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Lp-PLA$_2$ as a Marker of Cardiovascular Diseases

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Abstract Inflammation lies at the base of endothelial dysfunction, eventually leading to plaque formation. The degree of inflammation defines the "vulnerability" of plaque to rupture. Numerous strategies have been adopted to identify and eventually treat high-risk vulnerable plaque. Lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) has emerged as one such candidate marker of inflammation that may play a direct role in the formation of rupture-prone plaque. Epidemiologic studies have clearly demonstrated the prognostic ability of increased Lp-PLA$_2$ levels and their association with increased risk of future coronary and cerebrovascular events. Moreover, Lp-PLA$_2$ might have similar predictive power for both incident coronary heart disease in initially healthy individuals as well as for recurrent events in those with clinically manifest atherosclerosis. The latest evidence has also suggested its incremental value for risk determination over the well-established traditional risk factors and biomarkers in patients with congestive heart failure. These data support an integral role of Lp-PLA$_2$ activity in lipid peroxidation and cardiovascular risk assessment. This review summarizes the current body of evidence supporting the clinical utility of Lp-PLA$_2$ and its future applications in cardiovascular medicine.

Keywords Lp-PLA$_2$ · Phospholipase A_2 · Cardiovascular diseases

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Introduction

Atherosclerosis is a systemic disease, with rupture of vulnerable plaque responsible for major cardiovascular events. Recently there has been growing interest in defining, diagnosing, and managing vulnerable plaque because of its key role in acute coronary syndromes (ACS). Because established conventional risk factors do not explain all the changes in atherosclerotic vascular disease, efforts to identify vulnerable plaque have focused on development of novel imaging modalities and soluble biomarkers. Fueled by the discovery of inflammatory cells in the cap of atherosclerotic plaques, research has focused on whether markers of inflammation can aid in the earlier prediction of cardiovascular disease (CVD). Many novel markers of CVD, both inflammatory and noninflammatory, have been discovered and studied, including C-reactive protein (CRP), lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$), homocysteine, E-selectin, plasminogen, interleukin-6, and vitamin B_6.

Lp-PLA$_2$ is an intriguing biomarker that is being explored in the pathogenesis of vulnerable plaque and has emerged as a possible therapeutic target. This review summarizes the available evidence on the subject, providing insight into the pathobiology and the potential diagnostic and therapeutic role of this important biomarker.

Pathophysiology

The exact role of Lp-PLA$_2$ is unclear. Both proatherogenic and antiatherogenic mechanisms have been proposed. Oxidative stress is a key mechanism through which atherosclerosis and CVD develop. It is mediated by reactive oxygen species that alter the fundamental properties of

cholesterol, cholesteryl esters, and phospholipids on lipoproteins, as well as other proteins, to make them dysfunctional, immunogenic, and proatherogenic. Lp-PLA_2, a member of phospholipase A2 superfamily, is a 50-kDa protein produced by inflammatory cells of myeloid origin and is associated with circulating atherogenic lipoproteins. Lp-PLA_2 has been detected in atherosclerotic plaque, particularly in ruptured plaques with a large necrotic core [1]. It is expressed by macrophages and lymphocytes, and 80% of it circulates bound mainly to low-density lipoprotein (LDL) and partly to lipoprotein(a) (Lp(a)). Five major categories of PLA_2 have been described, including the secreted small molecular weight sPLA_2, the larger cytosolic Ca^{2+}-dependent cPLA_2, the Ca^{2+}-independent iPLA_2, the platelet-activating factor (PAF) acetylhydrolases, and lysosomal PLA_2. Lp-PLA_2 acts as a calcium-independent serine lipase specifically hydrolyzing the sn-2 fatty acids of 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. There is also evidence that lysophosphatidylcholine and oxidized fatty acids may be involved in plaque destabilization. The products of Lp-PLA_2 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 plaque. Kolodgie et al. [1] reported a study of progressively more advanced atherosclerotic lesions that were individually stained using antibodies for the novel inflammatory biomarker Lp-PLA_2. The investigators reported that Lp-PLA_2 is strongly expressed within the necrotic core and surrounding macrophages of ruptured plaques, with relatively weak staining in less advanced lesions. These findings, together with the association of Lp-PLA_2 in apoptotic macrophages, suggest that Lp-PLA_2 has a potential role in promoting plaque instability. Additionally, Lp-PLA_2 may be more of a marker of rupture-prone plaque than of early stable plaque [1].

