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Decreased circulating lipoprotein-associated phospholipase A2 levels are associated with coronary plaque regression in patients with acute coronary syndrome

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ABSTRACT

Objective: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a vascular-specific inflammatory enzyme, of which increases are associated with cardiovascular events. However, the relationship between circulating Lp-PLA2 levels and coronary plaque volume has not been clarified in patients with acute coronary syndrome (ACS).
Methods: We studied 40 patients with ACS (age, 61.4 ± 8.0 years; male, 87.5%; statin use, 45.0%) who had undergone successful percutaneous coronary intervention (PCI). Plaque volume (PV) in non-culprit sites of PCI lesions was precisely determined using grayscale intravascular ultrasound (IVUS) at onset and at six months later. We then analyzed associations among PV, lipid profiles and Lp-PLA2 levels.
Results: Circulating Lp-PLA2 levels and PV significantly decreased between baseline and six months of follow-up (458.6 ± 166.7 IU/L vs. 378.4 ± 158.5 IU/L, $p < 0.001$ and 82.2 ± 34.8 mm^3 vs. 77.3 ± 33.1 mm^3, $p < 0.001$, respectively). The % change in PV positively and significantly correlated with % change in LDL-C and in the LDL-C/HDL-C ratio ($r = 0.444$, $p = 0.004$ and $r = 0.462$, $p = 0.003$, respectively). Furthermore, % changes in Lp-PLA2 and in PV correlated even more closely ($r = 0.496$, $p = 0.001$). The absolute change in PV also significantly correlated with the change in Lp-PLA2 levels ($r = 0.404$, $p = 0.009$).
Conclusions: Circulating Lp-PLA2 levels are associated with changes in coronary plaque determined by IVUS in patients with ACS.

1. Introduction

Many studies with surrogate endpoints have shown that intensive lipid-lowering therapy improves the progression of atherosclerosis. An epidemiological relationship between lipid values, most notably those of LDL-C, and cardiovascular events has been proven in treatment trials showing that reducing LDL-C translates into improved cardiovascular outcomes [1,2]. Lipoprotein-associated phospholipase A2 (Lp-PLA2, also known as platelet-activating factor acetyl hydrolase) is an enzyme that belongs to the A2 phospholipase superfamily and it is produced by inflammatory cells that are involved in lipoprotein modification within atherosclerotic plaque [3]. This enzyme has also recently become regarded not only as a novel risk marker that appears specific to vascular inflammation but also as a complementary therapeutic target of LDL-C reduction in patients with advanced atherosclerosis [3–5].

Serial clinical intravascular ultrasound (IVUS) studies have demonstrated that reducing LDL-C levels and the LDL-C/HDL-C ratio using pharmacological intervention including statins retards the progression of atherosclerotic disease and might even achieve coronary plaque regression if very low LDL-C levels can be achieved [6–10]. We also identified a benefit of atorvastatin on plaque regression over a period of six months in patients with acute coronary syndrome (ACS) [11]. These results indicate a close linear relationship between the degree of LDL-C lowering and changes in plaque volume (PV). However, the association between Lp-PLA2 levels and coronary plaque changes during the clinical course is not understood. We therefore investigated the relationship between circulating Lp-PLA2 levels and PV in patients with ACS using serial IVUS measurements.

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2. Methods

2.1. Study design

The present observational longitudinal study estimates associations among Lp-PLA2 levels, coronary plaque and other established biomarkers, and analyzes information that was prospectively gathered during the Extended-ESTABLISH trial. The Extended-ESTABLISH trial (extended version of the ESTABLISH trial) has been described in detail [11,12]. In brief, the Extended-ESTABLISH trial evaluated associations among clinical prognosis, coronary plaque change and early intensive statin therapy in 180 patients with ACS who underwent PCI under IVUS guidance. Patients were randomized within 48 h of ACS onset to receive either intensive lipid-lowering therapy (atorvastatin 20 mg p.o. daily, $n = 90$) after PCI or standard care (lipid-lowering diet, $n = 90$).

