CAROTID PLAQUE BURDEN IN HIV IS ASSOCIATED WITH SOLUBLE MEDIATORS AND MONOCYTES

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Conference on Retroviruses & Opportunistic Infections 2020
March 8-11, 2020
Presentation: 0638 (P-L01)

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Abstract

Background
Despite antiretroviral therapy (ART), HIV-infected adults remain at risk cardiovascular disease (CVD) Maximal carotid plaque thickness (MCPT) is the measure of the largest plaque thickness in the carotid artery and reflects atherosclerosis plaque burden. MCPT may be a better predictor of CVD and cerebral vascular accidents (CVA) than carotid artery intima-media thickness (cIMT) because it identifies potential unstable arterial atherosclerotic plaques. We assessed the relationships of monocyte (MO),T-cell populations and plasma soluble mediators with MCPT.

Methods
Cross-sectional and longitudinal analysis of a cohort study of CVD risk in people living with HIV (PLWH) aged > 40 years on stable antiretroviral therapy (ART) > 6 months. High resolution B-mode ultrasound images of the right carotid artery were obtained to assess MCPT at baseline and year 2. At entry, peripheral blood mononuclear cells were obtained and immunophenotypes to assess the frequency of MO subsets [classical MO ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), non-classical ($CD14^{low/+}CD16^{++}$)] and levels of T-cell activation ($CD38^{+}HLA\text{-}DR^{+}$) by flow cytometry. Plasma mediator of inflammation and apolipoproteins were measured by Luminex. Spearman correlation and multivariable regression were used for statistical analysis to identify factors that associated with MCPT.

Results
We studied 125 HIV-infected individuals, 88% male, 55% Caucasian, with a median age of 51, median CD4 count of 477 cells/μL (Q1: 325, Q3: 612), 84% undetectable plasma HIV RNA (<50 copies/mL). Baseline MCPT correlated with non-classical monocyte (r=0.451, p=.046), MCP-1 (r=0.487, p=0.016), TNFα, (r=0.474 p=0.019), sVCAM1 (r=0.472, p=0.020), ApoB6 (r=-0.473, p=0.019) and IL-6 (r=0.455, p=0.025). In a multivariable regression model, MCP-1, TNFα, and sVCAM1 remained significant even after adjustment for age. Follow-up analysis of 15 HIV+ participants 2 years later revealed no correlation between changes in MCPT with types of ART; lipid lowering, hypertensive and antiplatelet medications, tobacco smoking or illicit drug use.

Conclusion
Carotid plaque burden was associated with increased inflammatory, monocyte and endothelial measures including MCP-1, TNFα, and sVCAM1 levels. Changes in MCPT were not associated with any specific therapy, such as antilipid medications. Further investigation into changes in monocyte and inflammatory cytokines on plaque burden is warranted.

Background

- People living with HIV (PLWH) are at an increased risk for cardiovascular disease (CVD), shown by increased prevalence of coronary artery disease and peripheral artery disease (PAD).
- 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 atherosclerosis plaques.
- Maximal carotid plaque thickness (MCPT) is the measure of the largest plaque thickness in the carotid artery and reflects atherosclerosis plaque burden.
- MCPT may be a better predictor of CVD and cerebral vascular accidents (CVA) than carotid artery intima-media thickness (cIMT) because it identifies potential unstable arterial atherosclerosis plaques.
- We assessed the relationships of monocyte (MO), T-cell populations and plasma soluble mediators with MCPT.

Statistical Methods

- Associations between baseline MCPT and soluble biomarkers were evaluated by Spearman correlation.
- Significant factors associated with MCPT were then analyzed using linear regression analysis adjusted for age.
- A two-sided p-value less than 0.05 was considered statistically significant.
- Analyses were performed by SPSS (IBM, Version 24, Armonk, NY).

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Methods

- This is a cross-sectional analysis of the baseline data obtained on participants in the Hawai'i Aging with HIV-Cardiovascular (HAHC-CVD) study.
- HAHC-CVD is a 5-year longitudinal cohort study that examined the role of oxidative stress and inflammation in HIV.
- Participants enrolled had documented HIV infection, > 40 years of age, and were on stable antiretroviral therapy for > 3 months.
- Follow-up assessment was obtained in a subset of participants.

