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Non-Classical Monocytes Predict Progression of Carotid Artery Bifurcation Intima-Media Thickness in HIV-infected Individuals on Stable Antiretroviral Therapy

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Abstract

BACKGROUND—Inflammation may contribute to cardiovascular disease (CVD) among antiretrovirally suppressed HIV-infected individuals. We assessed relationships of monocyte, CD8 T-cell activation and plasma biomarkers to changes in carotid artery intima-media thickness (CIMT).

METHODS—Longitudinal study of HIV-infected subjects ≥ 40 years and on stable antiretroviral therapy (ART) ≥ 3 months. Peripheral blood mononuclear cells were immunophenotyped by multiparameteric flow cytometry to quantify classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), non-classical ($CD14^{low/+}CD16^{++}$) and transitional (CD14+CD16−) monocyte subsets and activated ($CD38^{+}$HLA-DR^{+}) $CD8^{+}$ T-cells at baseline. Plasma biomarkers were assessed by multiplex Luminex assay. High resolution B-mode ultrasounds of right carotid arteries were obtained. Changes in CIMT over 2 years at the right common carotid artery ($CIMT_{CCA}$) and right bifurcation ($CIMT_{BIF}$) were outcome variables.

RESULTS—We studied 50 subjects: 84% male, median age 49 (Q1, Q3; 46, 56) years, median CD4 count 461 (317, 578) cells/mm^3, and with HIV RNA≤50 copies/mL in 84%. Change in $CIMT_{BIF}$ correlated with log values of baseline absolute count of non-classical monocytes (r=0.37,

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CONFLICT OF INTEREST

None of the authors have any relevant conflicts of interest to disclose.

p=0.020), and with MCP-1 (r=0.42, p=0.0024) and TNF-α (r=0.30, p=0.036) levels. In multivariable linear regression, only non-classical monocytes and MCP-1 predicted the change in $CIMT_{BIF}$, independent of Framingham Risk Score and baseline $CIMT_{BIF}$. No correlation was noted between CD8 T-cell activation and $CIMT_{BIF}$ change. Monocyte subsets, CD8 T-cell activation and biomarker concentrations were not correlated with changes in $CIMT_{CCA}$.

CONCLUSIONS—Our findings highlight the role of non-classical monocytes and MCP-1 in the progression of $CIMT_{BIF}$ in HIV-infected individuals on stable ART independent of traditional cardio-metabolic risk factors.

Keywords

HIV; cIMT; Monocytes; Biomarkers

INTRODUCTION

As human immunodeficiency virus (HIV)-infected individuals are living longer as a result of access to virally suppressive combination antiretroviral therapy (ART) regimens, cardiovascular disease (CVD) has become an important cause of morbidity and mortality in this population [1]. While the underlying pathophysiology for this phenomenon has yet to be determined, it is believed that HIV infection leads to chronic inflammation and pro-atherogenic processes [2]. Multiple studies have used high-resolution ultrasound to assess carotid artery intima-media thickness as a non-invasive measure of subclinical atherosclerosis. Assessment and progression of CIMT is predictive of future cardiovascular events [3], and has been well-studied amongst HIV-infected patients [4].

Although initial studies were mixed regarding the association of HIV-infection with CIMT, the limitations of these results included small sample sizes and inconsistent analyses of carotid artery segments [5]. Specifically, a majority of studies showed no differences between controls and HIV patients at the common carotid artery ($CIMT_{CCA}$) [6], whereas recent investigators have emphasized a greater difference in areas of low endothelial shear stress, such as the carotid artery bifurcation. Atherosclerotic lesions are more likely to occur in areas of low endothelial shear stress where factors including down-regulation of eNOS, increased uptake of LDL, and promotion of oxidative stress predispose to vascular lesions [7–9]. Low flow areas allow for the attachment of inflammatory cells and downstream up-regulation of pro-inflammatory cytokines and adhesion molecules [10]. Additionally, areas of low shear stress have been linked to the transition of a stable lesion to a vulnerable plaque due to a combination of vessel remodeling and inflammation [2]. Given that HIV-uninfected individuals are prone to vascular damage at the carotid artery bifurcation, HIV infection may further augment the inflammatory processes quantified by carotid artery intima-media thickness at the bifurcation ($CIMT_{BIF}$). Studies have shown an independent association of HIV infection with more rapid $CIMT_{BIF}$ progression relative to other segments of the carotid artery [11].

