Lp-PLA₂: A New Kid on the Block

ANDREW ZALEWSKI,1,4* JEANENNE J. NELSON,2 LISA HEGG,1 and COLIN MACPHEE3

Background: Atherosclerosis is a systemic disease with focal rupture of vulnerable plaque responsible for major clinical events. Several population-based studies indicate an association between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) and cardiovascular events. Lp-PLA₂ is emerging as a biomarker that may be a potential link between oxidized LDL cholesterol and multifocal plaque vulnerability.

Content: Lp-PLA₂ is produced by inflammatory cells of myeloid origin, is associated with circulating atherogenic lipoproteins (e.g., LDL), and is highly expressed in vulnerable plaques (de novo expression). Specificity of Lp-PLA₂ toward polar phospholipids in oxidized LDL may contribute to the formation of downstream products (e.g., lysophosphatidylcholine and nonesterified fatty acids) that mediate processes intimately involved in plaque vulnerability in situ, including proinflammatory cell phenotype and macrophage death. Recent studies in patients with acute coronary syndrome (ACS) demonstrate that Lp-PLA₂ and LDL measurements are not useful to assess the long-term cardiovascular risk shortly after the acute event, most likely because of the acute drop in LDL values that is commonly observed in ACS. However, when measured at later time points, Lp-PLA₂ emerges as an independent predictor of the long-term cardiovascular risk, according to multivariate analysis.

Summary: Lp-PLA₂ is an intriguing marker of cardiovascular risk and may also be a marker of plaque activity/vulnerability. Despite these findings, unanswered questions still exist with respect to this enzyme and its biologic role in atherosclerosis. Addressing these questions will help clarify the clinical utility of measuring Lp-PLA₂ in routine clinical practice in the context of other approaches for identifying high-risk patients.

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Atherosclerosis is a systemic disease with focal manifestations that are responsible for major clinical events (e.g., cardiovascular death, myocardial infarction, stroke). High-risk lesions are characterized by a large necrotic core and the presence of numerous inflammatory cells (monocyte-derived macrophages, T cells, and mast cells) and a thin fibrous cap. The breach of the fibrous cap and subsequent thrombus formation has been identified in ~60% of sudden death victims, with the remaining patients suffering fatal coronary thrombosis resulting from plaque erosion or protruding calcified nodules (1). In high-risk patients, plaque vulnerability is a multifocal phenomenon involving more than a single spot within the coronary circulation. Many plaques have both ruptured and healed without clinical sequelae or will rupture at some time in the future. In clinical practice, however, these pathologic characteristics cannot be easily discerned, hence an intense interest in improving the means for identifying vulnerable patients who are at high risk for events. These efforts have focused on 3 broad classes of biomarkers: (a) vascular imaging to identify the presence of atheroma or its composition; (b), functional assessments that reflect abnormal arterial homeostasis (e.g., arterial stiffness); and (c), measurements of soluble biomarkers. In recent years, several epidemiology studies have revealed an association between lipoprotein-associated phospholipase A₂ (Lp-PLA₂)—a biomarker that may be viewed as a potential link between noxious effects of oxidized LDL cholesterol and elusive plaque vulnerability—and cardiovascular events (2–16). Lp-PLA₂, a member of phospholipase A₂ superfamily, is produced by inflammatory cells of myeloid origin, is associated with circulating atherogenic lipoproteins, and is highly expressed in the diseased vessel (Fig. 1) (17). As with all putative markers of plaque