Patients were included in the present study if they fulfilled the following criteria: coronary plaque of a non-PCI site in a culprit vessel precisely evaluated by grayscale IVUS at ACS onset and six months later and adequate serum volume in conservatively frozen samples for various measurements. Acute coronary syndrome was defined as high-risk unstable angina, non-ST-elevated myocardial infarction (MI), or ST-elevated MI. An increase ($\geq$2-fold) in serum creatine phosphokinase and troponin T positivity indicated a diagnosis of MI. Both PCI and post-interventional management proceeded in the standard manner. We consequently enrolled 40 consecutive patients with ACS who were admitted to hospital between April 2003 and March 2005 during the entry period of the Extended-ESTABLISH trial. Our institutional review board approved the study and all patients provided written informed consent to participate. This manuscript report followed the STROBE guidelines [13].

2.2. IVUS examination and analysis

All IVUS images were acquired as described using a 40-MHz, 2.9 F system (Boston Scientific) at baseline and at follow-up [11]. After the intracoronary administration of nitroglycerin (0.2 mg), an ultrasound catheter was positioned $\geq$10 mm distal to the PCI site. The catheter was automatically retracted at 0.5 mm/s and IVUS measurements were recorded on super VHS videotape and quantified offline. Plaque volume was assessed by volumetric analysis using a Netra 3D IVUS system (ScImage, Los Altos, CA, USA). Baseline and follow-up IVUS images were simultaneously reviewed on a display and target segments were selected. One target segment was determined at a non-PCI site that was >5 mm proximal or distal to the PCI site with a reproducible index side branch. Segments with obvious calcification or tortuosity were avoided. An independent experienced IVUS investigator who was blinded to the patient groups and angiographic results quantified lesion length, vessel and lumen volumes in the standard manner and PV as vessel volume minus lumen volume. The % change in PV was defined as a change in PV (follow-up minus baseline plaque volume) divided by baseline PV.

2.3. Biomarker analysis

Serum lipid levels and inflammatory markers were measured in conservative frozen samples at baseline and at follow-up. Concentrations of Lp-PLA2 in the samples were determined spectrophotometrically as described [14]; platelet-activating factor acetylhydrolase activity was assayed using the substrate 1-myristoyl-2-(*p*-nitrophenylsuccinyl)phosphatidylcholine. The product, *p*-nitrophenyl succinate, was spectrophotometrically determined at 410 nm. Standard assays proceeded using a JCA-BM8040 automatic analyzer (JEOL, Tokyo, Japan) with the following parameters: method, Rate-A; measuring points, 40–56; sample volume, 60 μL; R1 volume, 60 μL; R2 volume, 20 μL; wavelength (main/sub), 410/505 nm; K-Factor, 12,890. Five-fold-diluted samples (60 μL) were mixed with 60 μL of Reagent 1 (200 mmol/L NaCl, 15 mmol/L EDTA, 9.6 mmol/L sodium 1-nonanesulfonate, 7 mmol/L CHAPS, and 100 mmol/L HEPES, pH 7.6) and incubated at 37 °C for 5 min. The reaction was then started by adding 20 μL of Reagent 2 (18 mmol/L citric acid monohydrate, 8.64 mmol/L sodium 1-nonanesulfonate, 10% ethanol, and 3.2 mmol/L 1-myristoyl-2-(4-nitrophenylsuccinyl)phosphatidylcholine, pH 4.5). Absorption was measured at 2 and 5 min after adding the substrate (Reagent 2). Activities were calculated using the difference between the absorbance of the above measurement points and the extinction coefficient ($\varepsilon = 12.3 \times 10^3$ L/mol/cm at pH 7.6) of 4-nitrophenol. This automated assay can measure the activities of thousands of samples within a few hours with excellent precision (coefficient of variation 0.5%, $n = 30$) and a good correlation ($r = 0.979$, $n = 100$) compared with conventional radioisotopic assays. The assays proceeded as standard laboratory tests; all samples were processed individually and randomly in a blinded fashion.

