

# The role of lipoprotein-associated phospholipase A<sub>2</sub> on cardiovascular disease risk assessment and plaque rupture: a clinical review

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**Abstract:** During the last several last decades, reduction in lipids has been the main focus to decrease the risk of coronary heart disease (CHD). Several lines of evidence, however, have indicated that lipids account only for the <50% of variability in cardiovascular risk in the United States. Therefore, for better identification of people at high cardiovascular risk, a more effective and complete approach is required. Our understanding of atherosclerosis has shifted from a focal disease resulting in symptoms caused by severe stenosis to a systemic disease distinguished by plaque inflammation with a potential to rupture and thrombosis, turning a substenotic atherosclerotic lesion into a complete occlusive lesion. Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) is a novel inflammatory biomarker that can provide much needed information about plaque inflammation and plaque stability. Lp-PLA<sub>2</sub> is among the multiple biomarkers that have been associated with increased CHD risk. In this present work, we review the evidence from previous studies addressing the effect of different therapies on decreasing Lp-PLA<sub>2</sub> and the role of direct Lp-PLA<sub>2</sub> inhibitors. This work also briefly reviews the evidence of Lp-PLA<sub>2</sub> clinical utility as a potential marker of vascular inflammation and formation of rupture prone plaques. Additionally, we also discuss the implication of available evidence in context of current cardiovascular inflammatory biomarkers recommendations and the evidence from epidemiologic studies addressing the relationship of Lp-PLA<sub>2</sub> and risk of cardiovascular disease.

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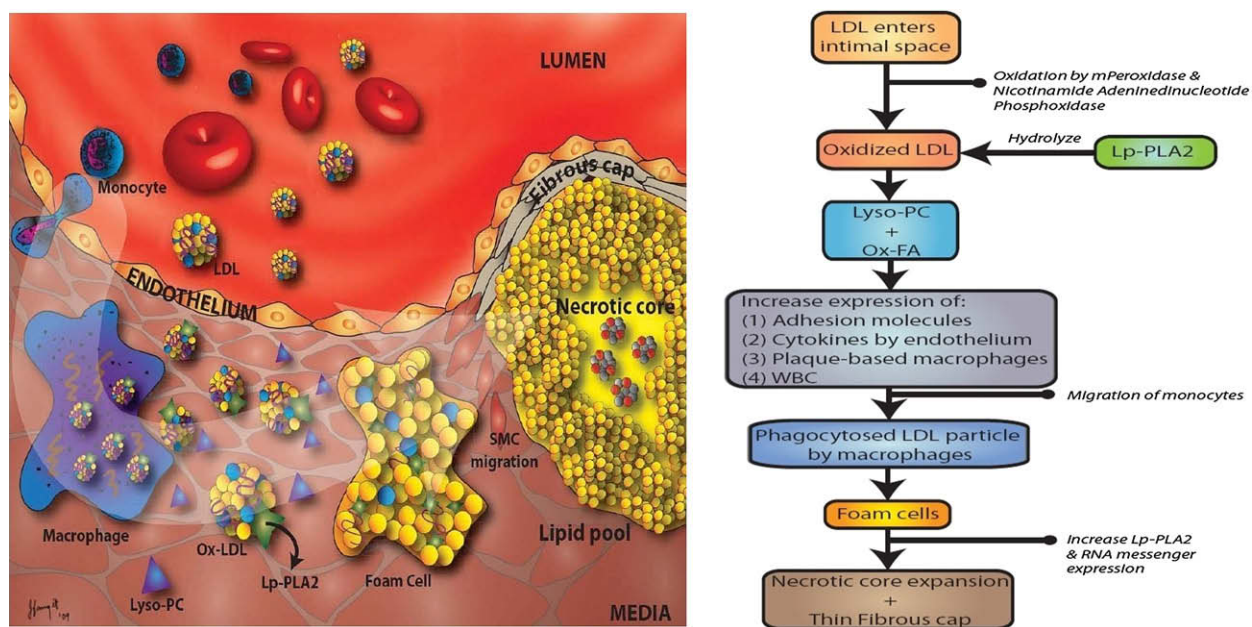
During the past several decades, substantial efforts have been directed toward reducing average blood lipids, specifically, the low-density lipoprotein (LDL) cholesterol levels of the American population. Although the level of success in the fight against the cardiovascular disease is appreciable, it has not been able to stem the tide of heart

disease completely. Our present understanding of cardiovascular risk assessment is not enough and is just an oversimplification of the complex issue of cardiovascular disease. With the advent of new technology and better understanding of plaque structure and pathophysiology, many new biomarkers that play an essential role in assessing cardiovascular risk have been recognized. Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) is one such inflammatory biomarker that may possibly help health care workers recognize rupture-prone plaque and the degree of inflammation present in the walls of coronary arteries.

