss P, Gasiorowski J, Ledergerber B, Kowalska J, Chiesi A, *et al*. **Serious fatal and nonfatal non-AIDS-defining illnesses in Europe.** *J Acquir Immune Defic Syndr* 2010; **55**:262–270.
2. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, *et al*. **HIV infection and the risk of acute myocardial infarction.** *JAMA Intern Med* 2013; **173**:614–622.
3. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, *et al*. **CD4+ count-guided interruption of antiretroviral treatment.** *N Engl J Med* 2006; **355**:2283–2296.
4. Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, El-Sadr W, *et al*. **Class of antiretroviral drugs and the risk of myocardial infarction.** *N Engl J Med* 2007; **356**:1723–1735.
5. Saves M, Chene G, Ducimetiere P, Leport C, Le Moal G, Amouyel P, *et al*. **Risk factors for coronary heart disease in patients treated for human immunodeficiency virus infection compared with the general population.** *Clin Infect Dis* 2003; **37**:292–298.
6. Baker JV, Henry WK, Patel P, Bush TJ, Conley LJ, Mack WJ, *et al*. **Progression of carotid intima-media thickness in a contemporary human immunodeficiency virus cohort.** *Clin Infect Dis* 2011; **53**:826–835.
7. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, *et al*. **Inflammation, coagulation and cardiovascular disease in HIV-infected individuals.** *PLoS One* 2012; **7**:e44454.
8. Triant VA, Meigs JB, Grinspoon SK. **Association of C-reactive protein and HIV infection with acute myocardial infarction.** *J Acquir Immune Defic Syndr* 2009; **51**:268–273.
9. Budoff MJ, Achenbach S, Blumenthal RS, Carr JJ, Goldin JG, Greenland P, *et al*. **Assessment of coronary artery disease by cardiac computed tomography: a scientific statement from the American Heart Association Committee on Cardiovascular Imaging and Intervention, Council on Cardiovascular Radiology and Intervention, and Committee on Cardiac Imaging, Council on Clinical Cardiology.** *Circulation* 2006; **114**:1761–1791.
10. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS. **Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study.** *Circulation* 1995; **92**:2157–2162.
11. Raggi P, Cooil B, Shaw LJ, Aboulhson J, Takasu J, Budoff M, *et al*. **Progression of coronary calcium on serial electron beam tomographic scanning is greater in patients with future myocardial infarction.** *Am J Cardiol* 2003; **92**:827–829.
12. Budoff MJ, Hokanson JE, Nasir K, Shaw LJ, Kinney GL, Chow D, *et al*. **Progression of coronary artery calcium predicts all-cause mortality.** *JACC Cardiovasc Imaging* 2010; **3**:1229–1236.
13. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, *et al*. **Coronary artery calcium score and risk classification for coronary heart disease prediction.** *JAMA* 2010; **303**:1610–1616.
14. Hermann DM, Gronewold J, Lehmann N, Moebus S, Jöckel KH, Bauer M, Erbel R, The Heinz Nixdorf Recall Study Investigative Group. **Coronary artery calcification is an independent stroke predictor in the general population.** *Stroke* 2013; **44**:1008–1013.
15. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. **Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **16**:83–92.
16. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, *et al*. **T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women.** *J Infect Dis* 2011; **203**:452–463.
17. Hearps AC, Maisa A, Cheng WJ, Angelovich TA, Lichtfuss GF, Palmer CS, *et al*. **HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy.** *AIDS* 2012; **26**:843–853.
18. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, *et al*. **Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome.** *Blood* 2012; **120**:4599–4608.
19. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, *et al*. **Nomenclature of monocytes and dendritic cells in blood.** *Blood* 2010; **116**:e74–e80.
20. Ziegler-Heitbrock L. **The CD14+ CD16+ blood monocytes: their role in infection and inflammation.** *J Leuk Biol* 2007; **81**:584–592.
21. Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, Luscinskas FW, *et al*. **Fractalkine preferentially mediates arrest and migration of CD16+ monocytes.** *J Exp Med* 2003; **197**:1701–1707.
22. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, *et al*. **The proinflammatory CD14+CD16+ DR++ monocytes are a major source of TNF.** *J Immunol* 2002; **168**:3536–3542.
23. van de Veerdonk FL, Netea MG. **Diversity: a hallmark of monocyte society.** *Immunity* 2010; **33**:289–291.
24. Gerszten RE, Tager AM. **The monocyte in atherosclerosis: should I stay or should I go now?** *N Engl J Med* 2012; **366**:1734–1736.
25. Vellozzi C, Brooks JT, Bush TJ, Conley LJ, Henry K, Carpenter CC, *et al*. **The study to understand the natural history of HIV and AIDS in the era of effective therapy (SUN study).** *Am J Epidemiol* 2009; **169**:642–652.
