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Lipoprotein-Associated Phospholipase A_2 Bound on High-Density Lipoprotein Is Associated With Lower Risk for Cardiac Death in Stable Coronary Artery Disease Patients

A 3-Year Follow-Up

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Objectives The aim of this study was to examine the prognostic value of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) associated with high-density lipoprotein (HDL) (HDL-Lp-PLA_2) in patients with stable coronary artery disease (CAD).

Background Lp-PLA_2 is a novel risk factor for cardiovascular disease. It has been postulated that the role of Lp-PLA_2 in atherosclerosis may depend on the type of lipoprotein with which it is associated.

Methods Total plasma Lp-PLA_2 and HDL-Lp-PLA_2 mass and activity, lipids, and C-reactive protein were measured in 524 consecutive patients with stable CAD who were followed for a median of 34 months. The primary endpoint was cardiac death, and the secondary endpoint was hospitalization for acute coronary syndromes, myocardial revascularization, arrhythmic event, or stroke.

Results Follow-up data were obtained from 477 patients. One hundred twenty-three patients (25.8%) presented with cardiovascular events (24 cardiac deaths, 47 acute coronary syndromes, 28 revascularizations, 22 arrhythmic events, and 2 strokes). Total plasma Lp-PLA_2 mass and activity were predictors of cardiac death (hazard ratio [HR]: 1.013; 95% confidence interval [CI]: 1.005 to 1.021; $p = 0.002$; and HR: 1.040; 95% CI: 1.005 to 1.076; $p = 0.025$, respectively) after adjustment for traditional risk factors for CAD. In contrast, HDL-Lp-PLA_2 mass and activity were associated with lower risk for cardiac death (HR: 0.972; 95% CI: 0.952 to 0.993; $p = 0.010$; and HR: 0.689; 95% CI: 0.496 to 0.957; $p = 0.026$, respectively) after adjustment for traditional risk factors for CAD.

Conclusions Total plasma Lp-PLA_2 is a predictor of cardiac death, while HDL-Lp-PLA_2 is associated with lower risk for cardiac death in patients with stable CAD, independently of other traditional cardiovascular risk factors. (J Am Coll Cardiol 2012;60:2053–60) © 2012 by the American College of Cardiology Foundation

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor (PAF) acetylhydrolase, expresses a Ca^{2+}-independent phospholipase A_2 activity and catalyzes the hydrolysis of the ester bond at the sn-2 position of PAF and oxidized phospholipids (1,2). These phospholipids are formed during oxidative modification of low-density lipoprotein (LDL) in the arterial intima and may play important roles in atherogenesis (1,2). Lp-PLA_2 is produced by inflammatory cells such as macrophages, foam cells, T cells, hepatic Kupffer cells, and mast cells (3,4). Lp-PLA_2 circulates in plasma in active form bound to various lipoproteins, primarily to LDL, whereas a minor proportion of circulating enzyme is also associated with high-density lipoprotein (HDL), lipoprotein(a), and remnant lipoproteins (1,5). Lp-PLA_2 is located in advanced rupture-prone plaques, from which it can be released into the circulation (2,6).

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Abbreviations and Acronyms

ACS = acute coronary syndrome
apo = apolipoprotein
CAD = coronary artery disease
CI = confidence interval
HDL = high-density lipoprotein
HDL-Lp-PLA$_2$ = high-density lipoprotein–associated lipoprotein-associated phospholipase A$_2$
HR = hazard ratio
hsCRP = high-sensitivity C-reactive protein
IPAQ = International Physical Activity Questionnaire
LDL = low-density lipoprotein
Lp-PLA$_2$ = lipoprotein-associated phospholipase A$_2$
MET = metabolic equivalent
PAF = platelet-activating factor

Several epidemiologic studies suggest that plasma Lp-PLA$_2$ is an independent predictor of cardiovascular events in primary and secondary prevention (7–15). A recent meta-analysis (9), which included 79,036 participants with or without coronary artery disease (CAD) from 32 prospective studies, showed that Lp-PLA$_2$ activity and mass each had a continuous association with the risk for CAD. Particularly, in the setting of stable CAD, it has been shown that Lp-PLA$_2$ is a significant predictor of nonfatal adverse cardiovascular outcomes independent of traditional clinical risk factors (10).