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**Figure 1** Schematic presentation of the lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) role in vascular inflammation.

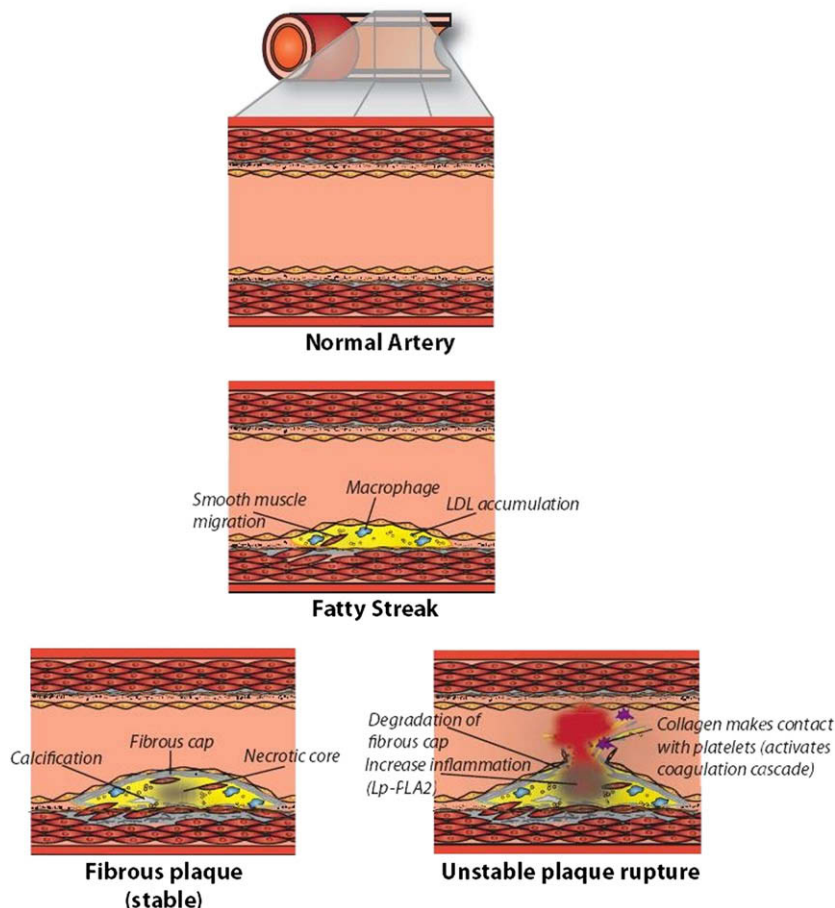
The purpose of this work is to review the evidence from previous studies addressing the effect of different therapies on decreasing Lp-PLA<sub>2</sub> and the role of a direct Lp-PLA<sub>2</sub> inhibitor, as well as a brief review of the evidence of Lp-PLA<sub>2</sub> clinical utility as a potential marker of vascular inflammation and of formation of rupture-prone plaques. Additionally, we also discuss the implication of available evidence in the context of current cardiovascular inflammatory biomarker recommendations and the evidence from epidemiologic studies addressing the relationship of Lp-PLA<sub>2</sub> and the risk of cardiovascular disease.

## Biomarker concept, definition and current available biomarkers

A biomarker is an indicator of a particular disease state or a particular state of organism. A National Institutes of Health working group defined biomarkers as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.<sup>1</sup> Ideally, an effective biomarker would be an indicator of disease etiology, independently predict the risk of developing disease, have prognostic value, be easily measured, and be a cost-effective therapeutic intervention target. Much effort, time, and available resources have been invested in this area of research in finding an ideal biomarker. As a result, numbers of emerging biomarkers have shown potential benefits.

High-sensitive C-reactive protein (hs-CRP) is by far the best tested proposed cardiovascular disease inflammatory biomarker. Numerous available prospective data suggests a twofold increase of events in individuals with high hs-CRP

