ORIGINAL RESEARCH

Echolucency of the Carotid Artery Intima-Media Complex and Intima-Media Thickness Have Different Cardiovascular Risk Factor Relationships: The Women's Interagency HIV Study

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***Background*—**Adults infected with HIV have increased atherosclerosis potentially associated with both HIV and non-HIV associated factors. We characterized risk factors for atherosclerosis as measured by noninvasive vascular imaging.

***Methods and Results*—**We used B-mode ultrasound to examine levels and correlates of echogenicity and vessel wall thickness of the carotid artery intima-media complex in 1282 HIV-infected and 510 HIV-uninfected women of the Women's Interagency HIV Study. Levels of gray scale median (GSM, a measure of echogenicity) did not vary between HIV infection groups. In both groups, smokers had increased GSM, whereas age, diabetes, elevated blood pressure, and high BMI were associated with lower (rather than higher) GSM. Each of these non-lipid CVD risk factors, especially age and blood pressure, was also associated with higher levels of carotid artery intima-media thickness (cIMT). Higher serum triglyceride levels were associated with lower GSM in both HIV-infected and HIV-uninfected groups. Additional lipid risk factors for low GSM including high LDL cholesterol and low HDL cholesterol levels were identified in HIV uninfected but not in HIV infected women. In contrast to findings for GSM, among the lipid parameters only LDL cholesterol level had an association with cIMT, which was observed only in the HIV uninfected group.

***Conclusions*—**Lipid and non-lipid risk factor associations with echolucency of the carotid artery and the thickness of the common carotid artery intima-media layer suggest that these measures capture different aspects of atherosclerosis. **(*J Am Heart Assoc*. 2015;4:e001405 doi: 10.1161/JAHA.114.001405)**

Key Words: carotid arteries • epidemiology • immune system • risk factors • ultrasonics

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Atherosclerosis is characterized by changes in the structure and composition of blood vessels. Noninvasive vascular imaging may allow ascertainment of underlying structural and compositional arterial changes in patients with and without clinical atherosclerotic vascular disease. Carotid artery intima-media thickness (cIMT) is one such measure that has been widely used. Changes in cIMT primarily reflect vascular remodeling in response to hemodynamic conditions, and accordingly, age and hypertension are the dominant risk factors for increased cIMT. A rationale has been developed for investigating the use of B-mode ultrasound to infer composition of the carotid arteries. Prior studies have measured echogenicity on B-mode ultrasound at regions with carotid artery plaques, and less commonly, echogenicity measurements have also been obtained within carotid artery segments relatively free of disease. Regions of the carotid arterial wall that appear echolucent (black) in B-mode ultrasound images may be indicative of lipid deposits. Regions that appear echodense (white) suggest calcification and presence of fibrous tissue.[1,2] Prior studies show that echogenic properties

of the carotid arterial wall on ultrasound images correlate with the presence of cardiovascular disease (CVD) risk factors[3–5] and risk of all-cause and CVD mortality. High-density lipoprotein cholesterol (HDL-c) level, body mass index (BMI), smoking, and markers of oxidative stress and inflammation are associated with carotid artery echogenicity.[3–5] Older adults who had low levels of arterial echogenicity had significantly increased CVD mortality risk in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study.[6]

The potential value is that a standardized noninvasive approach to measuring vessel echogenicity may interrogate a different aspect of the disease process versus other well-established sonographic measures such as arterial wall thickness (ie, cIMT). Carotid artery echogenicity and cIMT have only a modest correlation with one another (eg, $r<-0.20$).[6] In the PIVUS study, the ability of echogenicity to predict mortality was independent of cIMT. Moreover, patterns of classical CVD risk factor relationships may differ between cIMT and echogenicity of the intima-media complex, which would suggest that echogenicity and cIMT reflect different pathogenic processes that lead to atherosclerosis.

It is well established that HIV infection is associated with premature atherosclerosis likely due to multiple factors including HIV-related inflammation, HIV treatment-related lipodystrophy and hypertension, and immunodeficiency.[7,8] Traditional risk factors such as lipids and smoking appear to be more important than HIV disease-related variables in producing increased cIMT in HIV-infected adults.[9] To our knowledge, echogenicity of the carotid artery intima-media complex has not been studied in an HIV-infected population. We applied B-mode carotid artery ultrasound in the Women's Interagency HIV Study (WIHS) to address several research aims: (1) to characterize echogenicity of the intima-media complex of the common carotid artery wall (defined as gray-scale median, GSM) in HIV-infected women; (2) to identify risk factors for carotid artery echogenicity and cIMT in the same HIV-infected women; and (3) to examine whether HIV-infected and HIV-uninfected women differ in carotid artery ultrasound parameters or in risk factor relationships.

