Inflammatory Biomarkers and Cardiovascular Risk Assessment. Current Knowledge and Future Perspectives

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Abstract: Cardiovascular disease is the leading cause of morbidity and mortality in the Western world. However, it appears that currently available risk assessment tools often underestimate risk, especially for patients in the intermediate-risk category. Considering the socioeconomic cost, it is imperative to correctly identify patients in the intermediate-risk category who would benefit from more aggressive treatment. A plethora of experimental and observational studies provide support that lipoprotein associated phospholipase A2 (Lp-PLA2) and secretory phospholipases A2 (sPLA2) as well as high sensitivity C-reactive protein (hsCRP) are useful biomarkers of cardiovascular risk. Particularly, Lp-PLA2 has also been addressed as pharmacological target and we are eagerly awaiting the results of ongoing phase III clinical trials. In this review we discuss the current literature regarding the pros and cons of these biomarkers.

Keywords: Atherosclerosis, biomarkers, cardiovascular disease, hsCRP, inflammation, Lp-PLA2, PAF-acetylhydrolase, sPLA2.

INTRODUCTION

It has been suggested that a considerable number of patients with cardiovascular disease (CVD) lack any of the so called conventional risk factors (cigarette smoking, diabetes, hypertension and hyperlipidemia) [1]. Indeed, a meta-analysis of 14 clinical studies determined that overall among patients with coronary artery disease (CAD), the prevalence of those with at least 1 of the 4 conventional risk factors was 84.6% in women and 80.6% in men [2]. Moreover, a large number of CVD events occur in intermediate-risk patients, as defined using current global risk assessment tools [3]. In this regard, there is scarcity of non-invasive tools for the prediction of future events on an individual basis as well as of established treatment strategies for this group of patients. Therefore, this emerging demand boosts research towards the discovery and evaluation of cost-effective risk biomarkers to identify intermediate-risk patients who would benefit from more aggressive treatment after stratifying them into higher risk categories.

Since inflammation participates in atherogenesis, a plethora of biomarkers of inflammation have been evaluated, including white blood cell count, serum amyloid A, fibrinogen, von Willebrand factor, erythrocyte sedimentation rate, high sensitivity C-reactive protein (hsCRP), oxidized phospholipids/apolipoprotein B (apoB), interleukin (IL)-6, IL-8, circulating levels and enzymatic activity of phospholipases A2 [lipoprotein-associated phospholipase A2 (Lp-PLA2) and secretory phospholipases A2 (sPLA2s)]. However, the usefulness of these biomarkers and their incremental benefit in risk prediction remains ambiguous. In addition, since different biomarkers may reflect distinct aspects of atherogenesis, their combination may further improve risk prediction.

Among the whole spectrum of candidate biomarkers of cardiovascular risk, two families of phospholipase A2 enzymes and hsCRP will be discussed in detail. hsCRP is generally accepted as a classic risk biomarker in cardiovascular medicine, whereas the above phospholipases A2 have been chosen to be discussed in the present review, since there is substantial clinical evidence that they may represent not only biomarkers but also cardiovascular risk factors, while specific inhibitors are currently evaluated in clinical trials.

SEARCH STRATEGY

Reviewers independently searched the Medline (up to 1993) bibliographic database. Any article considered potentially relevant by any reviewer was retrieved for full review. The search strategy involved the use of the keywords “cardiovascular risk”, “inflammation”, “phospholipase A2”, “hsCRP” in any field (title, abstract and/or the main body) of papers. Inclusion criteria were studies: i. of any design (including original papers, systematic reviews, meta-analyses and case reports), ii. written in English language. A total of 663 papers were screened and 200 potentially relevant full-text articles were retrieved.

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (Lp-PLA2)

Biochemistry-Pathophysiology

Lp-PLA2 is a calcium-independent 45-kDa phospholipase A2 that is produced and secreted principally by macrophages, as well as monocytes, T-lymphocytes, mast and liver cells [4-7]. Lp-PLA2 in plasma is complexed to lipoproteins, primarily bound to low-density lipoprotein (LDL) (80%) whereas a small proportion is associated with high-density lipoprotein (HDL) [8,9]. We have shown that among LDL subfractions, Lp-PLA2 activity is preferentially associated with atherogenic small-dense LDL particles and beyond that, it actually represents a marker of these particles in human plasma [10,11]. Furthermore, Lp-PLA2 is bound to very low-density lipoprotein (VLDL) and likewise to intermediate density lipoprotein (IDL) and lipoprotein (a) [Lp(a)] [5]. In fact, the presence of Lp(a) in concentrations exceeding 30 mg/dl may influence the distribution of Lp-PLA2 between LDL and HDL [12].

Lp-PLA2 is comprised by a serine/aspartate/histidine catalytic triad at its active site, whilst it is closely related to neutral lipases and serine esterases [13,14]. Lp-PLA2 specifically hydrolyses short acyl chains (up to 9 methylene groups) at the sn-2 position of the phospholipid substrate. The first substrate described for Lp-PLA2 was platelet-activating factor (PAF) [15,16], a biologically active phospholipid that is implicated in atherogenesis and in several other pathophysiological conditions [5,8]. PAF is hydrolyzed and inactivated by Lp-PLA2, thus Lp-PLA2 is also denoted as PAF-acetylhydrolase [5].

Of paramount importance Lp-PLA2 also hydrolyzes oxidized phospholipids (oxPL) [17,18], generating oxidized free fatty acids.
Lp-PLA2 Gene Polymorphisms

Polymorphisms in the gene for Lp-PLA2 (PLA2G7), located on chromosome 6p12.1-12, significantly contribute to inter-individual variation in Lp-PLA2 activity and mass [32]. Indeed, several missense polymorphisms within the coding regions of PLA2G7 have been described. The Asia-specific PLA2G7 994G-T transversion leads to V279F substitution. The V279F variant is encountered in nearly 30% of the Japanese population resulting in absent catalytic activity of Lp-PLA2 in homozygotes and significantly reduced one in heterozygotes [33,34]. The prevalence of the V279F null allele was 11.5% in 2,690 cases with CAD (before the age 60) and 12.8% in 3,128 controls. The odds ratio (OR) for CAD for this allele was 0.80 (95% confidence interval [CI] 0.66-0.97, p=0.02), following adjustment for age, BMI, diabetes, smoking, glucose and lipid levels. In addition, natural deficiency in Lp-PLA2 activity due to carriage of V279F allele protects Korean men from CAD [35]. These results provide evidence for a causal relationship between Lp-PLA2 and CAD.

Association of Lp-PLA2 with CVD Risk

During the past decade, a number of studies carried out in primary and secondary prevention settings as well as meta-analyses have documented a strong relationship between Lp-PLA2 and CVD risk across a wide variety of subjects of both sexes, of different ethnic backgrounds and ages [36,37] (Table 1).