levels. However, this effect was attenuated with adjustment of classical risk factors. hs-CRP levels are confounded by other systemic inflammatory diseases and risk factors, eg, smoking, body mass index, overt hyperlipidemia, insulin resistance, obesity, and high blood pressure. Aside from clinical utility debate, the causal role of CRP in cardiovascular disease still remains uncertain. Mendelian randomization trials have failed to provide firm evidence that CRP is a causal factor in cardiovascular disease.<sup>2,3</sup> Recently, the Justification for the use of statin in prevention: An intervention trial Evaluating Rosuvastatin (JUPITER)<sup>4</sup> trial studied 17,802 apparently healthy men and women with LDL cholesterol levels of <130 mg/dL (3.4 mmol/L) and hs-CRP levels of 2.0 mg/L or higher who received rosuvastatin 20 mg/day or placebo. They were followed for the occurrence of the combined primary end points of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes. Rosuvastatin decreased the relative risk of the primary end point by 44% (hazard ratio [HR] for rosuvastatin compared with placebo, 0.56; 95% confidence interval [CI], 0.46–0.69). Those with a family history of premature coronary heart disease (CHD) had an even greater relative risk reduction in the primary end point of about 65%. The study observed similar reduction in the events rates in participants who had elevated hs-CRP, irrespective of their lower or higher risk. JUPITER did not include a control group with low levels hs-CRP. Moreover, it is now apparent that statins reduce cardiovascular risk even in those who do not have high blood cholesterol, and that reduction in LDL explains almost entirely the statin-induced reductions in events, and, interestingly, also reduction in CRP.<sup>5</sup> Its highly likely that the reduction achieved in JUPITER is one of the pleiotropic effect of statins on hs-CRP,<sup>6</sup> and



**Figure 2** Progression of normal artery to stable or unstable plaque. Unstable plaques have a large necrotic lipid pool. Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) has been found a great deal in ruptured plaques. Thus, elevated Lp-PLA<sub>2</sub> may be a sign that the plaque is ready to crack.

does not determine a causal correlation between the decrease in cardiovascular events and hs-CRP reduction. Thus, to establish association of hs-CRP with cardiovascular disease events, more prospective studies are needed.

Considering the strong intercorrelation of markers of inflammation with cardiovascular risk, a lot of focus has recently been put on interleukin-6 (IL-6). IL-6 is an acute-phase reactant secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation. IL-6 has shown robust association with cardiovascular risk in a recent meta-analysis and predicted cardiovascular events more strongly than CRP.<sup>7</sup> This finding is of interest as there are pathophysiologic reasons to consider IL-6 a potential factor in the atherosclerotic process. Irrespective of any causal role for IL-6, future studies are needed to address whether measurement of this acute-phase reactant will enhance the cost-effective risk prediction in addition to, and independent of, other risk factors.

In addition to inflammatory biomarkers, much interest has recently focused on N-terminal-pro-brain peptide (NT-pro-BNP): a marker of cardiac damage and ischemia. B-type natriuretic peptide, along with its inactive metabolite,

NT-pro-BNP, are used in the diagnosis of heart failure.<sup>8</sup> However, a number of studies suggest that NT-pro-BNP may predict risk of cardiovascular morbidity and mortality in a population without prevalent vascular disease.<sup>9,10</sup> Olsen et al<sup>11</sup> showed in a population-based study that NT-pro-BNP was associated with cardiovascular risk independent of classical risk factors and other markers of cardiac function; the odds ratio was 1.56 (95% CI, 1.33–1.83) per 1 standard deviation (SD) increase in plasma levels of NT-pro-BNP. Therefore, these findings warrant further investigation with larger samples to establish the role of NT-pro-BNP in predicting risk of cardiovascular events.

Metabolic biomarkers are another class of biomarkers that have been recently associated as a predictor of cardiovascular events. A meta-analysis of 19 western prospective studies suggests that a marker of  $\beta$ -cell dysfunction, proinsulin, may be more strongly associated with cardiovascular risk than insulin.<sup>12</sup> However, available data come from only three studies, thus requiring further studies to determine cardiovascular risk prediction. Adiponectin is a protein hormone whose high circulating levels are associated with reduced cardiovascular risk. A health professional study observed a 20%

reduction in myocardial infarction with a twofold increase of adiponectin levels even after adjusting for classical risk factors.<sup>13</sup> However, a meta-analysis of more than 1300 cases of heart disease showed no association with adiponectin levels.<sup>14</sup> Therefore, the relationship between this protein hormone and cardiovascular disease is complex and far from fully revealed.

### Lp-PLA<sub>2</sub>: a new inflammatory biomarker

Lp-PLA<sub>2</sub> is a subtype of the phospholipase A<sub>2</sub> superfamily—a family of enzymes that hydrolyzes phospholipids. Lp-PLA<sub>2</sub>, also known as platelet-activating factor acetylhydrolase, is a 50-KD Ca-independent phospholipase that is distinct from another macrophage product, secretory PLA<sub>2</sub>, a 14-kD Ca-dependent enzyme.<sup>15</sup> Lp-PLA<sub>2</sub> is expressed in atherosclerotic plaques and in macrophages within a fibrous cap of human rupture-prone lesions.<sup>16</sup> Lp-PLA<sub>2</sub> is attached to LDL and is the only enzyme responsible for the hydrolysis of oxidized phospholipid resulting in production of lysophosphatidylcholine and oxidized fatty acid.<sup>17</sup> The proinflammatory and atherogenic properties of lysophosphatidylcholine are well known (see Fig. 1).<sup>18</sup>

