Lipoprotein-Associated Phospholipase A2 Activity increase the Sensitivity of Framingham Score for detecting the incidence of Acute Myocardial Infarction in sub-population of Indonesian Male Patients

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Abstract

Background: Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an inflammatory biomarker of cardiovascular disease. Framingham score is currently a widely used risk stratification tool for detecting the incidence of acute myocardial infarction (AMI), although it is not very sensitive. Adding Lp-PLA2 to the Framingham score can raise the odds ratio (OR) for AMI, but requires further research. Objectives: In this study, we examine the role of Lp-PLA2 in increasing the sensitivity of the Framingham score for detecting the incidence of AMI. Methods: This study was a case-control study of 97 male patients, 60 of whom had AMI and were treated in the intensive care unit as test subjects, and 37 of whom were non-AMI patients with normal treadmill test used as controls. Results: Lp-PLA2 activity was found to be higher in the AMI group than that in the control group (83.97 ± 27.15 nmol/ml/min vs. 55.04 ± 31.99 nmol/ml/min). Eighteen patients (30%) with AMI were included in the high-risk category according to the Framingham score; this number increased to 36 patients after the addition of Lp-PLA2 activity to the Framingham score. Analysis using a receiver operating characteristic (ROC) showed a cut-off value for Lp-PLA2 activity associated with AMI of approximately 74.21 nmol/ml/min with an accuracy of 67%. The OR for detecting AMI incidence increased after the addition of Lp-PLA2 activity from 1:01 (CI 95%, 0.414–2.48) to 4.67 (CI 95%, 1.88-11.61).

Conclusions: The addition of Lp-PLA2 activity may increase the sensitivity of the Framingham score for detecting the incidence of AMI.

Keywords: Acute myocardial infarction, Framingham score, Lp-PLA2, Odds ratio, Sensitivity.

Introduction

Coronary heart disease (CHD) is a cardiovascular disease that is increasing in prevalence. In Indonesia, the death rate from CHD has been close to 200 per 100,000 deaths, the highest of all the Southeast Asian countries.\(^1\) Data released by the Jakarta Cardiovascular Study of 2008 showed an increase in the prevalence of acute myocardial infarction (AMI) of 5.29% over prior years.\(^2\) AMI is caused by atherosclerosis, which results in narrowing of coronary artery lumens, reduces coronary artery blood flow, and interferes with myocardial oxygen supply.\(^3\) Risk stratification for the incidence of AMI is needed to increase awareness of AMI mortality and morbidity. An often used method is the Framingham score, along with the European Systematic Coronary
Risk Evaluation (SCORE) or the Prospective Cardiovascular Munster (PROCAM).\textsuperscript{4}

There are shortcomings to the Framingham score, including a heavy focus on age range (<45 years for men and <65 years for women), which lowers the sensitivity of the AMI risk estimate.\textsuperscript{4-6} Furthermore, some experts state that certain tests, including imaging, physical examination, or biomarkers for atherosclerosis\textsuperscript{6}, may increase the sensitivity of the Framingham score.\textsuperscript{7,8}

Use of the Lp-PLA2 biomarker has been suggested to improve the sensitivity and accuracy of risk stratification for AMI incidence.\textsuperscript{7,9} It is a calcium-independent phospholipase enzyme with a molecular weight of 50 kD. Lp-PLA2 can hydrolyze fatty acids at the sn-2 platelet activating factor (PAF). This enzyme is mainly expressed by monocytes, macrophages, T lymphocytes, and mast cells. Lp-PLA2 is found abundantly in macrophages within the lipid core and fibrous capsules of vulnerable atherosclerotic plaques; it can be detected in the circulation and thereby can be used as a biomarker for plaque instability.\textsuperscript{10-12}

In plasma, approximately 80% of Lp-PLA2 is bound to low density lipoprotein (LDL), and 20% of high-density lipoprotein (HDL). Lp-PLA2 can hydrolyze oxidized LDL, which produces lysophosphatidylcholine (Lyso PC) and oxidized fatty acids (Ox NEFA), which are pro-inflammatory.\textsuperscript{11-17}

Nevertheless, how Lp-PLA2 acts as a risk biomarker for AMI, whether its activity is increased in patients with AMI compared with patients without AMI, and whether it can be integrated into the Framingham score to increase sensitivity for detection of AMI certainly requires further study.