Methods

Study Population

The Women's Interagency HIV Study (WIHS) cohort consists of over 4000 HIV-infected women and HIV-uninfected controls enrolled at multiple US urban sites.[10] WIHS participants complete study visits every 6 months for collection of biological specimens, questionnaire data, and clinical measurements. The Carotid Artery Ultrasound Substudy (CA US) was initiated in April 2004 and included 1865 women (1331 HIV-infected and 534 HIV-uninfected).[11] Of those in CA US, 1792 (1282 HIV-infected and 510 HIV-uninfected) were included in the present analysis and 73 women were excluded (62 participants were excluded in the present analysis for missing data on echolucency and another 11 were excluded for being an HIV seroconverter). Institutional Review Board approval and informed consent were obtained on all participants.

Carotid Artery Ultrasound

During 2004–2005, high-resolution B-mode ultrasound carotid artery images of the far wall of the right common carotid artery were obtained. Standardized carotid artery ultrasound images were centrally measured by automated edge detection software (Patents 2005, 2006, 2011).[12–15] Intima-media thickness of the right common carotid artery far wall (cIMT) was measured along a 1-cm segment determined by an electronic ruler just distal to the bifurcation. In the same common carotid artery segment, the echo-strength (based on the echo grey-white scale from 0 to 255) of each individual pixel within the intima-media complex was measured by the automated software. The grey scale median (GSM) was then calculated from all the pixels on the scale from 0 to 255 (unitless). Each image was calibrated to account for image contrast and brightness by using standardized nearby structures. Low GSM values represent echolucency (black), while high GSM values indicate echodensity (white). Measurements were determined at the same point in the cardiac cycle for each participant, namely the R-wave.

Assays

HIV infection was determined via serologic testing using enzyme-linked immunosorbent assay (ELISA) and confirmed using Western blot assays. Plasma HIV RNA levels were quantified using nucleic acid sequence-based amplification commercial assays with a lower limit of quantification of 80 copies/mL (bioMérieux, Boxtel, NC). Total peripheral CD4+ T cell counts were measured with standard flow cytometric methods. Circulating levels of total cholesterol (Tc), high-density lipoprotein cholesterol (HDL-c) and triglycerides were measured at central laboratories. Fasting values were used in all analyses. Low-density lipoprotein cholesterol (LDL-c) was estimated from Tc, HDL-c and triglycerides, when appropriate (eg, fasting specimens with triglycerides <4.5 mmol/L), or otherwise measured directly.

Variable Definitions

Study variables included age at visit (in years); LDL-c level (mmol/L); HDL-c level (mmol/L); triglycerides level (mmol/

L); current smoking status (smoker, non-smoker); race/ethnicity (African-American/Black, Hispanic, White/other); diabetes (defined as fasting glucose level ≥6.9 mmol/L, self-reported physician's diagnosis of diabetes or use of diabetes medications); body mass index (BMI, kg/m^2); and systolic and diastolic blood pressures (mm Hg). Analyses of HIV-infected individuals also included the following HIV-related variables: current and nadir CD4+ T cell count in cells/mm^3 (continuous as well as categories ≥500, 350 to 500, 200 to 350, and <200), current plasma HIV RNA in copies/mL (continuous on the $\log_{10}$ scale), highly active antiretroviral therapy (HAART) use (current, former and never), and duration of HAART use. HAART duration was quantified by summing the number of semi-annual study visits where a participant reported using HAART therapy at the time of visit.

