Lipoprotein-Associated Phospholipase A₂ and Prognosis After Myocardial Infarction in the Community

Yariv Gerber, Joseph P. McConnell, Allan S. Jaffe, Susan A. Weston, Jill M. Killian, Véronique L. Roger

Objective—We evaluated the role of lipoprotein-associated phospholipase A₂ (Lp-PLA₂), an inflammatory biomarker, in defining risk after myocardial infarction (MI).

Methods and Results—Olmsted County, Minn, residents who experienced an MI meeting standardized criteria between 2003 and 2005 (n=271) were prospectively identified and followed. Lp-PLA₂ levels were measured at baseline and evaluated along with traditional risk indicators. Lp-PLA₂ was modestly associated with total and low-density lipoprotein cholesterol, smoking, and age (inversely) but not with MI characteristics or severity, comorbidities, C-reactive protein, or the time from symptom onset to blood sampling. During the first year of follow-up, 42 deaths occurred. The survival estimates (95% confidence intervals [CI]) at 1 year were 92% (86% to 98%), 85% (78% to 93%), and 74% (65% to 84%) in the lowest, middle, and upper Lp-PLA₂ tertiles, respectively (P=0.007). After adjustment for age and sex, the hazard ratios for death in the middle and upper Lp-PLA₂ tertiles were 2.20 (95% CI: 0.88 to 5.54) and 4.93 (95% CI: 2.10 to 11.60), compared with the lowest tertile, respectively (P_trend<0.001). Further adjustment for other risk indicators resulted in even stronger associations. Lp-PLA₂ also contributed to risk discrimination as indicated by the increases in the area under the receiver operating characteristic curves obtained in each of the models examined (all P<0.05).

Conclusions—Among community subjects presenting with MI, increased Lp-PLA₂ levels measured early after MI are strongly and independently associated with mortality and provide incremental value in risk discrimination over traditional predictors. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

Key Words:

Elevations of inflammatory biomarkers are associated with increased cardiovascular disease risk.1,2 However, which biomarkers to use and their incremental value in risk stratification over traditional predictors are uncertain.3–5 Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is an inflammatory biomarker that offers several advantages relative to other markers, including specificity for vascular inflammation, minimal biovariability, and stability in states of myocardial ischemia.6–10 Observational studies carried out in primary prevention settings have shown a relationship between Lp-PLA₂ and cardiovascular risk, although the magnitude of the association has varied.11–16 However, data are sparse concerning the potential impact of Lp-PLA₂ levels in secondary prevention, particularly after myocardial infarction (MI). Two recent studies have suggested an independent association between Lp-PLA₂ measured several weeks after acute coronary syndromes and subsequent risk.17,18 Yet, when measured early after an event, no predictive value was found for Lp-PLA₂ in the PROVE IT–TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22) trial.19 However, the latter finding may have been affected by the randomization of participants to intensive versus moderate statin therapy soon after acute coronary syndromes, which differentially influenced both Lp-PLA₂ values and outcomes. In addition, clinical trial participants typically are different from community-dwelling individuals.19 Accordingly, the present study was undertaken to examine the association between plasma Lp-PLA₂ levels measured early after acute MI and mortality in a well-defined cohort of community patients and to determine the incremental value of Lp-PLA₂, if any, over traditional predictors of risk after MI.

Methods

The study was carried out in Olmsted County, Minn, the demographic characteristics of which are similar to those of white Americans. The Mayo Clinic and Olmsted Medical Center provide medical care for all county residents. These facilities use a unified system that accumulates comprehensive clinical records collected by physicians in a unit record system of high quality. The records are easily retrievable because the Mayo Clinic maintains extensive indices, which, through the Rochester Epidemiology Project, are extended to the records of other care providers to county residents.
resulting in the linkage of all medical records from all sources of care through a centralized system.\textsuperscript{20,21}