It has been postulated that the role of Lp-PLA$_2$ in atherosclerosis may depend on the type of lipoprotein with which it is associated (16,17). In contrast to total Lp-PLA$_2$ plasma enzyme, which mainly represents the LDL-associated Lp-PLA$_2$, several lines of evidence suggest that HDL-associated Lp-PLA$_2$ (HDL-Lp-PLA$_2$) may substantially contribute to the antiatherogenic activities of HDL (16). However, the clinical value of HDL-Lp-PLA$_2$ as a potent inhibitor of the atherosclerotic process remains to be established. Thus, the aim of the present study was to explore whether HDL-Lp-PLA$_2$ can predict future cardiovascular events in patients with stable CAD.

Methods

Study population. All patients with stable CAD who attended the outpatient cardiologic department of 3 large hospitals in Athens between 2006 and 2009 were asked to participate in the study. All patients were participants of the LAERTES (Lipoprotein-Associated phospholipasE A2 in stable coronary aRTEry diSease Study), which is an ongoing prospective, hospital-based study investigating the prognostic impact of Lp-PLA$_2$ in stable CAD. We initially screened 620 patients, of whom 524 satisfied the selection criteria and made up the final sample. Subjects were included in the study if they had been previously hospitalized for acute coronary syndromes (ACS), had undergone myocardial revascularization intervention, or had undergone coronary angiography for chest pain and there was documentation of CAD. Coronary artery stenosis was defined as ≥50% reduction in luminal diameter of any of the 3 coronary arteries or their primary branches.

Exclusion criteria were ACS or coronary artery bypass grafting within the previous 6 months, clinical evidence of heart failure, chronic renal failure (creatinine level >2 mg/dl), age >75 years, and coexistent neoplasm or inflammatory disease.

All patients underwent clinical examination and blood testing. The following definitions were used: hypertension, blood pressure ≥140/90 mm Hg and/or antihypertensive treatment; hypercholesterolemia, total cholesterol >200 mg/dl (5.2 mmol/l); diabetes mellitus, fasting plasma glucose >125 mg/dl (6.94 mmol/l) and/or glucose-lowering treatment. Smoking habits were recorded, and body mass index (weight in kilograms divided by the square of height in meters) and waist circumference were also evaluated.

Dietary habits were evaluated using a validated diet score that assesses adherence to Mediterranean dietary patterns (18). The score is derived from a questionnaire that includes 9 major food groups (nonrefined cereals, fruit, vegetables, legumes, potatoes, fish, meat and meat products, poultry, and full-fat dairy products), as well as olive oil and alcohol intake. Each question is scored on a scale of 0 through 5 according to the frequency of food consumption per week, and the diet score ranges between 0 and 55. Higher values of this diet score indicate greater adherence to the Mediterranean diet.

Physical activity was also assessed using a translated short version of the International Physical Activity Questionnaire (IPAQ), an index of weekly energy expenditure using the frequency (times per week), duration (minutes per time), and intensity of physical activity. The IPAQ measures are expressed as metabolic equivalent (MET)–minutes per week. One MET is defined as 3.5 ml oxygen · kg^{-1} · min^{-1}. We used the following MET estimates of the IPAQ: vigorous physical activity = 8 METs, moderate activity = 4 METs, walking on average = 3.3 METs, and sitting = 0 METs. For calculating the overall METs of physical activity, each category was multiplied by its special MET estimate value. The IPAQ has reasonable measurement properties for monitoring population levels of physical activity (19).

Finally, all patients underwent echocardiography, and the ejection fraction of the left ventricle was measured using the biplane method of discs (modified Simpson's rule) (20).

Measurement of Lp-PLA$_2$ activity and mass in total plasma and on HDL. Peripheral blood samples were collected from patients after an overnight fast between 8 AM and 10 AM. Lp-PLA$_2$ activity in total plasma and in apolipoprotein (apo) B–depleted plasma, after the sedimentation of all apo B–containing lipoproteins with dextran sulfate-magnesium chloride (HDL-Lp-PLA$_2$ activity), was determined using the trichloroacetic acid precipitation procedure using [^{3}H]-PAF (100 μmol/l final concentration) as a substrate (21,22). Fifty microliters of total plasma diluted 1:50 v/v with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4) or the apo B–depleted

plasma (diluted 1:3 v/v with 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid) was used as a source of the enzyme. The reaction was performed for 10 min at 37°C, and Lp-PLA$_2$ activity was expressed as nanomoles of PAF degraded per minute per milliliter of plasma.

