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Lipoprotein-associated Phospholipase A_2, Lp-PLA_2, and Age, are Predictors for Future Cardiovascular Events in Acute Coronary Syndrome Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author TTV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author LNH designed the study, performed the final statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and the final manuscript. Authors NVK and LXT managed the analyses of the study. Authors NCT and NQT managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: We investigated whether Lp-PLA_2 activity of blood samples collected 24 h after hospital admission could serve as a predictor of future cardiovascular events in Vietnamese patients with acute coronary syndrome (ACS). In addition, we correlated Lp-PLA_2 with common risk factors of

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ACS, such as age, HDL, diabetes, BMI, etc. and compared Lp-PLA_2 levels of ACS patients with those of a control group.

Methods and Results: Lp-PLA_2 activity was measured in serum collected in fasting state within 24 h of hospitalization in 293 ACS patients. The mean [SD] of Lp-PLA_2 activity (nmol/min/mL) was higher in ACS patients than in controls (212.7[57.8] versus 182.5[58], p<0.001), the mean difference was 14.2% of mean in ACS group. Both non-ST elevated myocardial infarction (NSTEMI) and STEMI had higher Lp-PLA_2 compared to unstable angina (UA) (p<0.017). The 3rd tertile of Lp-PLA_2 had a strong correlation with MI (NSTEMI/STEMI) showing ORs of 3.65 and 3.98 in logistic regression analysis compared to the 2 lower tertiles. There were only two factors, Lp-PLA_2 and age, serving as best independent predictors for future CV events in multivariate HR analysis. However their effects strongly began to start around 50 days after acute phase of ACS. The future CV event rates had an increasing trend from 1st to 3rd tertile (p_{trend}=0.011). The HR between 2nd to 1st tertile was 1.71 (p=0.075). The 3rd tertile had nearly a double of adjusted hazard ratio (HR) as 1.92 compared to the 1st tertile (p=0.029). There was weak association of Lp-PLA_2 activity with risk factors of CV diseases.

Conclusion: Lp-PLA_2 in acute phase of ACS was different between 3 groups of ACS. Both Lp-PLA_2 and age were independent predictors for future cardiovascular events in ACS patients.

Keywords: Lipoprotein-associated phospholipase A_2; acute phase; acute coronary syndrome; predictor.

1. INTRODUCTION

Lipoprotein-associated phospholipase A_2(Lp-PLA_2) is a calcium-independent member of the A_2 phospholipase superfamily [1], also known as platelet-activating factor acetylhydrolase (PAF-AH), which is produced mainly by macrophages and lymphocytes [2]. The oxidation of LDL to oxLDL yields oxidized phospholipids, that are hydrolyzed solely by Lp-PLA_2 producing lysophosphatidylcholine and non-esterified fatty acids. These 2 potent pro-inflammatory and proatherogenic mediators up-regulate the expression of adhesion molecules activating leukocytes and recruiting macrophages and monocytes to atherosclerotic plaques [3]. Importantly, Lp-PLA_2 has been found strongly expressed in the vicinity of macrophages of vulnerable and ruptured plaques [4,5]. Lp-PLA_2 is a potential novel inflammatory risk factor for coronary artery disease and has been suggested to provide information related and additional to that obtained from traditional lipid analyses [6] and complementary to C-reactive protein [7].

Many epidemiological studies have suggested Lp-PLA_2 as an independent predictor of cardiovascular events [7,8,9] and higher levels of Lp-PLA_2 have also been associated with stable coronary artery disease in case-control studies [7,10,11]. Those studies worked on subjects with Lp-PLA_2 collected at preexisting stable status of cardiovascular diseases. Few data are available concerning the clinical value of Lp-PLA_2 collected in acute phase of acute coronary syndrome (ACS). There were only 2 published studies on Lp-PLA_2 in acute phase of ACS patients [12,13]. The levels of Lp-PLA_2 in acute phase of ACS patients were slightly higher than that in healthy control subjects [12] or that collected at a delay time, i.e. 30 days, from the first days of ACS [13]. Thus it remains unclear whether Lp-PLA_2 could be different between three clinical groups of ACS. In addition, the risk of future cardiovascular events or mortality was not related to Lp-PLA_2 levels in ACS patients reported by those 2 studies. The biological role of Lp-PLA_2 in ACS patients still remains unclear. The ethnic variations in ACS were suggested to be present [14].

We investigated whether Lp-PLA_2 could represent both a biomarker for group classification in early ACS and a predictor for future cardiovascular events in Vietnamese ACS patients. We also examined the correlation between acute-phase Lp-PLA_2 and other known risk factors in ACS patients and compared Lp-PLA_2 levels of ACS patients with those of Vietnamese healthy control subjects.

2. MATERIALS AND METHODS

2.1 Study Population

The study was conducted at Cho Ray Hospital, Ho Chi Minh City, Viet Nam from January 2011 to February 2012.

In-patients were included in the study if diagnosed as first acute coronary syndrome (ACS). Emergency Department was the first department receiving patients, thereafter patients were referred to Cardiology Department or Cardiology Intervention. Patients were excluded if using lipid-lowering medicine about one year prior to hospitalization or having other diseases except diabetes and/or arterial hypertension.