### Patient Enrollment

All persons presenting to an Olmsted County facility with cardiac troponin T (cTnT) level $\geq 0.03$ ng/mL (the cut off value chosen for use at the Mayo Clinic, which is the value at which the coefficient of variation for the assay is $\leq 10\%$)\textsuperscript{22} between June 2003 and June 2005 were prospectively identified within 12 hours of the blood draw through the electronic files of the Department of Laboratory Medicine. Nurse coordinators sought written consent from all patients (or the next of kin if the patient could not grant consent) to measure cardiac and inflammatory biomarkers in unused blood samples initially stored for additional clinical need. If not available, an additional sample was drawn, in conjunction with a clinically indicated draw whenever possible. More than 90\% of the MI patients approached consented to participate in the study.\textsuperscript{23}

To determine MI status, the recommendations for Case Definition for Acute Coronary Heart Disease in Epidemiology and Clinical Research Studies\textsuperscript{24} were applied. MI was defined based on cardiac pain, ECG data (using Minnesota coding) and biomarker levels. The Mayo Clinic Institutional Review Board approved all aspects of the study.

### Lp-PLA\textsubscript{2} Measurement

Lp-PLA\textsubscript{2} levels were measured in plasma aliquots taken shortly after symptom onset and stored at $-70\degree C$ until assayed with an ELISA (PLAC test, diaDexus Inc, Calif.).\textsuperscript{7} Samples were incubated in microtiter plate wells with an immobilized monoclonal antibody (2C10) against Lp-PLA\textsubscript{2}. A secondary monoclonal antibody (4B4) labeled with horseradish peroxidase was used to identify the enzyme, and recombinant Lp-PLA\textsubscript{2} was used as the standard reference. The range of detection was 50 to 1000 ng/mL, and the inter-assay coefficients of variation were 7.8\% at 276 ng/mL, 6.1\% at 257 ng/mL, and 13.5\% at 105 ng/mL. The 2C10 monoclonal antibody against Lp-PLA\textsubscript{2} has been shown to have no cross-reactivity with other A\textsubscript{2} phospholipases.\textsuperscript{7} Lp-PLA\textsubscript{2} is stable in samples stored at 4\degree C and收益率 stability for at least 7 days and repeated freeze–thaw cycles (three cycles) do not affect the measured Lp-PLA\textsubscript{2} concentration.\textsuperscript{9} Long-term stability of Lp-PLA\textsubscript{2} has been demonstrated in frozen samples. All of the assays were performed by a single investigator who was blinded to the clinical characteristics and the outcomes of the patients.

### Risk Factor Assessment

The inpatient and outpatient medical record was used to ascertain risk factors at the time of the index MI. Measurements recorded at the index date or at the closest time before the index MI were used.

Smoking was classified into current versus noncurrent smoking. Obesity was defined as body mass index (BMI) $\geq 30$ kg/m\textsuperscript{2}. Clinical definitions were used to assess diabetes,\textsuperscript{25} hypertension,\textsuperscript{26} and dyslipidemia.\textsuperscript{27} Total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were recorded. High-sensitivity C-reactive protein (hs-CRP) was measured in stored serum from the first draw after symptom onset using a latex-enhanced immunoturbidimetric assay (Diasorin Inc, Stillwater, MN). The inter- and intra-assay coefficients of variation were 10\% and 8.8\% for the lower limit and 5\% and 0.4\% for the upper limit, respectively. Comorbidity was assessed by the Charlson index\textsuperscript{28} and analyzed categorically (severe comorbidity for 3 points or more versus no or moderate comorbidity for 2 points or less). Reperfusion therapy or revascularization included thrombolysis, percutaneous coronary intervention, or coronary artery bypass grafting performed during the index hospitalization. Ejection fraction (EF) was estimated echocardiographically within 30 days from the index date and dichotomized into below versus equal or above 50\%. Killip class was assessed within 24 hours of admission and analyzed categorically (class 2 or more versus class 1).