While studies of HIV-induced immune activation have traditionally focused on the role of CD8 T-cells, there is increasing interest in the role that monocytes may play in non-infectious complications seen among individuals with chronic HIV infection [12–14].

Monocytes are a heterogeneous population of cells, and are classified by international consensus into several subsets on the basis of their CD14 and CD16 surface expression: classical monocytes lacking CD16 expression ($CD14^{++}CD16^{-}$) and those expressing CD16 comprised of intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{low/+}CD16^{++}$) monocyte subsets [15]. We have recently published data reporting the identification of a fourth 'transitional' monocyte subset characterized by reduced but still detectable levels of CD14 ($CD14^{+}CD16^{-}$) that may represent an immature stage of monocyte development. Within our cohort of HIV-infected individuals on stable ART, expansion of transitional monocytes was found to be associated with increased $CIMT_{CCA}$ at baseline [16,17]. Classical monocytes lacking CD16 expression account for roughly 80–90% of circulating monocytes in normal healthy individuals. This population has been reported to increase in acute inflammation and to be rapidly recruited to sites of infection [18–20]. The CD16-expressing monocytes, on the other hand, increase with aging and in chronic inflammatory disorders. Compared to classical monocytes, they show higher expression of pro-inflammatory cytokines, higher potency in antigen presentation, and are more permissive for productive HIV infection [21–23]. CD16-expressing monocytes have also been shown to expand with HIV infection and acute coronary syndrome [24]. An increase in non-classical CD16-expressing monocytes has been reported to correlate with HIV disease progression in ART-naïve subjects [25]. Increases in both non-classical and intermediate CD16-expressing monocyte subsets similar in pattern to those in HIV-uninfected subjects with acute coronary syndrome have been reported in HIV-infected individuals with uncontrolled HIV disease [24]. In the general population, increases in circulating monocytes have been observed in diabetic subjects compared to non-diabetic controls [26–28]. CD16-bearing monocyte subsets have been reported to be increased in patients with type 2 diabetes and in particular in those with diabetic complications such as renal disease [13,26,29]. We have reported that increases in insulin resistance are associated with increases in circulating monocytes in HIV-infected subjects Taken together, these data indicate a potentially important role for monocyte populations in the pathogenesis of HIV-associated cardio-metabolic disorders.

This study sought to assess the relationship of monocyte subset phenotypes and T-cell activation with changes in CIMT among HIV-infected individuals on stable ART over time. We also assessed soluble plasma biomarkers of inflammation known to play a substantial role in HIV immune dysregulation.

MATERIALS AND METHODS

Subjects and Study Design

We analyzed entry data of participants enrolled into the Hawaii Aging with HIV-Cardiovascular Cohort, a 5-year longitudinal study investigating the role of immune activation and mitochondrial-specific oxidative stress on the pathogenesis of cardiovascular disease in HIV-infected patients on ART [30]. The study was approved by the Committee on Human Subjects of the University of Hawaii and written informed consents were obtained from all participants.

Entry criteria required subjects to have documented HIV infection, be at least 40 years of age, and be on stable ART for ≥ 3 months. Blood pressure measurements were obtained in

triplicate and averaged. Body mass index (kg/m^2) was calculated. CBC, T-cell subsets, plasma HIV RNA assessments, chemistries and metabolic labs (glucose, insulin, total cholesterol, directly measured LDL-C and HDL-C and triglycerides) were obtained at entry in a fasted state (nothing by mouth except water for 12 hours). Subjects were assessed for past and current tobacco use. Diabetes was defined as self-reported history of diabetes, use of diabetic medications, fasting blood glucose ≥ 126 mg/dL or a 2-hour OGTT glucose level >200 mg/dL. Participants without concurrent CIMT, monocyte subset and inflammatory measurements were excluded from the analysis.

Framingham Risk Score (FRS ATP III) was calculated based on a model comprising age, gender, total cholesterol, HDL-C, systolic blood pressure, treatment of hypertension, and any cigarette smoking in the past month as previously described [31]. FRS was used to categorize subjects into a Framingham Risk Class (FRC) defined as “low” (< 10% 10-year risk of CVD), “intermediate” (10–19% risk of CVD), and “high” risk (> 20% risk of CVD). Subjects with diabetes (as a CVD equivalent) or clinical CVD (history of myocardial infarction, angina, coronary disease-related cardiac surgery, or ischemic stroke) were automatically classified under high risk. Clinical CVD events were adjudicated by 2 physician-researchers.