Methods

This study was a case-control study involving 97 male patients aged 30-74 years who came to the Saiful Anwar Hospital. The study subjects were divided into two groups: a case and a control group. The case group consisted of 60 patients diagnosed with AMI with or without ST-segment elevation who were treated in the intensive care unit. The control group consisted of 37 patients without AMI, who presented without ischemic symptoms, and completed a treadmill test in the outpatient unit that showed no evidence of ischemia. All patients in both groups were assessed using the Framingham risk stratification score and divided into groups from low to moderate-risk to high-risk. Patients with diabetes mellitus, infection, malignancy, and those undergoing statin treatment for more than 12 days could not undergo treadmill testing, and were excluded from this study. Blood samples were taken with a vacutainer, and Lp-PLA2 activity was measured with an enzyme-linked immunosorbent assay (ELISA) using a PAF Acethylhidrolase Assay Kit from Cayman Chemical. The sampling technique used was the consecutive sampling method.

The research data is presented in the form of tabular distribution showing the mean ± SD to identify best the differences between the sample and control groups. Difference tests using Independent t-tests were conducted. The data obtained were analyzed statistically to determine differences in Lp-PLA2 activity between the two groups. The cut-off points for Lp-PLA2 activity and the Framingham scores were analyzed using receiver operating characteristics (ROC), which were then used to calculate the cross-tabulation odds ratio, sensitivity, and specificity of Lp-PLA2 activity and the Framingham score together for the detection of AMI, and for comparison against the use of the Framingham score alone for AMI detection. Statistical calculations were made using SPSS version 17 (SPSS Inc). A p-value of <0.05 was considered to be statistically significant. The study was approved by local committee on medical research ethics, the Saiful Anwar Hospital. Written informed consent was obtained from all study participant.

Results

The sample group consisted of 60 patients with AMI treated in the intensive care unit, and the control group consisted of 37 patients without AMI presenting to the outpatient unit with normal treadmill results. Basic characteristics of both groups of patients are shown in Table 1. There were significant differences between the two
groups in triglyceride levels, the presence of hypertension, and active smoking behavior. The average age in the group of patients without AMI was 55.04 ± 31.99 years, whereas in the AMI group the mean age was 83.97 ± 27.15 years. Active smoking was present in 86.7% of the AMI patients (52 patients), whereas active smoking was present in 51.4% of the non-AMI group (19 patients). The incidence of hypertension was higher in the non-AMI group than that in the AMI group, affecting 86.5% (32 patients) and 38.3% (23 patients) respectively. Age, HDL, LDL, and cholesterol did not differ significantly.

Table 1: Distribution and basic characteristics of research subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non AMI (n=37)</th>
<th>AMI (n=60)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53.62±10.44</td>
<td>55.80±10.90</td>
<td>0.334</td>
</tr>
<tr>
<td>Cholesterol total, mg/dl</td>
<td>191.81±42.28</td>
<td>185.17±45.17</td>
<td>0.473</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>43.92±11.11</td>
<td>40.23±10.2</td>
<td>0.098</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>117.41±24.05</td>
<td>120.75±37.94</td>
<td>0.596</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>179.73±154.64</td>
<td>126.32±76.58</td>
<td>0.018</td>
</tr>
<tr>
<td>Smoker(-),%</td>
<td>18 (48.6)</td>
<td>8 (13.3)</td>
<td>0.000</td>
</tr>
<tr>
<td>Smoker(+),%</td>
<td>19 (51.4)</td>
<td>52 (86.7)</td>
<td></td>
</tr>
<tr>
<td>Hypertension(-),%</td>
<td>5 (13.5)</td>
<td>37 (61.7)</td>
<td>0.000</td>
</tr>
<tr>
<td>Hypertension(+),%</td>
<td>32 (86.5)</td>
<td>23 (38.3)</td>
<td></td>
</tr>
</tbody>
</table>

Lp-PLA2 activity was significantly increased (p = 0.000) in the AMI group (83.97 ± 31.99 nmol/ml/min) compared with that in the non-AMI group (55.04 ± 31.99 nmol/ml/min). The ROC curve for the Framingham score was below the 50% line; thus, it has very weak diagnostic value (AUC = 0.486). AUC values obtained from the ROC curve for Lp-PLA2 activity were 74.5% (95% CI 63.5%–85.6%, p = 0.000). The Framingham score and Lp-PLA2 activity together had a ROC curve above 50%, demonstrating good diagnostic value (AUC = 0.745). AUC values obtained from the ROC curve for the Framingham Score together with Lp-PLA2 activity were 74.5% (95% CI 63.7%–85.4%, p = 0.000) (Figure 1).