### Lp-PLA<sub>2</sub>: an independent cardiovascular risk predictor

Lp-PLA<sub>2</sub> is a unique inflammatory biomarker, as it is highly vascular specific, has low biologic variability, and is directly related to the propensity of plaque rupture. Lp-PLA<sub>2</sub> is an enzyme that hydrolyzes the oxidized phospholipids present in the walls of arteries and releases lysophosphatidylcholine, which has proinflammatory properties. A recent meta-analysis of 14 prospective epidemiologic studies involving more than 20,000 patients established a high relative risk for cardiovascular events with high Lp-PLA<sub>2</sub>.<sup>19</sup> The risk estimates appear to be relatively unaffected by the adjustment for conventional cardiovascular risk factors. Results from the study by Tsimikas et al,<sup>20</sup> which was a prospective analysis of epidemiology and pathogenesis of atherosclerosis, analyzed the prognostic value Lp-PLA<sub>2</sub> activity. Lp-PLA<sub>2</sub> activity and baseline variables were measured in 765 subjects aged 45–84 years. Incident cardiovascular disease (cardiovascular death, myocardial infarction, stroke, and transient ischemic attack) and rates of noncardiovascular disease mortality were assessed between 1995 and 2005. Subjects with incident cardiovascular disease had higher levels of Lp-PLA<sub>2</sub> activity ( $884 \pm 196$  vs.  $771 \pm 192$   $\mu\text{mol}/\text{min}/\text{L}$ ,  $P < .001$ ). Increased Lp-PLA<sub>2</sub> activity was significantly related to incident cardiovascular disease (age- and sex-adjusted HR 2.9; 95% CI 1.6–5.5; third vs. first tertile group;  $P < .001$ ) and to vascular mortality but not to noncardiovascular disease mortality; thus, relating increased Lp-PLA<sub>2</sub> levels to fatal and nonfatal cardiovascular diseases.

The West of Scotland Coronary Prevention Study<sup>21</sup> (WOSCOPS) was a primary prevention trial in which Lp-PLA<sub>2</sub> was compared among 580 cases and 1160 age-matched controls. A twofold greater risk of coronary artery disease was observed for the patients with the highest Lp-PLA<sub>2</sub> levels. Similar to Lp-PLA<sub>2</sub>, the highest level of hs-CRP was also associated with a twofold increase in CHD risk. However, on a running multivariate analysis, the risk associated with hs-CRP was attenuated, but risk associated with Lp-PLA<sub>2</sub> remained statistically significant ( $P = .005$ ), displaying the strength of Lp-PLA<sub>2</sub> as an independent CHD risk predictor. Furthermore, WOSCOPS demonstrated that Lp-PLA<sub>2</sub> was the only inflammatory marker whose levels were not affected by smoking.

Similarly, in a post hoc analysis among the 934 healthy men aged 45–64 years in Monitoring Trends and Determinants in Cardiovascular Diseases<sup>22</sup> (MONICA), the relationship between the Lp-PLA<sub>2</sub> levels and coronary heart disease was evaluated. After 14 years of follow-up, 97 men who experienced a coronary event had significantly higher mean baseline levels of Lp-PLA<sub>2</sub>. Each SD increase in Lp-PLA<sub>2</sub> levels resulted in a 37% increase in the risk of a coronary event, even after controlling for the potentially confounding factors. Additionally, the study involving 504 patients at the Mayo Clinic<sup>23</sup> who underwent clinically indicated coronary angiography demonstrated that higher Lp-PLA<sub>2</sub> was associated with a greater coronary heart disease risk of events; the HR per SD was 1.28 (95% CI 1.06–1.54,  $P = .009$ ) and remained significant after adjusting for clinical and lipid variables and hs-CRP.

In another study, Lp-PLA<sub>2</sub> levels were evaluated in a clinical study of 148 men, 48 of whom had angiographically proven coronary artery disease; 46 had experienced myocardial infarction at least 1 year before the study and 54 were normal aged-matched controls.<sup>24</sup> Higher levels of Lp-PLA<sub>2</sub> were found in patients with angiographically proven coronary artery disease compared to normal subjects. On multiple regression analysis, this increase in Lp-PLA<sub>2</sub> was independent of LDL and other risk factors, including smoking.