Maximal Carotid Plaque Thickness (MCPT) Measurements

- High-resolution B-mode ultrasound images of the right carotid artery were obtained.
- A single reader measured the intima-media thickness of the far wall of the distal common carotid artery with automated edge detection (Images acquired at The Queen's Medical Center in Honolulu, and analyzed at University of Southern California Atherosclerosis Research Unit).
- Measurements were obtained at the common (CCA) and internal carotid artery (ICA),bifurcation regions of the carotid artery
- Maximal carotid plaque thickness was identified by measuring and choosing the largest plaque found in either the carotid bifurcation, internal carotid artery, or common carotid artery.

Cell Staining and Flow Cytometry Analysis

- Cryopreserved peripheral blood mononuclear cells (PBMC) were surface stained to identify monocyte subpopulations using conjugated monoclonal antibodies(mAb), V500-conjugated antiCD3, Qdot605-conjugated anti-CD14, Alexa700-conjugated anti-CD16, PE-Cy7-conjugated anti-CD56, PE-Cy7-conjugated anti-CD19, PE-Cy7-conjugated anti-CD20, and APC-H7- conjugated HLA-DR monoclonal antibodies (mAbs).
- Monocytes were identified in the HLA-DR positive gate monocyte subpopulations were further defined based on the expression of CD14 and CD16 as previously described(reference). All antibodies were from BD Biosciences, except for Q605-conjugated anti-CD14 and Live/Dead yellow amine-reactive dye (Life Technologies).
- 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.
- Plasma soluble biomarkers were tested with Milliplex Human Cardiovascular Disease panels (EMD Millipore, USA). Soluble biomarkers of inflammation including monocyte chemoattractant protein-1 (MCP-1), sE-selectin, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), matrix metallopeptidase-9 (MMP-9), myeloperoxidase (MPO), tPA-1, CRP, serum amyloid A (SAA), serum amyloid P (SAP), IL-1β, IL-6, IL-8, IL-10, VEGF, IFN-γ, NT ProBNP , TNF-α, and apolipoprotein B6 (ApoB6), were assessed as stated by the manufacturer's protocols.

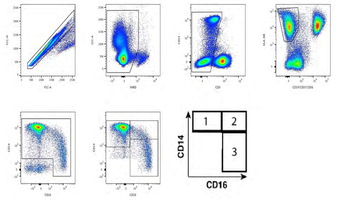

Figure. Multiparametric flow cytometry gating strategy to phenotype three distinct monocyte sub populations from peripheral blood based on CD16 and CD14 expression: (1) classical monocytes lacking CD16 expression (CD14++CD16-) and those expressing CD16 comprised of (2) intermediate (CD14++CD16+) and (3) non-classical (CD14low/+CD16++) monocytes.

Results

Clinical and Laboratory Characteristics of Study participants

	MCPT+	MCPT-	p-value
N	25	100	
Age, years	52 (45.5, 56)	49 (45, 55.75)	0.131
Male gender, n (%)	24 (98)	85 (85)	0.255
Race			
Caucasian, n (%)	14 (56)	55 (55)	>0.999
Native Hawaiian/Pacific Islander, n (%)	3 (12)	12 (12)	>0.999
BMI, kg/m²	25.91 (23.76, 30.19)	25.91 (23.97, 28.47)	0.997
Hypertension, n (%)	11 (44)	27 (27)	0.159
Diabetes mellitus, n (%)	0 (0)	7 (7)	0.381
Smoking History			
Current, n (%)	5 (20)	23 (23)	0.957
Past, n (%)	14 (56)	66 (66)	0.485
Never smoked, n (%)	11 (44)	35 (35)	0.547
Blood pressure			
Systolic Blood Pressure, mmHg	116 (112.5, 128.5)	114 (105, 123)	0.0758
Diastolic Blood Pressure, mmHg	72 (66.5, 73.5)	68 (63, 76)	0.409
Fasting plasma glucose, mg/dL	89 (80.5, 93.5)	86 (81, 93)	0.912
Total Cholesterol, mg/dL	196 (154, 211.5)	178 (157.25, 211.25)	0.573
LDL, mg/dL	122 (97.5, 139.5)	108 (89.25, 134.5)	0.263
HDL, mg/dL	44 (33, 49.5)	45 (34, 56)	0.451
HIV-specific characteristics			
Baseline CD4+ T-cell count, cells/mm³	466 (291, 718.5)	478.5 (339.25, 606.25)	0.946
Baseline HIV-1 viral load, copies/mL	48 (48, 48)	48 (48, 48)	0.938
Undetectable baseline HIV-1 viral load<50 copies/mL n, (%)	21 (84)	84 (84)	>0.999