1 GlaxoSmithKline, 1 Cardiovascular Medicine Development Centre; 2 Worldwide Epidemiology; and 3 Center of Excellence for Drug Discovery, Philadelphia, PA.
4 Thomas Jefferson University, Philadelphia, PA.
5 Nonstandard abbreviations: Lp-PLA₂, lipoprotein-associated phospholipase A₂; PAF, platelet activating factor; ACS, acute coronary syndromes; PROVE-IT, PRavastatin Or atorVastatin Evaluation and Infection Therapy; TIMI-22, Thrombolysis In Myocardial Infarction trial-22.
In humans, circulating Lp-PLA2 (small blue circles) is bound predominantly to LDL and is carried to the intima along with LDL. Lp-PLA2 acts on oxidized phospholipids within modified LDL to generate lysophosphatidylcholine and oxidized fatty acids. Both products have proinflammatory effects that contribute to the initiation and progression of atheroma, in large part, through the recruitment and activation of monocyte-macrophages. However, Lp-PLA2 is also produced de novo within atheroma by macrophages, and free Lp-PLA2 may leach out of advanced lesions into the systemic circulation. There is also evidence that lysophosphatidylcholine and oxidized fatty acids may be involved in plaque destabilization. The products of Lp-PLA2 activity induce apoptosis among macrophages, which may contribute to necrotic core expansion, thinning of the fibrous cap, and increased inflammatory infiltrate within the fibrous cap region—key characteristics of the so-called vulnerable plaques (17).

Fig. 1. This image summarizes the putative proatherogenic effects of Lp-PLA2 in atheroma.

In the context of this debate (i.e., “friend or foe”), recent findings in patients with prior acute coronary syndrome (ACS) add to the growing evidence that higher concentrations of circulating Lp-PLA2 activity are associated with increased risk of recurrent fatal and nonfatal cardiovascular event over the next 2 years, as opposed to lower cardiovascular risk (12, 14).

Could increased Lp-PLA2 concentrations among patients who go on to experience a recurrent event simply represent a compensatory mechanism for removing oxidized LDL? Although epidemiology studies cannot provide conclusive answers to this question, several studies in primary prevention populations (i.e., without manifested atherosclerotic vascular disease) illustrate a similar picture in which increased Lp-PLA2 concentrations (either mass or activity) are predictive of future cardiovascular events for as many as 14 years (5). In addition, mechanistic studies also suggest that higher Lp-PLA2 activity contributes to processes identified as pivotal to plaque vulnerability, including monocyte migration, proinflammatory effects of oxidized LDL, and macrophage death.
(18, 19). Recent findings by Stafforini et al. (20) further indicate that human Lp-PLA₂ activity contributes to the release of F2-isoprostanes, products of lipid peroxidation in vivo that possess several noxious biologic effects. Collectively, these experimental observations indicate that increased Lp-PLA₂ activity has the potential to exert harmful effects, thereby supporting the conclusions from epidemiologic studies.

**HOW CAN Lp-PLA₂ EPIDEMIOLOGY DATA BE RECONCILED WITH CONTRADICTORY RESULTS FROM STUDIES OF Lp-PLA₂ VARIANTS?**

Several reports purported that Japanese carriers of the mutation in the PLA2G7 gene (V279 variant) are at increased risk of cardiovascular events, despite the absence of circulating enzyme. The subsequent discovery that carriers of this variant are unable to secrete Lp-PLA₂ from monocytes/macrophages suggests a more complex picture than previously suggested (21). Rather than the lack of circulating Lp-PLA₂ being responsible for cardiovascular risk, the unfolded protein syndrome in individuals with V279 variant, with its effect on monocyte/macrophages survival (e.g., apoptosis), could offer 1 explanation for the disconnect between findings from many epidemiologic studies and those from genetic studies of Lp-PLA₂ in Japanese.