Patients were classified according to three groups of ACS: unstable angina (UA), non-ST elevation myocardial infarction (NSTEMI) and STEMI. UA was defined as unexpected chest pain, usually at rest, no elevation in troponin, with or without ECG changes indicative of ischemia. The diagnosis of NSTEMI was made when a patient had symptoms of unstable angina, had no ST-segment elevation on the ECG, and had an elevation in cardiac enzymes (troponin I and CK-MB).The ST-Elevation myocardial infarction (STEMI) was defined as the same as NSTEMI but "ST segment" on the ECG appeared "elevated," reflecting coronary artery total occlusion.

Healthy control subjects were persons who came to the Internal General Clinics of Out-patient Department for health check-up visit for their own reasons. The healthy control group was matched with above ACS patients by age and sex. Only persons who did not have any clinical or history of atherosclerosis and no evidence of pathological electrocardiogram were recruited into study.

The cross-sectional study compared levels of Lp-PLA_2 activity in ACS patients to those in healthy subject controls matched by age and gender. The cohort study was done in all ACS patients for recording the second cardiovascular events occurred within the maximum follow-up time of 2 years since the hospitalization.

All subjects gave written informed consent. The study was approved by the Medical Ethics Committee of Cho Ray Hospital.

2.2 Data Collection

During the time of hospitalization, patients were interviewed with a prepared questionnaire for information about smoking status, alcohol behavior, any history of diabetes mellitus, hypertension, hyperlipidemia, current drug use, and family history of early MI.

In all patients directly follow-up was conducted by researchers during the hospitalization until the hospital discharge. Thereafter, patients were followed as out-patients at the Cardiology Intervention Clinics or Cardiology Clinics of Out-Patient Department, Cho Ray Hospital in their own routine visit schedules. Patients could also conduct the health follow-up at local hospitals at their locations. The monitoring of patient follow-up was routinely performed on telephone interview by researchers with patients or their family relatives. The follow-up time was completed when a secondary cardiovascular event occurred and recorded, or censored at 2 years as the maximum time of follow-up.

Secondary cardiovascular events were defined either as cardiovascular disease (CVD) as the main cause of death, nonfatal myocardial infarction (UA, NSTEMI, STEMI), coronary revascularization, or ischemic cerebrovascular event (stroke). The source of data were hospital case records during hospitalization, out-patient record book of each clinics in the out-patient department if patients performed the follow-up at Cho Ray Hospital, or hospital discharge certificate from local hospitals, or treatment sheets reported by authorized primary care private physicians. Other event data were excluded as invalid data.

2.3 Laboratory Methods

Blood samples were collected in fasting state within the first 24 hours after hospital administration. Patients fasted around 10-12 hours after the last meal before measurement of lipid blood panel and phenotype analysis of LDL cholesterol with gradient-gel electrophoresis. All serums samples were collected in standard procedure, thereafter transferred to plain tubes for storing. Serum samples were stored at -80℃ until analysis for Lp-PLA_2 activity. Other tests, CK-MB, troponin, lipid blood panel were done within day of blood collection as routine performance.

The serum Lp-PLA_2 activity (nmol/min/mL) was determined by the PLAC® Test for Lp-PLA_2 Activity enzyme assay on automated clinical chemistry analyzers (Lp-PLA_2 Activity; diaDexusInc, South San Francisco, CA). This test was demonstrated to be equivalent to the legally marketed predicate device, diaDexus PLAC Test, measuring Lp-PLA_2 in concentration (ng/mL). PLAC® Test for Lp-PLA_2 Activity enzyme assay was approved by FDA in

December 2014. The Siemens Advia 1800® Clinical Chemistry Analyzer (Siemens Healthcare Diagnostics, NY, USA) was used to run the PLAC® Test for Lp-PLA_2 Activity enzyme assay.

Lp-PLA_2 in serum hydrolyzes the sn-2 position of the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, producing a colored reaction product, 4-nitrophenol. The rate of formation of 4-nitrophenol was measured spectrophotometrically for 8.5 minutes and the Lp-PLA_2 activity calculated from the rate of change in absorbance at 410 nm. A set of five Lp-PLA_2 calibrators was used to generate a standard curve fit of change in absorbance versus Lp-PLA_2 activity levels in nmol/min/mL. The kit of PLAC® Test for Lp-PLA_2 Activity was included two controls.

The clinical sensitivity (limit of quantification) of the assay was 10 nmol/min/mL with CV of 20%. The intra-assay and inter-assay variability, determined by testing five human pooled serum samples and two controls with Lp-PLA_2 activity in the calibration range of the assay (65-289 nmol/min/mL) were 0.3-0.8% and 1.6-4.2%, respectively. Linearity was assessed with a dilution series from 3 high level samples, 249-308 nmol/min/mL) to low level samples (73-74 nmol/min/mL), all R^2 were from 0.992 to 0.999.

CK-MB and troponin I (TnI-Ultra®) in serum were measured by chemiluminescent immunoassay on Advia Centaur Immunoassay System (Siemens Healthcare Diagnostics, NY, USA).

The blood lipid panel, including total cholesterol, triglycerides, HDL-C, LDL-C, and blood glucose were measured by spectrophotometry on Mindray BS-800 (Mindray, Shenzhen, China).