Carotid Artery Intima-Media Thickness

High-resolution B-mode ultrasound images of the right carotid artery were obtained. A single reader measured the CIMT of the far wall of the distal CCA and BIF with automated edge detection. Ultrasound images were acquired at the Queen’s Medical Center in Honolulu and analyzed at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center. Internal landmarks were identified and used at the subsequent visit to ensure that the measurements were performed at the same locations on follow-up visits. Images and measurements were performed at baseline and at 24 months.

Flow Cytometric Analysis

Cryopreserved PBMC cells were thawed and surface-stained with the following antibodies to identify monocyte sub populations as previously described [32] : V500-conjugated anti-CD3, Qdot605-conjugated anti-CD14, Alexa700-conjugated anti-CD16, PE-Cy7-conjugated anti-CD56, PE-Cy7-conjugated anti-CD19, PE-Cy7-conjugated anti-CD20, APC-H7-conjugated HLA-DR monoclonal antibodies (mAbs), All antibodies were from BD Biosciences, except for Q605-conjugated anti-CD14 and yellow Live/Dead (Life Technologies). CD8 T-cell activation was defined using the following antibodies: Alexa700-conjugated anti-CD3, APCH7-conjugated anti-CD8, V450-conjugated anti-CD38, APC-conjugated anti-HLA-DR all mAbs (all from BD Biosciences San Jose, CA). Stained PBMCs were acquired by flow cytometry, using a 4-laser custom BD-Fortessa instrument (Becton Dickinson) and analyzed with FlowJo software (Treestar Inc Ashland, OR) as previously described [32].

Total monocyte count and CD8 T-cell counts were calculated using white blood cell count (WBC). Monocyte frequencies were obtained on previous flow cytometric evaluations conducted on blood specimens utilized in our prior report [32]. We utilized the mean

fluorescence intensity of HLA-DR expression to confirm to separate monocyte subpopulations as previously reported [33]. See Figure 1 for the multiparametric flow cytometry gating strategy to phenotype four distinct monocyte sub-populations from peripheral blood based on CD16 and CD14 expression.

Plasma Soluble Biomarkers

Testing was conducted using Milliplex Human Cardiovascular Disease panels (EMD Millipore, USA) as outlined in the manufacturer's protocols. Soluble biomarkers assessed included MMP-9, tPAI-1, hsCRP, IL-6, IL-8, IL-10, TNF-α, soluble E-selectin (sE-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular cell adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and interferon-gamma (IFN-γ).

Statistical Analyses

Demographic, clinical and immunologic information was summarized by median and interquartile (IQR) for continuous variables and frequency (percentage) for categorical variables. Continuous variables (e.g., biomarker levels) were log-10 transformed to improve normality before further analyses were performed if their distributions were skewed. Correlations between variables were performed by Pearson correlation. The outcome variables were the changes in $CIMT_{CCA}$ and $CIMT_{BIF}$ between year 2 and baseline. The relationships between the change in CIMT, baseline CIMT, year 2 CIMT and various immune variables were assessed by simple and multivariable linear regression analyses, adjusting for immuno-virologic and HIV-specific factors. Soluble plasma biomarkers were examined by multivariable analysis if the simple regression analysis produced a $p<0.10$. All statistical analyses were conducted in SAS version 9.3 (SAS Institute Inc., Cary, NC). A two-sided p-value < 0.05 was regarded as statistically significant.

RESULTS

Patient Characteristics

A total of 50 subjects enrolled into the Hawaii Aging with HIV-Cardiovascular cohort who had available baseline monocyte phenotypic characterization data, concurrent plasma biomarkers previously assessed, and CIMT data 2 years apart were included in this analysis. The demographic, clinical, and immunologic characteristics of the subjects are summarized in Table 1. The majority of the subjects were male and of Caucasian ethnicity. Entry criteria for the cohort required subjects to be on ART and 84% of these subjects were virologically suppressed at a plasma HIV RNA level of < 50 copies/mL. Subjects aged 40 years or older were recruited and the median age of the selected subjects in this dataset was 49 years (Q1, Q3). The median current CD4 count was relatively high at 461 cells/μL (Q1, Q3) while the median nadir CD4 was relatively low at135 cells/μL (Q1, Q3). The monocyte percent and count were 8% and 416 cells/μL respectively. By subsets, the classical monocytes comprised the majority (78%) of the population, with intermediate (1.4%), non-classical (5%) and transitional (14%) monocytes comprising the rest based on our cellular exclusion/inclusion gating strategy.