The available data are more convincing when considering Lp-PLA<sub>2</sub> as a risk factor for strokes. Five important stroke studies<sup>25–29</sup> looked at Lp-PLA<sub>2</sub> and its involvement in stroke prediction. The studies are Atherosclerosis Risk in Communities (ARIC) study, Rotterdam, Northern Manhattan Stroke Study (NOMAS) study, Veterans Affairs HDL Intervention Trial (VA-HIT) study, and the Women's Health Initiative Observational Study. ARIC was a prospective study conducted in four United States communities. ARIC was designed to investigate the etiology and natural history of atherosclerosis, the etiology of clinical atherosclerotic diseases, variation in cardiovascular risk factors, and medical care and disease by race, gender, location, and date.<sup>25</sup> The study was divided into cohort and community surveillance components. Hospitalized strokes were investigated in cohort participants. The age group in cohort group was between 45 and 64 years of age at entry.

Lp-PLA<sub>2</sub> and hs-CRP were checked to determine their ability to predict strokes. The ARIC study found that Lp-PLA<sub>2</sub> was higher in individuals with strokes; lipid factors were not predictive of strokes and higher levels resulted in a two-fold increase in risk independent of traditional risk factors. Elevated Lp-PLA<sub>2</sub> and hs-CRP were completely beyond traditional risk factors in identifying strokes and had a synergistic effect, approximately increasing risk of strokes by elevenfold.

The Rotterdam study<sup>26</sup> was a case cohort study with 7983 participants with 6.4 years of follow-up for incidence of ischemic stroke. Quartiles of Lp-PLA<sub>2</sub> were created and the lowest quartile was taken as a reference range. The study found that there was a stepwise increase in the risk of stroke based on heart rate. Total cholesterol and non-high-density lipoprotein (non-HDL) were identical in the stroke patients compared to controls. Even in subjects with less than median non-HDL levels, Lp-PLA<sub>2</sub> emerged as an independent predictor for strokes; thus indicating that although Lp-PLA<sub>2</sub> is a part of the LDL particle, it conveys a separate risk.

The NOMAS<sup>27</sup> was a study that examined how high Lp-PLA<sub>2</sub> drawn at the time of first stroke might predict the risk of recurrent stroke. The study followed 467 patients, diagnosed as having a first stroke, for about 4 years. Results showed that those with the highest levels of Lp-PLA<sub>2</sub> had an increased risk of recurrent stroke, heart attack, or vascular death, even after adjusting for factors such as age, gender, ethnicity, and history of heart disease. CRP levels were not associated with the risk of recurrent stroke but provided important information about mortality; however, this association was nonsignificant in patients with LDL cholesterol levels <130 mg/dL.

The VA-HIT<sup>28</sup> examined whether increased Lp-PLA<sub>2</sub> would also predict cardiovascular events in the absence of high LDL cholesterol in a population with low HDL cholesterol. The study involved 1451 men with low HDL cholesterol (mean 32 mg/dL) and low LDL cholesterol (mean 110 mg/dL) who were randomized into placebo or treatment groups. Lp-PLA<sub>2</sub> levels were measured at baseline and 6 months after therapy. The VA-HIT study found that Lp-PLA<sub>2</sub> was an independent risk factor for cardiovascular events and that a 1 SD increase in Lp-PLA<sub>2</sub> levels resulted in a significant increase in all cardiovascular events.

Similarly, the Women's Health Initiative Observational study<sup>29</sup> investigated the role of elevated Lp-PLA<sub>2</sub> with stroke risk. This was one of the largest studies to look at Lp-PLA<sub>2</sub> and risk for strokes. The study assessed the relationship between Lp-PLA<sub>2</sub> and the risk of incident ischemic stroke in 929 stroke patients and 935 control subjects in the Hormones and Biomarkers Predicting Stroke Study,<sup>30</sup> a nested case-control study from the Women's Health Initiative Observational Study. The study found a statistically significant association between the risk incident of stroke and elevated Lp-PLA<sub>2</sub>, whereas hs-CRP did not show statistically significant association with stroke risk.

## Lp-PLA<sub>2</sub> and unstable rupture prone plaque

Atherosclerosis is main pathologic process that deposits fatty substances, cholesterol, cellular debris, and calcium in the inner layer of arteries. This buildup in arteries is called plaque, which narrows the arterial lumen. By age 50, there is 50% reduction in the lumen area of blood vessels reducing the blood supply. Plaques are divided into soft and hard plaques depending on their vulnerability to rupture and degree of stenosis. Hard plaques are stable, more stenotic, and are less active, whereas soft plaques are less stable and less stenotic, but are more active and rupture prone. It is clinically important to shift our focus to identify individuals with highly active inflamed soft plaques instead of those with hard lumen narrowing plaques. Hard plaques have small lipid rich cores and very thick fibrous caps that typically produce angina, which responds positively to local therapy or revascularization methods. In contrast, soft plaques are asymptomatic, inflamed, have large lipid rich cores, and a thin fibrous cap that most often result in sudden cardiac death. Most myocardial infarctions and sudden death occur from atherosclerotic lesions in minimally to modestly stenotic arteries. Falk et al<sup>31</sup> found that most acute myocardial infarctions present with <50% stenosis and most sudden cardiac deaths are related to thin fibrous cap and rupture-prone plaques. Only 16% of myocardial infarctions occur in >70% stenotic lesions. Similarly, in another study, necropsy samples found that in sudden death cases, 76% of the culprit lesion involved rupture-prone plaque and only 24% occurred at sites of severe luminal narrowing.<sup>32</sup>

