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Lipoprotein-associated phospholipase A₂ and cardiovascular disease risk in HIV infection

A Ross Eckard¹, CT Longenecker^{2,3}, Y Jiang², SM Debanne², D Labbato^{2,3}, N Storer^{2,3}, and GA McComsey^{2,3}

¹Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA

²Case Western Reserve University, Cleveland, OH, USA

³Rainbow Babies and Children's Hospital and University Hospitals Case Medical Center, Cleveland, OH, USA

Abstract

Objectives—HIV-infected patients on antiretroviral therapy (ART) have an increased cardiovascular disease (CVD) risk as a result of heightened inflammation and immune activation, despite at times having normal lipids and few traditional risk factors. Biomarkers are needed to identify such patients before a clinical event. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) predicts CVD events in the general population. This study investigated the relationship between Lp-PLA₂ and markers of CVD risk, systemic inflammation, immune activation, and coagulation in HIV infection.

Methods—One hundred subjects on stable ART with normal fasting low-density lipoprotein (LDL) cholesterol were enrolled in the study. Plasma Lp-PLA₂ concentrations were measured by enzyme-linked immunosorbent assay (ELISA; > 200 ng/mL was considered high CVD risk). Subclinical atherosclerosis, endothelial function, inflammation, immune activation and fasting lipids were also evaluated.

Results—The median age of the patients was 47 years and 77% were male. Median (range) Lp-PLA₂ was 209 (71–402) ng/mL. Fifty-seven per cent of patients had Lp-PLA₂ concentrations > 200 ng/mL. Lp-PLA₂ was positively correlated with soluble markers of inflammation or immune activation (tumour necrosis factor receptor-II, intercellular and vascular cellular adhesion molecules, and CD14; all $R = 0.3$; $P < 0.01$), and negatively correlated with coagulation markers (D-dimer and fibrinogen; both $R = -0.2$; $P < 0.04$). Lp-PLA₂ was not correlated with lipids, coronary artery calcium score, or flow-mediated vasodilation, but trended towards a significant correlation with carotid intima-media thickness ($R = 0.2$; $P = 0.05$).

Conclusions—In this population with stable ART and normal LDL cholesterol, Lp-PLA₂ was in the high CVD risk category in the majority of subjects. Lp-PLA₂ appears to be associated with inflammation/ immune activation, but also with anti-thrombotic effects. Lp-PLA₂ may represent a

valuable early biomarker of CVD risk in HIV infection before subclinical atherosclerosis can be detected.

Keywords

cardiovascular disease; carotid intima-media thickness; coagulation; HIV; immune activation; inflammation; lipoprotein-associated phospholipase A₂

Introduction

HIV-infected patients are at an increased risk of developing cardiovascular disease (CVD) compared with the general population [1,2]. This increased risk is attributable in part to a high prevalence of traditional risk factors in the HIV-infected population, such as smoking, hypertension and hyperlipidaemia [3]. However, some HIV-infected patients may have few or no traditional risk factors and are still at increased risk as a result of the independent effects of HIV infection despite virological suppression with combination antiretroviral therapy (ART) [4,5]. There is growing evidence that heightened inflammation and immune activation play key roles in the increased CVD risk in HIV infection. For example, levels of the pro-inflammatory cytokines high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) predict CVD events and mortality among HIV-infected individuals [6,7], and higher frequencies of CD8 T cells expressing CD38 and human leucocyte antigen (HLA)-DR antigens are associated with an increased prevalence of subclinical atherosclerosis [8,9].

Identifying HIV-infected patients who have few or no traditional CVD risk factors, but yet are still at an increased risk of CVD, is imperative so that they can be targeted for preventive measures before a clinical event occurs. Even in the general population, as many as 10–20% of those who experience a coronary heart disease event have only one or no traditional risk factors [10,11], and approximately 35% of CVD deaths occur in people with total cholesterol levels < 200 mg/dL [12]. There has been a particular interest, in both the general and HIV-infected populations, in identifying circulating biomarkers that could help further stratify CVD risk among those with and without traditional risk factors, particularly for primary prevention. One notable example is hsCRP, increased blood concentrations of which are significantly associated with the risk of a first CVD event, even among asymptomatic patients in the general population [13–15], and current guidelines suggest measuring hsCRP in older individuals to aid in therapeutic management [16]. However, hsCRP remains controversial with regard to the degree to which it improves the predictive accuracy of traditional risk stratification models [17]. Thus, additional cardiovascular biomarkers are needed to further characterize CVD risk. This is particularly important in virologically suppressed HIV-infected patients, where hsCRP levels are already increased relative to healthy controls [5].