The majority of subjects had an increase in $CIMT_{CCA}$ and $CIMT_{BIF}$ over the 2 year period. Eighty-eight percent of subjects had an increase in $CIMT_{CCA}$ with a median increase of .015 mm and 94% had increased $CIMT_{BIF}$ with a median increase of .016 mm.

Relationship between monocyte subsets, CD8 activation and CIMT

$CIMT_{CCA}$ at baseline and year 2 correlated with transitional monocytes (CD14+CD16−) but there was no correlation between the transitional monocyte subset and biomarkers with change in $CIMT_{CCA}$ (Tables 2 and 3). Classical, non-classical and intermediate monocytes were not correlated with baseline, year 2 or change in $CIMT_{CCA}$ (Table 2). No correlation was noted between $CD8^+$ T-cell activation ($CD38^+HLA\text{-}DR^+$) and $CIMT_{CCA}$ at each endpoint or $CIMT_{CCA}$ change (data not shown).

We observed that the frequency (%) of non-classical monocyte (CD14+/lowCD16++) were associated with change in $CIMT_{BIF}$ on simple regression analysis (r=0.36, p=0.025) (Table 2). Upon adjustment, the proportion of non-classical monocytes predicted change in $CIMT_{BIF}$ (β=0.13, p=0.028), independent of Framingham Risk Score (FRS) and baseline $CIMT_{BIF}$ (Table 3). (Similar regression coefficients were observed if further adjusted for CD4 count (β=0.14, p=0.022, data not shown). Change in $CIMT_{BIF}$ did not correlate with FRS or with baseline $CIMT_{BIF}$ in our study. The frequency of “transitional” monocytes (CD14+CD16−) was marginally correlated with baseline $CIMT_{BIF}$ (r=0.308, p=0.053) and year 2 $CIMT_{BIF}$ (r=0.300, p=0.060), but not with change in $CIMT_{BIF}$ (Table 2). The frequency of classical and intermediate monocytes did not correlate with either baseline, year 2 or change in $CIMT_{BIF}$. No correlation was noted between $CD8^+$ T-cell activation ($CD38^+HLA\text{-}DR^+$) and $CIMT_{BIF}$ at each endpoint or $CIMT_{BIF}$ change.

Correlations between Soluble Plasma Biomarkers and Change in CIMT

sVCAM-1 was correlated with $CIMT_{CCA}$ at baseline and year 2 but not with change in $CIMT_{CCA}$ Table 3). MCP-1 and TNF-α were correlated with change in $CIMT_{BIF}$ but not with $CIMT_{BIF}$ at baseline or year 2. MCP-1 predicted change in $CIMT_{BIF}$ (β=0.031, p=0.009), independent of FRS and baseline $CIMT_{BIF}$ (Table 4), while TNF-α did not. In multivariable linear regression of change in $CIMT_{BIF}$, MCP-1 remained significant after adjustment with non-classical monocytes, FRS and baseline $CIMT_{BIF}$.

DISCUSSION

The risk for CVD among HIV virally suppressed individuals may be higher than in HIV uninfected individuals [34–37]. An appreciation of the role of immune-mediated inflammation and immune activation is gathering tremendous interest as a causal modality in explaining the pathogenesis of this increased risk [38,39]. While our previous work found that baseline $CIMT_{CCA}$ was associated with an increase in transitional monocytes, this study assessing change in CIMT found that higher proportions and absolute levels at baseline of the non-classical monocyte subset were associated with increased $CIMT_{BIF}$, although not with $CIMT_{CCA}$ [17]. This association between non-classical monocyte subset and $CIMT_{BIF}$ remained significant even after adjustment for CD4, FRS, and baseline $CIMT_{BIF}$. Although the majority of study participants were in the low Framingham risk category, the

associations between non-classical monocyte subset and $CIMT_{BIF}$ were still seen in the intermediate and high Framingham risk category.