### Mortality Follow-Up

Follow-up was completed by passive surveillance through the community medical records. The comprehensive approach in place under the auspices of the Rochester Epidemiology Project ensures complete ascertainment of deaths as it incorporates several sources of information. First, all death certificates for Olmsted County residents are obtained every year from the County office. Second, the Mayo Clinic registration office monitors the obituaries and notices of death in the local newspapers to update the record. Finally, electronic files of death certificates are obtained from the State of Minnesota Department of Vital and Health Statistics.\textsuperscript{21,29}

### Statistical Analyses

Subjects were divided into 3 equal groups (tertiles) according to Lp-PLA\textsubscript{2} levels. Trends in baseline characteristics across tertiles were assessed using generalized linear models for continuous variables and Mantel–Haenszel $\chi^2$ tests for categorical variables. One-year survival was assessed by the Kaplan–Meier method with right-censoring at the time of last follow-up. Cox proportional hazards regression models were constructed to evaluate the unadjusted and covariate-adjusted hazard ratios (HRs) and 95\% confidence intervals (CI) for death associated with Lp-PLA\textsubscript{2} tertiles, with the first serving as a referent. The proportional hazards assumption was tested using the Schoenfeld residuals and found valid for the first year of follow-up, with evidence of disruption afterward. Accordingly, we restricted our analysis to 1 year post-MI.

No missing values were present in any of the variables, except for hs-CRP (13\%), blood lipids (7\%), and EF (18\%). In all analyses, indicator variables reflecting missingness for EF and hs-CRP were included, as well as imputed values for missing LDL-C. Sensitivity analyses compared the latter approach to the complete case analysis and yielded similar results. The incremental value of Lp-PLA\textsubscript{2} in risk discrimination was examined by the area under the receiver operating characteristic (ROC) curve (AUC), which ranges between 0.5 and 1 and represents the probability that a person who had died by a given follow-up time had a higher risk score than a person who survived to that time. Fitting the AUC to proportional hazards models\textsuperscript{30} was performed through a local SAS macro (E. Bergstrahl et al, 2004). Comparisons of the AUC before and after the addition of Lp-PLA\textsubscript{2} were carried out using the method of Hanley and McNeil\textsuperscript{31} and were 1 tailed. SAS version 8 was used for all statistical analyses.

### Results

Two hundred seventy-one consecutive patients with MI were enrolled (58\% men), among which 95\% were incident (first-ever) cases. The mean age $\pm$ SD of the cohort was 69 $\pm$ 15 years. Blood for Lp-PLA\textsubscript{2} measurements was taken shortly after symptom onset (mean $\pm$ SD, 43 $\pm$ 39 hours). There was no correlation ($r = -0.02, P = 0.78$) between the timing of the draws and Lp-PLA\textsubscript{2} levels. The mean $\pm$ SD Lp-PLA\textsubscript{2} levels were 198 $\pm$ 68 ng/mL in women and 208 $\pm$ 71 ng/mL in men ($P = 0.22$). Participants were divided into tertiles according to Lp-PLA\textsubscript{2} levels. Group characteristics are presented in Table 1. Higher Lp-PLA\textsubscript{2} levels were positively associated with age. No differences were detected with regard to sex, hypertension, diabetes, obesity, peak cTnT, ST-elevation MI, Q waves on ECG, Killip class, EF, hs-CRP, statin use, prior MI, and reperfusion therapy or revascularization. As a continuous variable, Lp-PLA\textsubscript{2} levels were modestly correlated with total cholesterol ($r = 0.21, P = 0.007$) and LDL-C ($r = 0.28, P = 0.001$) but not with peak cTnT ($r = -0.04, P = 0.53$), hs-CRP ($r = -0.02, P = 0.71$), EF ($r = 0.05, P = 0.45$), BMI ($r = 0.04, P = 0.56$), and HDL-C ($r = -0.02, P = 0.81$).
Association Between Lp-PLA2 and Mortality

During the first year of follow-up, 42 deaths had occurred. The survival estimates (95% CI) at 1 year were 84% (79% to 88%) overall and 92% (86% to 98%), 85% (78% to 93%), and 74% (65% to 84%) in the lowest, middle, and upper Lp-PLA2 tertiles, respectively (P = 0.007 for equality of survival distributions). After adjustment for age and sex, the HR (95% CI) for death were 2.20 (0.88 to 5.54) in the middle tertile and 4.93 (2.10 to 11.60) in the upper tertile, compared with the lowest Lp-PLA2 tertile (P = 0.001) (Table 2). Further adjustment for traditional risk factors, LDL-C, Killip class, EF, hs-CRP, and reperfusion or revascularization, resulted in an increase in the association (Table 2 and Figure), whereas the inclusion of postevent statin use, prior statin use, comorbidity, peak cTnT, ST-elevation MI, or any other baseline characteristic did not change the magnitude of the association materially (data not shown).