Recently, attention has turned to lipoprotein-associated phospholipase A₂ (Lp-PLA₂), which has shown promise as a cardiovascular risk marker both independent of and additive to traditional risk factors [18,19]. Lp-PLA₂ is an inflammatory enzyme produced by monocytes, macrophages and T lymphocytes and is up-regulated in atherosclerotic plaques.

It has high specificity for vascular inflammation, as opposed to systemic inflammation [20]. A vast number of prospective studies and meta-analyses have shown that increased Lp-PLA₂ concentration or activity predicts both primary and recurrent future coronary or cardiovascular events in the general population [21–25]. Like hsCRP, elevated Lp-PLA₂ doubles the risk for first and recurrent CVD events. Importantly, when both hsCRP and Lp-PLA₂ are elevated, they have an even greater predictive capacity [26–28]. In fact, current general population guidelines include Lp-PLA₂ testing in patients with hsCRP values ≥ 2 mg/L [29], indicating that this may be a particularly valuable marker in HIV-infected patients given that many have increased hsCRP levels.

A few studies have evaluated Lp-PLA₂ in HIV-infected patients [30–33], but no study has evaluated Lp-PLA₂ as a biomarker of subclinical vascular disease in this population. We hypothesized that plasma concentrations of Lp-PLA₂ would be associated with blood concentrations of other cardiac biomarkers and surrogate markers of subclinical vascular disease. Thus, the primary aims of this study were (1) to determine plasma levels of Lp-PLA₂ in HIV-infected patients on stable ART and with normal low-density lipoprotein (LDL) cholesterol, (2) to investigate the relationship between plasma concentrations of Lp-PLA₂ and other markers of inflammation, immune activation, and coagulation associated with CVD, and (3) to investigate the relationship between plasma concentrations of Lp-PLA₂ and endothelial function and measures of subclinical atherosclerosis. Secondary objectives included evaluating the relationship between plasma levels of Lp-PLA₂ and traditional CVD risk factors, HIV-related variables, and other variables that have been associated with Lp-PLA₂ in the general population.

Methods

Study design/population

This was a cross-sectional exploratory study of Lp-PLA₂ and CVD risk. The study enrolled subjects who underwent extensive cardiovascular assessment between March 2011 and August 2012 at the University Hospitals Case Medical Center, Cleveland, OH, USA. The first 100 subjects who qualified on the basis of fulfilling the following criteria were included in the analysis: HIV infection, age ≥ 18 years, fasting LDL cholesterol ≥ 130 mg/dL, cumulative ART duration ≥ 6 months, stable ART regimen for ≥ 3 months, HIV-1 RNA < 1000 copies/mL, and no known coronary artery disease or statin use. The study was reviewed and approved by the Institutional Review Board of University Hospitals Case Medical Center, Cleveland, OH. Written informed consent was provided by all participants.

Clinical assessments

Targeted physical examination, weight and height measurements (from the same clinic scale), and blood pressure (using an automated, calibrated machine) were obtained. An extensive review of the medical records was conducted and subject-reported data were collected, including demographics, past and current medical diagnoses, concomitant medications, and detailed ART history. HIV-1 RNA level and CD4 cell count were collected as part of routine clinical care.

Laboratory assessments

Blood was drawn after a 12-hour fast. Whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and underwent processing for flow cytometry in real time. Additional blood was collected for plasma and serum isolation and frozen at -70°C without prior thawing until analysis. For all laboratory assessments, laboratory personnel were blinded to clinical information.

Plasma Lp-PLA₂ concentrations were measured with a specific enzyme-linked immunosorbent (ELISA) assay (PLACTM Test; diaDexus, Inc., South San Francisco, CA) as per the manufacturer's product manual. The calculated means of the measurements were used in the analysis. Inter- and intra-assay coefficients of variance (CVs) were $< 5\%$ and $< 9\%$, respectively. Lp-PLA₂ concentrations > 200 ng/mL were considered higher CVD risk based on current general population guidelines [16] that take into account several studies that show that CVD events dramatically increase above this cut point [23,27,29,34,35].