Given the numerous studies demonstrating a relationship between increasing burden of CIMT and future cardiovascular events, one interpretation of our data is that an increase in the non-classical monocyte population may be part of HIV-driven immune dysregulation, which would subsequently drive the increased risk of CVD seen in chronic HIV infection [12]. Westhorpe, et. al. found an increase of CX3CR1 expression on monocytes associated with worse CIMT. Non-classical monocytes have a higher expression of CX3CR1 [40].

Interestingly, the baseline and longitudinal relationships were not the same. We found a relationship between transitional monocytes and baseline $CIMT_{BIF}$ [17]. However, there was no association between transitional monocytes and the change in $CIMT_{BIF}$. This previously undescribed population of monocytes may serve a unique role as an indicator of CVD shaping a different pathogenic process that is static over time. Further studies to characterize this population are needed.

Our study found an association between change in $CIMT_{BIF}$ and both MCP-1 and TNF-α. In HIV-infected individuals, higher levels of MCP-1 have been associated with increased CIMT [41] and with thoracic aorta vessel wall area and vessel wall thickness [42]. Monocyte subsets expressing CCR2, the receptor for MCP-1, have been linked to increased HIV associated co-morbidities [43,44]. Similarly, higher levels of TNF-α have correlated with IMT specifically for the internal carotid artery [45].

In the general population, multiple studies have reported associations between various soluble plasma markers of inflammation and CIMT, particularly at the bifurcation region. A systemic review that more broadly assessed the relationship of various inflammatory markers to CIMT, subsequently concluded that in most studies, the relationship between inflammatory markers and CIMT disappeared after appropriate correction for the presence of traditional risk factors[46].

An explanation for this loss of association after adjustment is that inflammatory markers simply function in the causal pathway between risk factors and atherosclerosis, mediating some of the effects of these traditional risk factors [47]. It is interesting to note that this was not the case in our study. The association with MCP-1 remained significant when adjusted for traditional CVD risk factors as well as for non-classical monocytes and baseline CIMTBIF, suggesting that the elevation of MCP-1 was not simply a biomarker in the causal pathway between traditional risk factors and atherosclerosis, and had additional predictive factor above and beyond levels of non-classical monocytes. We speculate that this increase in MCP-1 may be secondary to HIV-induced immune dysregulation and may partially mediate the increased risk of CVD in our HIV-infected population.

This study is limited by its relatively small size of the cohort, male predominance and no HIV sero-negative comparision group.. However, the strengths of the study are the careful clinical and metabolic characterization of the subjects in association with detailed phenotypic characterization of monocytes, as well as biomarker assays performed in plasma. Although $CIMT_{BIF}$ also has more variation compared to $CIMT_{CCA}$ especially on repeated

measures over time, the study attempted to reduce operator variation by restricting the CIMT measures to a single sonographer and reading center. The study was limited by its ability to assess effects of co-infections such as Hepatitis C and cytomegalovirus (CMV). Host genetics were also not examined in this study. We conclude that chronic HIV in subjects on stable ART is characterized not only by dysregulation and immune activation of CD8 T-cells but also by monocytes. Higher levels of monocytes, specifically non-classical monocytes, may contribute to the increased rates of atherosclerosis in this population and may play a substantial role in the increased risk of cardio-metabolic disease in chronic HIV infection.

Conclusion

The proportion of peripheral non-classical monocytes and MCP-1 predict progression of $CIMT_{BIF}$ in HIV-infected individuals on stable ART independent of traditional cardiometabolic and HIV immuno-virologic factors in our cohort. The role of non-classical monocytes in CVD risk in this vulnerable population requires further study.

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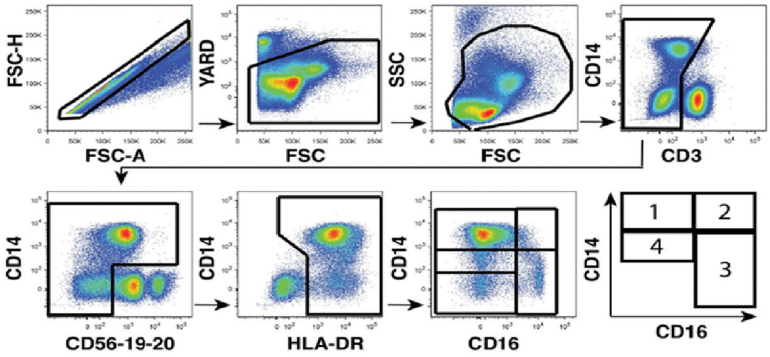

Figure.

