SUPPLEMENTARY MATERIAL

HIV indirectly accelerates coronary artery disease by promoting the effects of risk factors - longitudinal observational study

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Supplemental text

SUPPLEMENTAL METHODS

Definitions of demographic characteristics

At the time of each CT, patients underwent a detailed interview to obtain information about medical history and risk factors, including alcohol consumption, cocaine use and cigarette smoking. Regarding cardiovascular risk factors, hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or antihypertensive medication use verified by medical records. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL or antidiabetic medication use verified by medical records. Positive family history for CAD was considered positive if first-degree relatives had a clinical diagnosis of significant CAD. A physical examination was performed, and anthropometrics were recorded. Routine clinical laboratory blood chemistry tests were conducted. The following laboratory tests were performed: total cholesterol; triglycerides; high-density lipoprotein; low-density lipoprotein glucose and high-sensitivity C-reactive protein.

Native and coronary CTA acquisition protocols

Protocols were published earlier.[1,2] The participants' studies were performed at the Johns Hopkins Outpatient Center. Imaging was performed on a Sensation 64-slice Cardiac scanner (Siemens Medical Solutions, Erlangen, Germany) from May 2004 through June 2009 and a Siemens 128-slice dual-source CT scanner (Somatom Definition FLASH; Siemens Healthcare, Forchheim, Germany) from July 2009 through February 2015. Non-contrast examinations were performed using 120kVp with a slice thickness of 3 mm. For coronary CTA depending on patient size, 100 or 120 kVp was used with a rotation time of 0.33 seconds and a collimation of 64x0.6 mm for the 64-slice scanner and a rotation time of 0.28 seconds and a collimation of 128x0.6 mm for the 128-slice scanner. Scan data were reconstructed to a 512x512 matrix using a slice thickness of 0.75 mm at 0.4 mm increments for the 64-slice

scanner and 0.5 mm increments for the 128-slice scanner. The scan protocol used between 80mL and 100 mL of an iodixanol based contrast agent (Vispaque-320; GE Medical Systems, GE Healthcare Ireland, Cork, Ireland) injected at 5 to 6 cc/sec with test bolus data used to calculate the peak contrast enhancement to determine correct scan delay. All image data were transferred to a dedicated workstation for analysis (Syngo.via; Siemens Medical Solutions, Malvern, PA). All images were randomly interpreted by a level-3 certified specialist in cardiac CT imaging with 5 years of experience and blinded to all clinical data.

Statistical analysis

Continuous variables are presented as means and standard deviations, while categorical variables are shown as frequencies and percentages. Continuous variables were compared using the t-test or the paired t-test as appropriate, while categorical parameters were compared using the Fisher's-test or McNemar's-test as appropriate.

We used linear mixed models to analyze our longitudinal data. We first assessed the need to include a random intercept at a patient level. Next we evaluated the need to include a random slope term per-patient. For the random slope we used the time difference between the baseline and the follow-up scans.[3] We used the likelihood ratio test to assess the potential significant deterioration in fit if we were to remove one or both random terms when we only had a fixed intercept term. Our results show the need for both random slope and intercept for all outcomes (supplemental tables 5-7*)*. We then ran univariate models by adding each predictor, time difference between the two scans and their interaction as fixed effects. Restricted maximum likelihood was used to fit the models. We assessed the statistical significance of the fixed terms using Satterthwaite's method. Furthermore, we calculated the confidence intervals by computing a likelihood profile and finding the appropriate cutoffs based on the likelihood ratio test. If either the main effect or the interaction with time had a p value smaller than 0.10,

we included the parameter in the multivariate model. For the multivariate model, we included all significant parameters, time between the CT scans, and their interactions with time into the models. Linear mixed models were built using the lme4 (v.1.1-21) package.[4] P values were calculated using the lmerTest (v.3.1-0) package.[5] All calculations were performed in the R (v. 3.6.1) environment [6].

SUPPLEMENTAL TABLES

Supplemental table 1. Annual progression rates of coronary artery disease parameters stratified by HIV-serostatus.

Coronary artery disease parameters	Annual progression rates		
	HIV-infected	HIV-uninfected	p
Clinical characteristics			
Agatston-score (unit/year)	10.83±25.13	7.19±17.84	0.174
Number of plaques (unit/year)	0.23±0.28	0.33±0.47	0.106
Segment Stenosis Score (unit/year)	0.53±0.84	0.54±1.29	0.961

Data reported as average and standard deviation.