Plasma levels of inflammation markers, endothelial adhesion molecules, and coagulation markers that are associated with CVD in HIV-infected individuals and/or in the general population and that are elevated in HIV infection were selected [5–7,36–42], including the pro-inflammatory cytokines IL-6 and soluble receptors of tumour necrosis factor- α (sTNFR-I and sTNFR-II), the cellular adhesion molecules soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1), and the coagulation markers D-dimer and fibrinogen. Concentrations of sVCAM-1, sICAM-1, IL-6, sTNFR-I and sTNFR-II were determined by quantitative sandwich ELISAs (R&D Systems, Minneapolis, MN). hsCRP levels were determined by particle enhanced immunonephelometric assays on a BNII nephelometer (Siemens USA, Washington, DC). Concentrations of D-dimer were determined by immunoturbidometric assay on a STA-R Coagulation Analyzer (Diagnostica Stago, Inc., Parsippany, NJ). Concentrations of fibrinogen were determined by particle enhanced immunonephelometric assay on a BNII nephelometer (Siemens USA). All inter-assay CVs were $< 12\%$ except for very low D-dimer values (< 0.20 $\mu\text{g/mL}$) where the interassay CV was 21%. Intra-assay CVs were not calculated because of the small number of samples in each run.

Immune activation markers were also selected, including soluble CD14 (sCD14) and soluble CD163 (sCD163). Serum levels of sCD14 and sCD163 were measured using Quantikine ELISA kits (R&D Systems). Soluble CD14 inter-and intra-assay CVs were both $< 6\%$. Soluble CD163 inter-and intra-assay CVs were 5% and 3%, respectively. CD8 T-cell activation was determined by flow cytometry. Whole-blood samples were incubated for 15 min on ice with FACS Lyse buffer (BD Biosciences, San Diego, CA) and then washed in wash buffer (phosphate-buffered saline with 1% bovine serum albumin and 0.1% sodium azide). Cells were then stained for 30 min in the dark on ice and then washed in wash buffer, and fixed in 1% formaldehyde. Cell surface molecule expression was measured by staining cells with the following fluorochrome-labelled antibodies: anti-CD38 R-phycoerythrin (PE), anti-HLA-DR fluorescein isothiocyanate (FITC), anti-CD3 peridinin-chlorophyll-protein complex (PerCP), anti-CD8 PerCP (BD Biosciences), and appropriate isotype control monoclonal antibodies. Samples were then analysed or stored at 4°C until analysis.

Cells were analysed using an LSR II flow cytometer (Becton-Dickinson, San Jose, CA) and FACSDIVA software version 6.1.1 (BD Biosciences).

Cardiovascular assessments

Carotid intima-media thickness (IMT), coronary artery calcium (CAC) score and flow-mediated vasodilation (FMD) of the brachial artery were measured as well-established markers of subclinical vascular disease [16]. For carotid IMT, a high-resolution B-mode ultrasound scan of the bilateral common carotid arteries was performed using a Philips iU22 ultrasound system with an L9-3 MHz linear array transducer (Philips Healthcare, Andover, MA) according to the consensus protocol of the American Society of Echocardiography [43]. R-wave gated still frame images of the distal 1 cm of the common carotid artery (CCA) far wall were obtained at three separate angles bilaterally (anterior, lateral and posterior). CCA IMT was measured offline using semi-automatic edge detection software (Brachial Analyzer for Research; Medical Imaging Applications LLC, Coralville, IA). The mean-mean CCA IMT was used for analysis. CAC was quantified using a 64-slice multidetector computed tomography (CT) scanner (Somatom Sensation 64; Siemens Medical Solutions USA) with 30×0.6 mm collimation, 330 ms rotation time, and 120 kV tube voltage. Three-millimetre slices were obtained from the carina to the diaphragm with prospective electrocardiogram (ECG) gating at 60% of the R-R interval. One experienced radiologist blinded to clinical data quantified CAC using the Agatston method. Flow-dependent endothelial function testing was performed by brachial artery ultrasound using a 5-minute forearm occlusion method as previously described [44]. Brachial artery diameter was measured by semi-automated edge detection, and FMD was calculated as the percentage change in brachial artery diameter after the induction of reactive hyperaemia. All vascular ultrasound measurements were performed by a single blinded reader (CTL). The coefficient of variance was 1.8%.

