

HIV POSITIVE PATIENTS TREATED WITH PROTEASE INHIBITORS HAVE
VASCULAR CHANGES RESEMBLING THOSE OBSERVED IN
ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

John Lekakis¹, Sotirios Tsiodras², Ignatios Ikonomidis¹, John Palios¹, Garyfalia Poulakou², Loukianos Rallidis², Anastasia Antoniadou², Periklis Panagopoulos², Antonios Papadopoulos², Helen Giamarellou², Dimitrios T. Kremastinos¹.

¹. 2nd Department of Cardiology, Attikon University Hospital, University of Athens Medical School

². 4th Department of Internal Medicine, Attikon University Hospital, University of Athens Medical School

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Address correspondence to
Dr. Ignatios Ikonomidis, MD, PhD, FESC
Attikon University Hospital
Perikleous 19, 1434 N Chalkidona
Athens Greece
E-mail: ignoik@otenet.gr
Fax: +30 210 7299201

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ABSTRACT

A metabolic syndrome associated with atherosclerosis and cardiovascular disease has been described in HIV positive individuals. We investigated whether HIV individuals and CAD patients have similarities in their vascular function and structure.

In a case-control study, we compared measurements of carotid artery intima media thickness (IMT) and brachial artery flow-mediated dilatation (FMD) in HIV individuals with age and sex-matched controls with similar risk factors and patients with established CAD.

Seventy-one HIV patients (age 42 ± 13.9 yrs, 91 % male) were compared to 29 CAD patients and 25 controls. HIV patients had higher IMT than controls and similar IMT with CAD patients (0.64 ± 0.2 vs. 0.55 ± 0.05 vs. 0.66 ± 0.08 mm respectively, $F=4.2$, $p=0.01$). Patients on protease inhibitors, had higher IMT (0.69 ± 0.2 vs. 0.57 ± 0.15 mm $p=0.01$), blood pressure, cholesterol and triglycerides than those without ($p<0.05$). In multiple regression analyses increasing blood pressure (b: 0.37, $p=0.001$), glucose (b: 0.26, $p=0.016$), cholesterol (b: 0.24, $p=0.033$), duration of HIV disease (b: 0.33, $p=0.008$) and use of protease inhibitors (b: 0.27, $p=0.04$) were the most important determinants of IMT respectively. FMD was associated only with triglyceride measurements.

Patients with HIV present arterial changes resembling those found in patients with atherosclerotic cardiovascular disease. These vascular changes are closely related to protease inhibitor-induced changes of metabolic parameters. Thus, intensive treatment of metabolic parameters might retard atherosclerosis in HIV patients.

Key words: HIV, endothelial, HAART, coronary artery disease, IMT, FMD

Abbreviations

IMT=intimal media thickness

FMD= brachial artery flow-mediated dilatation

HAART= highly active antiretroviral therapy

PI=protease inhibitors

NNRTI=non-nucleoside reverse transcriptase inhibitors

NRTI=nucleoside reverse transcriptase inhibitors

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) dramatically reduced the HIV-associated morbidity and mortality [1] prompting increasing concerns related to the chronic management of these patients. Metabolic alterations, namely dyslipidemia, insulin resistance and lipodystrophy, are common side-effects of HAART [2] and there is increasing concern that these changes will translate in the long-term into an increased incidence of cardiovascular atherosclerotic disease [3]. Indeed, the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study Group [4] reported that HAART was associated with 26% increase in the rate of myocardial infarction per year of exposure to antiretroviral drugs.

Careful cardiovascular evaluation in the course of HIV disease can identify changes early enough to intervene. In addition to the routine blood lipids and glucose measurements, it seems reasonable to estimate surrogate markers for atherosclerosis in HIV positive subjects. Endothelial function and carotid artery intima-media thickness are surrogate markers commonly used in atherosclerotic patients and have been proven to be valuable for the prediction of acute ischemic events [5-7]. Both endothelial function indices and carotid IMT have been shown to be abnormal in HIV patients compared to normal controls [8,9]. To date no study has been published comparing patients with HIV disease to patients with atherosclerotic heart disease. We hypothesized that these two groups of patients might have similarities in their vascular function and structure and therefore we designed a study to investigate the carotid artery IMT and the flow-mediated dilatation of the brachial artery, a reliable estimate of endothelial function, in HIV patients, subjects with proven coronary artery disease and healthy individuals.