Statistical Analyses

GSM and cIMT each had distributions that approximated normality. Initial analyses examined age-adjusted comparisons of GSM and cIMT between HIV-infected and HIV-uninfected groups. Age-adjusted mean GSM and cIMT were calculated by nadir and current CD4+ T cell count category. Student's *t* tests tested for differences of GSM and cIMT across categories of CD4+ T cell count. After assigning the within-category median CD4+ T cell count value to each individual within a given CD4+ T cell count category, we tested for linear trend between CD4+ T cell count with GSM and cIMT in age-adjusted linear regression models. Possible non-linear relationship between nadir and current CD4+ T cell counts with GSM and cIMT were assessed using quadratic terms. We conducted parallel analyses among HIV-infected and HIV-uninfected groups to identify the association of standard CVD risk factors and HIV disease stage (eg, CD4+ T cell count, plasma HIV RNA) with cIMT and GSM. To assess the association between cIMT or GSM and continuous variables, we calculated Pearson partial correlation coefficients, adjusting for age. Log-transformations were applied to triglyceride levels and plasma HIV RNA to improve normality. To examine the association between cIMT or GSM and categorical variables (race/ethnicity, diabetes status, and current smoking status), we calculated age-adjusted means within each category. Statistical significance of differences in GSM and cIMT across categories was assessed by analysis of covariance. We ran separate regression models for each independent variable, adjusting for age.

Among HIV-infected women, all participants with complete covariate information were included in separate multivariable linear regression models, run separately with GSM or cIMT as the dependent variables, and the following independent variables: age, race/ethnicity, HDL-c level, LDL-c level, triglyceride level (log-transformed), BMI, systolic and diastolic blood pressure, diabetes status, current smoking status, and HIV disease and treatment status. In additional analyses, to examine whether associations between risk factors and GSM were independent of cIMT, and vice versa, we included cIMT and GSM as independent variables in the fully adjusted multivariable models. These did not produce substantially different results from the analyses presented in this manuscript (data not shown). We also assessed the possibility of a U-shaped relationship using a quadratic term for cardiovascular risk factors in linear regression models examining GSM and did not find any evidence of a curvilinear association between each examined risk factor and GSM. $P<0.05$ was considered statistically significant. While stratified analysis was the original approach, we also examined statistical tests of interaction between CVD risk factor and HIV serostatus in models adjusted for age and race.

All analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC).

Results

Subject Characteristics

Compared with 510 HIV-uninfected women, 1282 HIV-infected women were on average 3 to 4 years older, had lower BMI and HDL-c levels, and higher triglyceride levels ($P<0.0001$) (Table 1). In the overall sample, correlation between cIMT and GSM (echogenicity) was $r=-0.21$, $P<0.0001$. This correlation was $r=-0.17$ ($P<0.0001$) in HIV-infected women and $r=-0.28$ ($P<0.0001$) in HIV-uninfected women.

Association Between GSM and cIMT and HIV Infection Status

Mean age-adjusted GSM was not significantly different between HIV serostatus groups (63.1 in HIV-uninfected and 62.5 in HIV-infected women, $P=0.54$). Mean age-adjusted cIMT was higher in HIV-uninfected women (738.0 μm) than in HIV-infected women (718.8 μm, $P=0.0003$). Among HIV-infected women, current CD4+ T cell count had a weak correlation with GSM and with cIMT (age-adjusted $r=-0.08$ and $r=0.07$, respectively, both $P<0.05$, Table 2); nadir CD4+ T cell count was not associated with GSM but was significantly associated with cIMT (age-adjusted $r=0.06$, $P=0.04$, Table 2). Current HIV RNA was positively associated with GSM and not associated with cIMT after adjustment for age (Table 2). No association was observed between age-adjusted GSM or cIMT and use of HAART or cumulative duration of HAART. Category-based analyses did not reveal a dose-response pattern of increasing or decreasing age-adjusted GSM or cIMT across the range of CD4+ T cell counts (Tables 3 and 4). After

Table 1. Demographic Characteristics of HIV-Infected and HIV-Uninfected WIHS Participants