The ROC curve for Lp-PLA2 activity was above 50%, thereby showing superior diagnostic value (AUC = 0.745) than the Framingham score alone. AUC values obtained from the ROC curve for Lp-PLA2 activity were 74.5% (95% CI 63.5%–85.6%, p = 0.000). The Framingham score and Lp-PLA2 activity together had a ROC curve above 50%, demonstrating good diagnostic value (AUC = 0.745). AUC values obtained from the ROC curve for the Framingham Score together with Lp-PLA2 activity were 74.5% (95% CI 63.7%–85.4%, p = 0.000) (Figure 1).

Figure 1: ROC curves for the Framingham score, Lp-PLA2 activity, and a combination of the Framingham score with Lp-PLA2 activity with sensitivity and specificity associated with the incidence of AMI. The addition of Lp-PLA2 activity against Framingham score increases AUC associated with the incidence of AMI (AUC 0.7453, 95% CI 0.637–0.854; p = 0.000)
When a cut-off value for Lp-PLA2 of 74.21 nmol/ml/min was used, the Framingham score alone was associated with an AMI incidence of 2.5, whereas the fusion of the Framingham score and Lp-PLA2 activity was 0.656. Furthermore, when the cut-off activity of Lp-PLA2 was divided into 2 groups by the level of Lp-PLA2 activity, there was a group with high Lp-PLA2 activity (74.21 nmol/ml/min, n = 52) and a group with low Lp-PLA2 activity (<74.21 nmol/ml/min, n = 45).

However, Lp-PLA2 activity in 12 patients without AMI was increased (>74.21 nmol/ml/min), and 20 patients with AMI had a Lp-PLA2 activity cut-off of <74.21 nmol/ml/min. However, Lp-PLA2 activity in 12 patients without AMI was increased (>74.21 nmol/ml/min), and 20 patients with AMI had a Lp-PLA2 activity cut-off of <74.21 nmol/ml/min. After determining the cut-off for each group, cross-tabulation demonstrated 29 research subjects classified as high risk, whereas 68 study subjects were classified in the low to moderate risk categories on the basis of the analysis of the ROC cut-off against the Framingham score. The 29 high-risk subjects consisted of 18 patients with AMI and 11 patients without AMI. Of the 68 low to moderate-risk subjects, there were 42 patients with AMI and 26 patients without AMI (Table 2).

### Table 2: Cross-tabulations of risk stratification for the Framingham score based on a cut-off ROC curve

<table>
<thead>
<tr>
<th>Cut-off Framingham score (ROC based analysis)</th>
<th>Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>gh Risk (&gt; 2.5)</td>
<td>AMI</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Non AMI</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>w to moderate(&lt;2.5)</td>
<td>AMI</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Non AMI</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>total</td>
<td>AMI</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Non AMI</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97</td>
</tr>
</tbody>
</table>

Diagnostic test results showed that Lp-PLA2 activity by the ROC cut-off had the greatest sensitivity (66.67%) and specificity (67.57%), followed by the combination of the Framingham Score with Lp-PLA2 activity based on the ROC cut-off with a sensitivity of 60.0% and specificity of 75.68%.

The possibility of subjects who would get high risk for AMI, when a positive diagnostic test resulted in a positive predictive value (PPV), using the Framingham score + Lp-PLA2 activity that resulted in the greatest PPV of 80.0%, was followed by Lp-PLA2 activity based on the cut-off with ROC with PPV of 76.92%.

In addition, it can be seen from Table 3 that the magnitude of the prediction accuracy of each score to guess the possibility of the subject who would be at high risk for AMI, when diagnostic test result was positive with the accuracy, using Lp-PLA2 activity, which resulted in the greatest accuracy of 67.01%, was followed by the Framingham score + Lp-PLA2 activity based on the cut-off by the ROC with an accuracy rate of 65.98%.