METHODS

Study population

We prospectively enrolled 71 HIV positive patients (mean age 42 ± 13.9 yrs, 91 % male) attending a tertiary care center with no previous medical history of coronary artery disease. Twenty nine patients with angiographically documented coronary artery (positive control) and 25 subjects of similar age, sex and atherosclerotic risk factors to the HIV patients served as positive and control groups respectively. The HIV and control subjects had no history of cardiovascular disease, normal physical

examination and normal resting and exercise electrocardiogram. The presence of metabolic syndrome was assessed among the study groups according to the criteria proposed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [10]. The creatinine clearance was calculated as marker of renal function using the following formula: Creatinine Clearance = $[[140 - \text{age}(\text{yr})] * \text{weight}(\text{kg})] / [72 * \text{serum Cr}(\text{mg/dL})]$ (multiplied by 0.85 for women). The study was approved by the hospital's scientific committee and all patients gave informed written consent. The research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

Endothelial function

Endothelium-dependent, flow-mediated dilatation (FMD) of the right brachial artery was determined in all patients. All subjects were studied in the morning, having abstained from alcohol, caffeine and food for 8 hours before the study; all vasoactive medications were withheld for 48 hours before the study. Optimal imaging of the brachial artery was obtained and a resting scan was recorded using an Echo-Doppler ultrasound system (Vivid 7, GE Medical systems, Horton, Norway) and a 10 MHz transducer. Reactive hyperemia was then induced by inflation of a blood pressure cuff on the forearm at a pressure of 200 mmHg for 5 min and subsequent deflation; brachial artery was scanned continuously 30 sec before and 90 sec after cuff deflation. All images were recorded on super VHS videotape for offline analysis. Artery diameter measurements were made using electronic calipers from the anterior to the posterior m-line. FMD was calculated as the percent increase in arterial diameter during hyperemia as compared to resting scan, as previously described [11]. Inter and intra- observer variability of these measurements in our laboratory has been previously presented [12]. At the end of the protocol, images were obtained again 4 min after sublingual nitroglycerin (0.4 mg) for measurement of nitrate-induced, endothelium-independent vasodilation.

Measurement of carotid atherosclerosis

B-mode ultrasonographic examination was performed in all patients using the same Echo-Doppler ultrasound. Scanning included left and right carotid arteries for the measurement of the intima-media thickness (IMT); all scans were performed by the same operator. The carotid artery image was focused in the far wall and 2 segments were identified on each side: the distal 1.0 cm of the common carotid proximal to the

bifurcation and the carotid bulb [12]. The mean value of all 4 sites was used for analysis. All scans were digitally stored (Echopac, GE Medical systems, Horton, Norway), and recorded on S-VHS videotape for off-line analysis.

Statistical analysis

All variables are expressed as mean \pm SD. Skewed variables were log-transformed to normalize their distribution. Categorical data were compared by means of the χ^2 -test. Differences in IMT or FMD between the 3 study groups (HIV, CAD, healthy controls) were examined by full factorial ANOVA (General linear model) using as covariables BMI, smoking status, blood pressure (systolic or diastolic separately), cholesterol, triglycerides, serum glucose, creatinine clearance, use of β -blockers, diuretics, nitrates and statins. The interaction terms between the variable of the 3 study groups and the above covariables were also examined. Using ANOVA, differences in IMT or FMD between the 3 study groups were also examined in a model including the presence of metabolic syndrome as covariable. The interaction term between the variable of the 3 study subgroups and metabolic syndrome was also examined. Post hoc comparisons were performed with Bonferroni's correction. Differences in variables between patients with and without protease inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors or between the above patient subgroups and controls were assessed by two-tailed unpaired t-test. Using simple linear regression the association of IMT or FMD (dependent variables) with age, sex, BMI, atherosclerotic risk factors (blood pressure, smoking, serum cholesterol, triglycerides and glucose levels), presence of metabolic syndrome, creatinine clearance, duration of disease, minimum CD4 lymphocyte counts and maximum viral load, current viral load and current CD4 lymphocyte counts and duration of HAART was examined. Additionally, exposure to HAART, as well as a viral load of over 100,000 c/ml for a period of more than 6 months or CD4 count <200 for 6 months were examined as dichotomous variables. Variables with a P-value of <0.2 in univariate analysis or of major clinical significance (e.g. sex, BMI, blood pressure, glucose, cholesterol, triglycerides, smoking, pulse pressure) were entered in multiple regression models. All covariables included in the final models were tested for interactions. The following variables were entered in the model separately to avoid colinearity. A) systolic, diastolic or mean blood pressure, B) use of HAART (any regimen) PI, NRTI or NNRTI, c) minimum CD4 or current CD4 lymphocyte counts, maximum viral load, current viral load or viral load >10⁵ c/ml for more than 6 months, d) disease duration or HAART duration,