**IS Lp-PLA₂ SIMPLY A MARKER OF SMALLER AND DENSER LDL?**

Although circulating concentrations of Lp-PLA₂ (mass and activity) correlate with several clinical characteristics [e.g., sex (concentrations tend to be higher in men than in women), age, smoking status] and laboratory variables (e.g., LDL), they contribute to only a relatively small portion of its variation in plasma (22). Recently, Corsetti et al. undertook an exploratory factor analysis to define the association between Lp-PLA₂ activity and clusters of lipid measurements, hemostatic biomarkers, and inflammatory and glycemic control measures in post-ACS patients (14). The fact that Lp-PLA₂ was associated with apoB and an atherogenic phenotype (high triglycerides, low concentrations of HDL cholesterol, and small LDL particle size) is not surprising, given that ~80% of the enzyme is bound to apoB and the activity of Lp-PLA₂ is mainly associated with smaller LDL particles (23, 24). However, how does one explain that only ~40% of the variance in Lp-PLA₂ could be attributed to lipid-related measurements? The answer is possibly related to the fact that Lp-PLA₂ expression is dependent on leukocyte activation, with lipoproteins serving as a functional reservoir for the enzyme in circulation (19). An additional pool of Lp-PLA₂ is produced by the activated inflammatory cells within atheroma in situ, where enzyme expression is particularly high within the lipid core and co-localizes with apoptotic cells (17, 25). These findings imply that circulating Lp-PLA₂ may reflect the lipoprotein profile (e.g., higher concentrations of LDL or number of smaller denser LDL carrying most of Lp-PLA₂), the state of circulating leukocytes, and also the inflammatory composition of atheroma (Fig. 1). Consistent with this hypothesis, Lp-PLA₂ surpassed apoB as an independent predictor of cardiovascular risk in a multivariate model in the work by Corsetti et al. (14). Unfortunately, data from diabetic patients from the same cohort have not been provided, which is important information, because diabetics have higher concentrations of small size LDL particles, greater disease burden with plaques enriched with inflammatory cells, and carry increased risk of cardiovascular events.

**Lp-PLA₂ MEASUREMENTS IN POST-ACS PATIENTS?**

Analogous to LDL concentrations that lack predictive value when measured in the setting ACS, emerging data from FRISC-2, GUSTO IV, and PROVE-IT (TIMI-22) indicate that Lp-PLA₂ is not a useful biomarker to assess the long-term cardiovascular risk when measured shortly after the acute event (12, 15, 16). The changes in variables that likely influence Lp-PLA₂ measurements (e.g., decreased LDL concentrations) are likely explanations why Lp-PLA₂ concentrations add little to the risk assessment when measured within hours or days after ACS. These findings should not be misinterpreted, however, as evidence that a therapeutic intervention aimed at such biomarkers would be futile. The results from statin trials in ACS, such as MIRACL and PROVE-IT, have illustrated that LDL lowering is effective for reducing cardiovascular events despite no association of baseline LDL values with outcomes in these patients (26, 27). Interestingly, when Lp-PLA₂ activity is measured at later time points, when

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### Table 1. Stepwise validation of cardiovascular biomarker clinical utility.

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CV, cardiovascular; FRS, Framingham risk score.
the metabolic storm surrounding ACS has subsided [e.g., at 1 month: PROVE-IT (12) or at 2 months: Corsetti et al. (14)], the measurements emerge as an independent predictor of the long-term cardiovascular risk in the multivariate analysis. These recent data also raise a provocative question as to whether better adherence to statin therapy in post-ACS patients would sufficiently lower Lp-PLA2 to abrogate the risk that was conferred by the reported activity levels. In the PROVE-IT study, in which all post-ACS patients were treated with 1 of 2 statin regimens, pravastatin failed to decrease Lp-PLA2 activity despite lowering LDL by 12.5%. Even aggressive atorvastatin therapy lowered mean Lp-PLA2 activity by only 20% after 1 month of treatment in spite of concomitant decrease in LDL by 42.5% (12). These data illustrate the limits of statin therapy in terms of modest effects on the levels of this enzyme mediated mainly by lowering LDL. Other studies suggest a similar effect between statins and fibrates on reducing plasma Lp-PLA2 (i.e., ~25% reduction), again through effects on lowering LDL; (28–30) however, there are no robust data with respect to the effects of other pharmacologic interventions on Lp-PLA2 mass or activity.

