Lipoprotein-associated phospholipase A2 does not predict mortality or new ischaemic events in acute coronary syndrome patients

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Aims Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been suggested as an independent predictor of cardiovascular events in epidemiological studies. We sought to evaluate Lp-PLA2 as a risk factor for future cardiovascular events in patients with acute coronary syndromes (ACS) and to elucidate the relationship between Lp-PLA2 and other known risk markers in ACS patients and healthy control subjects.

Methods and results Blood samples were obtained at randomization in a random subset of ACS patients in the FRISC II (n = 1362) and GUSTO IV (n = 904) studies and in 435 apparently healthy controls of similar age and gender. Median Lp-PLA2 (mass) levels were 305 ng/mL (FRISC II), 373 ng/mL (GUSTO IV), and 254 ng/mL (healthy controls). Time delay from symptom onset did not influence Lp-PLA2 levels. In the FRISC II patients and healthy controls, Lp-PLA2 was significantly correlated with cholesterol (r = 0.3), low-density lipoprotein (r = 0.4 and r = 0.3, respectively), and C-reactive protein (r = 0.08 and r = 0.1, respectively), all P < 0.01. Lp-PLA2 was not correlated with age, interleukin-6, troponin T, or NT-proBNP in any of the three cohorts. There was no difference in the composite of death and myocardial infarction at 30 days (GUSTO IV) or 180 days (FRISC II) in relation to low, middle, and top tertiles of Lp-PLA2 at randomization. In FRISC II, the 1 year mortality was 4.2, 4.2, and 4.8% in the low, middle, and top Lp-PLA2 tertiles, respectively. In GUSTO IV, 1 year mortality was 7.0, 8.3, and 9.6% in the low, middle, and top Lp-PLA2 tertiles, respectively, P = 0.5.

Conclusion ACS patients had higher Lp-PLA2 levels than healthy controls. Lp-PLA2 was significantly correlated to lipid levels but only weakly correlated or unrelated to other well-established risk markers in ACS. The risk of future cardiovascular events or mortality was not related to Lp-PLA2 levels in ACS patients. The biological role of Lp-PLA2 and its role as a risk marker in ACS patients still remain unclear.

Introduction

Inflammation plays a major role in the development and progression of atherosclerosis.1 The atherosclerotic plaque contains large amounts of inflammatory cells, such as macrophages2 and T lymphocytes,3 which may destabilize the plaque, and inflammation may thus be involved in the initiation of acute coronary syndromes (ACS).4 Inflammation in ACS is evident by elevated levels of cytokines5 and acute phase proteins,6,7 which have been related to future increased mortality.8,9 The elevation of inflammatory markers in ACS may originate from the ruptured atherosclerotic plaque, reflect a widespread inflammation in the coronary tree,10 and represent an acute-phase reaction owing to myocardial cell necrosis.11

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a potential novel inflammatory risk factor for coronary artery disease and has been suggested to provide information related to and additional to that obtained from traditional lipid analyses12 and complementary to C-reactive protein.13 This enzyme is secreted by monocytes, macrophages, T-lymphocytes, and mast cells.13 In plasma, ~80% of Lp-PLA2 circulates bound to low-density lipoproteins (LDL) and 15–20% is bound to high-density lipoproteins. Increased Lp-PLA2 mRNA has been found in macrophages from human and rabbit arteriosclerotic lesions.14 A growing number of epidemiological studies have suggested Lp-PLA2 as an independent predictor of cardiovascular events13,15,16 and higher levels of Lp-PLA2 have also been associated with stable coronary artery disease in case–control studies.12,17,18 These observations have provided the rationale to suggest causal links between Lp-PLA2 and plaque vulnerability in ACS patients, although sparse data are available in this population.19

We sought to evaluate Lp-PLA2 as a risk factor for future cardiovascular events in ACS patients and to elucidate the relationship between Lp-PLA2 and other known risk markers in ACS patients and healthy control subjects.
Methods

The FRISC II substudy

The FRISC II trial was a Scandinavian prospective, randomized, multicentre study in which ACS patients were randomized in a factorial design to an early non-invasive or invasive strategy and to long-term treatment with dalteparin or placebo.\textsuperscript{20,21} Patients were eligible for inclusion if they had experienced symptoms of ischaemia within 48 h before the start of dalteparin or unfractionated heparin treatment. Myocardial ischaemia had to be verified by electrocardiogram or by elevated biochemical markers of myocardial necrosis. Randomization was scheduled within 72 h of the start of open-label dalteparin/heparin treatment. Thereafter, all patients were treated with subcutaneous injections of aspirin and dalteparin for 5–7 days, followed by aspirin and twice-daily subcutaneous injections of either dalteparin or placebo for a period of 3 months. Blood samples for analyses of Lp-PLA\textsubscript{2} were obtained at randomization and analysed in 1362 (out of 2457) randomly selected patients.