e) presence of metabolic syndrome or associated risk factors (glucose, cholesterol, triglycerides, blood pressure parameters).

Analysis was weighted for age. The associations between vascular indices and patients' clinical characteristics are presented by means of standardized regression coefficient (β). $P < 0.05$ was considered to be the level of statistical significance. All statistical tests were two tailed. SPSS 11.5 software (SPSS Inc. Chicago, IL) was used for statistical analysis.

RESULTS

Both control groups had similar age, sex, atherosclerotic risk factors, creatinine clearance, incidence of metabolic syndrome and use of statins with HIV patients (table 1). The CAD group had slightly higher mean age than the two other groups that did not reach statistical significance. All diabetic patients were on oral antidiabetic treatment. The CAD patients were treated to a higher percent with β -blockers than the other 2 groups ($p < 0.05$) and 4 of them were receiving long-acting nitrates. The remaining medication was similar among HIV patients and control groups. Among HIV patients those receiving protease inhibitors ($n = 37$) had higher mean and diastolic blood pressure and higher levels of cholesterol, triglycerides and glucose compared to patients on other antiretroviral or no treatment (table 1, for all comparisons, $p < 0.05$). As a result, patients of protease inhibitors had a higher incidence of metabolic syndrome than patients on other antiretroviral or no treatment ($p < 0.05$). Furthermore, patients receiving protease inhibitors had higher IMT ($p = 0.01$) and similar FMD values ($p = 0.07$) compared to patients on other antiretroviral or no treatment. Patients receiving protease inhibitors had higher maximal viral load and incidence viral load $> 10^5$ c/ml for more than 6 months as well as reduced minimum CD4 count compared to patients on other antiretroviral or no treatment ($p < 0.05$). However, the current CD4 count and viral load was similar between the 2 subgroups of HIV patients (table 1, $p = ns$).

Comparisons between HIV patients CAD patients and healthy controls

By ANOVA, HIV patients had higher IMT than controls and similar IMT with CAD patients (0.64 ± 0.2 vs. 0.55 ± 0.05 vs. 0.66 ± 0.08 mm respectively, $F = 4.2$, $p = 0.01$, table 1). However, this difference was driven by those patients treated with PI (0.69 ± 0.2 vs. 0.57 ± 0.15 mm, $p = 0.01$, table 1) as patients not receiving PI had similar IMT levels to those in controls ($p = 0.47$). HIV patients had lower FMD than controls but higher

FMD than CAD patients [5.4 ± 2.8 vs. 7.1 ± 3.7 vs. 2.8 ± 2.6 % respectively, $F=11.7$, $p < 0.01$]. NMD values were similar among the study groups. Differences remained significant after adjustment for the use of statins, other medication or presence of metabolic syndrome. Additionally, the interaction terms between the variable of the 3 study groups and use of medication or presence of metabolic syndrome were not significant (data not shown).