2.4. Statistical analysis

All data were statistically analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and JMP version 9.0 (SAS Institute Inc., Cary, NC, USA). We confirmed data normality using histograms or box plots and the results are expressed as means ± SD or as medians and interquartile ranges. Categorical data are presented as numbers and ratios (%). Absolute changes in biomarker levels were assessed during follow-up using a *t* test or the Wilcoxon signed-rank test. Correlations were searched using Spearman's rank correlation. Based on the assumption that the correlation coefficient between the % change in PV and in Lp-PLA2 was 0.45, 36 samples were sufficient to achieve 80% power to detect correlation coefficients with a two-sided significance level of 0.05. Effects and interactions among different variables on changes in plaque for six months were examined by multivariable analysis. The following variables were initially incorporated into the univariable model: age, sex, diabetes, hypertension and atorvastatin for six months, and % changes in LDL-C, hs-CRP and Lp-PLA2. Statistically significant variables in the multivariable regression analysis selected using the forward stepwise procedure were subsequently included in a new model. A two-tailed p value of <0.05 was considered statistically significant.

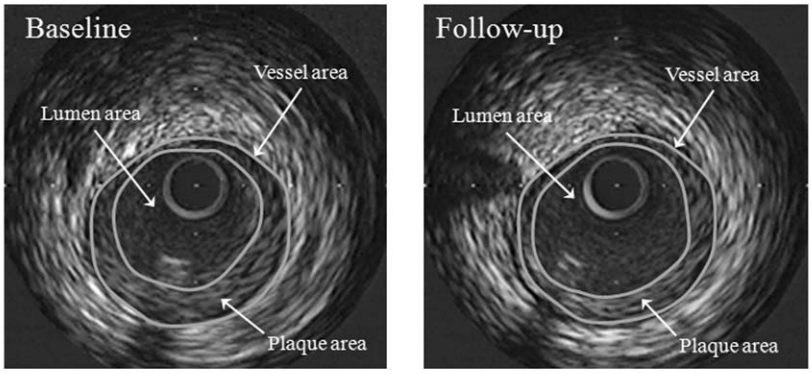
3. Results

3.1. Baseline clinical characteristics and IVUS measurements

Table 1 shows the baseline characteristics of the patients. The mean age was 61.4 ± 8.0 years, 35 were men and 62.5% of the patients had ST elevation MI. The left anterior descending artery was the culprit coronary artery in 42.5% of patients. The ACS in all patients was treated with bare metal stents. Eighteen patients were randomized to receive atorvastatin therapy after PCI for six months (atorvastatin group) and 22 were randomized to a control group (non-statin) in the Extended-ESTABLISH trial. Thus, 45.0% of the patients received statin treatment in the present study. The analyzed coronary plaques were all proximal to the PCI sites. Plaque volume significantly decreased between baseline and at 6-month follow-up (82.2 ± 34.8 mm^3 vs.77.3 ± 33.1 mm^3, $p < 0.001$; Table 2) (Fig. 1).

3.2. Serum Lp-PLA2 levels and lipid profiles in patients with ACS

Table 2 shows data regarding various biomarkers. Levels of Lp-PLA2 and LDL-C as well as the LDL-C/HDL-C ratios significantly decreased between baseline and six months



	Baseline	Follow up
Plaque volume (mm^3)	100.1	89.2
Lp-LPA2 (IU/L)	502	237
LDL-C (mg/dL)	118.1	53.2
HDL-C (mg/dL)	48.1	47.8
LDL-C / HDL-C ratio	2.46	1.11

Fig. 1. Example of baseline and 6-month follow-up IVUS images and biomarkers. Eccentric plaque is observed at non-PCI proximal site in left anterior descending artery. Lumen is significantly enlarged whereas plaque and circulating Lp-PLA2 levels are reduced in patients treated with statins for six months.