### Table 3: Table of diagnostic test results

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Framingham score</td>
<td>30.0</td>
<td>70.27</td>
<td>45.36</td>
<td>1.01</td>
</tr>
<tr>
<td>PLA2 activity</td>
<td>66.67</td>
<td>67.57</td>
<td>67.01</td>
<td>4.17</td>
</tr>
<tr>
<td>Framingham score + PLA2 activity</td>
<td>60.0</td>
<td>75.68</td>
<td>65.98</td>
<td>4.67</td>
</tr>
</tbody>
</table>

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There were 45 total study subjects classified in the high-risk category; 36 patients from the AMI group and 9 patients from the non-AMI group. There were 52 study subjects categorized in the low-risk category: 24 patients with AMI and 28 patients without AMI. 18 patients who were previously categorized in the mild to moderate risk for AMI group by the Framingham score alone were transferred into the high-risk category after adding Lp-PLA2 activity to the Framingham score (Table 4).

<table>
<thead>
<tr>
<th>Risk Stratification</th>
<th>Framingham score (n)</th>
<th>Framingham score+Lp-PLA2 activity (n)</th>
<th>Framingham score (n)</th>
<th>Framingham score+Lp-PLA2 activity (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low to moderate risk</td>
<td>26</td>
<td>28</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td>High risk</td>
<td>11</td>
<td>9</td>
<td>18</td>
<td>36</td>
</tr>
</tbody>
</table>

**Discussion**

Our patients’ demographic information shows that 86.7% (52 patients) of the patients with AMI were active smokers, while this was the case for only 51.4% (19 patients) in the non-AMI group. According to Tselepis et al., smoking induces Lp-PLA2 activity in cardiovascular disease-free adults. ATTICA reported that heavy smoking can trigger a proatherogenic effect, increasing both amount and Lp-PLA2 activity, and lowering the antiatherogenic effect of HDL.18

The incidence of hypertension was higher in the non-AMI group (32 patients, 86.5%) than in the AMI group (23 patients, 38.3%). Some studies indicate that Lp-PLA2 activity is significantly increased in patients with hypertension,19, 20 as well as the non-diabetic population who have hypertension and obesity with hypertriglyceridemia (metabolic syndrome).20

The fact that non-AMI patients with hypertension outnumbered AMI patients with hypertension in our study may have been due to the small number of population characteristics and risk factors that were investigated, as the control patients tended to have hypertension. Age did not differ significantly between the two groups in this study, although this finding differs from previous studies that have examined the existence of a significant correlation between age, lipid profile, alcohol drinking habits,21, 22 and dietary carbohydrates and proteins that affect the activity of Lp-PLA2.22 In several studies, low HDL and high LDL have been significant correlated with increased Lp-PLA2 activity, but this increase may also occur in populations with low or normal high HDL levels enough as reported by Robins et al. in Veterans Affairs HDL Intervention Trial (VA-HIT), who examined cardiovascular events with an increase in the activity of Lp-PLA2 in patients with low HDL levels.23 In our study, HDL and LDL levels did not differ significantly between subjects and controls.

In this study, the activity of Lp-PLA2 in patients with AMI was 83.97±27.15 nmol/ml/min, and 55.04±31.99 nmol/ml/min in patients without AMI. Lp-PLA2 activity in AMI patients was higher.
than in the control patients. These results were similar to the study done by Li Ning et al. of 152 patients with CHD and 142 patients without CHD in China (age <80 years), who were enrolled from February 2007 until March 2008 and followed for the next 6 months. Their study showed that Lp-PLA2 activity was higher in patients with ACS compared with patients without CHD (22.3 ± 1.23 mg/ml vs. 19.74 ± 3.85 mg/ml; p = 0.027).24

Several studies have reported an association between elevated levels of Lp-PLA2 and future cardiovascular events, regardless of the number of potential confounding factors. Using coronary and carotid tissue staining, Kolodgie et al. were able to show that Lp-PLA2 was strongly expressed in the necrotic core and surrounding macrophages from vulnerable and ruptured plaques. These findings suggest a potential role for Lp-PLA2 in encouraging plaque instability.25, 26

Lavi et al. reported an increase in Lp-PLA2 levels in atherosclerotic coronary arteries and the coronary sinus. High levels of Lp-PLA2 were also found in easily ruptured plaques, where seemingly Lp-PLA2 is released into the circulation.19, 25

In the Rotterdam study, Oei et al. studied a population cohort of 7983 subjects aged over 55 years. Lp-PLA2 activity was used as an independent predictor of coronary heart disease and ischemic stroke in the general population.36

Meanwhile, Liu et al. conducted a study of Lp-PLA2 activity on 146 patients with CAD who underwent coronary angiography and intravascular ultrasound (IVUS), which indicated that the activity of Lp-PLA2 in plasma was independently related to the incidence of plaque rupture. However, levels of Lp-PLA2 in plasma were also reported to be increased in stable CHD patients, thus the use of plasma Lp-PLA2 levels requires further study to determine the specific cut-off as a marker of plaque instability.24, 26, 28-33

The present study used a cut-off for Lp-PLA2 activity of 74.21 nmol/ml/min. In 40 patients with AMI and 12 patients without AMI, an increase of Lp-PLA2 activity >74.21 nmol/ml/min was found. Thus, we suspect that the increased activity of Lp-PLA2 above the cut-off causes the potential incidence of AMI.