Determinants of vascular indices among HIV patients

In univariate regression analyses age, systolic, diastolic and mean blood pressure, serum glucose, cholesterol and triglyceride levels, creatinine clearance, duration of HIV infection, use of PI and viral load $>10^5$ c/ml for more than 6 months were associated with IMT ($p < 0.05$ for all associations, table 2). The presence of metabolic syndrome had a borderline association with IMT ($\beta=0.24$, $p=0.049$) and no association with FMD ($\beta=0.043$, $p=0.68$). Maximal viral load, current viral load, minimum CD4 count, CD4 count <200 for >6 months were not associated with IMT or FMD ($p > 0.2$ for all associations).

In multivariate regression analysis, weighted for the effect of age, the most important determinants of increased IMT were serum glucose, cholesterol and blood pressure (mean, systolic or diastolic) levels, HIV duration, viral load $>10^5$ copies/ml for more than 6 months and use of PIs after adjustment for sex, BMI, pulse pressure, smoking status, triglycerides, duration of HAART (table 2). In a similar model including the presence of metabolic syndrome instead of other associated risk factors (glucose, cholesterol, triglycerides, blood pressure parameters), the most important determinants of increased IMT were metabolic syndrome ($\beta=0.29$, $p=0.01$), use of PIs ($\beta=0.24$, $p=0.046$) and viral load $>10^5$ copies/ml for 6 months ($\beta=0.23$, $p=0.049$). In univariate and multivariate regression analysis (weighted for the effect of age) triglyceride levels were the most important determinants of decreased FMD (table 2, $p=0.002$) after adjustment for sex, BMI, blood pressure (mean, systolic or diastolic separately) pulse pressure, smoking status, use of HAART (any regimen, PI, NRTI or NNRTI separately), duration of HAART or HIV disease (entered separately), viral load $>10^5$ c/ml for more than 6 months. In a similar multivariable model, including metabolic syndrome instead of other associated risk factors (glucose, cholesterol, triglycerides, blood pressure parameters), the association between metabolic syndrome and FMD remained not significant ($\beta=0.032$, $p=0.8$).

DISCUSSION

Our case-control study compared functional and structural arterial changes between HIV patients, age- and sex -matched coronary artery disease patients and controls with similar atherosclerotic risk factors. Patients with HIV had similar IMT values to those observed in patients with coronary artery disease, and higher than those found in individuals with similar atherosclerotic risk factors. Components of the HIV disease such as treatment with protease inhibitors and duration of disease as well as components of the metabolic syndrome such as higher blood pressure, cholesterol and glucose levels determined independently increasing IMT values. Treatment with protease inhibitors was related to impaired metabolic parameters and a higher incidence of the metabolic syndrome compared to other antiretroviral treatment and thus may have contributed to increasing IMT values. Regarding FMD, HIV patients showed values in-between those of matched patients with coronary artery disease and healthy controls.

This study confirms previous data evaluating IMT changes in HIV patients; however none of the studies had examined patients with diagnosed CAD as controls. Although such comparisons are affected by significant confounding they may provide significant clinical insight. In a previously reported study HIV positive subjects had higher IMT values than age matched controls, that also progressed rapidly after one year of follow-up [8]. In concordance with our findings this study found classical cardiovascular risk factors to be associated with increased IMT [8] ; however they also reported an association with low CD4 count-an index of immunodeficiency in HIV patients, that could not be verified in our patient population in univariate analysis. In addition higher IMT values were observed in patients with viral load $>10^5$ copies/ml for more than 6 months further suggesting a relation between severity of the disease and vascular abnormalities.

Another recently performed study compared HIV patients with healthy controls as well as diabetic patients [13]; IMT was similar in HIV and diabetic patients but in addition to metabolic parameters, factors relating to HIV infection and antiretroviral medications use were also related to IMT [13]. The role of duration of disease and

exposure to HAART needs further elucidation. We found a significant positive association between increasing HIV duration and higher IMT weighted for the ageing effect. Similarly a recent large case-control study emphasized the independent role of HIV duration as well as exposure to HAART in the development of higher IMT values [14]. In fact the study concludes that the “vascular age of HIV individuals is 4-5 years higher than age and sex-matched controls [14]. Thus, chronic inflammation during the course of the disease may cause the release of several cytokines and growth factors leading functional and structural vascular changes in HIV patients. In support of this mechanism, we have observed an independent association between a viral load $>10^5$ copies/ml for more than 6 months, a marker of the intensity of the inflammatory process, and IMT values in our HIV patients. In the present study, we have also found that exposure to protease inhibitor treatment was related to increased IMT values independently of the examined metabolic parameters. Furthermore, in our study patients receiving protease inhibitors had higher mean diastolic blood pressure and higher levels of cholesterol, triglycerides and glucose and thus, a higher incidence of the metabolic syndrome compared to patients on other antiretroviral therapy or no treatment in agreement with previous studies [2].