The age- and sex-adjusted HR for the upper (≥1.08 mg/L) versus lowest (≤0.27 mg/L) tertile of hs-CRP was 2.90 (95% CI: 1.16 to 7.30) and 2.00 (95% CI: 0.79 to 5.05) after further adjustment for comorbidities and Lp-PLA2.

Contribution of Lp-PLA2 to Risk Assessment

Improvement in the predictive accuracy of the models was obtained after the inclusion of Lp-PLA2 (Table 3). For example, the AUC increased from 0.73 to 0.78 in a model that included age and sex (P = 0.03) and from 0.82 to 0.85 in a model that included age, sex, traditional risk factors, EF, Killip class, hs-CRP, and reperfusion or revascularization (P = 0.05).

Discussion

There is a growing body of evidence linking inflammation to cardiovascular disease, leading to a scientific statement from
ical role has been controversial, with initial reports purporting it is responsible for the hydrolysis of oxidized LDL. Its biological function is thought to circulate bound primarily to small, dense LDL and some of them may reflect more directly plaque-related stratification. Thus, other novel inflammatory markers may provide different information and thereby enhance risk stratification.

Lp-PLA2 (also known as platelet-activating factor acetylhydrolase) is an enzyme produced by inflammatory cells. It is thought to circulate bound primarily to small, dense LDL and is responsible for the hydrolysis of oxidized LDL. Its biological role has been controversial, with initial reports purporting atheroprotective effects thought to be a consequence of degrading platelet-activating factor and removing polar phospholipids from modified LDL. Recent studies, however, have focused on the proinflammatory role mediated by products of the Lp-PLA2 reaction with lipids such as lysophosphatidylcholine and oxidized free fatty acids. These bioactive lipid mediators, which are generated in lesion-prone vasculature and, to a lesser extent, in the circulation, are known to elicit several potentially adverse proinflammatory responses, presumably within the plaque itself, where it is found in substantial abundance.

Compared with other inflammatory markers currently in use, Lp-PLA2 has several potential advantages. Lp-PLA2 is a specific marker of coronary atherosclerosis and, except for a moderate correlation with LDL-C, is only minimally associated with other risk factors. Further, Lp-PLA2 is not correlated with markers of systemic inflammation and is not elevated in unstable angina, non-ST-elevation MI, and ST-elevation MI.

Primary prevention studies in general support the concept that plasma Lp-PLA2 level is a risk marker for the development of cardiovascular disease. In the West of Scotland Coronary Prevention Study (WOSCOPS), there was an independent dose-response relationship between Lp-PLA2 levels and coronary event risk, whereas in the Women’s Health Study, the univariate association was attenuated after adjustment for other risk factors. In the Atherosclerosis Risk in Communities (ARIC) Study, after multivariable adjustment, an association was found only in subjects with LDL-C <130 mg/dL. Among the 934 participants of the MONICA (MONitoring of Trends and Determinants in Cardiovascular Disease) study, a 1 SD increment in Lp-PLA2 level was associated with ≈40% higher risk for future coronary events. This association was slightly attenuated in a multivariable analysis that included total and HDL-C. The Rotterdam Study has shown a strong linear association between Lp-PLA2 activity and risk of both coronary disease and stroke, even after risk factor adjustment. Support for the relationship between Lp-PLA2 and stroke has been provided by additional report from the ARIC Study, which indicated a strong independent association and a synergistic effect with hs-CRP.