In the overwhelming majority of studies Lp-PLA2 remained an independent predictor of future events even after adjustment for several conventional risk factors. A log-linear association of Lp-PLA2 activity and mass with risk of CAD and vascular death was observed in a meta-analysis of 32 prospective studies with 79,036
participants. Relative risks adjusted for conventional risk factors, were: 1.10 (95% CI 1.05-1.16) and 1.11 (1.07-1.16) for CAD; 1.08 (0.97-1.20) and 1.14 (1.02-1.27) for ischaemic stroke; 1.16 (1.09-1.24) and 1.13 (1.05-1.22) for vascular mortality; and 1.10 (1.04-1.17) and 1.10 (1.03-1.18) for non-vascular mortality, for Lp-PLA2 activity and for Lp-PLA2 mass respectively [36].

Primary Prevention Studies

Primary prevention studies, essentially sustain that Lp-PLA2 is a risk marker for CVD. In the West of Scotland Coronary Prevention Study (WOSCOPS), a trial evaluating the role of pravastatin in the prevention of coronary events in hyperlipidemic, middle-aged men (average age 56.8 years) followed-up for 5 years, an independent dose-related relationship between Lp-PLA2 plasma levels and CAD risk was demonstrated [38]. In this study, for 1-standard deviation (SD) increase in Lp-PLA2 an 18% increase in risk was observed, even after controlling for other traditional risk factors and hsCRP. In contrast to other inflammatory biomarkers, including white cell count, CRP and fibrinogen, the association between elevated Lp-PLA2 levels and coronary risk remained unaffected by classic cardiovascular risk factors including age, BMI, systolic and diastolic blood pressure (BP) and smoking. The Atherosclerosis Risk in Communities (ARIC) Study was a large prospective study, involving 12,819 middle-aged men and women followed-up for 6 to 8 years. In a prospective case-cohort study Lp-PLA2 plasma levels were higher in 608 cases with incident CAD compared with 740 controls [39,171]. Following multivariable adjustment, an association between elevated Lp-PLA2 levels and CAD was evident exclusively in subgroups with LDL-cholesterol <130 mg/dL [39]. In the MONICA (MONItoring of Trends and Determinants in Cardiovascular Dis-

### Table 1. Lp-PLA2 in Primary and Secondary Prevention Studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Year</th>
<th>Population</th>
<th>Years of Follow-up</th>
<th>Endpoints</th>
<th>Major Findings</th>
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<tbody>
<tr>
<td><strong>Primary Prevention Studies</strong></td>
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<tr>
<td>Packard et al. [38]</td>
<td>2000</td>
<td>Hypercholesterolemic men (cases=580, controls=1,160)</td>
<td>4.9</td>
<td>Cardiac death, nonfatal MI, coronary revascularization</td>
<td>RR 1.18 (1.05-1.33) per 1-SD increase</td>
</tr>
<tr>
<td>Ballantyne et al. [39,171]</td>
<td>2004</td>
<td>Apparently healthy middle-aged men and women (cases=608, controls=740)</td>
<td>6</td>
<td>Death, nonfatal MI, coronary revascularization</td>
<td>HR 1.15 (0.81-1.63) for Q3 vs Q1</td>
</tr>
<tr>
<td>Koenig et al. [57]</td>
<td>2004</td>
<td>Apparently healthy middle-aged men (N=934)</td>
<td>14</td>
<td>Fatal or nonfatal coronary events</td>
<td>HR 1.21 (1.01 to 1.45) per 1-SD increase</td>
</tr>
<tr>
<td>Blake et al. [41]</td>
<td>2001</td>
<td>Apparently healthy middle-aged women (cases=123, controls=123)</td>
<td>3</td>
<td>Cardiac death, nonfatal MI, and stroke</td>
<td>RR 1.17 (0.45-3.05) for Q4 vs Q1</td>
</tr>
<tr>
<td>Hatoum et al. [42]</td>
<td>2011</td>
<td>Apparently healthy women (cases=421, controls=842)</td>
<td>14</td>
<td>MI</td>
<td>RR 1.75 (1.09-2.84)</td>
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<tr>
<td><strong>Secondary prevention studies</strong></td>
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<tr>
<td>Brilakis et al. [44]</td>
<td>2005</td>
<td>Patients undergoing coronary angiography (N=466)</td>
<td>4</td>
<td>Death, MI, coronary revascularization and stroke</td>
<td>HR 1.30 (1.06-1.59) per 1-SD increase</td>
</tr>
<tr>
<td>Corsetti et al. [45]</td>
<td>2006</td>
<td>Postinfarction patients (N=766)</td>
<td>2.1</td>
<td>Cardiac death, MI and unstable angina</td>
<td>HR 1.90 (1.31-2.75)</td>
</tr>
<tr>
<td>Sabatine et al. [47]</td>
<td>2007</td>
<td>Patients with stable CAD (N=3,766)</td>
<td>4.8</td>
<td>Death, MI, coronary revascularization, hospitalization for unstable angina and stroke</td>
<td>HR 1.41 (1.17-1.70) for Q4 vs Q1</td>
</tr>
<tr>
<td>Gerber et al. [48]</td>
<td>2006</td>
<td>Patients with acute MI (N=271)</td>
<td>1</td>
<td>Death</td>
<td>HR 7.61 (2.88-20.01) for Q3 vs Q1</td>
</tr>
<tr>
<td>Koenig et al. [49]</td>
<td>2006</td>
<td>Patients aged 30 to 70 years with diagnosis of CAD within the past 3 months (N=1,051)</td>
<td>4</td>
<td>Cardiac death, nonfatal MI and stroke</td>
<td>mass: HR 2.09 (1.10-3.96) activity: 1.81 (0.94-3.49) for Q3 vs Q1</td>
</tr>
<tr>
<td>O'Donogue et al. [51]</td>
<td>2006</td>
<td>Patients with ACS [at baseline (N=3,648) and after 30 days (N=3,265)]</td>
<td>2</td>
<td>Death, MI, unstable angina requiring hospitalization, revascularization and stroke</td>
<td>At 30 days HR 1.33, (1.01-1.74)</td>
</tr>
<tr>
<td>May et al. [50]</td>
<td>2006</td>
<td>Patients undergoing coronary angiography (N=1493)</td>
<td>6.7</td>
<td>Death, MI and stroke</td>
<td>CAD death HR 2.18 (1.04-4.57) for Q1 vs Q3, HR 1.73 (0.84-3.61) for Q1 vs Q4</td>
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Abbreviations: ACS: acute coronary syndromes, CAD: coronary artery disease, HR: hazard ratio, MI: myocardial infarction, RR: relative risk
Lp-PLA2 plasma levels was associated with 40% higher risk for future coronary events in 934 middle-aged men free from CVD [40]. A nested case-control analysis of 246 low-risk women from the Women’s Health study (mean age 60 years) reported higher Lp-PLA2 plasma levels in women with increased CAD risk, but adjustment for other CVD risk factors eliminated this association [41]. Recently, one case-control study examined the association between Lp-PLA2 and CAD in a large sample of women free of CVD, followed-up for 14 years. In this study, Lp-PLA2 activity was significantly associated with myocardial infarction (MI) [relative risk (RR) 2.86 for extreme quartiles, 95% CI 1.98-4.12], even after adjustment for lipids, inflammatory and clinical risk factors (RR 1.75, 95% CI 1.02-2.84). This study also reported that the addition of Lp-PLA2 activity to a multivariate adjusted model increased the receiver operating characteristics (ROC) curves and significantly improved the net reclassification improvement index [42].