Multiparametric flow cytometry gating strategy to phenotype four distinct monocyte sub-populations from peripheral blood based on CD16 and CD14 expression: (1) classical monocytes lacking CD16 expression (CD14++CD16−) and those expressing CD16 comprised of (2) intermediate (CD14++CD16+) and (3) non-classical (CD14low/+CD16++) monocytes. A fourth MO subset which we have termed (4) ‘transitional’ monocyte subset is characterized by reduced but still detectable levels of CD14 (CD14+CD16−)

Table 1

Baseline characteristics

Variables	**All Subjects (N = 50)**
Demographics	
Age, median years (interquartile range)	49 (46, 56)
Male, n (%)	42 (84%)
Ethnicity, White, n (%)	27 (54%)
Clinical Data	
BMI, kg/m^2	26 (25, 29)
hsCRP, mg/L	0.9 (0.6, 2.1)
Framingham risk score (ATP 3)	0.045 (0.02, 0.10)
Framingham risk class	
Low, n (%)	38 (76)
Intermediate, n (%)	4 (8)
High, n (%)	8 (16)
History of CVD event, n (%)	6 (12)
Metabolic syndrome, n (%)	8 (16)
Systolic blood pressure, median mmHg (IQR)	122 (116, 133)
Total cholesterol, median mg/dL (IQR)	181 (155, 209)
HDL cholesterol, median mg/dL (IQR)	46 (34, 57)
LDL cholesterol, median mg/dL (IQR)	115 (97, 132)
Triglyceride, mg/dL (IQR)	106 (77, 167)
Diabetes, n (%)	1 (2)
Current smoker, n (%)	5 (10)
Protease Inhibitor use	26 (52)
Current Atazanavir use, n (%)	16 (32)
HIV data	
Nadir CD4 cell count cells/mm	135 (29, 235)
Undetectable plasma HIV-1 RNA, n (%)	42 (84)
CD4+ T cell count, median cell/ul	461 (317, 578)
Carotid Intima-media Thickness (cIMT) measures	
Right Common Carotid, mm	0.73 (0.66, 0.83)
Right Bifurcation, mm	0.80 (0.73, 0.87)
Maximal plaque thickness, mm	2.89 (2.48, 3.39)
Number of plaques (plaque > 2 mm), n (%)	9 (18)
Biomakers	
hsCRP, ng/mL (IQR)	0.60 (0.90, 2.10)
Log IFN-γ, pg/mL (IQR)	−0.21 (−0.44, 0.02)
Log IL-6, pg/mL (IQR)	0.14 (−0.07, 0.38)
Log IL-8, pg/mL (IQR)	0.54 (0.44, 0.63)
Log IL-10, pg/mL (IQR)	0.21 (−0.04, 0.65)
Log MCP-1, pg/mL (IQR)	2.13 (2.04, 2.21)

Variables	**All Subjects (N = 50)**
Log MMP-9, ng/mL (IQR)	1.71 (1.54, 1.86)
Log E-selectin, ng/mL (IQR)	1.49 (1.30, 1.66)
Log sICAM-1, ng/mL (IQR)	2.12 (2.03, 2.19)
Log sVCAM-1, ng/mL (IQR)	3.07 (3.00, 3.11)
Log TNF-α, pg/mL (IQR)	0.50 (0.27, 0.63)
log_tPAI-1, ng/mL (IQR)	1.96 (1.86, 2.06)
CD8 Activation %	10.5 (8.1, 16.7)
CD8 Activation count, cells/uL (IQR)	88 (39, 113)
log CD8 Activation count	1.95 (1.59, 2.05)
Monocyte Sub-Types	
Classical, % (IQR)	78.3 (72.6, 82.5)
Intermediate, % (IQR)	1.36 (0. 57, 4.41)
Transitional, % (IQR)	13.6 (10.5, 15.6)
Non-Classical, % (IQR)	5.44 (4.48, 8.61)
log Absolute total monocyte count (cells/L)	8.62 (8.51, 8.71)
log Classical (CD14++CD16−)	8.51 (8.36, 8.62)
log Intermediate (CD14++CD16+)	6.82 (6.38, 7.19)
log Transitional (CD14+CD16−)	7.76 (7.61, 7.89)
log Non-classical (CD14+/lowCD16++)	7.33 (7.16, 7.48)