	HIV-Infected (N=1282)		HIV-Uninfected (N=510)	
Continuous variables	N	Mean (SD) or median (Q1 to Q3)	N	Mean (SD) or median (Q1 to Q3)
Age, y	1282	41.6 (8.8)	510	37.9 (10.0)
GSM (unitless)	1282	62.1 (18.2)	510	64.2 (19.7)
cIMT, μm	1282	725.7 (114.1)	510	720.8 (121.0)
LDL cholesterol, mmol/L	1264	2.6 (0.8)	504	2.7 (0.9)
HDL cholesterol, mmol/L	1263	1.2 (0.4)	504	1.4 (0.4)
Triglycerides, mmol/L	1226	1.3 (0.9 to 1.9)	486	1.0 (0.73 to 1.34)
BMI, kg/m^2	1262	28.3 (7.3)	497	30.8 (8.5)
Diastolic blood pressure, mm Hg	1279	74.1 (9.0)	509	74.2 (9.0)
Systolic blood pressure, mm Hg	1279	116.7 (13.9)	509	118.1 (14.7)
Categorical variables	N	%	N	%
Race/ethnicity				
African-American/black	746	58.2	311	61.0
Hispanic	367	28.6	143	28.0
White/other	169	13.2	56	11.0
Lipid-lowering medication				
Yes	92	7.2	15	2.9
No	1190	92.8	495	97.1
Diabetes				
Present	234	18.3	88	17.3
Absent	1048	81.8	422	82.8
Current smoking status				
Smoker	564	44.3	253	49.6
Non-smoker	709	55.7	257	50.4
HIV-related variables	N	Mean (SD) or median (Q1 to Q3) or %		
Current CD4+ T cell count, $cells/mm^3$	1266	467.9 (286.1)		—
Nadir CD4+ T cell count, $cells/mm^3$	1266	252.2 (187.7)		—
Log_{10} HIV RNA, copies/mL	1274	2.9 (1.2)		—
HAART use				
Never	185	14.40		—
Former	305	23.80		—
Current	792	61.80		—
HAART duration, y	792	7.2 (5.4)		—

BMI indicates body mass index; cIMT, carotid artery intima-media thickness; GSM, grey scale media; HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WIHS, Women's Interagency HIV Study.

adjustment for potential confounders including age, race/ethnicity, serum lipids, blood pressure, and smoking in multivariable models, no significant association was observed between HIV serostatus and GSM or cIMT. After confounder adjustment, GSM was similar in the HIV-infected as compared with the HIV-uninfected women (adjusted difference or β comparing GSM in HIV-infected versus HIV-uninfected=−1.68, P=0.09). In confounder-adjusted models, the difference comparing cIMT in HIV-infected versus HIV-uninfected was 6.38 μm, P=0.24.[11]

Table 2. Age-Adjusted Association of Cardiovascular Disease Risk Factors With GSM and cIMT Among HIV-Infected Women, WIHS

	HIV-Infected (N=1282)				
		GSM, unitless		cIMT, μm	
Continuous variables		*r**	*P* value	*r**	*P* value
Age, y	1282	−0.17	<0.0001	0.49	<0.0001
LDL cholesterol	1264	−0.03	0.35	0.05	0.07
HDL cholesterol	1263	0.04	0.16	−0.01	0.67
Triglycerides†	1226	−0.14	<0.0001	−0.01	0.73
BMI	1262	−0.31	<0.0001	0.12	<0.0001
Diastolic blood pressure	1279	−0.05	0.09	0.16	<0.0001
Systolic blood pressure	1279	−0.09	0.001	0.21	<0.0001
Categorical variables		Mean GSM (95% CI)	*P* value	Mean cIMT (95% CI)	*P* value
Race/ethnicity			0.48		<0.0001
African-American/black	746	62.6 (61.3, 63.9)		741.5 (734.5, 748.6)	
Hispanic	367	61.6 (59.8, 63.5)		701.0 (690.9, 711.1)	
White/other	169	61.0 (58.2, 63.7)		709.3 (694.3, 724.3)	
Diabetes			0.003		0.0001
Present	234	58.9 (56.5, 61.2)		748.8 (735.9, 761.8)	
Absent	1048	62.8 (61.7, 63.9)		720.5 (714.4, 726.5)	
Current smoking status			0.004		<0.0001
Smoker	564	63.7 (62.2, 65.2)		742.3 (734.1, 750.5)	
Non-smoker	709	60.8 (59.5, 62.1)		712.4 (705.1, 719.7)	
HIV-related variables		*r**	*P* value	*r**	*P* value
Current CD4+ T cell count	1266	−0.08	0.005	0.07	0.01
Nadir CD4+ T cell count	1282	−0.03	0.28	0.06	0.04
Current HIV RNA†	1274	0.08	0.004	0.02	0.53
		Mean GSM (95% CI)	*P* value	Mean cIMT (95% CI)	*P* value
HAART use			0.08		0.75
Never	185	62.6 (60.0, 65.2)		730.8 (716.3, 745.2)	
Former	305	63.9 (61.9, 65.9)		724.7 (713.4, 735.9)	
Current	792	61.3 (60.0, 62.5)		724.9 (717.9, 731.8)	
		*r**	*P* value	*r**	*P* value
HAART duration, y	1282	−0.0009	0.75	−0.05	0.08