Negative results were reported in a large prospective case-control study, the European Prospective Investigation of Cancer (EPIC)-Norfolk, involving 1,002 cases who developed fatal or non-fatal CAD matched to 1,859 controls over a 10-year period [43]. In this study, following adjustment for waist circumference, physical activity, smoking, diabetes, systolic BP, LDL-cholesterol and HDL-cholesterol levels, and for hormone replacement therapy in women, Lp-PLA2 was not associated with an increased CAD risk, in contrast to CRP, myeloperoxidase (MPO) (in men only), sPLA2 and fibrinogen.

**Secondary Prevention Studies**

Brilakis et al. evaluated 466 consecutive men and women with stable CAD undergoing coronary angiography, and showed that high Lp-PLA2 plasma levels was a significant and independent risk factor for major adverse events (death, MI, coronary revascularization and stroke) over a 4 year period [44]. After multivariable adjustment, an 1-SD increase in Lp-PLA2 mass was associated with a RR of 1.30 for major adverse events [44]. Lp-PLA2 plasma levels correlated with the extent of angiographic CAD on univariate but not on multivariate analysis. In line were also the results of another study in 766 post-infarction patients of the Thrombogenic factors and recurrent Coronary Events (THROMBO) study which substan-
tiated that high Lp-PLA2 activity was a significant and independent predictor of recurrent coronary events [45]. Of importance, in this study, adding Lp-PLA2 activity to the multivariable model replaced apoB as an independent predictor of risk. In accordance, Caslake et al. evidenced that Lp-PLA2 plasma levels were stronger predictor of CVD risk than apoB, in patients with CAD [46].

Plasma Lp-PLA2 mass was measured in 3,766 patients with stable CAD from the Prevention of Events with Angiotensin Converting Enzyme Inhibition (PEACE) trial [47]. Patients were followed-up for a median of 4.8 years for adverse cardiovascular events (including, death, MI, coronary revascularization, hospitalization for unstable angina and stroke). After adjustment for baseline characteristics, patients in the higher quartiles of Lp-PLA2 remained at significantly greater risk for the primary endpoint (p<0.001 for trend, adjusted hazard ratio (HR) 1.41 (95% CI 1.17 to 1.7, for patients in higher quartile compared with the lowest quartile) [47]. Gerber et al. scrutinized 271 residents of Olmsted County to evaluate the role of Lp-PLA2 plasma levels as a predictor of death 1 year after acute MI. Following adjustment for age and sex, HRs for death in the middle and upper Lp-PLA2 tertiles were 2.20 (95% CI, 0.88-5.54) and 4.93 (95% CI, 2.1-11.6) compared with the lowest tertile, respectively. Patients with Lp-PLA2 mass < 218 ng/mL had a 95% 1-year survival rate and those with < 166 ng/mL had approximately a 98% 1-year survival rate [48]. Notably, in this study blood was drawn at 43 ± 39 h after acute MI.

An independent association between Lp-PLA2 plasma levels and activity measured within 3 months following a coronary event has been reported in a cohort of 1051 patients with CAD, followed-up for 4 years [49]. In multivariable analyses, Lp-PLA2 mass and activity were vigorously associated with cardiovascular events after controlling for traditional risk factors, severity of CAD, statin treatment, cystatin C, and N-terminal B-type natriuretic peptide (NT-proBNP). The HR for recurrent events was 2.65 (95% CI 1.47 to 4.76) for the top tertile of Lp-PLA2 mass compared with the bottom tertile and 2.40 (95% CI, 1.35 to 4.29) for Lp-PLA2 activity. After additional adjustment for LDL-cholesterol, the HRs were only moderately attenuated (mass: 2.09; 95% CI 1.10 to 3.96; activity: 1.81; 95% CI 0.94 to 3.49, respectively), but the Lp-PLA2 activity was no longer statistically significant. The authors concluded that Lp-PLA2 mass may be a promising biomarker for risk prediction in secondary prevention of CVD.

The Intermountain Heart Collaborative Study measured Lp-PLA2 mass and hsCRP in 1,493 consecutive patients undergoing coronary angiography, followed-up for 6.7 years. A stepwise progression in CAD death associated with elevated Lp-PLA2 plasma mass concentrations was observed. Lp-PLA2 showed an additive effect with hsCRP on CAD risk prediction [50].

Lp-PLA2 activity measured 30 days following an acute coronary event among 3,265 patients enrolled in the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE-IT-TIMI 22) trial was positively associated with adverse outcomes, even after adjustment for other risk factors. Patients with an Lp-PLA2 activity in the top quintile at day 30 had a 33% increased risk for recurrent events (RR 1.33; 95% CI 1.01 to 1.74) over 2 years of follow-up, compared with patients in the lowest quintile [51]. However, in this trial baseline Lp-PLA2 activity or mass was not associated with CVD risk, while the death rate was low.

The Myocardial Ischemia Reduction With Acute Cholesterol Lowering (MIRACL) trial investigated the relationship of Lp-PLA2 (and sPLA2 discussed in the next chapter) levels and activity with cardiovascular events in 2,587 patients with a recent MI, at baseline and after 16 weeks of treatment with atorvastatin 80 mg daily or placebo [52]. Baseline levels of Lp-PLA2 mass and activity were not associated with the primary endpoint (death, MI or unstable angina).