The GUSTO IV substudy

The GUSTO-IV trial included 7800 patients with non-ST-elevation ACS from 458 centres in 24 countries during 1999 and 2000.\textsuperscript{22} In summary, eligible patients were at least 21 years of age, with chest pain lasting more than 5 min, within 24 h of admission and either a positive cardiac Troponin test or ST-segment depression. The patients were randomly assigned to abciximab bolus and subsequent infusion for 24 or 48 h or corresponding placebo infusion. All patients received aspirin for long-term treatment as well as either unfractionated heparin infusion or subcutaneous dalteparin. Coronary angiography was discouraged during or within 12 h after the completion of study agent infusion. During 30 days of follow-up, mortality and rate of all adjudicated myocardial infarctions were recorded. At 12 months, only all-cause mortality was collected. Levels of Lp-PLA\textsubscript{2} were analysed from serum obtained at randomization in 904 randomly selected patients.

Healthy controls

The study population consisted of 435 men and women included in the SWISCH study\textsuperscript{23} during 2000-2003. The SWISCH study was a case–control study on risk factors for coronary artery diseases in elderly men and women. In this study, subjects were matched for age and sex with ACS patients included at six hospitals in the FRISC II study during 1996-1998. Controls were screened by history and clinical examination. Exclusion criteria were established cardiovascular disease, other chronic disease or acute illness, or cardiovascular medication. All controls had normal levels of haemoglobin, white blood cell count, platelet count, creatinine, and blood glucose. They provided written informed consent, and the Ethics Committees of all participating hospitals approved the study.

Blood sampling and analyses

In the FRISC II and in the GUSTO IV study, blood was obtained at randomization by a direct vein puncture. In the FRISC II, blood was anticoagulated with EDTA, and plasma was prepared within 30 min of collection by centrifugation at 2000 g at room temperature for 20 min. In GUSTO IV, samples were centrifuged and serum was stored in aliquots. In the healthy controls, venous blood samples were obtained at an outpatient visit, anticoagulated with EDTA, and plasma was prepared within 30 min of collection by centrifugation at 2000 g at room temperature for 20 min. All samples were frozen and stored in aliquots at −70 °C and sent to the Department of Clinical Chemistry, Uppsala, Sweden for analyses. Lp-PLA\textsubscript{2} was analysed with the commercially available PLAC test (diaDexus Inc.), an ELISA test with two specific monoclonal antibodies.\textsuperscript{24}

Statistics

Baseline characteristics for the three study populations are presented as means with standard deviations or proportions, as appropriate. Lp-PLA\textsubscript{2} levels are presented as medians with first and third quartiles. All statistical tests were two-sided and P-values of less than 0.05 were considered significant. The levels of Lp-PLA\textsubscript{2} in relation to baseline characteristics were compared with Mann–Whitney U-tests, and correlation of Lp-PLA\textsubscript{2} with other biomarkers were evaluated with Spearman correlations. In the statistical analyses, the three different study populations were analysed separately and presented in tables and figures accordingly, except for the analysis of Lp-PLA\textsubscript{2} levels in relation to time from symptom onset. The two ACS populations were pooled for the analysis of Lp-PLA\textsubscript{2} (natural logarithm) in relation to time from symptom onset to blood sampling (individually calculated for each ACS patient). This was evaluated by linear regression analyses and the interaction of the two ACS populations was evaluated with covariance analysis.

Clinical endpoints, i.e. death and myocardial infarction at 30 days (GUSTO IV) and 180 days (FRISC II) and mortality up to 2 years, were evaluated with log-rank test in relation to tertiles of Lp-PLA\textsubscript{2} at randomization. A Cox regression analysis was also performed with pooled data from the two ACS cohorts evaluating 1-year mortality in relation to the natural logarithm of Lp-PLA\textsubscript{2} at randomization and the interaction between study cohort and Lp-PLA\textsubscript{2}.

Results

The baseline characteristics were similar in the subjects studied in the FRISC II and GUSTO IV trials despite minor differences in inclusion criteria, Table 1. The median time from symptom onset to randomization was 38 h (interquartile range 28–53) in FRISC II and 15 h (interquartile range 9.4–20) in the GUSTO IV study cohort.

Lp-PLA\textsubscript{2} levels were slightly higher in the ACS patients, with median levels of 305 (interquartile range 244–371) ng/mL in the FRISC II study, 373 (297–454) ng/mL in the GUSTO IV study, compared with 254 (217–324) ng/mL in the SWISCH study.