PAF acetylhydrolase has been found to have anti-inflammatory properties by hydrolyzing PAF, which plays a role in the activation of platelets, monocytes, and macrophages. Additionally, some experimental evidence suggests that inhibition of Lp-PLA_2 may be atheroprotective, but evidence for the opposite effect also has been observed. For instance, Lp-PLA_2 inhibition diminished the rise of lysophosphatidylcholine levels that usually occurs with LDL oxidation, and it reduced the ensuing apoptosis of monocyte-macrophages [2]. In contrast, experimentally augmented expression of Lp-PLA_2 reduced spontaneous atherosclerosis in apolipoprotein E–deficient mice [3]. In addition, the effect of Lp-PLA_2 activity in the arterial wall may be different from its effect in the circulation. When oxidized phospholipids enter the circulation, they are mostly scavenged by Lp(a) and can subsequently be degraded by Lp-PLA_2 activity [4•]. The product lysophosphatidylcholine may then be transferred to albumin, where it is relatively innocuous. Thus, in plasma, Lp-PLA_2 appears to play a beneficial role in the incapacitation of potentially toxic oxidized phospholipids [2, 3, 4•].

Findings from epidemiologic studies also are not consistent and equivocal. Genetic deficiency of Lp-PLA_2, which is common in Japan, has been associated with an increased risk of coronary heart disease (CHD), stroke, and peripheral artery disease [5].

Medical Genetics of Lp-PLA_2

The gene for Lp-PLA_2 (*PLA2G7*) has 12 exons and is located on chromosome 6p21.2-12. The Val279Phe variant is associated with reduced levels of Lp-PLA_2 in 27% of heterozygous Japanese individuals and complete absence of Lp-PLA_2 in 4% of homozygous individuals, caused by a defect in enzyme secretion [6, 7]. Homozygosity for the V379 allele of the A379V polymorphism in the Lp-PLA_2 gene, shown to result in lower Lp-PLA_2 activity, was found to be associated with a reduced risk of CHD in a large European case-control study [8].

Epidemiologic Evidence Linking Lp-PLA_2 and Cardiovascular Disease

Several large population-based studies have demonstrated an association between Lp-PLA_2 and cardiovascular and cerebrovascular events. The West of Scotland Coronary Prevention Study (WOSCOPS), the Atherosclerosis Risk in Communities study (ARIC), and the Monitoring of Trends and Determinants in Cardiovascular Disease study (MONICA) identified higher circulating Lp-PLA_2 mass levels as an independent predictor for first-time acute myocardial infarction (AMI) or cardiac death in patients at risk but without angiographically confirmed coronary artery disease (CAD, adjusted attributable risk of 1.17–1.21) [15]. Likewise, the Rotterdam study showed a 40% to 100% higher risk of cardiac death and AMI in patients with systemic Lp-PLA_2 activity above the lowest quartile [10]. This study also highlighted a similarly elevated risk for ischemic stroke in the subgroup of patients with elevated systemic Lp-PLA_2 activity.

Lp-PLA_2 has emerged as an independent predictor of CHD and ischemic stroke. The associations are independent of classic cardiovascular risk factors such as age, hyperten-

sion, obesity, and CRP [9]. The association between Lp-PLA_2 and CHD has been shown to be present over a wide range of cholesterol levels [9, 11]. Lp-PLA_2 has also been found to be associated with fibrinogen and elevated creatinine levels. There are gender and age differences in the distribution of Lp-PLA_2 levels.