2.4 Statistical Methods

The body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the square of the patient's height inmeters. Patients were classified as underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2). Smoking was defined as current smoking or previously smoked. Alcohol behavior meant drinking alcohol almost everyday or being alcoholism. Family history of early MI was positive in the case of any documented CVD of one first-degree relative at aged less than 55 years. Diabetes mellitus was diagnosed if patients had history of previously undergone dietary treatment, additional oral antidiabetic or insulin medication, or had laboratory results of fasting plasma glucose (FPG) equal or higher than 126 mg/dL (7 mmol/L). Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or in using antihypertensive drugs.

The distribution of Lp-PLA_2 activity was normal, z scores of Skewness and Kurtosis less than 2.0, therefore Lp-PLA_2 activity was presented as mean (SD). Other quantitative data were also presented as mean (SD), else stated. Qualitative data were reported in number of cases and proportions. Sociodemographic, clinical, laboratory and Lp-PLA_2 activity characteristics of ACS patients and healthy control subjects were presented as means (SD) or proportions, as appropriate.

The parametric tests, t-test and ANOVA, were used for comparing means between 2 or ≥ 3 independent quantitative groups, respectively. For ANOVA test, if p values were less than 0.05, the post hoc Bonferroni test was used to identify different paired groups with p = 0.05/n[n-1]/2.

The associations of sociodemographic data and cardiovascular risk factors with Lp-PLA_2 activity were evaluated by Chi-quare $\chi 2$ test.

Partial Spearman correlation coefficients were calculated for Lp-PLA_2 activity and age; blood lipids (total cholesterol, triglycerides, HDL-C, LDL-C).

The odds ratios, 2x2 tables, were used for univariate analysis of correlations between Lp-PLA_2 in each of pairs of tertiles. Multinomial logistic regression was used for adjusted OR compared between 3rd tertile to 1st tertile in correlation with Lp-PLA_2 by gender and age groups.

The relation of Lp-PLA_2 activity with CVD events during the follow-up was assessed by the Kaplan-Meier and life table method and tested by the Log-rank test. The Cox proportional hazards model was used to assess the independent association of Lp-PLA_2 activity distribution with the risks of secondary CVD events. The main factors were Lp-PLA_2 activity (low, mid, top tertiles), ACS types (UA, NSTEMI, STEMI), age (y), gender (male/female). Besides these main factors a set of covariates, including BMI (overweight or obese/normal), smoking status (yes/no), hypertension (yes/no), diabetes mellitus

(yes/no), family history of early MI (yes/no), total cholesterol (mg/dL), HDL-C (mg/dL), triglycerides (mg/dL), LDL-C (mg/dL), was assessed in univariate analysis. Only those having the significant value ($p<0.05$) were included in multivariable analysis of hazard ratio (HR) Cox regression.

All statistical tests were two-sided and p-values of less than 0.05 were considered significant.

3. RESULTS

Overall, 323 ACS patients were enrolled into study. A total of 293 patients (90.7%) completed the follow-up after hospital discharge until the second cardiovascular event occurred or maximum of 2 year follow-up. There were 30 patients (9.3%) lost in follow-up including 9 who refused to be followed up, 13 who could not be contacted and 8 who did not obey a standard therapy. A total of 91 healthy control subjects were also included in the study.

Table 1 shows the sociodemographic, clinical, laboratory and Lp-PLA_2 activity characteristics between ACS patients and healthy controls. Triglycerides and HDL-cholesterol, but not total- and LDL-cholesterol, were different between ACS patients and controls. Lp-PLA_2 activity was statistically higher in ACS patients compared to controls, 212.7±57.8 versus 182.5±58 nmol/min/mL, respectively. The mean difference of 30 nmol/min/ml was lower than the SD, i.e. 58 nmol/min/mL, of data distribution of both groups. The mean difference was 14.2% compared to mean of Lp-PLA_2 activity in ACS group.

Table 2 shows the relationship of various cardiovascular risk factors (gender, age, smoking, drinking alcohol, BMI, hypertension, diabetes mellitus, family history of early MI) and 3 types of ACS with Lp-PLA_2 activity. There was no association between Lp-PLA_2 activity and any of cardiovascular risk factors. There was a strong and positive relationship between Lp-PLA_2 and 3 types of ACS. Post-hoc analysis (Bonferroni test) showed that Lp-PLA_2 activity was higher in both NSTEMI and STEMI than in UA ($p<0.017$).

Table 3 shows the weak correlations between lipid variables and LP-PLA_2 activity. There was no correlation between Lp-PLA_2 and triglycrides ($p=0.213$). Total cholesterol and LDL-cholesterol were positively associated with Lp-PLA_2 ($r=0.135$ and $r=0.145$, respectively). HDL-cholesterol had a negative correlation. All correlations coefficients were statistically significant ($p<0.02$) but of weak intensity ($r < 0.3$).

Table 4 shows that 3rd tertile of Lp-PLA_2 had a strong correlation with myocardial infarction MI (NSTEMI/STEMI) showing ORs of 3.65 in univariate analysis and 3.98 in multinominal logistic regression analysis adjusted by age and gender compared to that of the 2 lower tertiles. The sensitivity and specificity of 3rd tertile of Lp-PLA_2 for MI was 36.5% and 86.4%, respectively.