later (458.6 ± 166.7 IU/L vs. 378.4 ± 158.5 IU/L, $p < 0.001$; 102.6 ± 25.2 mg/dL vs. 86.4 ± 31.0 mg/dL, $p = 0.003$; 2.4 ± 0.8 vs. 2.0 ± 0.8, $p < 0.001$, respectively), whereas the HDL-C levels did not significantly change (44.9 ± 10.9 mg/dL vs. 45.5 ± 8.9 mg/dL, $p = 0.681$). Although the Lp-PLA2 levels at baseline were similar in patients who were treated or not with atorvastatin (463.1 ± 180.1 IU/L vs. 453.1 ± 153.6 IU/L, $p = 0.913$), the Lp-PLA2 levels were significantly lower at follow-up in patients who were treated with atorvastatin than in those who were not (319.8 ± 132.6 IU/L vs. 426.3 ± 164.5 IU/L, $p = 0.038$).

We also analyzed correlations between Lp-PLA2 levels and other markers and parameters. Levels of Lp-PLA2 positively and significantly correlated with LDL-C ($r = 0.344$, $p = 0.029$ and $r = 0.563$, $p < 0.001$, respectively) and the LDL-C/HDL-C ratio ($r = 0.531$, $p < 0.001$ and $r = 0.597$; $p < 0.001$, respectively) at both baseline and at follow-up. However, levels of Lp-PLA2 did not significantly correlate with those of either HDL-C or hs-CRP (all $p > 0.05$).

3.3. Correlation between biomarkers and coronary plaque volume

We assessed correlations between % changes in PV and in biomarkers at six months after ACS to determine which factors are associated with changes in coronary plaque volume. We found that % change in PV positively and significantly correlated with % change in LDL-C and in LDL-C/HDL-C ($r = 0.444$, $p = 0.004$ and $r = 0.462$, $p = 0.003$, respectively). Furthermore, % change in Lp-PLA2 correlated more closely with % change in PV ($r = 0.496$, $p = 0.001$). However, % changes in PV and in HDL-C did not significantly correlate ($r = 0.101$, $p = 0.542$; Fig. 2A–D).

Table 1
Patients' characteristics.

Age (year)	61.4 ± 8.0
Men/women	35/5
Body mass index (kg/m^2)	24.1 ± 3.2
Systolic blood pressure (mmHg)	137.2 ± 24.9
Diastolic blood pressure (mmHg)	77.2 ± 15.2
History	
Diabetes mellitus, *n* (%)	16 (40.0)
Hypertension, *n* (%)	23 (57.5)
Current smoker, *n* (%)	18 (45.0)
Medication	
Statins, *n* (%)	18 (45.0)
Aspirin, *n* (%)	40 (100.0)
Beta blockers, *n* (%)	17 (42.5)
ACEI/ARB, *n* (%)	31 (77.5)
Type of ACS	
AMI, *n* (%)	25 (62.5)
UAP, *n* (%)	15 (37.5)
Culprit coronary artery	
Left anterior descending, *n* (%)	17 (42.5)
Left circumflex, *n* (%)	5 (12.5)
Right coronary artery, *n* (%)	18 (45.0)
Laboratory	
Creatinine (mg/dL)	0.74 (0.56–0.90)
Troponin, T (pg/mL)	1471 (427–6725)

ACS, acute coronary syndrome; AMI, acute myocardial infarction; UAP, unstable angina; $p < 0.05$ was considered significant.

Table 2
Laboratory and IVUS findings.