Lp-PLA_2 levels increase with age and are lower in premenopausal women than in men [9]. Several studies have shown that higher Lp-PLA_2 levels were associated with more severe angiographic CAD on univariate but not on multivariate analysis. Also, higher Lp-PLA_2 levels were associated with a higher incidence of major adverse events during follow-up, independent of traditional CAD risk factors and CRP. Caslake et al. [10] demonstrated that Lp-PLA_2 levels were higher in 94 patients with CAD than in 54 controls. The association persisted after adjusting for LDL cholesterol and high-density lipoprotein cholesterol, smoking, and systolic blood pressure [10].

Investigators from the Bruneck study, which followed a prospective epidemiologic cohort of 765 individuals 45 to 84 years of age for 10 years, showed that increased baseline Lp-PLA_2 activity was associated with increased risk of future cardiovascular events [12]. The authors demonstrated a significant relationship between Lp-PLA_2 with vascular death but not with non-CVD mortality, consistent with a specific effect on the vessel wall; they also confirmed a significant relationship of Lp-PLA_2 with the metabolic syndrome.

Garza et al. [15••] reported a meta-analysis of more than 20,000 patients across 14 studies evaluating the association between Lp-LPA_2 and CVD. They computed an unadjusted odds ratio for the association between elevated Lp-PLA_2 levels and CVD risk of 1.51 (95% CI, 1.30–1.75), and the odds ratio adjusted for conventional CVD risk factors was 1.60 (95% CI, 1.36–1.89).

Lp-PLA_2 in Acute Coronary Syndromes

Lp-PLA_2 is not increased in AMI patients, in contrast to acute-phase reactants such as CRP and fibrinogen [9]. This is most likely because of the acute drop in LDL levels that is commonly observed in ACS patients. Analogous to LDL concentrations that lack predictive value when measured in the setting of ACS, data from the Fragmin During Instability in Coronary Artery Disease 2 (FRISC-2) study, the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries IV (GUSTO IV) study, and the Pravastatin or Atorvastatin Evaluation and Infection Therapy Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) study indicate that Lp-PLA_2 is not a useful biomarker to assess the long-term cardiovascular risk when measured shortly after the acute event [5].

However, when measured at later time points, Lp-PLA_2 emerges as an independent predictor of the long-term cardiovascular risk. In the PROVE-IT study, pravastatin failed to decrease Lp-PLA_2 activity despite lowering LDL by 12.5%. Even aggressive atorvastatin therapy lowered mean Lp-PLA_2 activity by only 20% after 1 month of treatment in spite of a concomitant decrease in LDL of 42.5% [5]. These data illustrate the limits of statin therapy in terms of modest effects on the levels of this enzyme mediated mainly by lowering LDL.

Is Lp-PLA_2 a Marker of Atherosclerotic Burden or a Vital Tool for Prognostication?

Although the predictive value of Lp-PLA_2 has been established in multiple large population-based studies, its utility in estimating atherosclerotic burden has not been ascertained. Coronary artery calcium (CAC) is a bona fide marker of atherosclerosis. The Rotterdam study [11] did not show a significant association between CAC and Lp-PLA_2 levels but did establish a strong association between Lp-PLA_2 levels and risk of CHD and stroke.