BMI indicates body mass index; cIMT, carotid artery intima-media thickness; GSM, grey scale media; HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WIHS, Women's Interagency HIV Study.
*Partial Pearson correlation coefficient adjusted for age.
†Variable was log-transformed.

Risk factors for GSM and cIMT among HIV-infected women

Among HIV-infected women, advanced age was correlated weakly with low GSM (r=−0.17) and correlated moderately with high cIMT (r=0.49, Table 2). In age-adjusted analyses among HIV-infected women, higher BMI and diabetes were also associated with lower GSM and higher cIMT. Systolic and diastolic blood pressures had weaker correlations with GSM (age-adjusted r=−0.09, P=0.001 and r=−0.05, P=0.09) than with cIMT (age-adjusted r=0.21 and r=0.16, P<0.0001). In contrast with the abovementioned variables, which had directionally opposite associations with GSM and cIMT, current smoking as compared with non-smoking was associated with significantly higher levels of both GSM and cIMT. Other variables were significantly associated with only one of the carotid artery measures in HIV-infected women: high

Table 3. Association of CD4+ T Cell Count With GSM Among HIV-Infected Women, WIHS

	N	Mean GSM* (95% CI)	*P* Value*	*P* Value*, Linear Trend	*P* value*, Non-Linear Trend
Nadir CD4+ T cell count, cells/mm^3				0.73	0.95
HIV+, ≥500	128	61.7 (58.5, 64.8)	Ref		
HIV+, 350 to 500	194	62.2 (59.6, 64.7)	0.81		
HIV+, 200 to 350	409	61.9 (60.2, 63.7)	0.89		
HIV+, <200	551	62.3 (60.8, 63.8)	0.72		
Current CD4+ T cell count, cells/mm^3				0.10	0.77
HIV+, ≥500	507	61.2 (59.7, 62.8)	Ref		
HIV+, 350 to 500	278	61.2 (59.1, 63.4)	0.99		
HIV+, 200 to 350	287	63.5 (61.5, 65.6)	0.08		
HIV+, <200	194	63.1 (60.5, 65.6)	0.23		

Age-adjusted correlation coefficient between nadir CD4+ T cells count with GSM=−0.03, *P*=0.28; age-adjusted correlation coefficient between current CD4+ T cells count with GSM=−0.08, *P*=0.005. GSM indicates grey scale median; WIHS, Women's Interagency HIV Study.
*Age-adjusted.

triglyceride level was associated with lower GSM but not with cIMT; African-American race was associated with higher cIMT but not with GSM.

Subsequent multivariable analyses among 1178 HIV-infected women included demographic variables, CVD risk factors, and HIV-related variables (ie, current CD4+ T cell count, plasma HIV RNA viral load, and HAART use). In multivariable analyses, lower GSM and higher cIMT were significantly associated with older age and higher BMI (Table 5). Lipid-related variables were associated with GSM but not with cIMT in multivariable analyses. Higher triglyceride level was associated with lower GSM (*P*=0.002), and higher LDL-c level had a significant association with higher GSM (*P*=0.04) (Table 5). Other variables in multivariable models were independently associated with higher cIMT but not with GSM: systolic blood pressure, smoking, and diabetes. The associations between CVD risk factors and GSM were similar after additional adjustment for cIMT, and likewise the variables associated with cIMT were similar after adjustment for GSM (data not shown).