Treadmill tests and other advanced modalities presently used in cardiovascular assessment protocol alone will not detect these actively inflamed plaques, as severe stenosis is not a requirement for a rupture prone plaque formation. This implies that patients still may be at high risk from plaque rupture despite having a normal stress test and angiographic testing. In other words, patient may have low grade stenosis but still have a high degree of atherosclerosis disease activity. Consequently, a noninvasive method or an inflammatory biomarker that could possibly identify these patients with minimal or substenotic lesions will be of great help. Testing for endothelial dysfunction is one modality that could possibly detect individuals with active unstable rupture-prone plaques. However, this technique has reproducibility problems and has not been established as an independent risk factor for coronary artery disease in all studies. hs-CRP is one of the currently recommended inflammatory biomarkers that is related to the degree of inflammation and may provide a clearer picture for plaque activity. However, this biomarker lacks specificity and its levels can be easily altered by other systemic inflammatory conditions as well as by blood pressure, smoking, obesity, insulin resistance, and overt hyperlipidemia.

Biovariability is another important clinical application of biomarkers that should be taken into account before a biomarker is used to determine plaque activity and

monitoring specific therapy. A biovariability study was done on 364 pairs of plasma and serum samples from 43 non-fasting healthy adults. Venous blood was drawn at least 7 times over 4 weeks. Lp-PLA<sub>2</sub>, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and CRP were measured in the corresponding samples. The study reported interindividual biologic variation for Lp-PLA<sub>2</sub> of 32.8% compared to 122.4%, 23.5%, and 50.5% for CRP, LDL cholesterol, and triglycerides, respectively.<sup>33</sup> Thus, a reproducible, specific, noninvasive test with low to modest biovariability, which could identify individuals with rupture-prone soft plaques, would definitely fill an important, unmet clinical need. In this regard, investigators have reported that Lp-PLA<sub>2</sub> is strongly expressed within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques, with relatively weak staining in less advanced lesions, suggesting that Lp-PLA<sub>2</sub> may be a marker of rupture-prone plaque. As Lp-PLA<sub>2</sub> is produced by macrophages and foam cells of atherosclerotic plaques that are numerous in unstable plaque, the differentiation between stable versus unstable plaque could be established by the presence of elevated Lp-PLA<sub>2</sub>. In addition to this, Lp-PLA<sub>2</sub> is mainly associated with LDL particles in the blood and is the sole enzyme responsible for the hydrolysis of oxidized phospholipids on LDL particles producing oxidized fatty acid and lysophosphatidyl choline, which are well-established triggers of inflammation cascade. Thus, Lp-PLA<sub>2</sub> may represent a tool to identify rupture-prone plaques, and inhibiting Lp-PLA<sub>2</sub> may stabilize the plaque and may prevent heart attacks and strokes.

### Lp-PLA<sub>2</sub>, darapladib, and lipid-modifying medications

It has been established that Lp-PLA<sub>2</sub> is expressed abundantly in the necrotic core of coronary lesions and products of its activity may contribute to inflammation and cell death, rendering plaque vulnerable to rupture. Darapladib is a direct inhibitor of Lp-PLA<sub>2</sub> enzyme activity that has been shown to prevent necrotic expansion of human coronary atherosclerotic plaque, resulting in reduction of inflammation and cell death. Recently, a study compared the effects of 12 months of treatment with 160 mg darapladib or placebo on coronary atheroma deformability (intravascular ultrasound palpography) and plasma hs-CRP in 330 patients with angiographically documented coronary disease.<sup>34</sup> Changes in necrotic core size, atheroma size, and blood biomarkers were also determined. Background therapy was comparable between groups with no difference in LDL cholesterol at 12 months. Lp-PLA<sub>2</sub> activity was inhibited by 59% with darapladib ( $P < .001$  vs. placebo). Darapladib prevented necrotic core expansion ( $-0.5 \pm 13.9$  mm<sup>3</sup>;  $P = .71$ ) versus the placebo-treated group that showed an increase in necrotic core volume ( $4.5 \pm 17.9$  mm<sup>3</sup>,  $P = .012$ ). However, after 12 months of therapy, there were no significant differences noted between groups

in plaque deformability ( $P = .22$ ) or plasma hs-CRP ( $P = .35$ ).