**Lp-PLA2 as a Predictor of Ischemic Stroke**

Lp-PLA2 has emerged as a predictor of ischemic stroke based on the outcomes of several large clinical trials. A positive association between plasma Lp-PLA2 concentration and the risk for ischemic stroke was substantiated in the Rotterdam study. In this cohort of 7,983 subjects above the age of 55 years, ischemic stroke occurred in 110 individuals during a median follow-up time of 6.4 years. The age- and sex-adjusted HR was 2.0 between the first and fourth quartiles for Lp-PLA2 activity [53]. Likewise, 194 cases of ischemic stroke were identified in the ARIC study during a 6-year follow-up. Baseline Lp-PLA2 plasma levels differed significantly between stroke and control groups (443 and 374 μg/L, respectively, p<0.001) [39]. Lp-PLA2 and CRP concentrations were complementary in the determination of stroke risk in this study.

**Lp-PLA2 in Subclinical Atherosclerosis**

Regarding the association of Lp-PLA2 with subclinical atherosclerosis, data from a large cross-sectional study using carotid artery intima media thickness reported no independent association with Lp-PLA2 activity after adjustment for cholesterol levels [54]. In the Rotterdam Coronary Calcification Study the OR of having a total calcium score above 1,000 per SD of Lp-PLA2 activity (measured 7 years before scanning) was 1.6 (95% CI 1.1-2.4), an association which was eliminated after adjustment for non-HDL-cholesterol and HDL-cholesterol [55].
Inflammatory Biomarkers of CVD Risk

_Lp-PLA2 as a Negative CVD Predictor_

Low Lp-PLA2 levels may be negatively correlated with CVD, which in turn may indicate plaque stabilization in patients with CAD. In a cohort of 1,051 patients (aged 30-70 years) with CAD, 95% of high-risk patients with Lp-PLA2 levels below 223 ng/mL remained free of cardiovascular events if, over 4-6 years of follow-up [49]. In one study involving 504 patients with CAD, followed-up for 7 years, Lp-PLA2 mass in the bottom tertile (<200 ng/mL) was associated with a cardiovascular event rate of 5% compared with an event rate above 10% for patients in the higher tertile for Lp-PLA2 [44].

_Lp-PLA2 Correlations with Traditional Risk Factors_

In nearly all studies, correlations with traditional risk factors were negligible, excluding LDL-cholesterol, while no correlation was observed with CRP [56-58]. In contrast, CRP has been shown to correlate with most traditional risk factors such as smoking and BMI. Thus, from this point of view Lp-PLA2 is advantageous and may be more specific for vascular inflammation in patients with abdominal obesity or the metabolic syndrome. As widely known, metabolic syndrome is highly correlated with CVD risk, especially in the intermediate risk category of patients [59]. Since Lp-PLA2 has been shown to be independent of insulin resistance, it could be additive to the metabolic syndrome as a risk predictor [60,61]. Finally, it should be noted that plasma levels of Lp-PLA2 activity and mass are increased in uremic patients compared with controls [62,63].

A synergistic effect between Lp-PLA2 and hsCRP in CVD risk prediction has been reported [57]. In the MONICA study, the combination of both elevated Lp-PLA2 and elevated CRP was consistently associated with an increased risk for future coronary events and was superior to either marker alone in predicting risk, with an HR of 1.93 (95% CI 1.09-3.40). This is also in line with the findings of another study, which showed an additive effect between the two markers [50].

_Lp-PLA2 Mass or Enzyme Activity?_

Lp-PLA2 can be evaluated via assessment of either mass or activity of the enzyme and it is not clear-cut which of these parameters is the best marker. In this regard, a mass assay for Lp-PLA2 measurement has gained Food and Drug Administration (FDA) approval (PLAC assay; diaDexus), and may be feasible in the clinical setting whilst all activity assays are currently used only for research purposes [64].

_Predictive value of Lp-PLA2 in Patients Receiving Hypolipidemic Therapy_

We and others have demonstrated that lipid lowering agents, and particularly statins, significantly reduce plasma Lp-PLA2 mass and activity [52,65-68]. However it has not been established yet whether Lp-PLA2 mass or activity retains its predictive value after LDL-cholesterol reduction by statin therapy. In this regard, Lp-PLA2 mass and activity were recently evaluated before and after treatment with 20 mg rosuvastatin or placebo in the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial in 17,802 men and women without CVD [69]. Rosuvastatin induced a 33.8% reduction in Lp-PLA2 mass and a 33.2% reduction in Lp-PLA2 activity. Among patients allocated to placebo, increasing quartiles of Lp-PLA2 activity [p(trend)=0.04] but not Lp-PLA2 mass [p(trend)=0.92] were associated with incident cardiovascular events after adjustment for LDL-cholesterol and other conventional risk factors. On the other hand, among those allocated to rosuvastatin no significant relationship between Lp-PLA2 levels and subsequent vascular events was shown. The ability of rosuvastatin to reduce vascular events was not significantly modified by baseline Lp-PLA2 levels. Nonetheless, patients with Lp-PLA2 activity in the higher second, third and fourth quartiles had a somewhat greater RR reduction compared with patients at the first quartile [69]. Similarly, in the ATPIII guidelines for the use of inflammatory markers, recommendations were made that Lp-PLA2 mass or activity retain its predictive value after LDL-cholesterol reduction by statin therapy [70]. In addition, the American Association of Clinical Endocrinologists (AACE) published new guidelines in March/April 2012 that endorse the use of Lp-PLA2 as a vascular specific inflammatory biomarker [72].

**HDL-Associated Lp-PLA2**

All the above mentioned studies concern the total plasma Lp-PLA2, which mainly represents the LDL-associated enzyme. However, the role of Lp-PLA2 in atherosclerosis may depend on the type of lipoprotein with which it is associated [8]. Thus, in contrast to the LDL-associated Lp-PLA2, several lines of evidence suggest that HDL-associated enzyme (HDL-Lp-PLA2) may substantially contribute to the HDL antiatherogenic activities [8]. In this regard, we recently published the results of a prospective study assessing the prognostic value of HDL-Lp-PLA2 in a cohort of 524 consecutive patients with stable CAD, followed-up for a median of 34 months [9]. Primary endpoint consisted in cardiac death, while secondary endpoints in hospitalisation for ACS, myocardial revascularization, arrhythmic event or stroke. HDL-Lp-PLA2 mass and activity were associated with lower risk for cardiac death (HR 0.972; 95% CI 0.952 to 0.993; p=0.010; and HR 0.689; 95% CI 0.496 to 0.957; p=0.026, respectively) after adjustment for traditional risk factors for CAD [9]. These results suggest that HDL-Lp-PLA2 may be a clinically useful tool in the assessment of the residual cardiovascular risk, a hypothesis that needs to be further supported by large scale clinical studies.