Lp-PLA$_2$ mass was determined by a dual monoclonal antibody immunoassay standardized to recombinant Lp-PLA$_2$ (PLAC Test, diaDexus, Inc., South San Francisco, CA), following the manufacturer's instructions (23). Lp-PLA$_2$ mass was measured in plasma and in apo B–depleted plasma, prepared as described earlier (HDL-Lp-PLA$_2$ mass), as we have previously described (24). We used 10 μl of undiluted plasma or apo B–depleted plasma as the source of the enzyme. It should be noted that in all apo B–depleted plasma samples, there were no detectable amounts of apo B, whereas the apo A-1 concentration was similar to that determined in whole plasma of each individual patient (data not shown). Furthermore, according to our previously published results, the Lp-PLA$_2$ measured in apo B–depleted plasma is exclusively associated with HDL (24,25). The intra-assay and interassay coefficients of variation of Lp-PLA$_2$ measurements in the apo B–depleted plasma were 6.4% and 8.9%, respectively, for activity and 5.5% and 7.5%, respectively, for mass.

Biochemical measurements. Serum levels of total cholesterol, HDL cholesterol, and triglycerides were measured enzymatically using an automatic analyzer (Dimension RXL; Dade Behring, Marburg, Germany). LDL cholesterol was calculated according to the Friedewald formula: LDL cholesterol = total cholesterol − (triglycerides/5 + HDL cholesterol). Serum levels of apo A-1 and apo B were measured by immunonephelometry using a BN Prospect analyzer (Dade Behring). C-reactive protein was assayed using high-sensitivity particle-enhanced immunonephelometry (N Latex, Dade Behring), with a range of 0.175 to 1,100 mg/l. The intra-assay and interassay coefficients of variation of all measured biochemical parameters were <5%.

Follow-up. After the initial appointment, patients were followed up at 6-month intervals by telephone interview by trained cardiologists. In addition, between September and December 2010, all patients were contacted to assess their status. When a patient was not found, data were obtained by telephone interview with family members or the treating physician. The primary endpoint was cardiovascular death, and the secondary endpoint was readmission for ACS, arrhythmic events, stroke, or myocardial revascularization (percutaneous coronary intervention or coronary artery bypass grafting) due to clinical deterioration. Noncardiac deaths were not included in the analysis. In case of death, death certificates were obtained and the cause of death was verified by verbal or written contact with the treating physician.

The study was approved by the ethics committee of our institution, and all subjects gave their informed consent.

Statistical analysis. Continuous variables are presented as mean ± SD, while non-normally distributed variables are presented as medians and interquartile ranges. We used Student *t* tests for independent samples to compare means for normally distributed variables and Mann-Whitney tests for skewed variables and chi-square tests for qualitative variables. The Shapiro-Wilk test for normality was used to evaluate the *t*-test assumption. Bivariate correlations were performed using Pearson correlation coefficients and linear regression analysis using beta regression coefficients and corresponding SE. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated using Cox proportional-hazards models. The natural logarithm of high-sensitivity C-reactive protein (hsCRP) was used because of its skewed distribution. Proportionality of hazards was assessed using Schoenfeld residuals and the linear assumption using Martingale residuals. Crude survival curves were constructed using the Kaplan-Meier method to describe the incidence of death over time, and log-rank tests were applied to evaluate differences between groups.

A p value <0.05 was considered significant. SPSS version 13 (SPSS, Inc., Chicago, Illinois) and Stata version 9.1 (StataCorp LP, College Station, Texas) were used.

Results

Baseline characteristics. We initially recruited 524 consecutive patients with stable CAD. Follow-up data were not obtained in 39 patients for several reasons (declined to participate, wrong addresses, etc.). In addition, 8 patients whose deaths were considered noncardiovascular were excluded. Therefore, analysis was confined to 477 patients with CAD. Baseline characteristics according to occurrence of death are shown in Table 1.

Cardiac events during follow-up. The median period of follow-up was 34 months (interquartile range: 24 to 40 months). One hundred twenty-three patients (25.8%) presented with cardiac events during the follow-up period. Of these, 24 died (cardiac death) (5%), 47 (9.9%) developed ACS, 28 (5.9%) underwent myocardial revascularization procedures (percutaneous coronary intervention or coronary artery bypass grafting) because of clinical deterioration, 22 (4.6%) had arrhythmic events, and 2 (0.42%) had strokes. Patients who underwent revascularization procedures during ACS were assigned once in the group with ACS.