Table 1. Sociodemographic, clinical, laboratory and Lp-PLA_2 activity characteristics between ACS patients and healthy control subjects

Parameters		ACS patients n = 293	Healthy control subjects n = 91	P[!]
Gender: men, n (%)		195 (66%)	54 (59%)	0.21
Age, y (μ, SD)		62.6±12.7	63.6±11.1	0.48
Smoking status: yes, n (%)		145 (50%)	37 (41%)	0.14
Alcohol drinking: yes, n (%)		45 (16%)	11 (12%)	0.44
Family history of early MI, n (%)		40 (14%)	0 (0%)	NA
Arterial hypertension, n (%)		159 (54%)	0 (0%)	NA
Diabetes mellitus, n (%)		47 (16%)	0 (0%)	NA
BMI, kg/m^2, n (%)	<18.5	36 (12%)	8 (9%)	0.24
	18.5-24.9	208 (71%)	77 (85%)	
	≥25	49 (17%)	6 (7%)	
Total cholesterol, mg/dL (μ, SD)		190±50	183±39	0.21
Triglycerides, mg/dL (μ, SD)		211±61	189±54	0.002*
HDL-C, mg/dL (μ, SD)		34.1±10.0	39.9±9.6	0.001*
LDL-C, mg/dL (μ, SD)		115±43	116±37	0.78
Lp-PLA_2 activity, nmol/min/mL (μ, SD)		212.7±57.8	182.5±58.0	0.001

*! Chi-square for proportions, t-test for means, * Statistically significant difference, NA: not applicable*

Table 2. Lp-PLA$_2$ activity in relation to baseline characteristics and types of ACS

Variables		Proportions in tertiles of Lp-PLA$_2$ activity (nmol/min/mL)			Lp-PLA$_2$ activity (nmol/min/mL) Mean (SD)
		1st tertile 98-181 n= 98	2nd tertile >181-244 n=98	3rd tertile >244-346 n=97	
Gender	male, n=195	70 (71%)	69 (70%)	56 (58%)	209.0±57.6
	female, n=98	28 (29%)	29 (30%)	41 (42%)	220.2±57.8
	p	0.078			0.118
Age (y)	<= 39, n=12	4 (33.3%)	3 (25%)	5 (41.7%)	227.9±67.0
	40-49, n=35	16 (45.7%)	7 (20%)	12 (34.3%)	207.2±64.6
	50-59, n=79	24 (30.4%)	34 (43.0%)	21 (25.6%)	209.1±57.2
	60-69, n=77	24 (31.2%)	23 (29.8%)	30 (39.0%)	215.9±60.5
	70-79, n=56	19 (33.9%)	19 (33.9%)	18 (32.2%)	213.2±56.8
	>=80, n=34	11 (32.4%)	12 (35.2%)	11 (32.4%)	213.2±45.3
	p	0.56			0.93
Smoking	Yes, n=145	48 (49%)	57 (58.2%)	40 (41.2%)	216.1±57.5
	No, n=148	50 (51%)	41 (41.8%)	57 (58.8%)	209.2±58.1
	p	0.061			0.311
Drinking alcohol	Yes, n=45	15 (15.3%)	18 (18.4%)	12 (12.4%)	212.8±57.4
	No, n=248	83 (84.7%)	80 (81.6%)	85 (87.6%)	212.4±60.8
	p	0.51			
Hypertension	Yes, n=159	56 (57.1%)	55 (56.1%)	48 (49.5%)	217.2±60.6
	No, n=134	42 (42.9%)	43 (43.9%)	49 (50.5%)	208.9±55.3
	p	0.508			0.218
Diabetes mellitus	Yes, n=47	21 (21.4%)	12 (12.2%)	14 (14.4%)	215.4±57.4
	No, n=246	77 (78.6%)	86 (87.8%)	83 (85.6%)	198.7±58.3
	p	0.188			0.07
BMI (kg/m^2)	<18.5, n=36	6 (6.1%)	15 (15.3%)	15 (15.5%)	223.6±52.8
	18.5-24.9, n=208	74 (75.5%)	64 (65.3%)	70 (72.2%)	212.6±57.8
	≥25, n=49	18 (18.4%)	19 (19.4%)	12 (12.3%)	205.1±60.9
	p	0.137			0.345
Family history of early MI	Yes, n=40	11 (11.2%)	13 (13.3%)	16 (16.5%)	210.9±58.3
	No, n=253	87 (88.8%)	85 (86.7%)	81 (83.5%)	222.6±53.9
	p	0.538			0.235
Types of ACS	UA, n=44	19 (19.4%)	19 (19.4%)	6 (6.2%)	187.6±49
	NSTEMI, n=26	4 (4.1%)	13 (13.3%)	9 (9.3%)	223±51
	STEMI, n=223	75 (76.5%)	66 (67.3%)	82 (84.5%)	216.5±59
	p	0.007*			0.006*
Bonferroni test, p critical=0.017	UA vs NSTEMI				0.012*
	UA vs STEMI				0.002*
	NSTEMI vs STEMI				0.581

**statistically significant difference*

Table 3. Spearman correlation coefficients (R) between lipid variables and Lp-PLA$_2$ activity

Lipid variables	R	p
Total cholesterol, mg/dL	0.135	0.021*
Triglycerides, mg/dL	-0.073	0.213
HDL-cholesterol, mg/dL	-0.158	0.007*
LDL-cholesterol, mg/dL	0.145	0.013*

** statistically significant difference*

During a follow-up of 2.0 years, 86 major cardiovascular events occurred in 293 patients (the Kaplan–Meier estimated event rate was 29.4% at 2 years) including: 29 dead cases (5 death cases occurred during hospitalization), 21 with recurrent myocardial infarction, 5 with ischemic strokes, 7 with coronary revascularization and 24 with UA.