**Darapladib as a Selective Lp-PLA2 Inhibitor**

Darapladib (SB-480848) binds to the serine residue of Lp-PLA2, active site and selectively and efficiently inhibits enzyme activity [73]. In the porcine coronary model, darapladib significantly decreased necrotic core areas [74]. In the first published human study
the effect of darapladib 40, 80 or 160 mg or placebo and atorvastatin 20 or 80 mg for 12 weeks on plasma Lp-PLA₂ activity, IL-6, and hsCRP levels, in 959 CHD patients was assessed. Darapladib 40, 80, and 160 mg inhibited Lp-PLA₂ activity by approximately 43, 55, and 66% compared with placebo (p<0.001 weeks 4 and 12). Darapladib 160 mg also reduced IL-6 (p=0.028) by 12.3% but failed to alter hsCRP levels compared with placebo [75]. In the Integrated Biomarker and Imaging Study 2 (IBIS-2) trial darapladib 160 mg inhibited Lp-PLA₂ activity by 59% compared with placebo in 330 patients with angiographically documented CAD followed up for 12 months [76]. Nevertheless, no significant difference in the primary outcome of plaque deformability as assessed via intravascular ultrasound (IVUS) was observed. However, darapladib prevented the increase of necrotic core volume, which was significantly augmented in the placebo group. No major safety concerns were noted with darapladib. Malodor of the urine and feces was reported in 16% of darapladib-treated subjects compared with 3% of the placebo group.

We are currently expecting the results of 2 ongoing phase III trials: Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy Trial (STABILITY), which includes more than 15,000 patients with stable CAD [77], and the Stabilization of Plaques Using Darapladib–Thrombolysis in Myocardial Infarction 52 Trial (SOLID-TIMI 52), which includes 11,500 patients with acute coronary syndromes (ACS) [78]. These studies will determine whether inhibition of Lp-PLA₂ activity with darapladib safely reduces adverse cardiovascular events and death.

SECRETORY PHOSPHOLIPASE A₂ (sPLA₂)

Biochemistry-Pathophysiology

The sPLA₂ family consists of 10 disulfide-rich low molecular mass isoenzymes, namely sPLA₂-IB, -IIA, -IIC, -IID, -IIE, -IIF, -III, -V, -X and -XIIA, that are involved in a variety of biological processes [79]. sPLA₂ enzymes share a common His-Asp catalytic dyad. A subset of sPLA₂ isoforms have been associated with atherosclerosis, namely sPLA₂-IIA (13.9 kDa), -III (18.3 kDa), -V (13.8 kDa), and -X (13.6 kDa), as described by animal and in vitro studies [80-88]. sPLA₂-IIA, V, X and III have been detected in human and mouse atherosclerotic lesions [89-93]. sPLA₂-IIA is an acute phase reactant and has been shown to be induced by cytokines such as IL-1 and TNF-α [89,94,95]. sPLA₂-V, but not sPLA₂-X, is also highly upregulated during inflammation while their expression is downregulated by antiinflammatory cytokines [96]. In addition, sPLA₂ is also increased several hours after an ACS, and thereby precedes increases in CRP [97]. Unlike CRP, sPLA₂ may play an important role in the pathogenesis of CAD, since sPLA₂ßerote the production of proinflammatory mediators via their catalytic activity (Fig. 2). *In vitro* incubation of LDL and HDL lipoproteins with several sPLA₂S provokes phosphatidylcholine hydrolysis in the sn-2 position yielding lyso-PC and unsaturated fatty acids (FA) [84,85,92,93,98,99]. sPLA₂-IIA by hydrolyzing the phospholipids on LDL particles, induces formation of small-dense LDL particles that have been shown to be highly atherogenic (Fig. 2). Additively, through the hydrolysis of of LDL phospholipids, sPLA₂ alters the conformation of apoB thus enhancing LDL retention to matrix pro-

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**Fig. (2).** Schematic representation of the proatherogenic effects induced by secretory phospholipase A₂ (sPLA₂). In addition, sPLA₂ hydrolyzes phospholipids (PL) on LDL particles yielding lysophosphatidylcholine (lyso-PC) and unsaturated fatty acids (FA). Thus, sPLA₂ promotes the modification of LDL, inducing the formation of the proatherogenic small dense LDL particles (sdLDL). sdLDL bind with higher affinity to matrix proteoglycans compared with buoyant LDL particles and are more susceptible to oxidation thus promoting foam cell formation. Furthermore, lyso-PC formed under the action of sPLA₂, exerts proinflammatory actions and may elicit events promoting atherogenesis.
teoglycans [100,101] (Fig. 2). In addition, sPLA₂ enhances lipid peroxidation in lipoproteins [102,103].

Besides proatherogenic functions associated with their enzymatic activity, sPLA₂s may also stimulate inflammatory responses by nonenzymatic mechanisms mediated by binding of sPLA₂s to specific membrane receptors, including the M-type receptor [104]. The sPLA₂-mediated hydrolysis of phospholipids leads to the production of free unsaturated FA such as arachidonic acid, the precursor of various proinflammatory mediators such as leukotrienes and prostaglandins [105]. Of note, individual enzymes exert distinct functions in vivo. Group X sPLA₂ preferentially hydrolyses arachidonate and linoleate containing phospholipids, while group V sPLA₂ hydrolyses the linoleate containing phospholipids in preference to polyunsaturated containing ones. The group IIA enzyme appears to hydrolyze invariably all diacyl phospholipids [93]. In addition to LDL, sPLA₂s hydrolyze the phospholipids on HDL particles thus reducing the capacity of this antiatherogenic lipoprotein to promote cholesterol efflux from lipid-rich foam cells [93,106].

Association of sPLA₂ with CVD Risk

**Primary Prevention Studies**

Several studies indicate an independent association of sPLA₂ with CVD (Table 2). In 2 nested case-control studies from the population participating in the EPIC-Norfolk trial, increased levels of sPLA₂ mass and activity were associated with the risk of a coronary event during 6 years of follow-up in otherwise healthy individuals [107,108]. sPLA₂ levels were higher in cases than controls among men and women. sPLA₂ plasma levels significantly correlated with age, BMI, systolic BP, HDL-cholesterol, and CRP levels [107]. After adjustment for traditional cardiovascular risk factors and CRP levels, those in the highest sPLA₂ quartile had a 34% increased risk compared with those in the lowest quartile. In this study, CRP was poorly correlated with sPLA₂ activity suggesting that the 2 biomarkers reflect distinct pathophysiological pathways, sPLA₂ activity showed weak correlation with sPLA₂ type IIA concentration (r=0.20), since that measurement of sPLA₂ activity encompasses several types of sPLA₂, including IIA, V and X. It was recently shown that measurement of circulating sPLA₂ enzyme activity, which encompasses several types of sPLA₂, including sPLA₂ type IIA, V, and X, provides better prognostic value than sPLA₂ type IIA concentration while combined with CRP provides additive prognostic value than either biomarker alone [109].