Associations of Lp-PLA$_2$ and HDL-Lp-PLA$_2$. There was a significant correlation between Lp-PLA$_2$ activity and Lp-PLA$_2$ mass ($r = 0.526$, $p < 0.001$). Lp-PLA$_2$ activity was also positively correlated with LDL cholesterol and apo B levels ($r = 0.486$, $p < 0.001$, and $r = 0.529$, $p < 0.001$, respectively). Similarly, Lp-PLA$_2$ mass was positively correlated with LDL cholesterol and apo B ($r = 0.383$, $p < 0.001$, and $r = 0.260$, $p < 0.001$, respectively). Both Lp-PLA$_2$ activity and Lp-PLA$_2$ mass were positively cor-

Table 1 Baseline Characteristics of the Study Population According to the Occurrence of Events (Cardiac Death)

Parameter	Event (n = 24)	No Event (n = 453)	p Value
Age (yrs)	67 ± 9	61 ± 9	0.001
Men	21 (87.5%)	384 (84.8%)	0.712
Current smokers	6 (25.0%)	129 (28.5%)	0.712
Hypertension	17 (70.8%)	285 (62.9%)	0.433
Diabetes mellitus	12 (50.0%)	150 (33.1%)	0.089
Family history of CAD	8 (33.3%)	158 (34.9%)	0.877
LV ejection fraction	39.4 ± 13.2	52.7 ± 9.5	<0.001
Previous ACS	20 (83.3%)	366 (80.8%)	0.756
Body mass index (kg/m^2)	29.2 ± 4.4	28.7 ± 4.3	0.546
Waist circumference (cm)	107 ± 12.6	102.5 ± 10.9	0.058
Dietary score	33.1 ± 4.9	31.5 ± 5.2	0.244
Physical exercise (METs-min/week)	986 (331–3,875)	1,600 (612–3,612)	0.275
Total cholesterol (mg/dl)	169.1 ± 63.2	173.2 ± 38.4	0.624
Triglycerides (mg/dl)	99.0 (79.0–160.2)	119.0 (87.0–168.0)	0.151
LDL cholesterol (mg/dl)	110.7 ± 51.9	104.8 ± 32.7	0.407
HDL cholesterol (mg/dl)	37.9 ± 8.1	44.5 ± 10.2	0.002
Apo A-1 (mg/dl)	119.3 ± 17.7	137.3 ± 22.5	0.001
Apo B (mg/dl)	90.9 ± 33.9	90.6 ± 22.8	0.957
Lp-PLA$_2$ mass (ng/ml)	354.3 ± 70.8	311.8 ± 55.9	0.001
Lp-PLA$_2$ activity (nmol · ml^{-1} · min^{-1})	39.1 ± 13.1	33.8 ± 11.5	0.032
HDL-Lp-PLA$_2$ mass (ng/ml)	65.8 ± 27.9	80.9 ± 24.9	0.006
HDL-Lp-PLA$_2$ activity (nmol · ml^{-1} · min^{-1})	3.1 ± 1.5	3.9 ± 1.5	0.024
Lp-PLA$_2$/HDL-Lp-PLA$_2$ (mass)	6.7 ± 3.9	4.3 ± 1.7	<0.001
Lp-PLA$_2$/HDL-Lp-PLA$_2$ (activity)	12.5 (9.1–19.5)	8.8 (6.4–12.3)	0.001
hsCRP (mg/l)	3.5 (1.8–19.4)	1.9 (1.0–4.4)	0.004
Drug therapy			
Antiplatelet treatment	22 (91.7%)	421 (92.9%)	0.814
Lipid-lowering medication	19 (79.2%)	373 (82.3%)	0.692
Statins	19 (79.2%)	355 (78.4%)	0.926
Ezetimibe*	2 (8.3%)	33 (7.3%)	0.848
Fibrates*	0	7 (1.5%)	0.540
Nicotinic acid*	0	5 (1.1%)	0.605
Beta-blockers	20 (83.3%)	359 (79.2%)	0.629
ACE inhibitors or AT1 blockers	15 (62.5%)	270 (59.6%)	0.778
Thyroid substitution therapy	1 (4.1%)	15 (3.3%)	0.819

Values are mean ± SD, n (%), or median (interquartile range). *Alone or in combination with statins.

ACE = angiotensin-converting enzyme; ACS = acute coronary syndromes; apo = apolipoprotein; AT1 = angiotensin II type 1; CAD = coronary artery disease; HDL = high-density lipoprotein; HDL-Lp-PLA$_2$ = high-density lipoprotein–associated phospholipase A$_2$; hsCRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; Lp-PLA$_2$ = lipoprotein-associated phospholipase A$_2$; LV = left ventricular; MET = metabolic equivalent.

related with hsCRP ($r = 0.179$, $p < 0.001$, and $r = 0.190$, $p < 0.001$, respectively). In addition, Lp-PLA$_2$ activity was positively associated with smoking (beta = 0.168, SE = 0.113, $p = 0.028$).

