Non-Classical Monocyte, MCP-1 Predict Subclinical Atherosclerosis Progression in HIV

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Conference on Retroviruses & Opportunistic Infections 2016
February 22-25, 2016
Abstract Number: 652

Abstract

BACKGROUND: Persistent inflammation and immune dysregulation can contribute to cardiovascular disease (CVD) risk in patients with chronic HIV infection.

Methods: We conducted a longitudinal analysis utilizing HIV-infected subjects on stable antiretroviral therapy (ART) in the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study to determine the correlation of peripheral monocyte subsets and biomarkers of inflammation to progression of atherosclerosis using 2 year change in carotid artery intima-media thickness (CIMT) and coronary artery calcium (CAC). Peripheral blood mononuclear cells (PBMC) were immunophenotyped by multiparametric flow cytometry to quantify classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), non-classical ($CD14^{low/+}CD16^{++}$) and transitional ($CD14^{+}CD16^{-}$) monocyte subsets. Biomarkers (sE-selectin, sVCAM-1, sICAM-1, MMP-9, tPA-1, hsCRP, IL-6, IL-8, IL-10, TNF-α, MCP-1, IFN-α) were assessed by multiplex Luminex assay. The primary outcome variables were change in CIMT (right common carotid artery (CCA) and bifurcation (BIF)) and CAC over 2 years.

Results: 105 subjects: 91% male, median age 51 (Q1, Q3; 47, 57) years, median CD4 count 491 (352, 660) cells/mm^3, and 87% with HIV RNA≤50 copies/mL. The rate of change in CCA, BIF, and CAC was 0.010 mm/year, 0.0097 mm/year, and 16.2 Agatston units/year, respectively. Change in CCA correlated with TNFα (r= -0.28, p=0.026) but not with any monocyte subset. Change in BIF correlated with non-classical monocytes (r=0.29, p=0.038) and MCP-1 (r=0.34, p=0.005). Change in CAC correlated with non-classical monocytes (r=0.27, p=0.015) and MCP-1 (r=0.46, p<0.001). Non-classical monocyte and MCP-1 remained significantly associated with CAC and BIF progression after adjustment for age, hypertension, diabetes mellitus, total/HDL cholesterol ratio, and smoking history.

Conclusion: Non-classical monocytes and MCP-1 were associated with progression of atherosclerosis. The role of monocyte subsets in atherosclerosis warrants further investigation.

Background

- Cardiovascular disease (CVD) is a leading cause of death in people with HIV infection.
- Chronic HIV-1 infection is characterized by systemic immune activation and inflammation and may contribute to increased CVD risk.
- Circulating peripheral monocytes and macrophages are involved in the pathogenesis of atherosclerotic plaques.
- Carotid intima-media thickness (CIMT) has been shown to predict cardiac events.
- Coronary Artery Calcium (CAC) score has been shown to predict cardiac events.
- The objective of this study was to determine the correlation of peripheral monocyte subsets and biomarkers of inflammation to progression of atherosclerosis using 2 year change in carotid artery intima-media thickness (CIMT) and coronary artery calcium (CAC).

Statistical Methods

Pearson correlation test was used to assess the association of CIMT with monocyte subsets and soluble biomarkers. Due to the highly skewed distribution of CAC, spearman correlation test was used to assess the association of CAC with monocyte subsets and soluble biomarkers.

Multivariable linear regression was used to predict progression of CIMT from non-classical monocytes and MCP-1, separately, adjusting for age, hypertension, diabetes mellitus, total/HDL cholesterol ratio, and smoking history. Partial spearman correlation was used to assess the association of CAC with non-classical monocytes and MCP-1, separately, adjusting for the same risk factors.

A two-sided p-value < 0.05 was considered statistically significant.

Methods

- Longitudinal data from Hawaii Aging with HIV - Cardiovascular Disease (HAHC-CVD) Study, a study examining the role of oxidative stress and inflammation in HIV cardiovascular risk.
- HAHC-CVD enrolled HIV-infected adults ≥ 40 years old on stable ART > 6 months.

Carotid Intima-Media Thickness (CIMT) Measurements

- High-resolution B-mode ultrasound images of the right carotid artery was obtained.
- A single reader measured the intima-media thickness of the far wall of the distal carotid artery distal to the carotid artery bulb with automated edge detection (Images acquired at Queens Medical Center in Honolulu, and analyzed at University of Southern California Atherosclerosis Research Unit).
- Measurements were obtained at each segment: common (CCA) and bifurcation regions(BIF).
- Both external and internal landmarks were then identified and used at the subsequent visit to ensure that the measurements were performed at the same locations on the follow-up visits.
- Repeat scans and measurements were performed at baseline and again 24 months later.