Values reported as median (Q1, Q3), except for frequency count, n (%)

Table 2

Correlation of monocyte and monocyte subtypes with Carotid Intima-Media Thickness, baseline and year 2

	All Monocytes (from CBC)	Classical Monocytes, % (CD14++CD16−)	Intermediate Monocytes, % (CD14++CD16+)	Transitional Monocytes, % (CD14+CD16−)	Non-Classical Monocytes, % (CD14+/lowCD16++)
CIMT at bifurcation region (BIF)					
$CIMT_{BIF}$, baseline	0.224	−0.270	0.181	0.308	−0.105
$CIMT_{BIF}$, year 2	0.221	−0.277	0.187	0.300	−0.078
Change in $CIMT_{BIF}$	−0.015	−0.106	0.086	−0.086	0.355 *
CIMT at the common carotid artery (CCA)					
$CIMT_{CCA}$, baseline	0.113	−0.300	0.067	0.366 *	−0.042
$CIMT_{CCA}$, year 2	0.137	−0.278	0.070	0.350 *	−0.066
Change in $CIMT_{CCA}$	0.075	0.155	0.038	−0.096	−0.230

* $p < 0.05$

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Table 3

Correlation between monocyte subtypes and biomarkers

	log hsCRP	log IFN-γ	log IL-10	log IL-6	log IL-8	log MCP-1	log MMP-9	log selectin	log sICAM-1	log sVCAM-1	log TNF-α	log tPa-1
$CIMT_{CCA}$, Baseline	0.181	−0.179	−0.157	0.044	0.149	0.030	0.058	0.068	−0.116	0.327 *	0.079	0.114
$CIMT_{CCA}$, Year 2	0.123	−0.169	−0.132	0.039	0.145	0.025	0.045	0.044	−0.130	0.346 *	0.070	0.080
$CIMT_{CCA}$, Change	−0.159	0.134	0.202	−0.046	−0.089	−0.039	−0.089	−0.152	−0.025	−0.049	−0.079	−0.225
$CIMT_{BIF}$, Baseline	0.214	−0.056	−0.017	0.006	0.002	−0.072	0.080	−0.142	−0.168	0.160	0.035	0.034
$CIMT_{BIF}$, Year 2	0.173	−0.043	−0.013	0.006	−0.008	−0.041	0.080	−0.142	−0.161	0.176	0.056	0.016
$CIMT_{BIF}$, Change	−0.257	0.174	0.053	0.008	−0.133	0.421 *	0.006	−0.022	0.082	0.240	0.298 *	−0.248

* $p < 0.05$

Table 4

Multivariable linear regression analysis of the association of non-classical monocyte subset percentage and MCP-1 to the change of $CIMT_{BIF}$ from baseline adjusting for Framingham Risk Score and baseline $CIMT_{BIF}$

	Regression coefficient	Standard Error	T-value	p-value	95% Confidence Interval	
Regressions by individual biomarker						
$CIMT_{BIF}$, baseline	0.005	0.012	0.45	0.652	−0.018	0.029
Framingham Risk Score	0.026	0.027	0.98	0.336	−0.028	0.080
Non-classical monocyte, %	0.132	0.058	2.30	**0.028** *	0.016	0.249
$CIMT_{BIF}$, baseline	0.010	0.010	0.98	0.331	−0.010	0.029
Framingham Risk Score	−0.002	0.024	−0.08	0.939	−0.050	0.047
MCP-1	0.031	0.009	3.24	**0.002** *	0.012	0.050
Regression by both biomarkers						
$CIMT_{BIF}$, baseline	0.010	0.011	0.90	0.374	−0.012	0.032
Framingham Risk Score	0.023	0.025	0.93	0.361	−0.027	0.073
Non-classical monocyte, %	0.087	0.056	1.56	0.128	−0.026	0.201
MCP-1	0.025	0.010	2.63	**0.013** *	0.006	0.045

* $p < 0.05$