Supplemental table 2. Demographic, behavioral, clinical, laboratory and coronary artery disease characteristics at baseline and follow-up stratified by cocaine use.

Characteristic	Cocaine users (n=174)			Cocaine non-users (n=126)			Cocaine users vs. Cocaine non-users	
	Baseline	Follow-up	p	Baseline	Follow-up	p	Baseline p	Follow-up p
Anthropometrics								
Age (year)	48.6 ± 6.2	53.1 ± 6.5	<0.0001	47.1 ± 8.4	50.3 ± 8.1	<0.0001	0.083	0.001
Male sex (n, %)	126 (72.4%)	126 (72.4%)	1.000	84 (66.7%)	84 (66.7%)	1.000	0.308	0.308
BMI (kg/m^2)	26.4 ± 5.5	26.9 ± 5.7	0.075	26.8 ± 4.9	27.0 ± 5.3	0.511	0.509	0.900
Follow-up time (years)	-	4.5 ± 2.6	-	-	3.2 ± 1.6	-	-	<0.0001
Cardiovascular risk factors								
ASCVD risk (%)	7.7 ± 5.9	10.9 ± 7.4	<0.0001	7.4 ± 8.7	9.2 ± 8.3	0.017	0.796	0.085
Hypertension (n, %)	25 (14.4%)	49 (28.2%)	<0.0001	18 (14.3%)	40 (31.7%)	<0.0001	1.000	0.524
Diabetes (n, %)	6 (3.4%)	13 (7.5%)	0.023	3 (2.4%)	8 (6.3%)	0.074	0.739	0.820
Positive family history (n, %)	45 (25.9%)	58 (33.3%)	<0.001	34 (27.0%)	45 (35.7%)	0.003	0.894	0.712
Report of cigarette use (n, %)	160 (92.0%)	162 (93.1%)	0.48	88 (69.8%)	89 (70.6%)	1.000	<0.0001	<0.0001
Report of alcohol use (n, %)	161 (92.5%)	168 (96.6%)	0.023	94 (74.6%)	103 (81.7%)	0.008	<0.0001	<0.0001
Statin users (n, %)	15 (8.6%)	21 (12.1%)	0.041	10 (7.9%)	10 (7.9%)	1.000	1.000	0.337
Lipid profiles and laboratory results								
Total cholesterol (mg/dL)	174.5 ± 41.1	171.0 ± 40.7	0.156	173.2 ± 37.0	174.3 ± 36.4	0.637	0.776	0.468
LDL-C (mg/dL)	94.3 ± 36.9	86.5 ± 31.9	0.002	96.1 ± 34.1	96.8 ± 31.2	0.997	0.659	0.007
HDL-C (mg/dL)	55.9 ± 18.2	59.1 ± 23.2	0.01	50.6 ± 18.4	52.6 ± 18.6	0.075	0.015	0.009
Triglycerides (mg/dL)	125.3 ± 79.2	130.7 ± 86.1	0.575	124.2 ± 75.2	132.9 ± 87.0	0.232	0.901	0.829
Fasting glucose (mg/dL)	88.4 ± 15.8	91.9 ± 21.2	0.021	91.7 ± 40.0	100.1 ± 62.4	0.068	0.388	0.167
hsCRP (mg/dL)	3.4 ± 5.2	5.4 ± 9.9	0.005	3.1 ± 4.5	4.2 ± 10.6	0.253	0.596	0.338
HIV associated factors								
HIV-infected (n, %)	124 (71.3%)	124 (71.3%)	1.000	102 (81.0%)	102 (81.0%)	1.000	0.059	0.059
Time since HIV diagnosis (year)	21.5 ± 8.8	26.2 ± 9.6	<0.0001	22.0 ± 8.8	25.0 ± 9.0	<0.0001	0.692	0.310
Antiretroviral therapy users (n, %)	106 (89.1%)	110 (94.0%)	0.134	94 (90.4%)	97 (98.0%)	0.134	0.827	0.184
NRTI users (n, %)	101 (84.9%)	104 (88.9%)	0.248	89 (85.6%)	93 (93.9%)	0.074	1.000	0.233
Duration of NRTI use (year)	6.2 ± 4.9	9.4 ± 6.3	<0.0001	5.1 ± 5.6	7.1 ± 5.9	<0.0001	0.163	0.008
NNRTI users (n, %)	46 (38.7%)	51 (43.6%)	0.074	54 (51.9%)	59 (59.6%)	0.074	0.059	0.021
Duration of NNRTI use (year)	4.9 ± 4.0	7.0 ± 5.1	<0.0001	5.2 ± 5.3	6.9 ± 5.8	<0.0001	0.740	0.922
PI users (n, %)	90 (75.6%)	97 (82.9%)	0.023	68 (65.4%)	69 (69.7%)	0.480	0.105	0.024
Duration of PI use (year)	5.8 ± 5.4	8.6 ± 6.6	<0.0001	6.4 ± 8.9	8.4 ± 9.1	<0.0001	0.603	0.889
Visual characteristics of coronary artery disease								
Agatston-score (unit)	71.0 ± 219.1	130.6 ± 315.1	<0.0001	23.7 ± 69.2	41.8 ± 122.7	0.003	0.008	<0.001
Number of plaques (unit)	2.2 ± 2.2	3.1 ± 2.3	<0.0001	1.9 ± 1.6	2.5 ± 1.6	<0.0001	0.268	0.01
Segment Stenosis Score (unit)	3.2 ± 3.6	5.4 ± 5.4	<0.0001	2.9 ± 3.2	4.0 ± 3.6	<0.0001	0.420	0.122