	Baseline	Six months later	*p* Value
IVUS measurements			
Total length (mm)	85.8 ± 24.7	85.7 ± 24.6	0.982
Vessel volume (mm^3)	177.8 ± 66.1	169.3 ± 59.9	0.001
Lumen volume (mm^3)	95.5 ± 46.0	92.0 ± 45.1	0.076
Plaque volume (mm^3)	82.2 ± 34.8	77.3 ± 33.1	<0.001
Percent change in plaque volume		−4.9 ± 13.7	
Laboratory findings			
Lp-PLA2 (IU/L)	458.6 ± 166.7	378.4 ± 158.5	<0.001
% change in Lp-PLA2		−15.6 ± 20.6	
LDL-C (mg/dL)	102.6 ± 25.2	86.4 ± 31.0	0.003
% change in LDL-C		−13.7 ± 30.9	
HDL-C (mg/dL)	44.9 ± 10.9	45.5 ± 8.9	0.681
% change in HDL-C		3.4 ± 17.2	
LDL-C/HDL-C	2.4 ± 0.8	2.0 ± 0.8	<0.001
Triglyceride (mg/dL)	72.4 ± 41.3	128.9 ± 59.9	<0.001
Hs-CRP (mg/dL)	0.60 ± 1.96	0.15 ± 0.25	0.161

IVUS, intravascular ultrasound; Lp-PLA2, lipoprotein-associated phospholipase A2; $p < 0.05$ was considered significant.

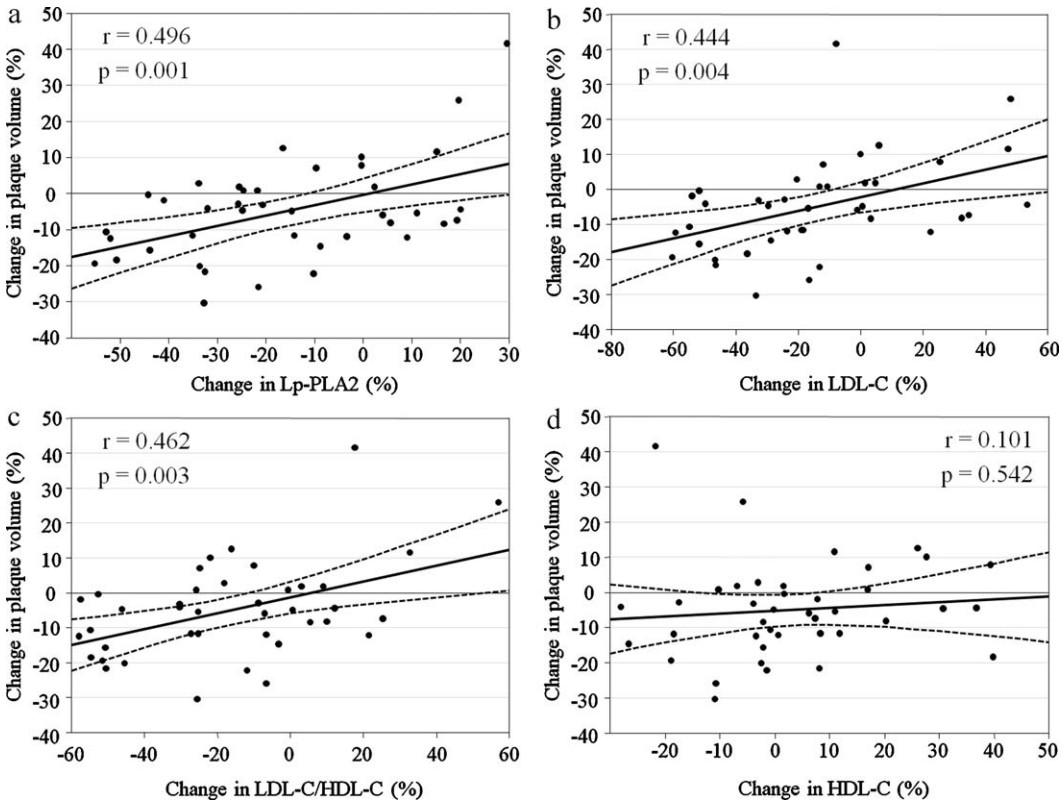


Fig. 2. Correlation between % change in plaque volume (PV) and in levels of various biomarkers in patients with ACS. Correlations were significant between % change in PV and levels of Lp-PLA2 (A) and LDL-C (B) and % change in LDL-C/HDL-C ratio (C), but not between % change in PV and HDL-C levels (D).