There was a significant association between HDL-Lp-PLA$_2$ activity and HDL-Lp-PLA$_2$ mass ($r = 0.765$, $p < 0.001$). HDL-Lp-PLA$_2$ activity was also positively correlated with HDL cholesterol and apo A-1 levels ($r = 0.586$, $p < 0.001$, and $r = 0.374$, $p < 0.001$, respectively). Similarly, HDL-Lp-PLA$_2$ mass was positively correlated with HDL cholesterol and apo A-1 ($r = 0.451$, $p < 0.001$, and $r = 0.308$, $p < 0.001$, respectively). Finally, HDL-Lp-PLA$_2$ activity was inversely correlated with waist circumference ($r = -0.107$, $p = 0.043$) and current smoking (beta = −0.373, SE = 0.168, $p = 0.028$), while an inverse association was observed between HDL-Lp-PLA$_2$ mass and diabetes mellitus (beta = −6.443, SE = 2.754, $p = 0.02$).

Predictors of cardiovascular events during follow-up. Univariate Cox regression analysis revealed that total plasma Lp-PLA$_2$ activity and mass, HDL-Lp-PLA$_2$ activity and mass, ratios of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ activity and mass, and the natural log of hsCRP predicted the occurrence of cardiac death (Table 2). Multivariate Cox regression analysis showed that Lp-PLA$_2$ activity and mass, HDL-Lp-PLA$_2$ activity and mass, the ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass, and the natural log of hsCRP were independent predictors of cardiac death after adjustment for conventional risk factors (age, sex, diabetes mellitus, total cholesterol, hypertension, smoking, and family history of CAD) (Table 2).

Table 2 Univariate and Multivariate Associations Between Total Plasma Lp-PLA$_2$ Mass and Activity, HDL-Lp-PLA$_2$ Mass and Activity, Ratios of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ Mass and Activity, Natural Log of hsCRP, and Cardiac Death at Follow-Up

	Univariate			Multivariate*		
Variable	HR	95% CI	p Value	HR	95% CI	p Value
Lp-PLA$_2$ (mass)	1.015	1.007–1.023	<0.001	1.013	1.005–1.021	0.002
Lp-PLA$_2$ (activity)	1.034	1.003–1.067	0.033	1.040	1.005–1.076	0.025
HDL-Lp-PLA$_2$ (mass)	0.974	0.955–0.993	0.007	0.972	0.952–0.993	0.010
HDL-Lp-PLA$_2$ (activity)	0.691	0.504–0.946	0.021	0.689	0.496–0.957	0.026
Lp-PLA$_2$/HDL-Lp-PLA$_2$ (mass)	1.432	1.268–1.619	<0.001	1.476	1.217–1.791	<0.001
Lp-PLA$_2$/HDL-Lp-PLA$_2$ (activity)	1.042	1.008–1.076	0.014	1.036	0.998–1.075	0.064
ln(hsCRP)	1.851	1.321–2.593	<0.001	1.731	1.234–2.429	0.002

*Adjusted simultaneously for age, sex, smoking status, presence of hypertension, diabetes mellitus, total cholesterol, and family history of coronary artery disease.
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

To address the problem of overfitting, that is, few events (cardiac deaths) for the covariates used, a new model was fitted using age as the unique covariate, because in univariate analysis, it was the only risk factor significantly associated with the outcome. All tested variables were independent predictors of cardiac death. In particular, the HR for HDL-Lp-PLA$_2$ mass was 0.967 (95% CI: 0.948 to 0.988; p = 0.002) and for HDL-Lp-PLA$_2$ activity was 0.685 (95% CI: 0.508 to 0.952; p = 0.023).

We applied Cox proportional-hazards models to assess the incidence of cardiac death according to tertiles of HDL-Lp-PLA$_2$ mass and the ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ (mass) after adjustments for classic risk factors. Importantly, patients in the upper tertile of HDL-Lp-PLA$_2$ mass (>90.78 ng/ml) had 75% less possibility of cardiac death compared with patients in the lower tertile (<68.15 ng/ml) (HR: 0.250; 95% CI: 0.080 to 0.785; p = 0.018), while patients in the upper tertile of the ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ (mass) (>4.62) had 4 times higher risk for cardiac death compared with patients in the lower tertile (<3.47) (HR: 4.243; 95% CI: 1.193 to 15.092; p = 0.026). Figure 1 presents unadjusted Kaplan-Meier survival curves stratified according to tertiles of HDL-Lp-PLA$_2$ mass and ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass. Finally, in the subgroup of patients with low HDL cholesterol levels (men with HDL cholesterol <40 mg/dl and women with HDL cholesterol <50 mg/dl), HDL-Lp-PLA$_2$ mass was an independent predictor of cardiac death after adjustment for conventional risk factors (HR: 0.951; 95% CI: 0.908 to 0.996; p = 0.033).