Average and standard deviation for continuous variables, frequencies and proportion (%) for categorical variables are reported. P-values are based on t-test or paired t-test as appropriate or chi-square test or McNemar-test as appropriate.

Abbreviations: ASCVD risk: cardiovascular risk defined by the ACC/AHA Guideline on the Assessment of Cardiovascular Risk; BMI: body mass index (kg/m2); LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HIV: human immunodeficiency virus; hsCRP: high-sensitivity C-reactive protein; NRTI: nucleoside reverse-transcriptase inhibitors; NNRTI: non-nucleoside reverse-transcriptase inhibitors; PI: protease inhibitors.

Supplemental table 3. Predictors of coronary artery disease outcomes among cocaine-users

Outcome	Predictor	Univariate models						Multivariate models					
		Overall effect on outcome			Effect on progression rate			Overall effect on outcome			Effect on progression rate		
		β	95% CI	p	β	95% CI	p	β	95% CI	p	β	95% CI	p
Agatston-score (unit)	HIV*	39.05	[-32.85-110.96]	0.289	5.39	[-2.75-13.53]	0.196	26.73	[-46.21-99.57]	0.471	1.55	[-5.90-9.00]	0.681
	ASCVD (%)	0.11	[-1.71-2.01]	0.906	**0.98**	**[0.43-1.52]**	**<0.001**	0.06	[-1.58-1.80]	0.944	**0.73**	**[0.21-1.25]**	**0.006**
	Positive family history	16.53	[-23.04-56.10]	0.415	**8.73**	**[0.68-16.77]**	**0.035**	25.62	[-11.44-62.35]	0.171	3.52	[-4.01-11.01]	0.356
	Statin use	**101.51**	**[50.18-152.65]**	**<0.001**	**11.92**	**[0.60-23.29]**	**0.041**	**95.91**	**[45.47-145.66]**	**<0.0001**	6.98	[-4.53-18.58]	0.235
	hsCRP (mg/dL)	0.22	[-1.08-1.53]	0.742	-0.11	[-0.71-0.50]	0.731						
Number of coronary plaques (unit)	HIV*	0.07	[-0.65-0.80]	0.843	0.00	[-0.08-0.08]	0.995	-0.05	[-0.73-0.63]	0.882	-0.02	[-0.11-0.06]	0.604
	ASCVD (%)	**0.06**	**[0.02-0.09]**	**<0.001**	**-0.01**	**[-0.01-0.00]**	**0.064**	**0.06**	**[0.03-0.09]**	**<0.0001**	0.00	[-0.01-0.00]	0.203
	Positive family history	**0.59**	**[0.08-1.10]**	**0.024**	-0.07	[-0.14-0.01]	0.081	**0.61**	**[0.12-1.09]**	**0.015**	**-0.08**	**[-0.16-0.00]**	**0.039**
	Statin use	**1.16**	**[0.42-1.90]**	**0.002**	-0.05	[-0.16-0.05]	0.276	**1.05**	**[0.34-1.76]**	**0.004**	-0.03	[-0.14-0.07]	0.540
	hsCRP (mg/dL)	**0.03**	**[0.01-0.06]**	**0.003**	**-0.01**	**[-0.01-0.00]**	**0.010**	**0.04**	**[0.02-0.06]**	**<0.0001**	**-0.01**	**[-0.01-0.00]**	**0.003**
Segment Stenosis Score (unit)	HIV*	0.16	[-1.17-1.49]	0.814	0.13	[-0.11-0.38]	0.280	-0.13	[-1.32-1.05]	0.825	0.08	[-0.16-0.32]	0.487
	ASCVD (%)	**0.12**	**[0.05-0.20]**	**<0.001**	0.01	[-0.01-0.02]	0.423	**0.12**	**[0.05-0.18]**	**<0.0001**	0.01	[-0.01-0.02]	0.398
	Positive family history	0.94	[-0.19-2.07]	0.104	-0.01	[-0.24-0.22]	0.958						
	Statin use	**3.34**	**[1.73-4.93]**	**<0.0001**	0.11	[-0.19-0.40]	0.478	**3.21**	**[1.73-4.68]**	**<0.0001**	0.05	[-0.26-0.36]	0.755
	hsCRP (mg/dL)	**0.13**	**[0.08-0.19]**	**<0.0001**	**-0.02**	**[-0.03-0.00]**	**0.045**	**0.15**	**[0.10-0.20]**	**<0.0001**	**-0.02**	**[-0.04-0.00]**	**0.013**