We also evaluated correlations between absolute changes in PV and in each biomarker. The change in PV positively and significantly correlated with changes in levels of LP-PLA2 and LDL-C as well as the LDL-C/HDL-C ratio ($r = 0.404$, $p = 0.009$; $r = 0.428$, $p = 0.006$ and $r = 0.366$, $p = 0.020$, respectively). Changes in PV and in HDL-C did not significantly correlate ($r = 0.116$, $p = 0.474$).

3.4. Regression analysis of % change in plaque volume

Regression analysis revealed that sex and atorvastatin, as well as % changes in LDL-C, and Lp-PLA2 were significantly associated (all $p < 0.05$). Stepwise multivariable regression analysis demonstrated that the independent variables associated with % change in PV over a period of six months in patients with ACS were % change in Lp-PLA2 ($p = 0.011$), atorvastatin for six months ($p = 0.079$) and sex ($p = 0.014$; Table 3).

4. Discussion

The present study demonstrated a significant association between serial changes in the Lp-PLA2 levels and changes in coronary plaque in patients with ACS. The relationship between baseline and on-trial Lp-PLA2 levels that resulted in changes in plaque was closer than the relationships between LDL-C levels and the LDL-C/HDL-C ratio that are presently considered significant correlative factors associated with plaque regression or progression. In addition, our multivariable analysis showed that the independent risk factor for coronary plaque change was % change in Lp-PLA2 levels even when statins were included as a variable.

We demonstrated significant correlations between IVUS measurements of plaque volume and laboratory parameters including LCL-C, LDL-C/HDL-C and Lp-PLA2 levels after ACS over six months. The % change in Lp-PLA2 levels was more closely correlated with % change in PV in this study. Based on these findings, we believe that Lp-PLA2 levels could serve as a novel surrogate marker for plaque regression in patients with ACS. The calcium-independent phospholipase Lp-PLA2 has specificity for oxidatively modified fatty acids located at the sn-2 position of oxidized phospholipids [15,16]. It is secreted by inflammatory cells and is primarily attached to circulating LDL and to a lesser degree with HDL and lipoprotein (a) [17]. The activity of Lp-PLA2 is upregulated in atherosclerotic lesions, particularly in complex plaque [18]. In addition, Lp-PLA2 plays an active role in LDL oxidation and it is significantly upregulated in atherosclerotic plaques [19]. The oxidative process transforms phosphatidylcholine (PC) to oxidative modified PC. This molecule acts as a substrate for Lp-PLA2. Thus, the interaction between oxidative modified PC and Lp-PLA2 generates oxidized fatty acids and lysophosphatidylcholine (Lyso-PC) [20]. Lyso-PC and oxidized fatty acids exert many proinflammatory actions that lead to atherosclerotic plaque formation. Molecules of Lp-PLA2 are expressed in and around the necrotic core of advanced human atheroma [21]. Several histopathological studies have also found Lp-PLA2 within plaques that are either prone to rupture or that already have fissured fibrous caps and have developed localized thrombus. The local intensity of Lp-PLA2 staining is apparently related to plaque vulnerability in a graded, dose-dependent fashion [17]. Therefore, we consider that to understand the association between ACS complicated with unstable plaque and changes in plaque volume and Lp-PLA2 levels is valuable for clinical management. On the other hand, consistent with the ATP III clinical guidelines for the use of inflammatory markers, Lp-PLA2 is recommended as a diagnostic test for vascular inflammation to better identify patients at high or very high risk who will benefit from intensified lipid-lowering therapy [22]. Traditional risk factors such as lipid measurement and cardiac imaging do not directly assess whether or not plaques are rupture-prone, or have a thin, fibrous

Table 3
Results of regression analysis of % change in plaque volume.