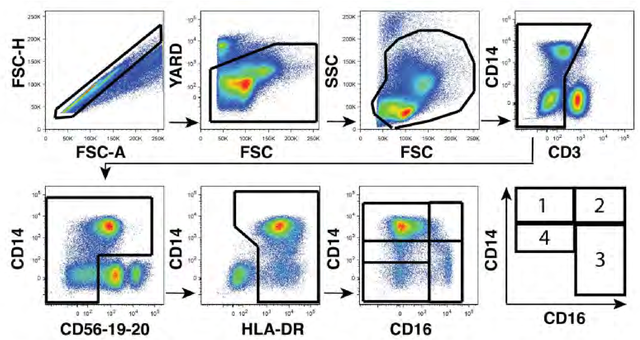
Coronary Artery Calcium (CAC) Score Measurements

- Baseline imaging for this study included measurement of coronary artery calcium (CAC) in both studies.
- Both cohorts had CAC scores measured in the same cardiac CT core laboratory.
- A dual source CT (DSCT) scanner for HAHC-CVD participants.
- For MESA, each of the six centers measured CAC with either a cardiac-gated electron-beam CT scanner (Chicago, Los Angeles, New York), or a multidetector CT (Baltimore, Forsyth County, St. Paul).
- Images were interpreted at the MESA CT reading center (Los Angeles Biomedical Research Institute at Harbor-University of California Los Angeles, Torrance, California), where a radiologist or cardiologist blinded to clinical data quantified CAC score, using an interactive scoring system to calculate Agatston score, with any Agatston score >0 defining the presence of CAC.

- Plasma biomarker multiplexing was performed on HAHC-CVD subjects by Milliplex Human Cardiovascular Disease panels (EMD Millipore, USA) which included sE selectin, sVCAM-1, sICAM-1, MMP-9, MPO, tPA-1, CRP, SAA, SAP, IL-1β, IL-6, IL-8, IL-10, TNF-α, MCP-1, VEGF, IFN-γ, and NT ProBNP.
- CHD risk profiles including the CVD risk factors, clinical profile, and lipid panel were assessed in all participants.

Change in CAC and CIMT (CCA and BIF)over 2 years were analyzed as the primary outcome variable with regards to monocyte subsets, biomarkers, and traditional CHD risk factors.

Cell Staining and Flow Cytometric Analysis



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-)

Results

Baseline Characteristics

N	105
Age, years [median (Q1, Q3)]	51 (47, 57)
Male gender, n (%)	91 (86.6%)
Ethnicity	
Caucasian, n (%)	57 (54.3%)
African American, n (%)	4 (3.8%)
Native Hawaiian/Pacific Islander, n (%)	15 (14.3%)
Asian, n (%)	9 (8.6%)
Others, n (%)	20 (19.0%)
BMI, kg/m^2 [median (Q1, Q3)]	26.0 (24.5, 28.5)
Hypertension, n (%)	42 (40%)
Blood pressure	
Systolic Blood Pressure, mmHg [median (Q1, Q3)]	121 (113, 133)
Diastolic Blood Pressure, mmHg [median (Q1, Q3)]	76 (69, 81)
Fasting plasma glucose, mg/dL [median (Q1, Q3)]	89 (81, 95)
Diabetes mellitus, n (%)	11 (10.6%)
LDL cholesterol, mg/dL [median (Q1, Q3)]	109 (83, 128)
Total/HDL cholesterol ratio [median (Q1, Q3)]	4.1 (3.5, 5.4)
Smoking history, n (%)	66 (63.5%)
10-year CHD risk estimated by Framingham risk score, % [median (Q1, Q3)]	0.06 (0.03, 0.15)
History of clinical CVD events, n (%)	
Myocardial infarction, n (%)	5 (4.7%)
Coronary artery bypass graft, n (%)	2 (1.9%)
Stroke, n (%)	3 (2.8%)
Current Cardiovascular Medications	
ACEI/ARB, n (%)	24 (22.9%)
Beta blocker, n (%)	9 (8.6%)
Statin, n (%)	30 (28.6%)
CD4+ T-cell count, cells/mm^3 [median (Q1, Q3)]	491 (352, 660)
CD4+ T-cell percent [median (Q1, Q3)]	29 (22, 36)
CD8+ T-cell count, cells/mm3 [median (Q1, Q3)]	741 (578, 1069)
CD8+ T-cell percent [median (Q1, Q3)]	45 (35, 51)
CD4/CD8 Ratio [median (Q1, Q3)]	0.68 (0.42, 0.97)
CD4+ T-cell nadir, cells/mm^3 [median (Q1, Q3)]	160 (34, 266)
Undetectable HIV RNA (≤ 50 copies/mL), n (%)	87 (82.8%)
Current Antiretroviral Medications	
Nucleoside reverse transcriptase inhibitor, n (%)	
Non-nucleoside reverse transcriptase inhibitor, n (%)	
Protease inhibitor, n (%)	
Integrase Inhibitor, n (%)	
Hepatitis C infection, n (%)	13 (12.4%)
Soluble biomarkers	
sE-Selectin, ng/mL [median (Q1, Q3)]	30.6 (20.6, 45.9)
sVCAM-1, ng/mL [median (Q1, Q3)]	1126.4 (925.2, 1286.5)
sICAM-1, ng/mL [median (Q1, Q3)]	132.8 (106.5, 156.7)
MMP-9, ng/mL [median (Q1, Q3)]	55.4 (38.3, 86.7)
MPO, ng/mL [median (Q1, Q3)]	16.0 (11.6, 21.2)
TPAI-1, ng/mL [median (Q1, Q3)]	88.9 (71.0, 115.2)
CRP, ng/mL [median (Q1, Q3)]	8023.2 (2576.6, 27488.2)
SAA, ng/mL [median (Q1, Q3)]	11700.4 (3122.9, 37608.4)
SAP, ng/mL [median (Q1, Q3)]	71487.3 (48141.7, 123621.0)
IL-1b, pg/mL [median (Q1, Q3)]	0.305 (0.305, 0.310)
IL-6, pg/mL [median (Q1, Q3)]	1.63 (0.97, 2.51)
IL-8, pg/mL [median (Q1, Q3)]	3.54 (2.77, 4.23)
IL-10, pg/mL [median (Q1, Q3)]	2.52 (1.13, 5.10)
TNF-α, pg/mL [median (Q1, Q3)]	3.05 (1.79, 4.26)
MCP-1, pg/mL [median (Q1, Q3)]	143.1 (118.5, 171.3)
VEGF, pg/mL [median (Q1, Q3)]	24.6 (13.8, 49.5)
IFN-γ, pg/mL [median (Q1, Q3)]	0.61 (0.36, 1.29)
Total monocyte count, cells/L [median (Q1, Q3)]	4.1 x 10^8 (3.3 x 10^8, 5.4 x 10^8)
Monocyte subsets	
Classical monocytes ($CD14^{++}CD16^{-}$) count [median (Q1, Q3)]	3.0 x 10^8 (2.3 x 10^8, 4.1 x 10^8)
Intermediate monocytes ($CD14^{++}CD16^{+}$) count [median (Q1, Q3)]	5.2 x 10^6 (2.3 x 10^6, 1.4 x 10^7)
Non-classical monocytes (CD14low/+CD16++) count [median (Q1, Q3)]	2.4 x 10^7 (1.4 x 10^7, 3.5 x 10^7)
"Transitional" monocytes ($CD14^{dim}CD16^{-}$) count [median (Q1, Q3)]	5.9 x 10^7 (4.3 x 10^7, 9.2 x 10^7)
Right Common Carotid, mm	0.73 (0.67, 0.82)
Right Bifurcation, mm	0.79 (0.72, 0.92)
Non-zero Coronary Artery Calcium, Agatston units	36.5 (15.6, 171.0)