**Secondary Prevention Studies**

Increased sPLA₂ levels are linked to increased CVD risk and have been suggested to be an independent CVD risk factor in secondary prevention patients, as evidenced by several epidemiological studies (Table 2). In a prospective study, involving 142 CAD patients and 93 control subjects, high levels of sPLA₂ (> 246 ng/dL; 75th percentile of sPLA₂ distribution in controls) were a significant independent risk factor for the presence of CAD in multivariate logistic regression analysis. In multivariate Cox hazard analysis, the higher levels of sPLA₂ were a significant predictor of developing coronary events during a 2-year follow-up period in patients with CAD, independently of other risk factors, including CRP [110]. Similar results were obtained in patients with unstable angina [97,111]. Plasma levels of sPLA₂-IIA were measured in 52 patients with unstable angina, in 107 patients with stable angina, and in 96 control subjects. This study showed that unstable angina patients had significantly higher sPLA₂ levels than patients with stable angina and control subjects [111].

### Table 2. sPLA₂ in Primary and Secondary Prevention Studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Year</th>
<th>Population</th>
<th>Years of Follow-up</th>
<th>Endpoints Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Prevention Studies</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Boekholdt et al. [107]</td>
<td>2005</td>
<td>Apparently healthy men and women (cases=1,105, controls=2,209)</td>
<td>6</td>
<td>Fatal and nonfatal CAD OR 3.34 (1.02-1.71) per 1-SD increase Q4 vs Q1</td>
</tr>
<tr>
<td>Mallat et al. [108]</td>
<td>2007</td>
<td>Apparently healthy men and women (cases=991, controls=1,806)</td>
<td>6</td>
<td>Fatal and nonfatal CAD Activity: OR 1.56 (1.21-2.02) for Q4 vs Q1</td>
</tr>
<tr>
<td><strong>Secondary prevention studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mallat et al. [109]</td>
<td>2005</td>
<td>Patients with ACS (N=466)</td>
<td>0.5</td>
<td>Death or MI Activity: HR 3.08 (1.37-6.9) for Q3 vs Q1</td>
</tr>
<tr>
<td>Kugiyama et al. [110]</td>
<td>1999</td>
<td>Patients with CAD (cases=142, controls=93)</td>
<td>2</td>
<td>Cardiac death, revascularization, nonfatal MI OR 3.46 (1.4-8.3) for Q3 vs Q1</td>
</tr>
<tr>
<td>Kugiyama et al. [111]</td>
<td>1999</td>
<td>Patients with unstable angina (N=52), with stable angina (N=107) and 96 controls</td>
<td>2</td>
<td>Cardiac death, revascularization, nonfatal MI OR 5.08 (1.4-18.6) for Q4 vs Q1</td>
</tr>
<tr>
<td>O’Donogue et al. [112]</td>
<td>2011</td>
<td>Patients with stable CAD (N=3,708)</td>
<td>4.8</td>
<td>Cardiac Death, MI, stroke HR 1.47 (1.06-2.04) for Q4 vs Q1</td>
</tr>
<tr>
<td>Liu et al. [113]</td>
<td>2003</td>
<td>Patients undergoing PCI (cases=247, controls=100)</td>
<td>2</td>
<td>Cardiac death, nonfatal MI, recurrent angina pectoris and revascularization For sPLA₂ &gt;450 ng/dL; OR 2.1 (1.4-7.0)</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACS: acute coronary syndromes, CAD: coronary artery disease, HR: hazard ratio, MI: myocardial infarction, PCI: percutaneous coronary intervention, RR: relative risk.
Recently, the prognostic utility of sPLA2 activity was evaluated in 3,708 subjects with stable CAD, followed-up for 4.8 years in the PEACE randomized trial [112]. A stepwise increase in the risk of cardiovascular death, MI, or stroke by quartile of sPLA2 activity (p(trend)<0.001) was observed. Subjects with sPLA2 activity levels in the highest quartile had a 2-fold higher risk of cardiovascular death (HR 2.00, 95% CI 1.18-3.37, p=0.01) compared with patients with sPLA2 in the lowest quartile. A modest correlation between sPLA2 activity and concentrations of hsCRP (r=0.26, p<0.001) was observed. A weak correlation was also found between sPLA2 activity and Lp-PLA2 mass (r=0.05, p<0.001). After inclusion of hsCRP, Lp-PLA2 as well as traditional risk factors in a multivariable model, sPLA2 activity in the highest quartile remained significantly associated with an increased risk of cardiovascular death, MI, or stroke (adjusted HR 1.47, 95% CI 1.06-2.04, p=0.019) compared with the lowest quartile. Three additional studies have examined the prognostic role of sPLA2 in patients with stable CAD. In a retrospective case-control study of 142 patients with CAD, sPLA2 mass was significantly higher in cases than controls and independently associated with a higher risk of major adverse cardiovascular events [110]. In a second study of 247 patients undergoing percutaneous coronary intervention (PCI), increased sPLA2 concentration post-PCI was associated with increased risk of coronary events during 2 years of follow-up [113]. sPLA2 concentration and activity was measured in a cohort of 1024 subjects between the ages of 30 and 70 with CAD. In the multivariable model, higher tertiles of sPLA2 mass and activity were associated with HRs of secondary CVD events of 2.07 (95% CI 1.17-3.66) and 1.65 (95% CI 0.96-2.84) for mass and activity, respectively, compared with lowest quartiles [114]. However, when cystatin C, N-proBNP, CRP, and Lp-PLA2 were included in the model the associations were attenuated. Of note, few fatal events occurred during study follow-up (2.8% incidence of cardiovascular death after median follow-up of 4.6 years).

In the MIRACL trial (also discussed above) [52], baseline levels of sPLA2 mass and activity were not associated with the primary endpoint (death, MI or unstable angina). Following multivariable adjustment, baseline sPLA2 mass predicted risk of death (HR for 2-fold increase, 1.30; 95% CI 1.09-1.56; p=0.004) during a 16-week period after ACS. When analyzed separately, this association lost its significance in the atorvastatin group. Compared with placebo, atorvastatin reduced median sPLA2 mass (-32.1 vs -23.1%, p<0.001), sPLA2 activity (-29.5 vs -19.2%; p<0.001 for all). Atorvastatin reduced the hazard of death associated with elevated sPLA2 mass and activity by approximately 50% [52].

Varespladib as a Direct sPLA2 Inhibitor
Varespladib methyl (1H-indole-3-glyoxamide A-002, from Anthera Pharmaceuticals) specifically inhibits sPLA2-IIA, -V, and -X with high affinity [115,116]. Varespladib significantly reduced atherosclerosis as well as aneurysm formation in apoE-/- mice fed a high-fat diet [117,118]. In addition, varespladib and pravastatin reduced atherosclerosis by 50%, significantly more than varespladib monotherapy indicating that they may act in a complementary manner [118].