Variable	Univariable				Multivariable			
	β Coefficient	SE	95%CI	*p*	β Coefficient	SE	95%CI	*p*
Age	0.005	0.003	−0.001 to 0.010	0.105	NS			
Sex	0.066	0.031	0.002–0.129	0.043	0.067	0.026	0.014–0.120	0.014
Diabetes	−0.013	0.022	−0.057 to 0.032	0.566	NS			
Hypertension	0.008	0.021	−0.036 to 0.053	0.710	NS			
Atorvastatin for six months	0.062	0.019	0.022–0.102	0.003	0.035	0.019	−0.004 to 0.075	0.079
% Change in LDL-C	0.197	0.064	0.067–0.327	0.004	NS			
% Change in hs-CRP	−0.001	0.003	−0.007 to 0.005	0.769	NS			
% Change in Lp-PLA2	0.290	0.083	0.122–0.458	0.001	0.227	0.085	0.054–0.400	0.011

SE, standard error; CI, confidence interval; Lp-PLA2, lipoprotein-associated phospholipase A2; NS, not selected; $p < 0.05$ was considered significant.

cap. In contrast, higher levels of Lp-PLA2 at least indicate plaque inflammation and endothelial dysfunction, and thus preventive treatments should be intensified [22]. Thus, we consider that Lp-PLA2 levels could be a more useful marker of plaque instability than simple values of lipids including LDL-C and HDL-C in clinical practice.

Some clinical epidemiological and case control studies have shown that elevated Lp-PLA2 levels predict future cardiovascular events [23,24]. Furthermore, specific Lp-PLA2 inhibitors (such as darapladib) have recently been developed because Lp-PLA2 inhibition is thought to reduce phospholipid oxidation and the expression of adhesion molecules that promote atherogenesis. Darapladib reduces the lysophosphatidylcholine content and the expression of 24 genes associated with macrophage and T-lymphocyte function, along with a considerable decrease in plaque and necrotic core areas in the porcine coronary model [25]. Furthermore, Serruys et al. recently reported the findings of Integrated Biomarkers and Imaging Study-2 (IBIS-2), which was an international, multicenter, randomized control investigation of the effects of darapladib on coronary plaque deformability and composition using IVUS-based radiofrequency analysis. The results showed that the necrotic core continues to expand in patients with coronary artery disease who are treated with optimal standard-of-care treatment whereas darapladib halts increases in necrotic core volume [26]. With respect to these results, we believe that circulating Lp-PLA2 levels reflect the systemic instability of atherosclerosis in patients with coronary artery disease and that Lp-PLA2 inhibition might offer a novel therapeutic approach. Furthermore, our finding of coronary plaque regression after ACS indicates a decrease in the necrotic core. Hence, we consider that decreasing the circulating Lp-PLA2 levels helps to attenuate PV and stabilize plaques in patients after ACS.

Several limitations are associated with the present study. Firstly, the sample size of this observational study was small and the advantages conferred by this study design will be difficult to replicate. Considerable positive evidence supporting the value of statins for treating coronary artery disease has recently been published and finding untreated patients for future comparisons with statin-treated patients who have ACS will probably become challenging. We feel that this factor outweighs the issue with the small study size. Secondly, the study population comprised only Japanese patients with ACS. Thus, our results might not be applicable to other circumstances. We therefore believe that further study is needed to clarify the external validity and clinical significance of circulating Lp-PLA2 levels.

In conclusion, the present study discovered an important relationship between circulating Lp-PLA2 levels and changes in coronary plaque determined by serial IVUS in patients with ACS. That a decrease in Lp-PLA2 in patients with ACS might play a significant role in promoting plaque regression provides further impetus for the concept that controlling Lp-PLA2 levels is atheroprotective.

Conflict of interest

No authors report any conflict of interest.

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