Correlations Between Monocyte Subsets and Change in CIMT, CAC

	All Monocytes	Classical Monocytes	Intermediate Monocytes	Transitional Monocytes	Non-Classical Monocytes
CIMT at bifurcation region (BIF)					
Change in $CIMT_{BIF}$	0.067	0.051	0.094	-0.124	0.285*
CIMT at the common carotid artery (CCA)					
Change in $CIMT_{CCA}$	0.062	0.115	0.013	-0.144	-0.073
Coronary Artery Calcium (CAC)					
Change in CAC	0.031	-0.035	0.123	-0.051	0.274*

*P < 0.05

Correlations Between Soluble Biomarkers and Change in CIMT, CAC

	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, Change	-0.07	-0.08	-0.13	-0.19	-0.04	-0.02	0.25*	0.05	0.001	0.18	-0.28*	-0.19
CIMT-BIF, Change	0.15	0.17	-0.02	0.03	0.03	0.34**	0.01	0.025*	0.10	0.15	0.14	-0.12
CAC, Change	-0.01	-0.02	-0.007	0.22	-0.12	0.45**	0.02	-0.08	-0.03	0.09	0.09	0.02

*P < 0.05
**P < 0.01

Multivariable Models predicting Progression of CIMT and CAC from Non-Classical Monocytes and MCP-1, Separately

Model	Variable	Coefficient	P-value
	Outcome: 2 Year Change in CAC[ł]		
1	Non-Classical Monocyte Count	0.277	0.020
2	MCP-1	0.444	< 0.001
	Outcome: 2 Year Change in CIMT-BIF[‡]		
3	Non-Classical Monocyte Count	0.491	0.046
4	MCP-1	0.829	0.010

ł Coefficient is partial correlation coefficient from partial spearman correlation.
‡ Coefficient is adjusted regression coefficient.

Conclusions

Our findings highlight the role of non-classical monocytes and MCP-1 in the progression of cIMT and CAC in HIV-infected individuals on stable ART independent of traditional cardio-metabolic and HIV virology factors.

Levels of non-classical monocytes level parallel increases in the pro-inflammatory cytokine MCP-1.

Non-classical monocytes may serve as diagnostic index of CVD risk and a potential therapeutic target to ameliorate CVD risk in this vulnerable population.

Acknowledgements

We thank our study participants and community physicians for their roles in this study.

This work was supported by NIH grants U54RR026136, U54MD007584, R01HL095135 , K23 HL088981, G12MD007601, and P20GM103466