Risk factors for GSM and cIMT among HIV-uninfected women

Age-adjusted analyses of risk factors among HIV-uninfected women mirrored several of the findings among the HIV-

Table 4. Association of CD4+ T Cell Count With cIMT Among HIV-Infected Women, WIHS

	N	Mean cIMT* (95% CI)	*P* Value*	*P* Value*, Linear Trend	*P* Value*, Non-Linear Trend
Nadir CD4+ T cell count, cells/mm^3				0.04	0.31
HIV+, ≥500	128	737.5 (720.1, 754.9)	Ref		
HIV+, 350 to 500	194	722.4 (708.3, 736.5)	0.18		
HIV+, 200 to 350	409	735.1 (725.4, 744.7)	0.81		
HIV+, <200	551	717.1 (708.7, 725.5)	0.04		
Current CD4+ T cell count, cells/mm^3				0.06	0.44
HIV+, ≥500	507	732.6 (723.9, 741.3)	Ref		
HIV+, 350 to 500	278	723.5 (711.7, 735.3)	0.22		
HIV+, 200 to 350	287	719.3 (707.6, 730.9)	0.07		
HIV+, <200	194	721.1 (707.0, 735.3)	0.18		

Age-adjusted correlation coefficient between nadir CD4+ T cells count with cIMT=0.06, *P*=0.04; age-adjusted correlation coefficient between current CD4+ T cells count with cIMT=0.07, *P*=0.01. cIMT indicates carotid artery intima-media thickness; WIHS, Women's Interagency HIV Study.
*Age-adjusted.

Table 5. Multivariable Analysis of Cardiovascular Disease Risk Factors With GSM and cIMT in HIV-Infected Women, WIHS

	HIV-Infected (N=1178)			
	GSM, unitless		cIMT, μm	
	β (95% CI)	*P* Value	β (95% CI)	*P* Value
Age, per y	−0.3 (−0.4, −0.2)	<0.0001	5.6 (4.8, 6.3)	<0.0001
Race group				
Black	2.7 (−0.5, 5.8)	0.10	16.6 (−1.4, 34.5)	0.07
Hispanic	2.0 (−1.3, 5.3)	0.24	−9.0 (−27.9, 10.0)	0.35
LDL cholesterol, per mmol/L	1.3 (0.1, 2.5)	0.04	6.7 (−0.3, 13.8)	0.06
HDL cholesterol, per mmol/L	−0.6 (−3.2, 1.9)	0.62	−5.3 (−19.8, 9.1)	0.47
Triglycerides*, per unit	−3.1 (−5.2, −1.1)	0.002	−4.2 (−15.8, 7.5)	0.48
BMI, per kg/m^2	−0.8 (−0.9, −0.6)	<0.0001	1.0 (0.1, 1.8)	0.02
Diastolic BP, per 5 mm Hg	0.2 (−0.7, 1.0)	0.68	0.9 (−3.9, 5.7)	0.71
Systolic BP, per 5 mm Hg	−0.3 (−0.8, 0.3)	0.37	5.8 (2.6, 9.0)	0.0005
Diabetes	0.1 (−2.6, 2.7)	0.96	18.3 (3.4, 33.3)	0.017
Smoking	1.6 (−0.4, 3.6)	0.13	28.0 (16.3, 39.6)	<0.0001
Current CD4+ T cell count, per 50 cells/mm^3	−0.06 (−0.26, 0.13)	0.52	1.0 (−0.1, 2.1)	0.09
HIV RNA*, per unit	0.01 (−1.11, 1.14)	0.98	3.13 (−3.32, 9.57)	0.34
HAART use				
Former (vs never)	1.38 (−1.93, 4.71)	0.41	0.02 (−19.01, 18.97)	0.99
Current (vs never)	−0.8 (−3.9, 2.3)	0.62	12.1 (−5.5, 29.6)	0.18

Analyses are adjusted for all variables in table. BMI indicates body mass index; BP, blood pressure; cIMT, carotid artery intima-media thickness; GSM, grey scale media; HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WIHS, Women's Interagency HIV Study.
*Log-transformed.