This finding suggests that protease inhibitor treatment may contribute to the impairment of metabolic parameters in HIV patients leading to increasing IMT values. The significant adverse effects of antiretroviral treatment on cardiovascular indices have been reported in very young HIV patients without traditional risk factors; indeed in a study of 83 children under treatment, higher IMT values were noted in HIV positive compared to healthy children [15]. These observations further highlight that the importance of antiretroviral therapy cannot be ignored.

The finding that IMT values in HIV patients were comparable to those observed in CAD patients is of major concern. Measurement of IMT is useful in studying atherosclerosis; it is associated with the extent of coronary atherosclerosis [16] as well the occurrence of cardiovascular events [5,17,18]. Recently the value of using IMT as a predictor for cardiovascular events has been extended to younger patients i.e less than 50 years of age [19]. Also IMT is a popular surrogate marker for the evaluation of various interventions for the retardation or even the regress of the atherosclerotic process [20]. It is reasonable to hypothesize that all applications of IMT in CAD patients can also be used to HIV patients for risk stratification and evaluation of antiatherosclerotic therapies, but this hypothesis has to be proved.

The independent association of IMT with blood pressure measurements, serum glucose and cholesterol values or with the presence of metabolic syndrome in our study highlights the significance of these parameters in the genesis of atherosclerosis in HIV individuals. Further it underscores the necessity for intense treatment of these risk factors and metabolic syndrome, especially in patients with higher values of IMT.

Endothelial dysfunction appears to be an early step to the atherosclerotic process and may be present even in children with cardiovascular risk factors [11]. Flow-mediated dilatation of the brachial artery is a reliable estimate of endothelial function, which is commonly used in clinical studies since it is noninvasive, correlates with the endothelial function in the coronary arteries and can predict cardiovascular events in high risk populations [21-24]. Endothelial dysfunction assessed by flow-mediated dilatation was modest in our HIV patients, worse than in normal individuals and better compared to CAD patients. Endothelial dysfunction has been previously reported in HIV patients [9,13] and may be caused by HIV infection itself as well as could be the result of the antiretroviral agents used to treat HIV. Infection with HIV is associated with a systemic inflammatory environment that could affect the vascular endothelium [25,26]. Moreover infections with opportunistic pathogens in patients with advanced HIV disease may exacerbate endothelial damage [27]. From the available antiretroviral medications, specific protease inhibitors have been associated with metabolic changes that may lead to endothelial dysfunction [2,26], while a direct cytotoxic effect on endothelial cells has also been described [28,29]. Nevertheless, an association with either protease inhibitors or viral load and FMD was not found in our study. Only triglyceride levels were an independent study predictor of decreased FMD in our HIV population suggesting that an intense treatment of elevated triglyceride levels might improve endothelial function in these patients. It is well known that hypertriglyceridemia impairs endothelial function while vasodilator function improves after lowering plasma triglycerides even without changes in confounding lipoproteins or insulin resistance [30]. Triglycerides may cause endothelial dysfunction through several mechanisms including enhancement of oxidative stress, reduction of high-density lipoprotein cholesterol, influence on low-density lipoprotein (LDL) size distribution, induction of cell adhesion molecule expression, direct effects of TG-rich lipoproteins (TRLs) on the vessel wall, and increase of fatty acids [30]. The triglycerides were the only independent determinant of FMD in our patients likely because of the extensive use of protease-inhibitors in our study cohort (52%).