Table 5 shows the Cox proportional hazard (HR) in univariate analysis between each of risk factors with cardiovascular events and the adjusted HR in multivariate analysis for those having the significant value of HR ($p < 0.05$) in univariate analysis. There were 3 factors having positive HR in univariate analysis: age, gender and Lp-PLA_2. The factor of gender was totally attenuated in multivariate analysis. The only 2 independent risk factors for cardiovascular events in 2-year follow-up were age (adjusted HR: 1.036, p=0.001) and Lp-PLA_2 activity tertiles (adjusted HR: 1.376, p=0.027). This means that the increase of age of one year will increase HR 3.6%, and the change of one tertile will increase HR 37.6%.

Table 6 shows the future cardiovascular event rates having a trend to increase from 1st tertile to 3rd tertile of Lp-PLA_2 (p_{trend}=0.011). The HR compared between 2nd tertile to 1st tertile (as reference) was so high as 1.71 (increased 71% HR of events) but not reaching the level of statistical significance (p=0.075). The 3rd tertile had nearly double of risk, adjusted HR of 1.92 (increased 92% HR of events) when compared to the 1st tertile of Lp-PLA_2 (p=0.029).

Fig. 1 shows the cumulative probability (cum hazard) of major cardiovascular events during 2-year follow-up according to tertiles of Lp-PLA_2 levels in acute phase of ACS in 293 patients. The overall comparison between 3 levels of tertiles showed a significant difference (p=0.043, Log-Rank test). The pairwise comparisons between each pair of tertiles were as follows: 1st tertile vs. 2nd tertile (p=0.05); 1st tertile vs. 3rd tertile (p=0.014); and 2nd tertile vs. 3rd tertile (p=0.628). In Fig. 1, the curves showed an increase in the cumulative probability of a second cardiovascular event during 2-year follow up. This trend was particularly pronounced in the 2nd and 3rd tertiles of Lp-PLA2 activity after 50 days of follow up. However, before 50 days, the cumulative probability is independent of Lp-PLA2 activity.

Table 4. The correlation (OR) between 3 types of ACS and Lp-PLA_2 activity

Tertiles of Lp-PLA_2 activity	Types of ACS (n)		Univariate analysis			Multinomial logistic regression analysis, adjusted by gender and age groups		
	UA	MI (NSTEMI/STEMI)	OR	CI 95%	p	OR	CI 95%	p
Tertile 1st	19	79	1					
Tertile 2nd	19	79	1	0.49-2.03	1			
Tertile 3rd	6	91	3.65	1.39-9.60	0.006*			
Tertile 1st + 2nd	38	158	1					
Tertile 3rd	6	91	3.65	1.48-8.96	0.03*	3.98	1.6-9.8	0.003*

statistically significant difference

Table 5. Association of Lp-PLA_2 activity in acute phase of ACS with major cardiovascular events during 2-year follow-up

Parameters	Units	Univariate analysis		Multivariate analysis	
		HR (95% CI)	p	Adjusted HR (95% CI)	p
Age	year	1.031 (1.013-1.049)	0.001*	1.036 (1.015-1.057)	0.001*
Gender	male	0.613 (0.4-0.94)	0.025*	0.697 (0.403-1.207)	0.198
Smoking	yes	0.84 (0.549-1.284)	0.421	1.222 (0.696-2.145)	0.484
Drinking alcohol	yes	0.959 (0.531-1.729)	0.888	1.316 (0.676-2.561)	0.419
BMI	≥25 kg/m^2	0.911 90.505-1.644)	0.757	1.079 (0.59-1.973)	0.806
Hypertension	yes	0.916 90.6-1.4)	0.686	0.716 (0.446-1.150)	0.167
Diabetes mellitus	yes	1.116 90.639-1.948	0.7	1.189 90.633-2.231)	0.59
Family history early MI	yes	0.94 (0.499-1.772)	0.849	1.214 (0.623-2.367)	0.569
Total cholesterol	mg/dL	1.0 90.995-1.004)	0.882	0.988 (0.973-1.004)	0.139
HDL-cholesterol	mg/dL	1.002 90.981-1.023)	0.874	1.005 (0.98-1.031)	0.679
LDL-cholesterol	mg/dL	1.001 (0.996-1.006)	0.694	1.014 (0.996-1.031)	0.126
Triglycerides	mg/dL	0.998 (0.995-1.002)	0.29	1.0 (0.996-1.005)	0.877
Lp-PLA_2	tertile	1.374 91.056-1.789)	0.018*	1.376 (1.037-1.826)	0.027*

statistically significant difference

Fig. 2 shows the cumulative probability (cum hazard) of major cardiovascular events during 2-year follow-up according to 5 age groups, <= 39, 40-49, 50-59, 60-69, 70-79 and ≥ 80 y, in acute phase of ACS in 293 patients. The overall comparison between 5 age groups showed a significant difference (p=0.021Log-Rank test). The pairwise comparisons between each pair of age groups showed only the difference between age group ≥80 y versus age groups ≤ 59 y ($p < 0.05$).