<table>
<thead>
<tr>
<th></th>
<th>FRISC II</th>
<th>GUSTO IV</th>
<th>SWISCH</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>1362</td>
<td>904</td>
<td>435</td>
</tr>
<tr>
<td>Gender (male, n (%))</td>
<td>958 (70)</td>
<td>579 (64.0)</td>
<td>295 (68)</td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>66.7 (9.7)</td>
<td>65.4 (11.3)</td>
<td>63.9 (7.6)</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2} (mean ± SD)</td>
<td>26.7 (3.6)</td>
<td>25.5 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Smoker (or stopped &lt;1 month), n (%)</td>
<td>318 (23.4)</td>
<td>196 (22)</td>
<td>63 (14)</td>
</tr>
<tr>
<td>angina &gt;3 months, n (%)</td>
<td>570 (42)</td>
<td>445 (49)</td>
<td></td>
</tr>
<tr>
<td>Previous PCI, n (%)</td>
<td>55 (4.0)</td>
<td>85 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Previous CABG, n (%)</td>
<td>108 (7.9)</td>
<td>71 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Previous AMI, n (%)</td>
<td>392 (29)</td>
<td>290 (32)</td>
<td></td>
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<tr>
<td>Cardiac failure, n (%)</td>
<td>67 (4.9)</td>
<td>75 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>446 (33)</td>
<td>491 (54)</td>
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<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>172 (13)</td>
<td>282 (31)</td>
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<tr>
<td>Diabetes mellitus, n (%)</td>
<td>191 (14)</td>
<td>174 (19)</td>
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<tr>
<td>Previous stroke, n (%)</td>
<td>75 (5.5)</td>
<td>26 (2.9)</td>
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<tr>
<td>ST-depression on admission ECG, n (%)</td>
<td>649 (48)</td>
<td>757 (84)</td>
<td></td>
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<tr>
<td>Troponin T &gt;0.1 μg/L, n (%)</td>
<td>801 (59)</td>
<td>446 (49)</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; AMI: acute myocardial infarction; ECG: electrocardiogram.
the healthy controls (SWISCH study). No significant differences in Lp-PLA2 levels were found in relation to gender, smoking, previous myocardial infarction, hypertension, or diabetes mellitus, Table 2, except for significantly lower Lp-PLA2 levels in females and higher levels in smokers in the GUSTO IV population. Lp-PLA2 levels were unrelated to the severity of coronary artery disease as judged by coronary angiography in 883 FRISC II patients, Table 2.

There were weak but statistically significant correlations between Lp-PLA2 and C-reactive protein in the FRISC II and SWISCH cohorts, Table 3. The correlations for Lp-PLA2 with cholesterol and LDL were stronger. Oxidized LDL was correlated with Lp-PLA2 in the FRISC II but not in SWISCH cohort. Lp-PLA2 was also weakly, but significantly, correlated with fibrinogen in the FRISC II patients and with creatinine in GUSTO IV patients. There were no significant correlations between Lp-PLA2 and interleukin-6, troponin T, NT-proBNP, or triglycerides in any of the study cohorts.

The time delay from symptom onset to blood sampling (up to 84 h) seemed not to influence the levels of Lp-PLA2 in the total cohort of ACS patients, Figure 1, with a slope coefficient of only $-0.003$, although significant ($P < 0.001$) because of the large number of observations. There was no significant interaction between the two study populations.

In the GUSTO IV study, 8.3, 6.0, and 8.3% had experienced a clinical event, i.e. composite of death or myocardial (re-)infarction, at 30 days in the low ($<324$ ng/mL), middle ($324-430$ ng/mL), and top tertiles ($>430$ ng/mL), respectively, $P = 0.5$. Similarly, in the FRISC II study, 12.1, 12.8, and 13.4% had experienced a clinical event at 6 months in the low ($<265$ ng/L), middle ($265-344$ ng/mL), and top tertiles ($>344$ ng/mL), respectively, $P = 0.8$. One year mortality in relation to tertiles of Lp-PLA2 is presented in Figures 2 and 3, with no significant differences in any of the two study cohorts. Two year follow-up data were available in 1087 of the 1362 patients in the FRISC II cohort, with 5.2, 2.7, and 4.9% mortality in the low, middle, and top tertiles, respectively, $P = 0.2$. There were no differences in clinical outcome in relation to Lp-PLA2 in different subgroups based on treatment assignment, i.e. invasive vs. non-invasive, dalteparin vs. placebo, or abciximab vs. placebo.
In a Cox regression analysis using the natural logarithm of Lp-PLA2 in pooled data from the 2266 patients in both ACS cohorts, the hazard ratio for 1 year mortality was 1.4 (95% C.I. 0.77–2.5), $P = 0.3$. There was no significant interaction observed between study cohort and Lp-PLA2, $P = 0.9$.