Statistical analysis

Continuous measures are described by medians and ranges, and nominal variables are described by frequencies and percentages. Plasma concentrations of Lp-PLA₂ were evaluated using both ordinal categories based on CVD risk (i.e. > 200 ng/mL versus ≤ 200 ng/mL) and continuous measures. Spearman correlation coefficients were used to assess correlations between Lp-PLA₂ in a continuous fashion and other continuous variables, and the point-biserial correlation coefficient was used to estimate the correlation between Lp-PLA₂ in a continuous fashion and other dichotomous variables.

After the primary results were known, we conducted two post hoc exploratory analyses using multivariable linear regression with the following outcomes: (1) CCA IMT restricted to values in the lower two tertiles and (2) Lp-PLA₂ values. Variables for each model were chosen based on clinical relevance.

The statistical evaluation between plasma levels of Lp-PLA₂ and CVD risk in this study was a post hoc analysis and considered exploratory; therefore, no power calculations were determined.

All statistical tests were two-sided with a 0.05 nominal significance level. Analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC).

Results

Study population

The subject characteristics are presented in Table 1. The majority of subjects were male and of black race, with a median age of 47 years. Eighty per cent of subjects had undetectable HIV-1 RNA. Forty-seven per cent and 52% of subjects were on a protease inhibitor and a nonnucleoside reverse transcriptase inhibitor (NNRTI), respectively, at the time of the study. Six per cent were on the nucleoside reverse transcriptase inhibitor abacavir. Twenty-two per cent, 13% and 6% were on anti-hypertensives, antihypertriglyceridaemics and low-dose aspirin, respectively. Fifty-three per cent of subjects had hsCRP ≥ 2 mg/L.

Lipoprotein-associated phospholipase A₂ analysis

The median (range) Lp-PLA₂ was 209 (71–402) ng/mL. Fifty-seven per cent of subjects were above the current recommended cut point indicating higher CVD risk (> 200 ng/mL). If the concentration was > 200 ng/mL, the patient's risk category increased from moderate to high or from high to very high. Thirty-one per cent of subjects with an hsCRP value ≥ 2 mg/L also had an Lp-PLA₂ value > 200 ng/mL.

Eighty-five, 15 and 1% of the subjects had Framingham risk scores in the low [$< 10\%$ coronary heart disease (CHD) risk equivalent], intermediate (10–20%), and high risk categories ($> 20\%$), respectively. Of the subjects who were in the low CHD risk category according to Framingham risk score, 59% had Lp-PLA₂ concentrations > 200 ng/mL. For the subjects in the intermediate- and high-risk Framingham categories, 47% and 0% had Lp-PLA₂ concentrations > 200 ng/mL, respectively.

Correlations of lipoprotein-associated phospholipase A₂ with CVD and HIV risk factors

The correlations between Lp-PLA₂ and measures of surrogate markers of subclinical atherosclerosis, endothelial function, and traditional CVD risk factors are presented in Table 2.

Neither FMD nor CAC was significantly correlated with Lp-PLA₂ ($P = 0.70$). Even when the analysis was restricted to subjects with detectable versus undetectable calcium score (CAC > 0 versus CAC = 0), there was no correlation between CAC and Lp-PLA₂.

CCA IMT was marginally positively correlated with Lp-PLA₂ ($R = 0.2$; $P = 0.05$). CCA IMT was then considered by tertiles. Within each of the tertiles, CCA IMT did not correlate with Lp-PLA₂. However, when the bottom two tertiles were combined, there was a significant correlation between mean CCA IMT and Lp-PLA₂ ($R = 0.27$; $P = 0.03$). Lastly, we limited the correlation analysis to subjects with plaque (defined as IMT > 1.5 mm at any measured point) versus those without plaque. Among those subjects without plaque ($n = 60$), CCA IMT was significantly associated with Lp-PLA₂ ($R = 0.26$; $P = 0.03$), while this was not seen for subjects with plaque.

Among traditional CVD risk factors, body mass index (BMI) and male sex were negatively and positively correlated with Lp-PLA₂, respectively. There were no significant correlations between Lp-PLA₂ and age, systolic blood pressure, LDL cholesterol, HDL cholesterol or triglycerides.