Regarding the secondary endpoints, in univariate analysis, HDL-Lp-PLA$_2$ activity and mass were associated with lower risk for ACS (for HDL-Lp-PLA$_2$ activity: HR: 0.771; 95% CI: 0.618 to 0.962; p = 0.021; for HDL-Lp-PLA$_2$ mass: HR: 0.987; 95% CI: 0.974 to 1.000; p = 0.044). However, this association was abolished when adjustment for conventional risk factors was performed. Importantly, when we examined the prognostic value of HDL-Lp-PLA$_2$ for the combined endpoint of cardiac death and ACS (n = 71) adjusted for conventional risk factors, a significant inverse association was observed for HDL-Lp-PLA$_2$ mass (HR: 0.986; 95% CI: 0.975 to 0.998; p = 0.021) and for HDL-Lp-PLA$_2$ activity (HR: 0.796; 95% CI: 0.654 to 0.968; p = 0.023).

Finally, the ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass was also associated with the development of any cardiovascular event (HR: 1.114; 95% CI: 1.015 to 1.224; p = 0.023), which remained even after adjustments to conventional risk factors (HR: 1.110; 95% CI: 1.001 to 1.230; p = 0.048).

Discussion

In this prospective study of patients with stable CAD, we demonstrate for the first time that HDL-Lp-PLA$_2$ (mass or activity) is inversely associated with future cardiac mortality or the combined endpoint of cardiac death and ACS independently of the classic risk factors. In addition, HDL-Lp-PLA$_2$ mass was an independent predictor of cardiac death after adjustment for conventional risk factors in the patients with low HDL cholesterol levels. The present study further shows that the ratio of the total plasma Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass is also an independent predictor of cardiac death.

In accordance with previously published data, the total plasma Lp-PLA$_2$ (mass or activity) is associated positively with future cardiac mortality independently of the classic risk factors. Indeed, previous studies have consistently demonstrated that the total plasma Lp-PLA$_2$, which primarily represents the LDL-associated enzyme (16,21), is associated with cardiovascular events in subjects both with and without documented CAD, and these data support the hypothesis that this enzyme may be a causal mediator of atherosclerosis and plaque instability (7–15).

In our study, we recruited patients with stable CAD. The majority of previous clinical studies have reported data on patients with ACS (11,12) or on mixed populations comprising patients with ACS and stable CAD (13,14). Thus, there are limited data concerning only patients with stable CAD. In this regard, Sabatine et al. (10) studied 3,766 patients with stable CAD who were followed for a median of 4.8 years. They found that increased levels of Lp-PLA$_2$ mass were significant predictors of nonfatal adverse cardiovascular outcomes, independent of traditional clinical risk factors and hsCRP. However, they failed to find an associ-