**Discussion**

In our two large ACS cohorts, we found slightly higher levels of Lp-PLA2 than in healthy control subjects, similar to findings in previous case–control studies in stable coronary artery disease.\(^{12,17,18}\) There seems to be no acute-phase elevation of Lp-PLA2, as the levels of Lp-PLA2 were unaffected by time from symptom onset up to 84 h, in contrast to other inflammatory markers such as interleukin-6 and C-reactive protein, which have a dynamic time-course with a peak at 36–42 h and 48–54 h after symptom onset, respectively.\(^{11}\) In the FRISC II study, Lp-PLA2 was weakly correlated to C-reactive protein and also to fibrinogen, which is regarded as a marker of a more chronic low-grade inflammation in ACS.\(^{7,11}\) As expected by previously published data, we found significant correlations between Lp-PLA2 and total cholesterol and LDL\(^{25–27}\) but no correlation with age or other established risk markers in ACS, such as interleukin-6, troponin T, NT-proBNP, or triglycerides (in any of the three study cohorts). Neither was Lp-PLA2 related to the severity of coronary artery disease, as judged by early angiograms obtained in a large subset of FRISC II patients.

We found no signs of an association with Lp-PLA2 in the acute phase and risk of mortality or future cardiovascular events in these two large cohorts of ACS patients. Similarly, Lp-PLA2 (neither mass nor activity) in the acute phase was unrelated to the risk of a composite of future cardiovascular events in recently published results from the PROVE IT-TIMI22 trial.\(^{19}\) The authors found Lp-PLA2 activity in the highest quintile significantly associated with lower mortality. In contrast, top quintile of Lp-PLA2 activity measured after 30 days was independently associated with higher rate of a composite of future cardiovascular events.\(^{19}\)

Large-scale epidemiological studies have previously demonstrated that Lp-PLA2 levels (mass or activity) are higher in those in whom future cardiovascular events develop.\(^{13,15,16}\) The results have not been entirely consistent and risk estimates of death, coronary events, or stroke have in some studies been attenuated and not statistically significant after full adjustment for several cardiovascular risk factors including LDL.\(^{25,28}\)

The biological role of this enzyme in humans has not been clarified.\(^{29}\) Lp-PLA2 is expressed in greater concentrations on atherosclerotic lesions.\(^{30}\) The enzyme is able to hydrolyse oxidized fatty acids from oxidized phospholipids in LDL, thereby releasing lysophosphatidylcholine and oxidized non-esterified fatty acids, both of which are potent pro-inflammatory products that may contribute to the formation of atherosclerotic plaques.\(^{29}\) Lp-PLA2, at least in vitro, is suppressed by pro-inflammatory cytokines and endotoxin\(^{31}\) but has not, or only weakly, been correlated with C-reactive protein levels in humans\(^{13,15,26,28}\) as in the present study. Initially, Lp-PLA2 was identified as platelet-activating factor (PAF) acetylhydrolase. PAF is a molecule with pro-inflammatory and pro-thrombotic properties.\(^{32}\) Hence, Lp-PLA2 was viewed to have a protective role in atherosclerosis by deactivating PAF. Deficiency of Lp-PLA2 has mainly been described in the Japanese population and is associated with increased risk of developing atherosclerosis and its clinical manifestations, e.g. myocardial infarction and stroke,\(^{30}\) thereby supporting the concept of a protective effect by Lp-PLA2.

Limitations to the present study include that it contains retrospective analyses on patients recruited in two different clinical trials. Nevertheless, despite minor differences in inclusion and exclusion criteria, baseline characteristics were strikingly similar in the two cohorts from FRISC II
and GUSTO IV, except for the time from symptom onset to randomization. Lp-PLA2 was analysed in a randomly selected subset of FRISC II and GUSTO IV patients. The numbers of patients were based on previous experiences with risk predictors. It thus seems unlikely that analyses of Lp-PLA2 in a larger subset or all of the FRISC II or GUSTO IV patients would have identified Lp-PLA2 as an independent risk predictor in multivariable analyses including other relevant and already established strong risk predictors, such as C-reactive protein, NT-proBNP, or troponins. Another limitation is the use of an ELISA test evaluating only Lp-PLA2 mass, although Lp-PLA2 activity can also be assessed. Only a modest correlation have previously been shown between Lp-PLA2 activity and Lp-PLA2 mass, the latter assessed by the commercially available ELISA method used in the present study.19,26

In summary, the levels of Lp-PLA2 were higher than in healthy controls in these two large cohorts of ACS patients, but there were no signs of an acute-phase response. Lp-PLA2 was related to lipid levels but only weakly related or unrelated to age or other well-established risk markers in ACS, including interleukin-6, C-reactive protein, troponin T, NT-proBNP, and creatinine. In ACS patients, the risk of future cardiovascular events or mortality was not related to Lp-PLA2 levels in the acute phase, similar to previously presented data.19 We conclude that the biological role of Lp-PLA2 and its potential as an independent, clinically useful risk marker in ACS patients still remains to be proved.

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disease, and major adverse events at follow-up. Eur Heart J 2005;26:137–144.