We have previously shown, that the use of these drugs [2] is related with 2 -fold higher incidence rate for hypertriglyceridemia than for hypercholesterolemia. Thus, the use of protease-inhibitors has a stronger association with elevated triglyceride levels than with other lipoprotein levels through mechanisms which are not fully defined and include a direct enhancement of the formation of VLDL lipoproteins [31] or reduction of lipoprotein lipase activity (LPL) [32]. LPL is an enzyme located at the capillary endothelial surface which hydrolyzes triglycerides to make fatty acids available to cells [30,33]. Thus, reduction of its activity by protease inhibitors may contribute to elevation of triglyceride levels and consequently, to endothelial dysfunction as observed in the present study.

The lack of the assessment of oxidative stress and inflammatory markers such as C-reactive protein may be considered a limitation of our study because oxidative stress and low-grade inflammation may contribute in the development of vascular changes. However, in the present study we have demonstrated an independent association between IMT and a viral load $>10^5$ copies/ml for more than 6 months which is a marker of the intensity of the inflammatory process in HIV patients.

In conclusion, patients with HIV present functional and structural arterial changes resembling those found in patients with atherosclerotic cardiovascular disease. Characteristics of the HIV infection itself e.g. viral load treatment, duration of the disease, treatment with protease inhibitors as well as parameters of the metabolic syndrome e.g. serum glucose, cholesterol and triglyceride values and blood pressure measurements appear to influence the pathogenesis of these vascular changes. Therefore intensive treatment of such changes might be helpful in preventing or retarding the atherosclerotic process in HIV patients.

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Table 1. Clinical characteristics of the study groups

	HIV (n=71)	CAD (n=29)	CONTROLS (n=25)	P*	PI (n=37)	No PI (n=34)	P#
Age (years)	42±13.9	46±5.7	40±5.6	0.1	44±12	40±13	0.1
Males	65 (91%)	24 (83%)	23 (92%)	0.2	32 (86%)	34 (97%)	0.24
Cholesterol (mg/dl)	200±60	220±70	223±40	0.14	224±60*	175±48	<0.001
Triglycerides (mg/dl)	200±154	137±70	182±100	0.11	256±166*	139±116	<0.001
Glucose (mg/dl)	94±44	90±45	88±39	0.9	104±59*	83±112	<0.05
Creatinine (mg/dl)	0.7±0.04	0.8±0.03	0.7±0.03	0.9	0.8±0.04	0.68±0.04	0.9
Creatinine clearance(ml/min)	142±25	138±27	150±15	0.6	140±27	144±24	0.9
Body mass index (kg/m ²)	24±3	28±4	25±3	0.9	24±3	24.5±3	0.8
Smoking	52 (73%)	24 (82%)	18 (72%)	0.7	26 (71%)	26 (76%)	0.7
Hypertension	14 (20%)	11 (37%)	6 (24%)	0.5	11 (32%)	3 (9%)	<0.05
Diabetes	5 (7%)	4 (13%)	2 (8%)	0.6	4(10%)	1(3%)	0.19
Hyperlipidemia	15 (21%)	6 (21%)	5 (20%)	0.9	11 (30.6%)	4 (11.8%)	<0.05
Metabolic syndrome	13 (18%)	6 (21%)	5 (20%)	0.8	10 (27%)	3 (9%)	0.04
Current CD4 (cells/ml)	443.6±246.2	N/A	N/A		420.2±263.9	470.1±226.2	0.4
Minimum CD4	232.2±152.4	N/A	N/A		160.4±107.2	310.2±157.1	<0.001