infected women (Table 6): (1) older age was modestly correlated with lower GSM and robustly correlated with higher cIMT; (2) higher BMI and diabetes were associated with lower GSM as well as higher cIMT; (3) smoking was associated with higher GSM, contrasting with other risk factors that were associated with lower GSM; and (4) blood pressure was more strongly associated with cIMT than with GSM. In addition, among HIV-uninfected women, the classic CVD-related dyslipidemic patterns including high LDL-c level and low HDL-c level were associated at a moderate-to-high degree of statistical significance with low GSM (age-adjusted P=0.04 and P=0.01, for LDL-c and HDL-c, respectively). High triglyceride level also correlated with low GSM at marginal levels of statistical significance (age-adjusted r=−0.08, P=0.06). LDL-c level also had a significant correlation with cIMT (P=0.02) but HDL-c level and triglyceride level did not (P=0.49 and P=0.20, respectively). The ability to conduct multivariable analyses in the HIV-uninfected group was limited by the relatively small numbers of HIV-uninfected women (N=473) as compared with the size of the HIV-infected group. Interaction terms did not yield P values indicating that risk factors for GSM and cIMT had significantly different effect estimates in HIV-uninfected and HIV-infected groups (P>0.05 for all, except for diabetes-GSM, which had P=0.007 suggesting a stronger association in the HIV-uninfected group), although power to detect interaction is likely poor and multiple tests were performed.

Discussion

In the WIHS cohort of HIV-infected and HIV-uninfected women we observed a trend suggesting lower cIMT in the HIV-infected group as compared with the HIV-uninfected group, confirming our previously published results.[11] In addition, HIV-infected and HIV-uninfected groups did not differ significantly in measures of arterial echogenicity (GSM) defined at a 1-cm segment of the far wall of the common carotid artery. As expected, elevated blood pressure was a predominant risk factor for cIMT in both HIV serostatus groups. Thus, the comparison of cIMT, moreso than GSM, across HIV serostatus groups may reflect the influence on blood pressure of HIV infection and treatment.[16] In both HIV serostatus groups, GSM was lower and cIMT was higher with advancing age. Associations of several non-lipid risk factors such as smoking, BMI, and diabetes with measures of GSM and cIMT were also similar across HIV-infected and HIV-uninfected groups. Finally, the associations between lipid

Table 6. Age-Adjusted Association of Cardiovascular Disease Risk Factors With GSM and cIMT Among HIV-Uninfected Women, WIHS

	HIV-Uninfected (N=510)				
		GSM, unitless		cIMT, μm	
Continuous variables		*r**	*P* value	*r**	*P* value
Age	510	−0.28	<0.0001	0.58	<0.0001
LDL cholesterol	504	−0.09	0.04	0.1	0.02
HDL cholesterol	504	0.12	0.01	−0.03	0.49
Triglycerides†	486	−0.08	0.06	−0.06	0.20
BMI	497	−0.36	<0.0001	0.07	0.10
Diastolic BP	509	0.01	0.79	0.24	<0.0001
Systolic BP	509	−0.02	0.63	0.25	<0.0001
Categorical variables		Mean (95% CI)	*P* value	Mean (95% CI)	*P* value
Race/ethnicity			0.38		0.11
African-American/black	311	65.1 (62.9, 67.2)		731.2 (720.3, 742.0)	
Hispanic	143	63.4 (60.3, 66.5)		706.4 (690.4, 722.4)	
White/other	56	61.6 (56.7, 66.6)		700.3 (674.7, 725.9)	
Diabetes			<0.0001		0.003
Present	88	56.6 (52.6, 60.6)		750.5 (729.5, 771.4)	
Absent	422	65.8 (64.0, 67.6)		714.6 (705.3, 724.0)	
Current smoking status			0.02		0.19
Smoker	253	66.1 (63.8, 68.5)		726.7 (714.4, 738.9)	
Non-smoker	257	62.3 (60.0, 64.6)		715.1 (703.0, 727.2)	

BMI indicates body mass index; BP, blood pressure; cIMT, carotid artery intima-media thickness; GSM, grey scale media; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WIHS, Women's Interagency HIV Study.

*Partial Pearson correlation coefficient adjusted for age.

†Variable was log-transformed.

variables and carotid artery parameters differed between HIV serostatus groups. Higher LDL-cholesterol levels and lower HDL-cholesterol levels were associated in particular with lower GSM in HIV-uninfected women. High serum triglyceride levels were consistently associated with low GSM in both HIV-infected and HIV-uninfected groups, although this may be more important in the HIV-infected group, which had higher average triglyceride levels.

We observed no significant difference in mean GSM by HIV serostatus. In addition, no consistent associations between HIV disease parameters such as total CD4+ T cell count or antiretroviral treatment status or duration and GSM were observed within the HIV-infected group. The cross-sectional nature of the carotid artery echogenicity assessments is an inherent limitation that impeded our ability to study the association of HIV disease history with echogenicity. Features of long-term HIV infection may affect arterial parameters in complex ways, leading to either increases or decreases in measures over time as HIV disease status and treatment exposures evolve.