Phospholipase Levels And Serological Markers of Atherosclerosis (PLASMA) study, a phase II, randomised, double-blind, placebo-controlled, parallel-arm, dose-response study evaluated the effect of varespladib methyl treatment (50–500 mg twice daily for 8 weeks) on circulating sPLA2-IIA levels and various cardiovascular biomarkers in 393 patients with stable CAD [119]. The primary end point was the change in sPLA2-IIA mass or activity from baseline to week 8. The authors reported a dose-dependent decrease in sPLA2-IIA mass levels, which reached an 86.7% reduction in varespladib group compared with a 4.8% reduction in the placebo group. Mean LDL-cholesterol was reduced by 8% from baseline to week 8 in varespladib treated group, compared with a 1.7% increase in the placebo group. Varespladib treatment had no effect on hsCRP, arachidonic acid, or leukotriene B4 concentrations.

PLASMA II evaluated the effect of varespladib methyl in patients with stable CAD [120]. Stable coronary heart disease patients (n=135) were treated with either varespladib methyl 250 mg once daily, varespladib methyl 500 mg once daily, or placebo for 8 weeks. Varespladib methyl treatment dose-dependently reduced sPLA2 concentrations compared with placebo. When compared with placebo, varespladib methyl 500 mg once daily reduced LDL-cholesterol by 15% (p<0.001), non-HDL-cholesterol by 15% (p<0.001), VLDL particle concentration by 14% (p=0.022), and small VLDL particle concentration by 24% (p=0.030). No major safety concerns were noted with varespladib.

The Fewer Recurrent Acute Coronary Events With Near-Term Cardiovascular Inflammation Suppression-ACS (FRANCIS) trial investigated the effects of varespladib vs placebo as adjunctive therapy to atorvastatin 80 mg daily on cardiovascular biomarkers, major adverse cardiovascular events (unstable angina, MI, death), and safety in ACS patients. Varespladib therapy effectively reduced LDL-cholesterol and inflammatory biomarkers in ACS patients treated with conventional therapy including atorvastatin 80 mg daily. At 6 months, respective reductions with varespladib and placebo were as follows: LDL-cholesterol 43.5 vs 37.6% (p<0.05), hsCRP 79.8 vs 77.0% (p=0.02) and sPLA2-IIA 78.5 vs 64.4% (p<0.0001). However, major adverse cardiovascular events were not different from placebo 6 months post-randomization (7.3 vs 7.7% placebo).

The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome (VISTA-16) attempted to explore the hypothesis that varespladib methyl reduces cardiovascular risk in patients with ACS. In this a phase III clinical trial 6,500 patients were randomized to either varespladib methyl 500mg daily or placebo for 16 weeks, in addition to background treatment with atorvastatin and standard care. The primary efficacy point was the combination of cardiovascular death, nonfatal MI, nonfatal stroke or hospitalization for unstable angina [121]. However, the study was unexpectedly halted on March 2012 after the data safety monitoring board found that the drug was ineffective and recommended the study's premature termination. Results have not yet been published.

HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP)
hsCRP is the most well studied and validated inflammatory marker of CVD risk [122-127]. A great number of epidemiological studies and meta-analyses have firmly and consistently established a moderate association of hsCRP with an increased risk of cardiovascular events in primary as well as secondary risk prevention, independently of established risk factors [125,127-138]. In addition, assays for hsCRP are sensitive, reproducible, internationally standardized, relatively inexpensive, and widely available. However, several unresolved issues remain, chiefly among them whether hsCRP is causally involved in atherogenesis and its value as a therapeutic target.

The JUPITER Trial
Following the publication of the JUPITER trial, the interest for hsCRP has intensified [139]. In JUPITER 17,802 men (≥50 years of age) and women (≥60 years of age) with LDL-cholesterol levels <130 mg/dL and hsCRP ≥2 mg/L, free of CVD or diabetes were randomized to 20 mg rosuvastatin daily or placebo. During the follow-up (median follow-up period 1.9-year, maximum 5 years), rosuvastatin reduced LDL-cholesterol by 50% and hsCRP by 37%, and this result was associated with a 44% reduction in the primary end point (first occurrence of MI, stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death) (p<0.0001; 95% CI 0.46-0.69). The authors calculated that 95 people would need to be treated with rosuvastatin for 2 years to prevent one event. In a prespecified analysis, a 65% reduction in
major events was noted in patients who achieved LDL-cholesterol <70 mg/dL and hsCRP <2 mg/L vs a 33% reduction in patients who achieved only one or neither target (p<0.0001 across treatment groups). Accordingly, JUPITER substantiated the potential utility of hsCRP as a target for therapy in primary prevention of CVD. However, other investigators cast doubts on the conclusions of JUPITER since it is advocated that the great reduction of LDL-cholesterol levels (below normal) could have confounded the effect of hsCRP on the lower coronary event rate [140]. Furthermore, one could argue that JUPITER’s generalizability is limited, since approximately 100 people need to be treated with rosuvastatin for 2 years to prevent one event. Additionally, only 20% of all patients screened were eligible for the trial [140].

hsCRP and the Framingham Risk Score

CRP measurement was shown to significantly contribute to coronary event risk prediction independent of the Framingham risk score, particularly in those in the intermediate range, in 3435 white men of German nationality, 45 to 74 years of age [141]. In their recommendations in 2004 the American Heart Association and Centers for Disease Control and Prevention underscores that in individuals with moderate 10-year risk for cardiovascular morbidity (Framingham risk score between 10 and 20%), additional measurement of hsCRP - at the discretion of the physician - may be reasonable (Class IIA recommendation) [124]. Patients can be categorized using CRP-based risk categories of low (<1 mg/L), average (2 to 3 mg/L), and high (>3 mg/L) on the basis of the average of 2 measurements taken optimally at least 2 weeks apart. However, reliability and utility of hsCRP as an add-on to Framingham risk score has been disputed, and has been characterized as small and inconsistent [142,143]. Shah et al. attempted to evaluate hsCRP as a risk marker extracting data from 2 large population-based prospective studies [the Northwick Park Heart Study (NPHS-II) and the Edinburgh Artery Study (EAS)] using all 3 methods for evaluation of a risk marker, namely 1) discrimination, 2) calibration and 3) reclassification. This study demonstrated that hsCRP alone provided modest discrimination for CHD (AUC 0.61 and 0.62 in NPHS-II and EAS, respectively) and only modest improvement in the discrimination of a Framingham-based risk score (FRS) (increment in AUC 0.04 and -0.01, respectively). The addition of hsCRP to models based on established markers included in the Framingham equation did not substantially enhance the calibration. When hsCRP was added to a Framingham-based model using four categories of 10-year CAD risk, the proportion of subjects correctly reclassified (risk upgraded in eventual cases and risk downgraded in people remaining healthy) was almost matched by the proportion incorrectly reclassified. The authors concluded that although hsCRP is consistently associated with CAD and CVD risk, the incremental predictive performance of hsCRP in CAD is limited when added to conventional risk factors, regardless of the metric utilized and that former guidance on the clinical use of hsCRP measurement may require updating in the light of these findings.