Far less is known about the potential prognostic value of Lp-PLA2 measurements after MI. Koenig et al have recently reported an independent association between Lp-PLA2 values (both levels and activity) measured within 3 months following a coronary event (mean 43 days) and recurrent ischemic events in a cohort of 1051 patients who participated in an in-hospital rehabilitation program. Furthermore, among 3265 patients with acute coronary syndromes enrolled in the PROVE IT trial, Lp-PLA2 activity measured 30 days postevent was positively associated with adverse outcomes, even after adjustment for various risk indicators. However, no association was found between baseline Lp-PLA2 values and subsequent risk. Several limitations, largely inherent to post hoc analyses of trials, impact the validity of the latter finding. The randomization of patients to moderate (pravastatin 40 mg daily) versus intensive (atorvastatin 80 mg daily) lipid-lowering therapy shortly after acute coronary syndromes is responsible for the hydrolysis of oxidized LDL. Its biologic function is thought to circulate bound primarily to small, dense LDL and is responsible for the hydrolysis of oxidized LDL. Its biological function is thought to circulate bound primarily to small, dense LDL and is responsible for the hydrolysis of oxidized LDL. Its biological function is thought to circulate bound primarily to small, dense LDL and is responsible for the hydrolysis of oxidized LDL.
might have biased the associations because it differentially affected both Lp-PLA₂ levels and outcomes. Additionally, although most previous studies have examined Lp-PLA₂ levels (ie, mass),⁹,¹¹–¹⁴,¹⁶ the PROVE IT trial focused primarily on Lp-PLA₂ activity. Unlike previous reports, where there was a strong correlation between Lp-PLA₂ mass and activity (correlation coefficients in the range of 0.6 to 0.9),¹⁷,¹⁹,³⁷ there was only a modest correlation (r<0.4) in PROVE IT. Finally, the death rate was low at 4% over a 2-year follow-up period, which is considerably less than the 20% to 25% post-MI mortality rates reported in community cohorts for such time periods²⁹,³⁸ and the 16% at 1 year reported herein.

Thus, the present data bring novel information on the prognostic value of Lp-PLA₂ after MI. First, we assessed the relationship between Lp-PLA₂ levels and mortality and observed a strong graded association, which was not attenuated after taking into account several known markers of post-MI risk. Subsequently, by ROC analyses, we demonstrated that Lp-PLA₂ provides important incremental information to predict death over known clinical indicators. These 2 distinct and complementary analytical steps are essential for the assessment of a new risk marker.³,³⁹

A clinical cutoff for Lp-PLA₂ of 235 ng/mL in healthy populations and 225 ng/mL in clinical populations has been recently proposed.⁴⁰ The latter cutoff is congruent with the upper Lp-PLA₂ tertile in our study, which was consistently associated with increased mortality irrespective of the model used and can therefore be supported by the present data. Our data may have therapeutic implications as well, because statins have been shown to reduce Lp-PLA₂ levels¹⁷,⁴¹,⁴² and specific inhibitors of Lp-PLA₂ are currently under development.⁴³–⁴⁵

Several potential limitations are important to consider. Our sample size is relatively modest, and although Olmsted County is becoming more diverse, the study population consists primarily of US whites. Thus, our findings require confirmation in other data sets and different racial and ethnic groups. In addition, Lp-PLA₂ was based on 1 measurement at a single time point, which may cause some misclassification.

Strengths of our study include its prospective community-based design, whereby all consecutive consenting patients in a geographically defined population were included and among whom Lp-PLA₂ was measured promptly after acute MI. Participation rate in this study was high at 90%, which minimizes selection bias inherent in lower participation studies.²³ Additional strengths include the consistent and rigorous ascertainment approaches, which relied on standardized criteria to define MI, and the complete follow-up. These important methodological strengths optimize the robustness of our findings.

Conclusions
In this community cohort of persons with acute MI, high Lp-PLA₂ levels are strongly associated with mortality independently of other indicators of post-MI risk. Further, Lp-PLA₂ provides incremental predictive value over traditional risk markers, thereby suggesting that it can offer useful information for post-MI risk stratification.

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References


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