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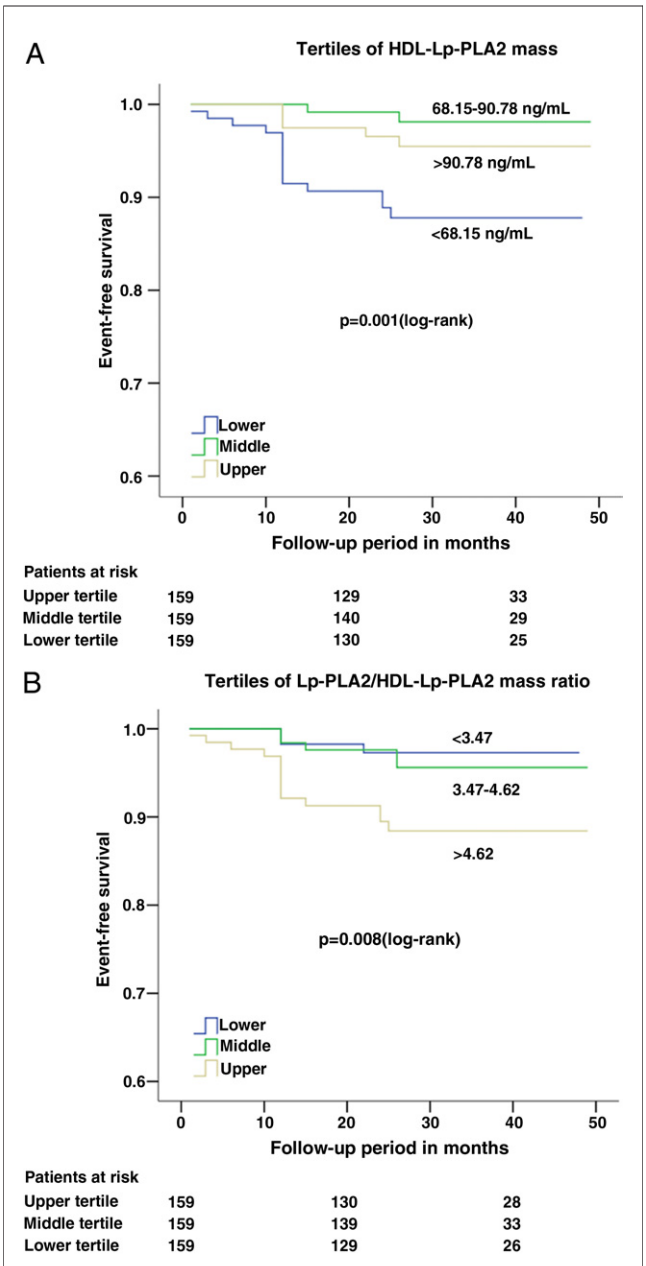

Figure 1 **Unadjusted Kaplan-Meier Survival Curves**

Unadjusted Kaplan-Meier survival curves for cardiac death classified by tertiles of high-density lipoprotein–associated phospholipase A_2 (HDL-Lp-PLA$_2$) mass **(A)** and ratio of Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass **(B)**.

ation with cardiovascular deaths after multivariate adjustments. In the present study, there was an independent association of total plasma Lp-PLA$_2$ with cardiac deaths.

A small proportion of Lp-PLA$_2$ in plasma is bound on HDL and previous studies have suggested that HDL-Lp-PLA$_2$ may exert antiatherogenic and anti-inflammatory effects. This assumption is based on findings from in vitro experiments as well from in vivo studies in animal models. In vitro studies have shown that Lp-PLA$_2$ contributes to the HDL-mediated inhibition of cell stimulation induced by oxidized LDL (26). Furthermore, adenoviral transfer of the human plasma Lp-PLA$_2$ gene in atherosclerosis-prone apo $E^{-/-}$ mice induced a 3.5-fold reduction of macrophage adhesion ex vivo and a 2.6-fold reduction in macrophage homing in vivo. These inhibitory effects were independent of the function of HDL as a cholesterol acceptor (27). Gene transfer of Lp-PLA$_2$ in apo $E^{-/-}$/mice also reduced the deposition of oxidized lipoproteins and the accumulation of macrophages and smooth muscle cells in the arterial wall, in parallel with the inhibition of neointima formation induced by endothelial denudation and reduction of spontaneous atherosclerosis (28). Consistent with these results, local adenovirus-mediated Lp-PLA$_2$ gene transfer at the site of injury resulted in a significant reduction in neointima formation in balloon-denuded aorta in cholesterol-fed rabbits (29). Similarly, local expression of Lp-PLA$_2$ reduces the accumulation of oxidized lipoproteins and inhibits inflammation, shear stress-induced thrombosis, and neointima formation in balloon-injured carotid arteries in normolipidemic rabbits (30). It should be emphasized that all these studies were conducted in animal models exhibiting a reverse lipoprotein profile compared with humans (higher HDL than LDL levels), while the vast majority of plasma Lp-PLA$_2$ in these animals is associated with HDL (31), so these results may not be relevant for the situation in humans.

There are very few published data regarding HDL-Lp-PLA$_2$ in humans. Previous data published by our group showed that the distribution of Lp-PLA$_2$ among HDL subclasses is heterogenous, and this enzyme is primarily associated with a small HDL subfraction denoted as HDL-3c or very high-density lipoprotein–1 (21,24,25). This subfraction contains only a small proportion of the total plasma HDL cholesterol (21). In patients with metabolic syndrome, HDL-Lp-PLA$_2$ activity was lower compared with those without metabolic syndrome and was inversely associated with the degree of insulin resistance (32,33). Low plasma levels of HDL-Lp-PLA$_2$ are observed in dyslipidemic type IIB and type IV patients characterized by elevated triglyceride levels, a defect that is significantly improved with fenofibrate treatment (25,34). Consistent with these suggestions are the results of the present study, the first prospective study to evaluate whether HDL-Lp-PLA$_2$ can predict future cardiovascular events in patients with stable CAD. Our results show for the first time that HDL-Lp-PLA$_2$ mass or activity is associated with lower risk for cardiac death independently of other traditional risk factors for CAD.