hsCRP and Traditional Risk Factors

CRP is closely associated with classic risk factors such as hypertension, dyslipidemia and insulin resistance [38,144,145]. Subcutaneous adipose tissue is another source of IL-6 and therefore plasma levels of CRP, IL-6 and TNF-a are all related to measures of obesity [126]. CRP is produced in the liver in response to several inflammatory stimuli. Since CRP tends to be elevated in patients with a wide range of inflammatory conditions, its specificity as a marker for CHD is narrow. CRP may produce false positive results in patients with CAD because of infection, extravascular inflammation, injury, abdominal adiposity, or recent MI [146]. It may also be of limited utility in postmenopausal women, in whom it can be confounded by hormone replacement therapy, which in turn stimulates hepatic production of CRP [147,148]. In addition, hsCRP is also influenced by kidney function, and increases in uremic patients [149].

hsCRP, a Marker or a Factor of CVD Risk?

The majority of evidence regarding CRP as a CVD marker is derived from observational epidemiological studies, including cohort and case-control studies. However, the reliability of observational studies vs randomized studies has been scrutinized in a recent meta-epidemiology study across 31 meta-analyses [150]. In this study it was shown that epidemiological studies may differ from randomized controlled ones variously (including the risk of confounding, the extent of susceptibility to publication and selective reporting biases, and the characteristics of participants). Because of such differences, the prognostic effect derived from observational studies may be significantly stronger compared with randomized controlled trials [150]. Controversy remains as to whether hsCRP participates mechanistically in plaque formation and plaque rupture or is merely a marker. Accumulating evidence derived primarily from experimental studies (in vivo and in vitro) yields towards a mechanistic role of CRP in plaque formation [151-154]. Histological staining of atherosclerotic lesions show that CRP is localized within the atheroma, while CRP may also be produced locally by smooth muscle cells and macrophages [155,156]. Several potential proinflammatory mechanisms by which CRP may promote atherosclerosis have been proposed, derived from in vitro experiments testing the addition of exogenous CRP to cultured endothelial cells, smooth muscle cells, and monocytes/macrophages [157-160]. Exogenous CRP induced the expression of adhesion molecules (such as intracellular and vascular cellular adhesion molecules and E-selectin), decreased levels of endothelial nitric oxide synthase (NOS) and prostacyclin while increased levels of endothelin-1, upregulated angiotensin I receptors on smooth muscle cells and increased tissue factor release from macrophages [157,158,161]. In vivo administration of CRP promoted the uptake of oxidized LDL and intracellular cholesterol ester accumulation in rat macrophages in vivo [159]. Nevertheless, the purity of the exogenous CRP used in these experiments may have influenced these findings [162].

Genome-wide Association Studies

Studying the human genome may offer novel ways to examine the relationship between biomarkers and common, chronic human diseases. In this regard, recent genetic studies maintain that hsCRP lies outside the mechanistic path of atherosclerosis [163,164]. A large Mendelian randomization study examined the association of genetic loci with plasma CRP concentrations and risk of CAD. The authors found that genetic variants expected to lower the CRP expression by about 20% did not reduce CAD risk by the predicted amount of 6%. This discordance argued against a causal relationship between CRP and CAD [164]. In the same line another large study tested the association of 4 single-nucleotide polymorphisms in the CRP gene and the risk of vascular disease. The principal finding of this study is that CRP polymorphisms are associated with markedly increased CRP levels but not with an increased risk of ischemic heart disease or ischemic cerebrovascular disease [163].

Although there is evidence that CRP may be directly involved in the pathogenesis of atherosclerosis, the question of whether reduction in CRP levels and/or its associated downstream effects will provide novel therapeutic avenues to reduce cardiovascular risk requires further investigation [165,166].

Guidelines for the Use of hsCRP in CVD Risk Assessment

In the recently published guidelines on CVD prevention in clinical practice hsCRP measurement is recommended as part of refined risk assessment in patients with an unusual or moderate CVD risk profile while it should not be measured in asymptomatic low-risk patients and high-risk patient to assess 10 year risk of CVD. However, several weak points are underlined namely de-
CONCLUSION AND FUTURE PERSPECTIVES

In view that inflammation plays a key role in the development of atherosclerosis and CVD, a large number of inflammatory biomarkers have been proposed for CVD risk prediction. However, it is imperative that each biomarker is assessed using the appropriate metrics and not simply extracting results from observational studies, which may provide insufficient evidence. In addition, prior studies have reached differing conclusions regarding the utility of inflammatory biomarkers for cardiovascular risk prediction. Some reports indicate that such biomarkers aid risk prediction [169], whereas other studies conclude that these biomarkers contribute relatively minor information [170]. Among all inflammatory biomarkers, most of the existing data provide support that Lp-PLA₂ and sPLA₂ as well as hsCRP are valuable biomarkers of cardiovascular risk. The real value of these biomarkers is in patients at intermediate risk (10-20% by Framingham Risk Score), since patients with a low CVD risk (<10% 10 year risk) do not require further evaluation beyond traditional risk factors.

Importantly, the above-mentioned phospholipases have also been addressed as therapeutic targets and may have an important role in CVD prevention, risk stratification and personalised medicine. In this regard, varespladib, a specific sPLA₂ inhibitor, was demonstrated by several studies to be an effective antiatherosclerotic agent, since it showed a promising reduction in biomarkers and effects on surrogate end points. However, the phase III VISTA-16 varespladib trial was recently unexpectedly halted after the data safety monitoring board found no evidence that the drug was effective in ACS patients. To our knowledge, there are no other clinical trials are evaluating the cardiovascular benefits of varespladib and there are no other sPLA₂ inhibitors in advanced stages of clinical development for cardiovascular indications. In contrast, two phase III clinical trials STABILITY and SOLID-TIMI 52 for darapladib, a specific Lp-PLA₂ inhibitor are currently in progress. They will determine whether specific inhibition of Lp-PLA₂ activity safely reduces adverse cardiovascular events and death.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Inflammatory Biomarkers of CVD Risk


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