*: HIV was forced into the multivariate model. Parameters showing an association with a given outcome with p<0.10 were included in the multivariate model. Bold indicates statistical significance.

Abbreviations: β: unstandardized beta coefficients from linear mixed model; ASCVD risk: cardiovascular risk defined by the ACC/AHA Guideline on the Assessment of Cardiovascular Risk; CI: confidence interval; HIV: human immunodeficiency virus; hsCRP: high-sensitivity C-reactive protein.

Supplemental table 4. Predictors of coronary artery disease outcomes among cocaine non-users

Outcome	Predictor	Univariate models						Multivariate models					
		Overall effect on outcome			Effect on progression rate			Overall effect on outcome			Effect on progression rate		
		β	95% CI	p	β	95% CI	p	β	95% CI	p	β	95% CI	p
Agatston-score (unit)	HIV*	6.28	[-24.57-37.12]	0.691	2.88	[-6.57-12.33]	0.552	0.98	[-29.28-31.34]	0.949	-2.73	[-11.23-5.78]	0.527
	ASCVD (%)	**1.23**	**[0.29-2.17]**	**0.011**	**1.04**	**[0.62-1.46]**	**<0.0001**	**1.21**	**[0.25-2.16]**	**0.012**	**1.07**	**[0.64-1.50]**	**<0.0001**
	Positive family history	1.96	[-19.65-23.27]	0.856	6.04	[-1.96-13.99]	0.140						
	Statin use	-14.56	[-59.32-30.20]	0.525	-2.03	[-15.77-11.71]	0.773						
	hsCRP (mg/dL)	0.23	[-1.21-1.66]	0.759	-0.20	[-0.87-0.48]	0.573						
Number of coronary plaques (unit)	HIV*	0.20	[-0.52-0.91]	0.592	0.07	[-0.03-0.17]	0.148	0.11	[-0.60-0.81]	0.767	0.08	[-0.02-0.18]	0.108
	ASCVD (%)	**0.02**	**[0.00-0.04]**	**0.020**	0.00	[-0.01-0.00]	0.512	**0.02**	**[0.00-0.04]**	**0.016**	0.00	[-0.01-0.00]	0.329
	Positive family history	0.21	[-0.19-0.62]	0.304	0.03	[-0.08-0.13]	0.594						
	Statin use	0.85	[-0.18-1.88]	0.109	0.09	[-0.06-0.23]	0.258						
	hsCRP (mg/dL)	0.01	[-0.01-0.04]	0.244	0.00	[-0.01-0.01]	0.512						
Segment Stenosis Score (unit)	HIV*	0.60	[-0.88-2.09]	0.426	**0.24**	**[0.01-0.47]**	**0.045**	0.40	[-1.02-1.83]	0.577	0.10	[-0.11-0.30]	0.343
	ASCVD (%)	0.03	[-0.01-0.07]	0.107	**0.02**	**[0.00-0.03]**	**0.008**	0.04	[0.00-0.08]	0.061	**0.01**	**[0.00-0.03]**	**0.036**
	Positive family history	**1.01**	**[0.07-1.96]**	**0.037**	0.12	[-0.12-0.36]	0.319	**0.99**	**[0.10-1.89]**	**0.030**	0.04	[-0.18-0.26]	0.714
	Statin use	0.62	[-1.56-2.80]	0.579	**0.56**	**[0.21-0.90]**	**0.001**	0.43	[-1.63-2.50]	0.680	**0.51**	**[0.19-0.84]**	**0.002**
	hsCRP (mg/dL)	0.02	[-0.03-0.07]	0.435	0.01	[-0.01-0.03]	0.332						