Maiolino et al., in a prospective cohort study of 712 Caucasian patients, showed a Lp-PLA2 activity cut-off as high as 136.1 nmol/ml/min (AUC 0.707, 95% CI 0.663 to 0.749, p <0.0001). However, until now, there has been no recommendation for the cut-off value of Lp-PLA2 activity for CHD. An increase in activity above the cut-off will lower survival rates for cardiovascular events and ACS.34

The accuracy of our cut-off value to estimate the incidence of AMI is 65.98%, with a sensitivity of 60% and specificity of 75.68%, using the combined Framingham score and models of Lp-PLA2 activity. Lp-PLA2 activity increases the risk of AMI 5-fold when combined with the Framingham score (OR 4.67, 95% CI 1.88-11.61) compared with that of Framingham score alone (OR 1:01; CI 95% 0:41–2:48).

Tsimikas et al. in the Bruneck study showed that the addition of Lp-PLA2 activity levels to the Framingham score resulted in a slight increase in the AUC of the ROC curve for the risk of cardiovascular events (0.737 vs. 0.717, Δ0.020, P = 0:31).

Reclassification improvement for the 10-year risk category <10%, 10-20%, and> 20% was 9.5% (P = 0:11).35 According to Koro et al., the addition of Lp-PLA2
activity to Framingham scores can reclassify as much as 36% factor risk subjects on the basis of data from the trial STABILITY. 36

In our study, 45 subjects were classified as high-risk, including 36 patients from the AMI group and 9 patients from the non-AMI group. In addition, 52 study subjects were categorized in the low-risk category; 24 of these were patients with AMI and 28 patients did not have AMI.

18 patients from the original AMI group classified in the mild-moderate category by their Framingham scores were reclassified as high-risk after adding Lp-PLA2 activity to the Framingham score. It would be interesting to conduct further prospective research to determine if the 36 AMI patients have higher reinfarction rates in the future compared with the 61 patients categorized as having mild to moderate risk.

In the PROVE-IT TIMI study, O'Donoghue showed that Lp-PLA2 activity was not useful for risk stratification when measured at the beginning of the acute coronary syndrome event. After 30 days, Lp-PLA2 activity was significantly decreased by the administration of high-dose statins and was associated with an increased risk of cardiovascular incidents. 37

Discourse on Lp-PLA2 as a specific inflammatory biomarker is very interesting. This enzyme is specific because it is produced in atherosclerotic plaques and is allegedly associated with atherosclerotic plaque rupture events. There is less variability in Lp-PLA2 than there is in Hs CRP, so Lp-PLA2 biomarkers could potentially be employed to determine cardiovascular risk. 38, 39

This study showed that Lp-PLA2 activity was significantly increased in patients with AMI. The cut-off activity of Lp-PLA2 that was correlated with the incidence of AMI was approximately 74.21 nmol/ml/min. Lp-PLA2 activity had a sensitivity, specificity, and OR which were relatively high. Also, it could be integrated into the Framingham score, making it possible to use it either simultaneously with the Framingham score or separately. From our study of Indonesian males who came to Dr. Saiful Anwar Hospital with symptoms of AMI as sample patients, and non-AMI patients as controls, we suspect that Lp-PLA2 activity has a role in increasing the sensitivity and specificity of the Framingham score’s prognostic value.

Patients classified in the high-risk category for AMI using the cut-off activity of Lp-PLA2 and the Framingham score together indicated that the trend for reinfarction was comparable with that of patients with mild to moderate category. Broadly, a prospective study with a larger population is needed to prove the role of Lp-PLA2 activity as a predictor of AMI incidence when integrated with the Framingham score.

Conclusion

The Lp-PLA2 activity was correlated with the incidence of AMI that increasing the sensitivity and specificity of the Framingham score’s prognostic value in Malang, Indonesian males population.

Acknowledgement

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