**Lp-PLA2 Quo Vadis and Challenges Ahead?**

To what extent residual plasma Lp-PLA2 activity is causally responsible for death and recurring cardiovascular events in aggressively treated post ACS patients—rather than being merely associated with such risk—cannot be answered from epidemiology studies. This is more than an academic question, given that intensively treated patients in the PROVE-IT study still had >20% risk of death or coronary events over the next 2 years (27). Clearly the answer to this query must await future randomized trials exploring the therapeutic value of selective Lp-PLA2 inhibition in conjunction with the standard of care (e.g., statins). Determining whether Lp-PLA2 inhibition improves clinical outcomes beyond that which can be achieved with high-dose statin therapy will be helpful in addressing the clinical utility of this novel biomarker.

At this time, the role of Lp-PLA2 measurements in routine clinical practice has not been fully established. Despite the growing number of epidemiologic studies assessing Lp-PLA2 activity or mass in primary or secondary prevention populations, the transition of this biomarker from research to clinical practice arena will require further work (Table 1). An important issue is to determine whether to measure the mass of Lp-PLA2 or to measure its enzyme activity. Several of the epidemiology studies measured only Lp-PLA2 mass (2–5; 7–9; 15,16), whereas others measured only enzyme activity, (6, 10, 11, 14) and a few have measured both in the same study population (12, 13). The relatively low correlation ($r = 0.36$) between Lp-PLA2 mass and activity in recent studies has raised the question of precisely what is being measured with the respective assays (12). The findings from one study (24), suggest that the activity assay measures enzyme associated with smaller, denser LDL, and the mass assay measures enzyme associated with both LDL and HDL. However, more research is needed to clarify potential differences between assays.

It is important to caution that neither hazard ratio (e.g., comparing the highest versus the lowest quartile or quintile of the study population) or accompanying $P$ values provide definitive answers regarding clinical utility of measuring the biomarker of interest (31). Key additional issues yet to be resolved include quantifying the incremental value of Lp-PLA2 measurements—over and beyond currently recommended multivariable tools used to quantify cardiovascular risk in primary prevention population—and defining the threshold values that warrant additional aggressive interventions in those with already manifested vascular disease. Recent observations from an ARIC study suggested that Lp-PLA2 measurements yielded only a small increase in the area under the ROC curve when added to traditional risk factors and failed to restratify patients from the intermediate to high risk (32). These caveats notwithstanding, it should be underscored that Lp-PLA2 is a modifiable risk marker and its utility as a therapeutic target can be addressed only through future clinical trials directly targeting this enzyme (Table 1). Addressing the remaining gaps in our understanding of the utility and pitfalls of measuring Lp-PLA2 is needed before this promising biomarker could be fully integrated into daily practice for prognostic or therapeutic purposes (31, 32).

In summary, the efforts toward better understanding the predictors of clinical instability have resulted in numerous studies of cardiovascular biomarkers and other intermediate endpoints in populations at risk. In a broad sense, poor correlations between circulating inflammatory biomarkers (e.g., hsC-reactive protein and Lp-PLA2) and vascular imaging highlight biological variability of the analyte in a disease with a long period of an indolent course or technology-related challenges related to such measurements (33, 34). Results of several epidemiology investigations could suggest that novel inflammatory biomarkers reflect the activity of atherosclerotic vascular disease, rather than plaque size or lumen narrowing. The reported lack of correlation between Lp-PLA2 activity and C-reactive protein levels also illustrates differences between the pathways, in which a growing number of inflammatory biomarkers are involved. The ultimate goal of these studies is to refine our current clinical tools for estimating cardiovascular risk and for selecting optimal therapy for individual patients. It is unlikely that a single test will overcome imprecision of looking into the future. Instead, the array of three biomarkers mentioned above: imaging (what type of plaque is present?), functional assessments (how abnormal is the vessel function?) and circulating biomarkers (how active is the disease process?) will be likely required to assess risk and guide treatment decisions.
References


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