Duration CD4 < 200 (months)	9.6±22.5	N/A	N/A		9.8±24	9.5±21.5	0.9
Current Viral load (lg10)	2.94±1.4	N/A	N/A		3.01±1.5	2.9±1.4	0.9
Max. Viral load (lg10)	4.83±0.99	N/A	N/A		5.1±0.89	4.5±1	<0.05
Viral Load >10 ⁵ x6months	35 (49.3%)	N/A	N/A		25 (67.5%)	10(29.4%)	<0.001
Ever HAART	48 (68%)	N/A	N/A		N/A	N/A	
Ever PI	37(52%)	N/A	N/A		N/A	N/A	
Ever NNRTI	33 (46%)	N/A	N/A		N/A	N/A	
Ever NRTI	48 (68%)	N/A	N/A		N/A	N/A	
SBP (mmHg)	117±20	117±16	120±9	0.8	120±25	115±13	0.1
DBP (mmHg)	74±10	74±11	74±8	0.9	78±10*	71±9	<0.05
MBP (mmHg)	88±12	89±11	89±7	0.9	92±13*	86±10	<0.05
IMT (mm)	0.64±0.2	0.66±0.08	0.55±0.05	0.01	0.69±0.2*	0.57±0.15	<0.05
NMD(%)	15.0±5.7	13.7±5.9	16.5±5.5	0.5	14.5±6.4	15.2±4.9	0.6
FMD (%)	5.43±2.7	2.76±2.6	7.07±3.7	<0.001	5.34±3.2	5.5±2.3	0.8
Medication							
B-blockers†	6 (8%)	25 (86%)	2 (8%)	<0.001	5 (13%)	1 (3%)	<0.05
Diuretics	8 (11%)	5 (17%)	4 (12%)	0.5	6 (16%)	2 (6%)	<0.05
Nitrates	0	4 (13%)	0		N/A	N/A	
Statins	9 (13%)	4 (14%)	3 (12%)	0.9	7 (19%)	2 (6%)	<0.05

IMT intima media thickness, FMD flow mediated dilatation–brachial index, NMD nitrate mediated dilatation–brachial index, HAART, highly active antiretroviral therapy (any regimen). Viral load: patients with viral load >10⁵ for more than 6 months duration. PI, protease inhibitors, NNRTI, non-nucleoside reverse transcriptase inhibitors, NRTI, nucleoside reverse transcriptase inhibitors, SBP, Systolic, DBP, diastolic or MBP mean blood pressure, N/A =not applicable *P value for ANOVA between 3 groups, # P value for comparison between PI vs. non-PI, †P=ns for HIV vs. normal controls

Table 2. Linear regression models assessing the association of IMT and FMD with clinical characteristics in HIV patients

	IMT		Multivariate*		FMD		Multivariate	
	Univariate				Univariate			
	β coefficient	p	β coefficient	p	β coefficient		β coefficient	p
Systolic blood pressure	0.38	0.01	0.32	0.005	0.01			
Diastolic blood pressure	0.45	<0.01	0.31	0.006	-0.009			
Pulse pressure	0.17	0.15			0.019			
Mean blood pressure	0.46	<0.01	0.36	0.001	0.001			
Age	0.51	<0.01			-0.16			
Male gender	0.18	0.12			-0.04			
BMI	0.007	0.9			-0.08			
Cholesterol	0.32	0.008	0.23	0.037	0.03			
Triglycerides	0.24	0.043			0.37		0.36	0.003
Glucose	0.37	0.002	0.25	0.02	-0.012			
Creatinine clearance	-0.03	0.8			-0.012			
Smoking	0.19	0.12			-0.09			
Duration of disease	0.25	0.04	0.32	0.005	0.17			
Use of HAART	0.17	0.14			-0.097			
Use of PI	0.28	0.018	0.27	0.04	-0.036			
Use of NNRTI	0.19	0.11			-0.038			
Use of NRTI	0.17	0.14			-0.13			
Duration of HAART	0.19	0.09			0.12			
Viral load >10 ⁵ x 6months	0.27	0.02	0.25	0.02	0.084			
Current CD4	0.17	0.14			0.11			

BMI, body mass index, IMT intima media thickness, FMD flow mediated dilatation-brachial index HAART, highly active antiretroviral therapy (any regimen). Viral load: patients with viral load $>10^5$ for more than 6 months duration. PI, protease inhibitors, NNRTI, non-nucleoside reverse transcriptase inhibitors NRTI, nucleoside reverse transcriptase inhibitors The multivariate models are weighted for age. The following variables were entered in the model separately to avoid colinearity: a) systolic, diastolic or mean blood pressure. b) use of HAART (any regimen) or use of PI, NNRTI or NRTI. c) viral load $>10^5$ c/ml for more than 6 months and current CD4 d) disease duration or HAART duration.

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Stage 2(a) POST-PRINT