26. Anderson KM, Wilson PW, Odell PM, Kannel WB. **An updated coronary risk profile. A statement for health professionals.** *Circulation* 1991; **83**:356–362.

27. Ford ES, Greenwald JH, Richterman AG, Rupert A, Dutcher L, Badralmaa Y, *et al*. **Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection.** *AIDS* 2010; **24**:1509–1517.
28. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. **Quantification of coronary artery calcium using ultrafast computed tomography.** *J Am Coll Cardiol* 1990; **15**:827–832.
29. Hokanson JE, MacKenzie T, Kinney G, Snell-Bergeon JK, Dabelea D, Ehrlich J, *et al*. **Evaluating changes in coronary artery calcium: an analytic method that accounts for interscan variability.** *Am J Roentgenol* 2004; **182**:1327–1332.
30. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. **Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).** *JAMA* 2001; **285**:2486–2497.
31. Neuhaus J, Jacobs DR Jr, Baker JV, Calmy A, Duprez D, La Rosa A, *et al*. **Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection.** *J Infect Dis* 2010; **201**:1788–1795.
32. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, *et al*. **Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy.** *J Infect Dis* 2008; **197**:126–133.
33. Triant VA, Regan S, Lee H, Sax PE, Meigs JB, Grinspoon SK. **Association of immunologic and virologic factors with myocardial infarction rates in a US healthcare system.** *J Acquir Immune Defic Syndr* 2010; **55**:615–619.
34. Sabin C and the D:A:D Study Group. **Associations between markers of immunosuppression and the risk of CVD**. In: *19th Conference on Retroviruses and Opportunistic Infections*. Seattle, WA; 2012.
35. Kaplan RC, Kingsley LA, Gange SJ, Benning L, Jacobson LP, Lazar J, *et al*. **Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men.** *AIDS* 2008; **22**:1615–1624.
36. Lichtenstein KA, Armon C, Buchacz K, Chmiel JS, Buckner K, Tedaldi EM, *et al*. **Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study.** *Clin Infect Dis* 2010; **51**:435–447.
37. Longenecker CT, Funderburg NT, Jiang Y, Debanne S, Storer N, Labbato DE, *et al*. **Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals.** *HIV Med* 2013; **14**:385–390.
38. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, *et al*. **T cell activation predicts carotid artery stiffness among HIV-infected women.** *Atherosclerosis* 2011; **217**:207–213.
39. Armah KA, McGinnis K, Baker J, Gibert C, Butt AA, Bryant KJ, *et al*. **HIV status, burden of comorbid disease and biomarkers of inflammation, altered coagulation and monocyte activation.** *Clin Infect Dis* 2012; **55**:126–136.
40. Martin GE, Gouillou M, Hearps AC, Angelovich TA, Cheng AC, Lynch F, *et al*. **Age-associated changes in monocyte and innate immune activation markers occur more rapidly in HIV infected women.** *PLoS One* 2013; **8**:e55279.
41. Ellery PJ, Tippett E, Chiu YL, Paukovics G, Cameron PU, Solomon A, *et al*. **The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo.** *J Immunol* 2007; **178**:6581–6589.
42. Aspinall AI, Curbishley SM, Lalor PF, Weston CJ, Miroslava B, Liaskou E, *et al*. **CX(3)CR1 and vascular adhesion protein-1-dependent recruitment of CD16(+) monocytes across human liver sinusoidal endothelium.** *Hepatology* 2010; **51**:2030–2039.
43. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, *et al*. **CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography.** *J Am Coll Cardiol* 2012; **60**:1512–1520.
44. 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**:589–598.
45. 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**:1558–1567.
46. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, *et al*. **Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients.** *J Infect Dis* 2011; **204**:1227–1236.
47. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, *et al*. **Plasma levels of soluble CD14 independently predict mortality in HIV infection.** *J Infect Dis* 2011; **203**:780–790.
48. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, *et al*. **Microbial translocation is a cause of systemic immune activation in chronic HIV infection.** *Nat Med* 2006; **12**:1365–1371.
49. Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F, *et al*. **Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study.** *J Infect Dis* 2010; **201**:318–330.
50. Monforte AD, Reiss P, Ryom L, El-Sadr W, Dabis F, De Wit S, *et al*. **Atazanavir is not associated with an increased risk of cardio or cerebrovascular disease events.** *AIDS* 2013; **27**:407–415.
51. Kronmal RA, McClelland RL, Detrano R, Shea S, Lima JA, Cushman M, *et al*. **Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA).** *Circulation* 2007; **115**:2722–2730.
52. Naghavi M, Falk E, Hecht HS, Jamieson MJ, Kaul S, Berman D, *et al*. **From vulnerable plaque to vulnerable patient – Part III: executive summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force report.** *Am J Cardiol* 2006; **98**:2H–15H.