Lp-PLA_2, Congestive Heart Failure, and Risk of Incident Congestive Heart Failure

There has been an emerging role ascribed to inflammation in the pathogenesis of congestive heart failure (CHF). Recently, investigators from the Cardiovascular Health Study [13], a prospective observational study of adults 65 years of age or older, established an epidemiologic link between Lp-PLA_2 and incident CHF. In almost 4000 individuals followed prospectively for a mean of 12 years, Lp-PLA_2 had an adjusted hazard-ratio (HR) of 1.44 (95% CI, 1.16–1.79) for incident CHF. Adjustment for incident coronary disease attenuated the HR for Lp-PLA_2 to 1.26 (95% CI, 1.02–1.57), whereas adjustment for CRP levels had minimal impact. Gerber et al. [14] recently reported the prognostic utility of Lp-PLA_2 in CHF patients. In their study of 646 residents of Olmstead County, Minnesota with CHF, they found that Lp-PLA_2 was strongly and independently associated with mortality and contributed incrementally to risk discrimination in patients (covariate-adjusted HR of 3.83; 95% CI, 1.93–7.61; $P<0.001$).

Measurement of Lp-PLA_2

Lp-PLA_2 can be measured by enzyme activity or mass assay. The systematic review by Garza et al. [15••] showed that the method of measurement appeared to make no

difference. Both ways of measuring Lp-PLA$_2$ (activity vs mass assay) resulted in similar estimates of association. The study by Caslake et al. [10] showed that the lipoprotein distribution of Lp-PLA$_2$ mass corresponded precisely to the activity assay. However, in the Coronary Artery Risk Development in Young Adults (CARDIA) study, the association between Lp-PLA$_2$ and CAC was statistically significant only with the mass assay [16]. Of note, the US Food and Drug Administration (FDA) approved the immunoassay test PLAC (diaDexus Inc, South San Francisco, CA) for screening of patients with high CVD risk.

Clinical Applications of Lp-PLA$_2$

Lp-PLA$_2$ levels may be useful to further stratify patients with an intermediate probability of developing cardiovascular events by the Framingham score. Although the use of serial measurement of Lp-PLA$_2$ in patients on lipid-modifying treatment to assess for plaque stabilization has not been formally studied and cannot be currently endorsed, this application represents a promising approach in the future. Currently, however, routine screening with Lp-LPA$_2$ of patients with intermediate likelihood of CHD or those already with known CHD cannot be recommended. The operating characteristics of the FDA-approved test for Lp-PLA$_2$ (the PLAC test) will need to be adequately established. Test characteristics are known to vary significantly between patient populations. The positive and negative likelihood ratios (not necessarily crude cut-off values) of the PLAC test for patients at low risk, intermediate risk, and high risk for various cardiovascular outcomes would need to be clarified before the test is used in specific patient populations. Most importantly, clinical studies evaluating the utility of LP-PLA$_2$ (using the PLAC test or any other test) in impacting patient management and outcomes will need to be undertaken before clinicians can incorporate this test into their routine clinical practice.

Lp-PLA$_2$ as a Therapeutic Target

If the association between Lp-PLA$_2$ and CVD is causal, decreasing Lp-PLA$_2$ levels would be expected to reduce the rate of cardiovascular events by 20% to 50%, a risk reduction similar to that achieved with aspirin, statins, and angiotensin-converting enzyme inhibitors. As specific inhibitors of Lp-PLA$_2$ have been developed and shown to be orally active in animal models [17, 18], Lp-PLA$_2$ has the potential to be a therapeutic target in patients with CVD. Although specific Lp-PLA$_2$ inhibitors such as azetidinones are being studied [19], larger trials are needed. Darapladib (GlaxoSmithKline, London, United Kingdom) is a novel oral therapeutic agent with potential anti-inflammatory properties that inhibits Lp-PLA$_2$ [20•].

Thus, until further studies are performed, it needs to be acknowledged that our understanding of the role of Lp-PLA$_2$ in atherogenesis, inflammation, and oxidative stress remains incomplete, and that most of the knowledge to date is founded more on evidence of association than causation.

Conclusions

Lp-PLA$_2$ is significantly associated with CVD and may be useful in CVD risk stratification as a new biomarker as well as for identification of vulnerable plaque. It may represent a therapeutic target for cardiovascular risk stratification, and several Lp-PLA2 inhibitors are currently being studied.

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