Association of Variables on MCPT in Study participants

Variable	Unadjusted*		Adjusted coefficient**	
	rho	p	B coefficient	p
MCP-1	.487	.016	.488	.016
TNFα	.474	.019	.444	.030
sVCAM1	.472	.020	.521	.009
Non-Classical Monocytes	.409	.073	.351	.137
ApoB6	-.473	.019	-.280	.211
IL-6	.455	.025	.150	.485

Follow-up Assessment of 15 Participants

	Participant	Baseline MCPT, mm	Year 3 MCPT, mm	Age, yr	Gender	Current Smoker	Hx Smoking	Substance Abuse	Change in ART Medication	Change Chol, mg/dL	Change LDL, mg/dL	Change HDL, mg/dL	Change TG, mg/dL	Baseline CVD Meds
MCPT increase	1	0	2.365	46	Male	No	Yes	None	None	-11	-2	18	-4	Diltiazem Fenofibrate Pravastatin
	2	0	2.192	45	Male	No	No	None	None	-7	8	17	24	Lisinopril
	3	2.096	3.041	48	Male	No	Yes	None	None	-9	-11	21	-77	Irbesartan
	4	2.47	2.517	43	Male	Yes	Yes	Crystal Meth	None	-31	-64	34	-42	
	5	3.469	3.513	69	Male	No	Yes	Alcohol	None	32	-1	31	29	Pravastatin
	6	2.64	2.666	57	Male	No	No	None	None	-3	13	5	-18	Lisinopril Terazosin
MCPT decrease	7	4.614	4.608	54	Male	No	Yes	Alcohol -Nitrates	None	0	9	3	-90	Carvedilol Gemfibrozil Niacin ER Nifedipine Benazepril
	8	6.732	6.724	60	Male	No	No	None	None	24	3	-1	145	Metoprolol Fenofibrate
	9	2.006	1.951	51	Male	Yes	Yes	None	None	21	22	2	175	
	10	2.894	2.807	43	Male	No	No	Nitrates	None	-26	-5	-9	-49	Metoprolol Atorvastatin
	11	2.482	2.254	60	Male	No	No	None	None	-11	-110	3	-46	Lisinopril Atorvastatin
	12	3.041	2.469	45	Male	No	No	Alcohol	Change from Artipla to tenofovir,emtricitabine, raltegravir, darunavir, ritonavir	29	37	6	-17	
	13	2.1765	0	47	Male	No	No	Marijuana	None	78	65	11	17	Atorvastatin
	14	3.0695	0	49	Male	Yes	Yes	Alcohol, Marijuana	None	-4	17	7	-79	Valsartan
	15	3.387	0	49	Male	No	Yes	Marijuana	None	26	10	7	100	

Conclusions

- Maximal carotid plaque thickness is associated with increased inflammatory, monocyte and endothelial biomarkers including MCP-1, TNFα, and sVCAM1 in people living with HIV on stable antiretroviral therapy.
- Changes in MCPT were not associated with any specific therapy, such as antilipid medications.
- We speculate the growth of a carotid plaque is associated with MCP-1, TNFα, and sVCAM1 after the carotid plaque is established.

Acknowledgements

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

NIH grants to now only mention: [U54 NS43049] , [R01HL095135], U54MD007584 and U54MD007601.

Hawaii Center for AIDS