*: HIV was forced into the multivariate model. Parameters showing an association with a given outcome with p<0.10 were included in the multivariate model. Bold indicates statistical significance.

Abbreviations: β: unstandardized beta coefficients from linear mixed model; ASCVD risk: cardiovascular risk defined by the ACC/AHA Guideline on the Assessment of Cardiovascular Risk; CI: confidence interval; HIV: human immunodeficiency virus; hsCRP: high-sensitivity C-reactive protein

Supplemental table 5. Model fit assessment regarding random effect terms for outcome: Ca-score.

Removal of term	Log Likelihood	AIC	–ΔLog Likelihood	Degrees of freedom	p
–	-3729.4	7466.7	–	–	–
Random slope	-3891.0	7788.0	161.6	1	$< 10^{-15}$
Random intercept	-4056.7	8119.4	327.3	1	$< 10^{-15}$
Random intercept and slope	-4083.7	8171.4	354.3	2	$< 10^{-15}$

Supplemental table 6. Model fit assessment regarding random effect terms for outcome: Number of coronary plaques.

Removal of term	Log Likelihood	AIC	–ΔLog Likelihood	Degrees of freedom	p
–	-1104.8	2217.5	–	–	–
Random slope	-1117.7	2241.5	12.9	1	$< 10^{-7}$
Random intercept	-1285.9	2577.8	181.1	1	$< 10^{-15}$
Random intercept and slope	-1289.0	2582.1	184.2	2	$< 10^{-15}$

Supplemental table 7. Model fit assessment regarding random effect terms for outcome: Segment stenosis score.

Removal of term	Log Likelihood	AIC	–ΔLog Likelihood	Degrees of freedom	p
–	-1104.8	2217.5	–	–	–
Random slope	-1117.7	2241.5	12.9	1	$< 10^{-7}$
Random intercept	-1285.9	2577.8	181.1	1	$< 10^{-15}$
Random intercept and slope	-1289.0	2582.1	184.2	2	$< 10^{-15}$

SUPPLEMENTAL REFERECES

1 Lai, S. *et al.* Long-term cocaine use and antiretroviral therapy are associated with silent coronary artery disease in African Americans with HIV infection who have no cardiovascular symptoms. *Clin Infect Dis* **46**, 600-610, doi:10.1086/526782 (2008).

2 Lai, H. *et al.* HIV Infection Itself May Not Be Associated With Subclinical Coronary Artery Disease Among African Americans Without Cardiovascular Symptoms. *J Am Heart Assoc* **5**, e002529, doi:10.1161/JAHA.115.002529 (2016).

3 Morrell, C. H., Brant, L. J. & Ferrucci, L. Model choice can obscure results in longitudinal studies. *J Gerontol A Biol Sci Med Sci* **64**, 215-222, doi:10.1093/gerona/gln024 (2009).

4 Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *2015* **67**, 48, doi:10.18637/jss.v067.i01 (2015).

5 Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package: Tests in Linear Mixed Effects Models. *2017* **82**, 26, doi:10.18637/jss.v082.i13 (2017).

6 R: A Language and Environment for Statistical Computing v. 3.5.2 (R Foundation for Statistical Computing, 2018).