We found that GSM and cIMT had different relationships with CVD risk factors. Smokers had higher GSM than non-smokers, whereas other risk factors such as age, diabetes, elevated blood pressure, and high BMI were associated with lower (rather than higher) GSM. These same established CVD risk factors including cigarette smoking also were associated with higher levels of cIMT. Age and blood pressure level were associated more strongly with cIMT than with GSM. These patterns of association were consistent across both HIV-infected and HIV-uninfected groups.

Our results are consistent with the notion that reduced arterial echogenicity may be a measure of triglyceride-rich lipoprotein content in the vessel wall.[17–19] We observed an association between higher serum triglyceride level and lower GSM in both HIV-infected and HIV-uninfected groups. We also observed associations between other atherogenic lipid phenotypes (low serum HDL-c and high serum LDL-c) with low GSM. These associations of HDL-c and LDL-c levels with carotid artery echogenicity were seen more strongly and consistently in the HIV-uninfected women as compared with

the group of HIV-infected women. In contrast, lipid risk factors for cIMT had relatively weak effects and only achieved significance in the HIV-uninfected group (LDL-c level, P=0.02).

The present findings lend plausibility to the hypothesis that vessel wall echogenicity and thickening of the vessel wall may reflect different pathogenic pathways to CVD. Echogenicity may provide a description of tissue composition of the intima layer of the vascular wall and thus be a sensitive marker of vascular disease pathophysiology above and beyond cIMT in which lipids play an integral role. Arterial wall thickening, as reflected in a high cIMT, is a strongly age- and blood pressure-dependent process. cIMT is affected by systemic hypertension, reflecting adaptive remodeling in response to hemodynamic parameters. This is of importance because HIV-infected patients may have decreased blood pressure prior to receiving treatment, while antiretroviral medication use over time may be related to increase in systolic and diastolic blood pressures.[16,20] The overall effect on carotid artery parameters, and cIMT in particular, may reflect the cumulative effect of hypertension status over years of infection, which in the case of the WIHS cohort, established in 1994, includes a longer duration of untreated infection than may be the case in contemporary patient groups.

The risk factor associations observed here among both HIV-infected and HIV-uninfected women provide additional insight into the interpretation of the carotid artery ultrasound measurements. Smoking, typically the most prevalent CVD risk factor in US HIV-infected patient populations, was shown to increase carotid artery echogenicity, possibly reflecting a fibrotic or sclerotic process initiated by constituents of tobacco smoke. In the PIVUS cohort, while low carotid artery echogenicity was the predominant risk factor for CVD mortality, individuals at the highest levels of echogenicity also tended to have increased mortality, which may be explained by the high smoking prevalence and long-term cumulative smoking exposure in that European cohort of older adults.[6] Associations of carotid artery parameters with lipid levels were also of interest, because as is well known, HIV infection produces lower LDL-c and HDL-c levels and elevated serum triglycerides.[21] Based upon prior results, and also as suggested here, it is uncertain whether the canonical associations of lipid levels with CVD risk that are found in non-HIV-infected populations may differ in HIV-infected adults, or in subsets defined by antiretroviral treatment or duration of infection.[22]

As these data are cross-sectional, we are unable to infer causality between risk factors and carotid artery wall measurements. Longitudinal follow-up of the cohort for progression of echogenicity is in progress. Additionally, we studied fewer HIV-uninfected than HIV-infected women, and we therefore may have lacked power to detect associations in stratified and interaction analyses and had insufficient sample size for multivariable analyses of HIV-uninfected women. The WIHS cohort includes only women, so we are unable to generalize these findings to men.

In conclusion, we confirmed that the echogenicity of B-mode ultrasound images obtained from a standardized, 1-centimeter region of the common carotid artery wall is associated with several CVD risk factors in HIV-infected persons. Our findings extend this approach to noninvasive carotid artery imaging to the setting of HIV infection.[3,5] Echolucency of carotid artery plaques and the thickness of the common carotid artery intima-media layer may capture different aspects of atherosclerosis.[4] The potential utilization of these distinct measures as risk assessment and research tools for identifying atherosclerosis in earlier stages should be explored.[3,4]

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Disclosures

None.

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