A number of HIV variables were also tested but none was significant, including HIV duration, current CD4 count, nadir CD4 count, HIV-1 RNA (< 50 HIV-1 RNA copies/mL versus ≥ 50 copies/mL), current or cumulative protease inhibitor use, current abacavir use, current NNRTI use and cumulative stavudine use. The current use of antihypertensives ($n = 22$), fish oil ($n = 10$) and fibrates ($n = 5$) were also tested but were not significant.

Correlations of lipoprotein-associated phospholipase A₂ with markers of inflammation, immune activation and coagulation

Correlations of Lp-PLA₂ with inflammation, immune activation, and coagulation markers are shown in Table 2 and Figures 1 and 2. Several markers of inflammation and immune activation were significantly positively correlated with Lp-PLA₂, including sTNFR-II, sICAM-1, sVCAM-1 and sCD14. The coagulation markers D-dimer and fibrinogen were both negatively correlated with Lp-PLA₂.

Multivariable regression

Two multivariable linear regression analyses were used to identify variables associated with CCA IMT values from the lower two tertiles and Lp-PLA₂. Variables included in the first model included Lp-PLA₂, age, male sex, current smoking, systolic blood pressure and hsCRP. Only age was significant ($P < 0.001$).

Variables in the second model investigating factors associated with Lp-PLA₂ included age, male sex, current smoking, systolic blood pressure, hsCRP, HDL cholesterol and LDL cholesterol. Only male sex was significant ($P = 0.01$).

Discussion

In this study, we evaluated for the first time Lp-PLA₂ concentrations in ART-treated, virologically suppressed HIV-infected subjects with normal LDL cholesterol. We found that the majority of subjects were above the recommended cut point for determining high CVD risk [29]. Similarly, more than one-third of subjects had elevated concentrations of both hsCRP and Lp-PLA₂. Notably, the majority of subjects in the low risk category by Framingham risk score had Lp-PLA₂ values that placed them in a higher CVD risk category, supporting the finding of previous studies that this modality may underestimate CVD risk in the HIV-infected population [45]. Lp-PLA₂ concentrations were also positively correlated with a number of key inflammatory markers and endothelial adhesion molecules that are associated with CVD in the general population and/or HIV-related vascular disease. These data emphasize the increased CVD risk among HIV-infected patients who have normal LDL cholesterol levels and few or no other traditional risk factors, and demonstrate the potential utility of Lp-PLA₂ in further characterizing CVD risk in HIV-infected patients.

In fact, Lp-PLA₂ concentrations may represent a very promising vascular inflammation biomarker in the HIV-infected population, as a result of some of its distinctive characteristics. Foremost among these, Lp-PLA₂ has a high specificity for vascular inflammation, as opposed to systemic inflammation. For example, Lp-PLA₂ levels are low in patients with systemic lupus erythematosus who do not have a history of CVD [46]. As a result of this specificity for vascular inflammation, Lp-PLA₂ may discriminate CVD risk in the HIV-infected population better than hsCRP, which is a marker of systemic inflammation and is elevated in many HIV-infected patients [5], even those with otherwise low CVD risk. In addition, Lp-PLA₂ is not associated with insulin resistance in people without diabetes [47]. Insulin resistance without frank diabetes is very common in HIV-infected patients [48]. The association of Lp-PLA₂ with CVD events maintains its significance even when adjusted for traditional risk factors [19], in contrast to some studies evaluating hsCRP [49]. Finally, studies show that Lp-PLA₂ is independently predictive of CVD in people with LDL cholesterol levels ≥ 130 mg/dL, similar levels to those in the current study [26].

Despite significant relationships with several inflammatory and cellular adhesion molecules, plasma concentrations of Lp-PLA₂ were not associated with coronary atherosclerosis as defined by the presence of CAC or brachial artery FMD; however, there was a trend towards an association with carotid IMT. Although the association between Lp-PLA₂ and FMD has been demonstrated in the general population [50], there are conflicting data regarding associations of Lp-PLA₂ with CAC score and carotid IMT [51,52]. Some have suggested that this dichotomy between Lp-PLA₂ as a predictor of CVD events attributable to plaque rupture and its capacity to determine atherosclerotic burden may be one of its limitations [53]. However, the trend toward a significant relationship between CCA IMT and Lp-PLA₂ may also be due to the fact that most subjects in this study had elevated Lp-PLA₂ concentrations, precluding an adequate separation of concentrations to allow accurate measurement of associations. Alternatively, our study may have been under-powered to show a significant relationship. Importantly, however, when the evaluation of the association between Lp-PLA₂ and CCA IMT was restricted to either the lower two tertiles or to those without plaque, we found significant associations. This suggests that Lp-PLA₂ may be a valuable early biomarker of CVD risk in HIV infection before subclinical atherosclerosis can be detected or at the very early stages of development. Larger longitudinal studies are needed to evaluate these relationships within the HIV-infected population.