Our results further reveal that the ratio of total plasma Lp-PLA$_2$ to HDL-Lp-PLA$_2$ mass is also independently associated with the risk for development of any cardiovascular event. This finding supports our previously published data showing that this ratio may represent a potential marker of atherogenicity in dyslipidemic type IIA patients (22,35). Furthermore, other investigators have reported that an elevation in the ratio of LDL-associated Lp-PLA$_2$ to HDL-Lp-PLA$_2$ is associated with an increased incidence of

paroxysmal atrial fibrillation and may be a marker for inflammation in these patients (36).

These results may be of particular importance in view of the phase 2 clinical studies showing that the selective Lp-PLA$_2$ inhibitor darapladib significantly reduces plasma Lp-PLA$_2$ activity (37,38) and prevents expansion of the necrotic core volume in the coronary arteries of patients with CAD (38), suggesting that darapladib may be a potentially useful therapeutic agent for the reduction of residual cardiovascular risk, especially in patients with ACS (39). However, in these studies, total plasma Lp-PLA$_2$ but not HDL-Lp-PLA$_2$ was determined. Thus, the effect of darapladib on HDL-Lp-PLA$_2$ remains to be determined. The fact that darapladib reduces total plasma Lp-PLA$_2$ by 59% to 66% at an oral daily dose of 160 mg for a period 3 to 12 months (37,38) could suggest that it may not significantly reduce HDL-Lp-PLA$_2$ activity, which represents 4.9% of the total plasma enzyme activity (24). In this regard, it has been demonstrated that a selective Lp-PLA$_2$ inhibitor, SB-222657, does not significantly influence HDL-Lp-PLA$_2$ activity (40). The clinical significance of Lp-PLA$_2$ in cardiovascular disease awaits to be proved in 2 phase 3 clinical trials, STABILITY (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) (41) and SOLID–TIMI 52 (Stabilization of Plaques Using Darapladib–Thrombolysis in Myocardial Infarction 52) (42), which are currently in progress. These studies will determine whether inhibition of Lp-PLA$_2$ activity with darapladib safely reduces adverse cardiovascular events and death.

Study limitations. The main limitation of our study was the relatively small number of cardiac deaths. Although we were able to detect significant associations between HDL-Lp-PLA$_2$ and cardiac deaths, these results should be interpreted with caution. A larger study is necessary to confirm our results and improve the prognostic power of the studied inflammatory markers before definitive conclusions can be made. Another limitation is the reliance on death certificates to identify the exact cause of death, which is not always reliable.

Clinical perspective. Epidemiological studies support an association between plasma levels of Lp-PLA$_2$ (which represent mainly the LDL-associated enzyme) and the risk for occlusive coronary and vascular events. However, the role of Lp-PLA$_2$ in atherosclerotic diseases may depend on the type of lipoprotein with which it is associated. We examined the prognostic value of HDL-Lp-PLA$_2$ in patients with stable CAD. We showed that HDL-Lp-PLA$_2$ mass or activity is associated with lower risk for cardiac death independently of other traditional risk factors for CAD. This finding may be of particular importance in view of the expected results of the phase 3 Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy clinical trial, which will determine whether inhibition of Lp-PLA$_2$ activity with darapladib safely reduces adverse cardiovascular events and death. However, in this study as well as in the previous phase 2 clinical studies, the effect of darapladib on HDL-Lp-PLA$_2$ has not been determined. Further studies are necessary to support the potential pathophysiological role and clinical utility of HDL-Lp-PLA$_2$ in cardiovascular disease.

Conclusions

This prospective cohort of patients with stable CAD demonstrated that HDL-Lp-PLA$_2$ (mass or activity) is inversely associated with future cardiac mortality independently of the conventional risk factors, while HDL-Lp-PLA$_2$ mass preserves its predictive value even in patients with low HDL cholesterol levels. These findings suggest that HDL-Lp-PLA$_2$ may be a clinically useful tool in the assessment of the residual cardiovascular risk, a hypothesis that needs to be further supported by large-scale clinical studies.

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Key Words: atherosclerosis ■ coronary artery disease ■ HDL ■ lipoproteins ■ Lp-PLA$_2$.