Table 6. Kaplan-Meier event rates and adjusted HRs (95% CIs) by Lp-PLA$_2$ activity tertiles at 2-year follow-up

Lp-PLA$_2$ activity nmol/min/mL	N	Event rates by Tertile, %	Univariate analysis		Multivariate analysis	
		n (%)	Unadjusted HRs (95% CI)	p	Adjusted HRs (95% CI)**	p
1st tertile 98-181	98	19 (19.4%)	1 reference			
2nd tertile >181-244	98	32 (32.6%)	1.75 (0.992-3.008)	0.053	1.71 (0.947-3.091)	0.075
3rd tertile >244-346	97	35 (36.1%)	1.978 (1.131-3.458)	0.017*	1.92 (1.069-3.447)	0.029*
$p_{Chi\text{-}square}$		0.026				
p_{trend}		0.011				

*statistically significant difference

** adjusted by age, gender, smoking, drinking alcohol, BMI ≥ 25 kg/m2, hypertension, diabetes mellitus, family history of early MI, cholesterol total, HDL-cholesterol, LDL-cholesterol and triglycerides

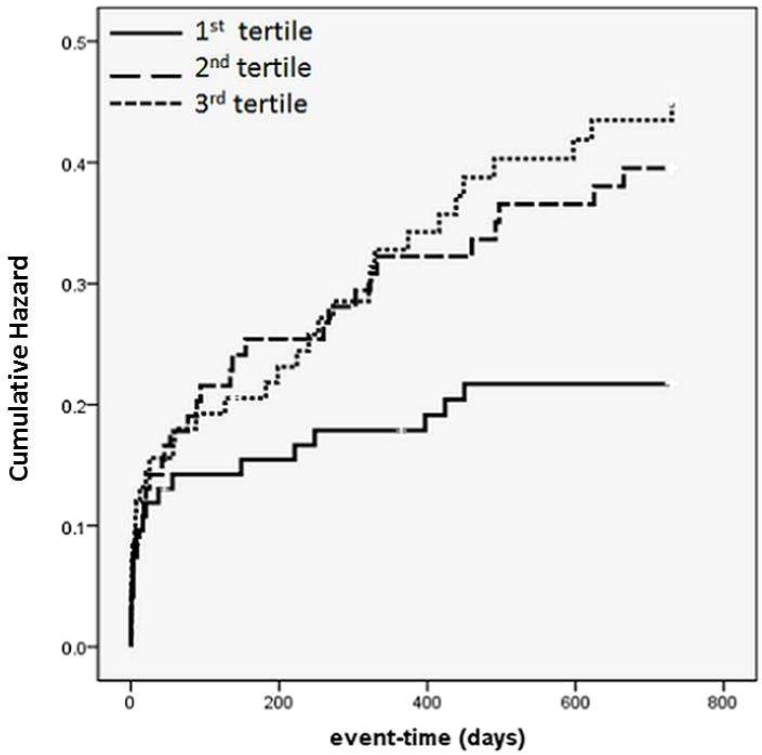

Fig. 1. Cumulative probability of second cardiovascular events during 2-year follow-up according to tertiles of Lp-PLA$_2$ activity in acute phase of ASC in 293 patients (p=0.043 by Log-Rank test)

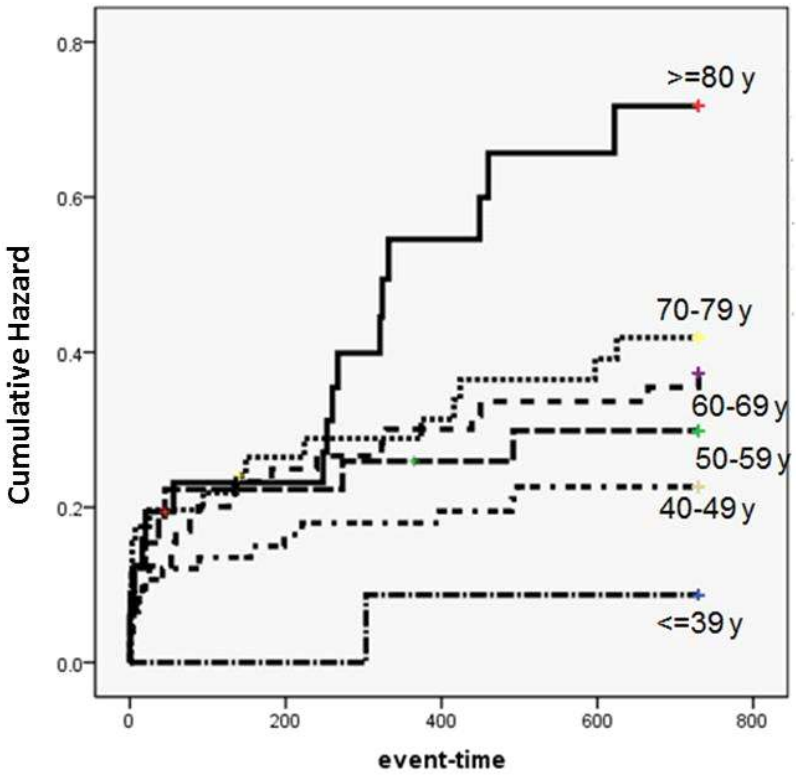

Fig. 2. Cumulative probability of second cardiovascular events during 2-year follow-up according to 5 groups of age in acute phase of ASC in 293 patients (p=0.021 by Log-Rank test)