Interestingly, plasma concentrations of Lp-PLA₂ were negatively associated with the coagulation markers D-dimer and fibrinogen. Both markers have been shown to be elevated in HIV-infected individuals and to be associated with CVD risk in both the general and HIV-infected populations [6,40–42]. Although the direction of this association may seem counterintuitive, Lp-PLA₂ is responsible for hydrolysing oxidized phospholipids in LDL and catalyses the degradation of the pro-thrombotic, pro-inflammatory platelet-activating factor to inactive products [54]. Few studies in the general population have assessed the relationship between Lp-PLA₂ and D-dimer or fibrinogen, and there have not been consistent findings [23,55]. However, because Lp-PLA₂ does have a potential anti-thrombotic effect [21], we believe that our observations in the HIV-infected population are novel and plausible.

Lp-PLA₂ was not significantly correlated with hsCRP, which is consistent with the findings of studies in the general population [56]. In addition, Lp-PLA₂ was negatively correlated with BMI in this study. The association between Lp-PLA₂ and BMI has been inconsistent in the general population, and the significance of this current finding is unclear [22,23,57]. Likewise, male sex was the only factor associated with Lp-PLA₂ in the multivariable regression, which has also been an inconsistent finding in the general population [22,23,57]. Further studies are needed in the HIV-infected population to further define these relationships.

As an exploratory study, this study investigated a number of associations between Lp-PLA₂ and variables of interest. Thus, there are statistical limitations to such a post hoc analysis, which limit the overall interpretation of the data. Likewise, because of the cross-sectional design, causality cannot be determined for the associations demonstrated, although the findings are interesting. This study also investigated a specific HIV-infected population: those on stable ART with low or undetectable HIV-1 RNA and normal LDL cholesterol. Thus, the generalizability to the HIV-infected population as a whole remains to be determined in further studies. This study also did not include an HIV-uninfected matched control group for comparison. Finally, we did not analyse the relationship between Lp-PLA₂ and lipoprotein subfractions in this study, which may offer additional insight into the role of Lp-PLA₂ in the HIV-infected population.

In conclusion, this study offers novel data which can form the substrate for future research. Prospective, longitudinal studies are needed to confirm and further define the relationship between Lp-PLA₂ and CVD in HIV-infected patients. Agents that reduce Lp-PLA₂ should also be investigated in this population. Statins, other lipid-lowering drugs, and darapladib, a selective oral Lp-PLA₂ inhibitor, have all been shown to decrease Lp-PLA₂ concentrations in the general population [58,59]. It remains to be seen if altering plasma concentrations of Lp-PLA₂ will affect CVD risk and plaque stability; however, it is clear that measuring Lp-PLA₂ levels in the general population, particularly in those who are already at an increased CVD risk, helps to predict CVD risk. Given the results of the current study, Lp-PLA₂ appears to be a promising biomarker for CVD risk in the HIV-infected population.

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Fig. 1.

Correlations between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) and markers of systemic inflammation and immune activation. Scatter plots depict the univariate relationship between levels of Lp-PLA₂ and systemic inflammation and immune activation. Lp-PLA₂ was significantly positively correlated with (a) soluble tumour necrosis factor receptor-II (sTNFR-II), (b) soluble intercellular adhesion molecule-1 (sICAM-1), (c) soluble vascular cell adhesion molecule-1 (sVCAM-1) and (d) soluble CD14 (sCD14). Note that two extreme outliers were removed from the sCD14 plot to more accurately reflect the relationship. *Spearman correlation coefficient.

Fig. 2. Correlations between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) and coagulation markers. Scatter plots depict the univariate relationship between levels of Lp-PLA₂ and coagulation markers. Lp-PLA₂ was significantly negatively correlated with (a) fibrinogen and (b) D-dimer. *Spearman correlation coefficient.