Similarly, another study examined the effects of 40, 80, and 160 mg once daily darapladib or placebo on biomarkers of cardiovascular risk in 959 patients with CHD and CHD-risk equivalent patients receiving atorvastatin (20 or 80 mg).<sup>35</sup> Blood samples were analyzed for Lp-PLA<sub>2</sub> activity and other biomarkers. Darapladib 40, 80, and 160 mg inhibited Lp-PLA<sub>2</sub> activity by approximately 43%, 55%, and 66% compared to placebo, respectively ( $P < .001$ , weeks 4 and 12). In addition to this, darapladib 160 mg decreased IL-6 by 12.3% (95% CI  $-22\%$  to  $-1\%$ ;  $P = .028$ ) and hs-CRP by 13.0% (95% CI  $-28\%$  to  $+5\%$ ;  $P = .15$ ) compared to placebo. The result from these studies suggests that the darapladib may reduce plaque inflammation and stabilize plaques as well as atherosclerotic disease activity. It also highlights that the fact Lp-PLA<sub>2</sub> inhibition interferes with necrotic core expansion, further establishes the presence of Lp-PLA<sub>2</sub> in unstable high-risk plaques, and supports the Lp-PLA<sub>2</sub> proatherogenic role. However, further prospective outcome studies using direct Lp-PLA<sub>2</sub> enzyme inhibitors are needed to establish their favorable effects on cardiovascular events.

Lipid-modifying medications stabilize plaques with lipid core regression, decreased macrophage infiltration, and thickening of the fibrous cap. This has been shown consistently in coronary plaques as well as with combination lipid-modifying therapy in carotid plaques.<sup>36</sup> Consequently, use of all lipid-modifying medications in modifying Lp-PLA<sub>2</sub> activity makes sense. Studies performed with paravastatin, fluvastatin, atorvastatin, simvastatin, and rosuvastatin have been shown to decrease levels of Lp-PLA<sub>2</sub>.<sup>37-39</sup> In the Pravastatin Atorvastatin and Infection Therapy (PROVE or IT) study, intensive therapy with atorvastatin resulted in a 25% reduction of Lp-PLA<sub>2</sub> mass concentration.<sup>37</sup> The decrease in Lp-PLA<sub>2</sub> was associated with a corresponding decrease in LDL cholesterol secondary to the stabilizing of plaque activity. In contrast, studies with fenofibrates and omega-3 fatty acids lower Lp-PLA<sub>2</sub> levels, even in the absence of any changes in LDL cholesterol levels.<sup>40</sup> The Combination of Prescription Omega-3 Plus Simvastatin (COMBOS) trial assessed if therapeutic doses of omega-3 fatty acids added to stable statin therapy improved non-HDL cholesterol levels, triglyceride levels, and lipid parameters. The study reported that a significant reduction occurred in the level of Lp-PLA<sub>2</sub>. Patients treated with prescription omega-3 fatty acid had an additional significant 10.7% decrease in Lp-PLA<sub>2</sub> levels compared to the group without omega-3 fatty acid.<sup>41</sup> Likewise, combining niacin with statin significantly lowered Lp-PLA<sub>2</sub>, even when statins decreased LDL cholesterol to optimal levels. Kuvin et al<sup>42</sup> added 1 g/day of extended-release niacin preparation to statins in patients with stable coronary artery disease. After 3 months, a significant additional 20% reduction in Lp-PLA<sub>2</sub> was reported with niacin, although the baseline level of LDL cholesterol of patients receiving statin monotherapy was already in the therapeutic range. Both

these studies highlighted the additional opportunity to reduce Lp-PLA<sub>2</sub> levels even in patients whose LDL levels are well within the reference range. In another recent study of patient with statin intolerance, the cholesterol absorption inhibitor ezetimibe lowered Lp-PLA<sub>2</sub> mass concentration by 18%. Finally, a study using combination therapy of simvastatin, ezetimibe, colesvalum, and omega-3 fatty acids with dietary modification reported a >30% reduction in Lp-PLA<sub>2</sub> mass concentration.<sup>43</sup> In summary, all lipid-modifying medications, used alone or in combination, lowered Lp-PLA<sub>2</sub> mass concentration.