4. DISCUSSION

Our study investigated Lp-PLA_2 activity in acute phase of ACS in 293 Vietnamese patients and found some relevant results. Lp-PLA_2 activity was a biomarker for group classification in ACS. Patients with Lp-PLA_2 activity in the 3rd tertile had risk of STEMI nearly 4 times (3.98 [1.69-9.8]: OR [95% CI]) compared to the 2 lower tertiles (Table 4). Lp-PLA_2 activity in acute phase was also an independent risk factor for future cardiovascular events in multivariate analysis, after adjustment for a wide range of routine risk factors, over 2-year follow-up after acute phase of ACS. There was about 2-fold increased risk, 1.92 [1.069-3.447] (adjusted HR [95% CI) (Table 6) of future CV events in patients with Lp-PLA_2 activity in the 3rd tertile compared to those in the 1st tertile. Finally, Lp-PLA_2 activity in acute phase of ACS patients was higher than that of healthy volunteer controls.

The Lp-PLA_2 activity in ACS patients was significantly higher than that in healthy controls (p=0.001), but the difference was not so wide, 212 versus 182 nmol/min/mL. The mean difference of 30 nmol/min/ml was lower than the SD, i.e. 58 nmol/min/mL, of data distribution of both groups. The slightly higher Lp-PLA_2 activity in ACS patients compared to healthy persons observed in our study was similar to that previously reported by Oldgren et al. [12] In ACS patients. The same findings were reported in previous case-control studies in stable coronary disease [11,15,16]. In the study by Blankenberg et al. [16] patients with ACS had higher Lp-PLA_2 activity than those with stable coronary disease. The mean levels of Lp-PLA_2 activity were slightly lower at 30 days of follow-up than at baseline, 35.7 vs 40.9 nmol/min/mL (p<0.001) in PROVE IT-TMTI 22 as reported by O'Donoghue et al. [13]. These data suggest that Lp-PLA_2 could not be as other acute-phase reactants (inflammatory markers) such as C-reactive protein (CRP) and interleulin-6 [6]. Lp-PLA_2 was weakly associated with CRP and fibrinogen in the FRISC II study, indicating that it could be a marker of more chronic low-grade inflammation in ACS [6,12,15]. In our study, we found the strong relationship between Lp-PLA_2 activity and the highest pathological type of ASC, i.e., STEMI. Patients with Lp-PLA_2 activity in the 3rd tertile had risk of

STEMI nearly 4 times higher compared to that of the other 2 lower tertiles. Thus, Lp-PLA_2 activity could be considered as a pathological_chronic inflammatory marker for ACS.

Lp-PLA_2 activity in acute phase of ACS was not related to the number of diseased vessels detected at coronary angiography and was considered to be not related with the severity of coronary artery disease in the study by Oldgren et al. [12]. Lp-PLA_2 was not useful for risk stratification when measured early after ACS in PROVE IT-TIMI 22 Trial [13]. The result in our study was different from these 2 studies. PL-PLA_2 activity was significantly different between the 3 types of ACS. Both NSTEMI and STEMI had higher PL-PLA_2 activity than UA. In addition, the multinominal logistic regression showed that patients with Lp-PLA_2 in the 3rd tertile had nearly 4 times STEMI compared to those in the 1st tertile. The reasons for difference could be due to the retrospective analyses in the study by Oldgren et al. [12], and the delay time for sample collection, around 7 days after the onset of ACS event, in the study by O'Donoghue et al. [13]. Another reason may be the exclusion criteria of no use of lipid-lowering substances at least one year before entering into our study. This matter could have been contributed to the higher study population homogeneity in our study compared to previously published studies. The level of Lp-PLA_2 activity in STEMI patients was higher than that in UA group in our study. In contrast, the mean level of Lp-PLA_2 was lowest in patients with STEMI in PROVE IT-TIMI 22 [13]. The Lp-PLA_2 activity has been reported to be higher in patients with ACS than with stable angina [16]. Thus Lp-PLA_2 activity may have clinical value in risk stratification in acute phase of ACS, as well as in stable coronary artery disease.

The other relevant finding of our study was the predictive value of Lp-PLA_2 in acute phase of ACS for future cardiovascular events. No previous studies on Lp-PLA_2 activity in ACS patients had the same conclusion as our study [12,13]. There was some explanation for our findings. Lp-PLA_2 was not considered as an acute-phase reactant, since its levels in acute phase were only slightly higher than those occurring in stable condition (about 16.5% higher). Thus, Lp-PLA_2 in acute phase of ACS may have the same role in prediction of future cardiovascular events compared to that in stable status of ACS. Our study showed that patients with 3rd tertile of Lp-PLA_2 activity had around 2-fold increase of relative hazard of cardiovascular events compared to patients with 1st tertile ($p=0.029$). Patients with Lp-PLA_2 in 2nd tertile had 75% of increase of hazard ratio of CV events compared to patients of 1st tertile, but not yet reaching the level of statistical significance ($p=0.075$). The predictive value of Lp-PLA_2 in stable coronary heart diseases had been reported in many previous studies [8,17,18,19]. Thus, our study suggests that Lp-PLA_2 may have in acute phase of ACS the same predictive value as in stable coronary artery disease.

Besides Lp-PLA_2, age was also an independent risk factor for future CV events ($p=0.021$, Log Rank test). However, the pairwise comparisons between each pair of age groups showed only the difference between age group ≥80 y versus age groups ≤ 59 y ($p < 0.05$).