Table 1

Subject characteristics

	Median (range) or n (%) (n = 100)
Age (years)	47 (25–68)
Male sex	77 (77)
Race	
Caucasian	29 (29)
Black	70 (70)
Other	1 (1)
Body mass index (kg/m ²)	27 (7–17)
Systolic blood pressure (mmHg)	120 (86–162)
Diastolic blood pressure (mmHg)	78 (57–102)
Current smoker	62 (62)
Self-reported family history of myocardial infarction	32 (32)
Total cholesterol (mg/dL)	175 (102–257)
HDL cholesterol (mg/dL)	45 (19–97)
LDL cholesterol (mg/dL)	98 (23–134)
Triglycerides (mg/dL)	117 (38–622)
Framingham score	4 (1–22)
hsCRP (mg/L)	2.01 (0.09–119)
Interleukin-6 (pg/mL)	2.9 (0.9–12.5)
sTNFR-I (pg/mL)	1761 (567–5181)
sTNFR-II (pg/mL)	2239 (850–5162)
sICAM-1 (ng/mL)	230 (31–574)
sVCAM-1 (ng/mL)	640 (298–2192)
Soluble CD14 (ng/mL)	2159 (936–16790)
Soluble CD163 (ng/mL)	669 (245–1979)
D-dimer (μg/mL)	0.18 (0.01–2.17)
Fibrinogen (mg/dL)	399 (240–854)
CD8 cell activation (%) [*]	27 (11–73)
CCA IMT (mm)	0.66 (0.53–1.54)
Coronary artery calcium score	
0	67 (67)
1–99	23 (23)
> 100	10 (10)
Flow-mediated vasodilation (%)	4.2 (0.7–18.3)
Current CD4 cell count (cells/μL)	633 (142–1683)
Nadir CD4 cell count (cells/μL)	199 (0–614)
HIV-1 RNA < 1000 copies/mL	100 (100)
Duration of known HIV infection (months)	156 (18–321)
Cumulative ART duration (months)	75 (10–260)

	Median (range) or n (%) (n = 100)
Cumulative PI duration (months)	47 (0–171)
Cumulative NNRTI duration (months)	30 (0–137)

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; hsCRP, high-sensitivity C-reactive protein; sTNFR-I, soluble tumour necrosis factor receptor-I; sTNFR-II, soluble tumour necrosis factor receptor-II; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cellular adhesion molecule-1; CCA IMT, common carotid artery intima-media thickness; ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; HLA, human leucocyte antigen.

* Percentage of CD8 cells expressing CD38 and HLA-DR antigens.

Table 2

Correlations between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) and measures of inflammation and immune activation, coagulation markers surrogate markers of subclinical atherosclerosis and endothelial function, and traditional cardiovascular disease risk factors

Variable	<i>R</i> *	<i>P</i>
Inflammation/immune activation markers		
hsCRP	-0.09	0.35
sTNFR-I	0.07	0.46
sTNFR-II	0.27	< 0.01
Interleukin-6	0.06	0.57
sICAM-1	0.33	< 0.01
sVCAM-1	0.32	< 0.01
Soluble CD14	0.31	< 0.01
Soluble CD163	-0.08	0.44
CD8 cell activation %**	-0.13	0.25
Coagulation markers		
D-dimer	-0.22	0.03
Fibrinogen	-0.21	0.04
Surrogate markers of atherosclerosis/endothelial function		
CCA IMT	0.20	0.05
Coronary artery calcium score	0.04	0.70
Flow-mediated vasodilation	-0.015	0.88
Traditional cardiovascular risk factors		
Age	0.14	0.17
Body mass index	-0.35	< 0.01
Male sex	0.23	0.02
Systolic blood pressure	-0.12	0.06
Current smoker	0.03	0.81
LDL cholesterol	0.14	0.16
HDL cholesterol	0.10	0.33
Triglycerides	-0.005	0.96

sTNFR-I, interleukin-6, soluble CD163, flow-mediated vasodilation and CCA IMT were log-transformed.

hsCRP, high-sensitivity C-reactive protein; sTNFR-I, soluble tumour necrosis factor receptor-I; sTNFR-II, soluble tumour necrosis factor receptor-II; sICAM-1, soluble intercellular adhesion molecule-I; sVCAM-1, soluble vascular cellular adhesion molecule-I; CCA IMT, common carotid artery intima-media thickness; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

* *R* is the correlation coefficient.

** Percentage of CD8 cells expressing CD38 and HLA-DR antigens.

Statistically significant correlation with *P* < 0.05 is in bold.