## Lp-PLA<sub>2</sub>, HDL cholesterol, and hepatitis C infection

Lp-PLA<sub>2</sub> is a marker of vascular inflammation predominantly bound to LDL particles, whereas 20% is bound to HDL.<sup>44</sup> However, this distribution of Lp-PLA<sub>2</sub> between HDL and LDL particles may be more variable.<sup>45</sup> Furthermore, the distribution of Lp-PLA<sub>2</sub> between LDL and HDL depends on the extent of its glycosylation, which can affect plasma Lp-PLA<sub>2</sub> activity. It has been shown that Lp-PLA<sub>2</sub> hydrolyzation of oxidized phospholipid on HDL contributes to the antioxidant function of HDL.<sup>44</sup> Consequently, it may suggest that the relationship of Lp-PLA<sub>2</sub> with HDL is more complex and is not fully understood at this time. For instance, in mice, in which the main component of cholesterol is HDL, Lp-PLA<sub>2</sub> is protective against the development of atherosclerotic disease and is bound exclusively to HDL particles, suggesting that maybe in humans high HDL–Lp-PLA<sub>2</sub> levels are needed for protection against atherosclerosis disease activity. However, to establish that this hypothesis of HDL-bound enzyme would be protective needs further investigation. Nevertheless, it puts forward several unanswered questions. First, does increased Lp-PLA<sub>2</sub> in patients with high HDL and low LDL contribute to antiatherosclerotic properties or is it a risk marker of atherosclerotic disease activity in these patients? Second, does the risk for heart attacks and strokes decrease in these patients even with high Lp-PLA<sub>2</sub>? Third, how does one differentiate that high Lp-PLA<sub>2</sub> in patients with high HDL and low LDL on aggressive medical therapy is coming from HDL but not from the plaque itself? Fourth, what would be the best treatment strategy for such patients, whether to treat them or not to treat them? Finally, is there a false-positive Lp-PLA<sub>2</sub> level?

Hepatitis C virus (HCV) is also linked to lipoproteins in serum. Lp-PLA<sub>2</sub> levels may become altered in the presence of high hepatitis viral load. A study evaluated whether chronic HCV infection could alter Lp-PLA<sub>2</sub> functions.<sup>46</sup> One hundred forty-five subjects were studied: 56 HCV and 52 HBV infected patients (pathological controls) and 37 healthy subjects as controls. This study reported that HCV infection may alter Lp-PLA<sub>2</sub> function and may contribute to HCV-related vascular damage. Similarly, another study also showed that HCV infection altered the platelet

activating factor/Lp-PLA<sub>2</sub> system, which improved after antiviral treatment.<sup>47</sup> Consequently, HCV infection should be considered while making treatment decisions for HCV-infected patients with altered levels of Lp-PLA<sub>2</sub>.

## Conclusion

The aim of preventive cardiology is to determine atherosclerotic disease activity, and shift our present focus from identification of stenosis, which is a focal disease, to identification of patients with inflamed and rupture-prone plaque. Numerous biomarkers have been proposed to better discern the vulnerability of plaque rupture, pathogenesis, or cardiovascular risk. Biomarkers like proinsulin, adiponectin, NT-pro-BNP, and IL-6 have either fallen by the wayside at least in enhancing cardiovascular risk prediction or warrant larger more robust studies. Although hs-CRP has somewhat shown enhanced cardiovascular risk prediction, it is less specific, more biovariable, and its effects are attenuated upon multivariate analysis with other inflammatory markers and traditional risk factors. Lp-PLA<sub>2</sub> is a biomarker that plays a critical role in the development of atherosclerosis and may be involved in the causal pathway of plaque inflammation and plaque rupture. The association of Lp-PLA<sub>2</sub> with cardiovascular risk among different population studies independent of classical risk factors makes the premise even stronger that Lp-PLA<sub>2</sub> is involved in progression of atherosclerosis to advanced rupture-prone unstable plaques. The inhibition of Lp-PLA<sub>2</sub> with darapal-dib prevented necrotic core expansion, further highlighting proatherogenic role of Lp-PLA<sub>2</sub>. In general, available clinical data suggest that risk conferred by an elevation of Lp-PLA<sub>2</sub> is about twofold for strokes as well as for coronary artery disease. Testing for Lp-PLA<sub>2</sub> appears to be a useful adjunctive tool to classical cardiovascular risk assessment, and intensification of therapy in individuals with elevated Lp-PLA<sub>2</sub> may offer additional benefits in stabilizing plaques and rendering them less rupture prone. Thus, elevated Lp-PLA<sub>2</sub> could be an alarming sign that the plaque is ready to crack. Furthermore, Lp-PLA<sub>2</sub> determination may provide a pivotal opportunity to appropriately classify the previous misclassified patients who are actually at high risk and in need of aggressive intervention. Nevertheless, further larger, more robust studies are needed to provide more statistical power to cardiovascular risk association with Lp-PLA<sub>2</sub>.

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