Finally, Lp-PLA_2 activity in acute phase of ACS had positive association with total cholesterol and LDL-cholesterol, and negative association with triglycerides and HDL-cholesterol ($p< 0.05$). However, the strength of association was weak with $r < 0.3$. This result was the same as reported with Lp-PLA_2 collected in stable phase of ACS or coronary artery diseases [12,16,19] or in acute phase of ACS [12,13]. The weak relationships between Lp-PLA_2 activity with other standard risk factors of cardiovascular diseases suggest that Lp-PLA2 is not a risk factor for ACS but a biomarker for severity classification of ACS.

Limitations to the present study included the use of questionnaire on investigation about history of taken lipid-lowering medication one year before entering into the study and the on-phone interview for cases lost from routine follow-up schedules. It could have a number of patients being used lipid-lowering drugs but not revealed. Furthermore, not all patients or their relatives had good knowledge on diseases as well as having proven documents for confirmation of cardiovascular events. However, researchers had to try all best ways to get the correct data and exclude all bias. Another limitation was the independence of CV events from Lp-PLA2 activity during early time of around 50 days from the first day of acute coronary syndrome (first day of hospitalization). Therefore the practical prognostic values of Lp-PLA_2 activity in ACS patients will be continued to investigate.

5. CONCLUSION

In conclusion, Lp-PLA_2 activity in acute phase of ACS can be a strong risk factor for coronary

heart disease in both risk stratification in acute phase of ACS and in prediction for future cardiovascular events.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tew DG, Southan C, Rice SQ, Lawrence MP, Li H, Boyd HF, Moores K, Gloger IS, Macphee CH. Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. Arterioscler Thromb Vasc Biol. 1996;16:591–599.
2. Asano K, Okamoto S, Fukunaga K, Shiomi T, Mori T, Iwata M, Ikeda Y, Yamaguchi K. Cellular source(s) of platelet-activating factor acetylhydrolase activity in plasma. Biochem Biophys. Res. Commun. 1999; 261:511-514.
3. Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase a(2), platelet-activating factor acetylhydrolase: A potential new risk factor for coronary artery disease. Atherosclerosis. 2000;150:413–419.
4. Epps KC, Wilensky RL. Lp-PLA2 – a novel risk factor for highrisk coronary and carotid artery disease. J. Intern. Med. 2011;269: 94-106.
5. Searle J, Danne O, Müller C, Möckel M. Biomarkers in acute coronary syndrome and percutaneous coronary intervention. Minerva Cardioangiol. 2011;59:203-23.
6. James SK, Oldgren J, Lindback J, Johnston N, Siegbahn A, Wallentin L. An acute inflammatory reaction induced by myocardial damage is superimposed on a chronic inflammation in unstable coronary artery disease. Am. Heart J. 2005;149: 619–626.
7. Tselepis AD, Dentan C, Karabina SA, Chapman MJ, Ninio E. PAF-degrading acetylhydrolase is preferentially associated with dense LDL and VHDL-1 in human plasma. Catalytic characteristics and relation to the monocyte-derived enzyme. Arterioscler. Thromb. Vasc. Biol. 1995;15:1764–1773.
8. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation. 2004;110:1903–1908.
9. Hakkinen T, Luoma JS, Hiltunen MO, Macphee CH, Milliner KJ, Patel L, Rice SQ, Tew DG, Karkola K, Yla-Herttuala S. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. Arterioscler. Thromb. Vasc. Biol. 1999;19:2909–2917.
10. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischaemic stroke: the Rotterdam study. Circulation. 2005;111:570–575.
11. Khuseyinova N, Imhof A, Rothenbacher D, Trischler G, Kuelb S, Scharnagl H, Maerz W, Brenner H, Koenig W. Association between Lp-PLA2 and coronary artery disease: Focus on its relationship with lipoproteins and markers of inflammation and hemostasis. Atherosclerosis. 2005; 182:181–188.
12. Oldgren J, James SK, Siegbahn A, Wallentin L. Lipoprotein – associated phospholipase A2 does not predict mortality or new ischaemic events in acute coronary syndrome patients. European Heart Journal. 2007;28:699–704.
13. O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E. Lipoprotein-associated phospholipase A2 and its association with cardiovascular outcomes in patients with

acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Orator Vastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction) Trial. Circulation. 2006;113: 1745–1752.

14. Lanza GA. Ethnic variations in acute coronary syndromes. Heart. 2004;90:595-597.
15. Oldgren J, Wallentin L, Grip L, Linder R, Norgaard BL, Siegbahn A. Myocardial damage, inflammation and thrombin inhibition in unstable coronary artery disease. Eur. Heart J. 2003;24:86–93.
16. Blankenberg S, Stengel D, Rupprecht HJ, Bickel C, Meyer J, Cambien F, Tiret L, Ninio E. Plasma PAF-acetylhydrolase in patients with coronary artery disease: Results of a cross-sectional analysis. J. Lipid Res. 2003;44:1381–1386.
17. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. Arterioscler. Thromb. Vasc. Biol. 2006;26:1586–1593.
18. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A2, high-sensitivity creactive protein, and risk for incident coronary heart disease in middle-aged men and women in the atherosclerosis risk in communities (ARIC) study. Circulation. 2004;109:837–842.
19. Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N. Engl. J. Med. 2000;343:1148–1155.

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