

Inflammatory markers in coronary heart disease

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Introduction: Inflammation plays a key role in the development of atherosclerosis and coronary heart disease (CHD).

Sources of data: Peer-reviewed studies published in English-language journals were reviewed with a focus on C-reactive protein (CRP) and lipoprotein-associated phospholipase A₂ (Lp-PLA₂).

Areas of agreement: Elevated levels of serum CRP and Lp-PLA₂ are associated with an increased risk of incident CHD events in both primary and secondary prevention studies across a wide range of age, gender and ethnic groups.

Areas of controversy: The utility of inflammatory markers in predicting CHD risk when added to traditional risk factors is under debate. They are most useful in subjects in the intermediate-risk category.

Growing points: Treatment with a statin in subjects with elevated CRP but without hyperlipidemia can reduce the risk of CHD.

Areas timely for developing research: Extensive research is under way to identify additional novel serum markers with higher specificity for coronary artery plaque inflammation. Specific inhibitors against vascular inflammation in combination with medications to lower low-density lipoprotein cholesterol, i.e. statins, may help prevent cardiovascular events in the future.

Keywords: inflammatory markers/C-reactive protein/lipoprotein-associated phospholipase A₂/coronary heart disease/epidemiology/prevention

Accepted: September 7, 2011

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Introduction

Atherosclerosis, the underlying pathology responsible for coronary heart disease (CHD), is an inflammatory disease.¹ Inflammation, at both focal and systemic levels, plays a key role in destabilization and rupture of atherosclerotic plaques, leading to acute cardiovascular (CV) events.² Current risk prediction models based on traditional risk factors have the ability to predict long-term CV risk in many individuals. Extensive research is under way to identify novel risk factors that can improve our ability to accurately predict CV risk, identify new targets for therapy and improve current prognostic algorithms.³

A role for inflammation in atherosclerosis was suggested in the early 20th century by Sir William Osler. In fact, elevated white blood cell counts have been known to be associated with CHD as early as the 1920s.⁴ Over the past few decades, much more attention has been paid to the role of inflammation in atherosclerosis. Pioneering work by Maseri and colleagues in the 1980s suggested that high-sensitivity C-reactive protein (hs-CRP) was increased in patients admitted for acute myocardial infarction (AMI).⁵ Subsequently, large epidemiological studies led by Ridker and colleagues established a role for CRP in the prediction of CHD events.^{6–9} A large number of additional inflammatory markers have been studied in this context, and the list is constantly growing (Table 1). Here, we review the evidence for the use of the two most-studied inflammatory biomarkers—CRP and lipoprotein-associated

Table 1 Inflammatory serum biomarkers associated with the risk of CHD in prospective studies.

CRP ⁶
Lp-PLA2 ²³
White blood cell counts ⁴⁵
Myeloperoxidase ⁴⁶
Serum phospholipase A2 (sPLA2) ⁴⁷
Soluble CD40 ligand ⁴⁸
Fibrinogen ⁴⁹
Serum amyloid A ⁵⁰
Pregnancy-associated plasma protein A (PAPP-1) ⁵¹
Matrix metalloproteinase 9 ⁵²
Tissue inhibitor of metalloproteinase-1 (TIMP-1) ⁵³
Placental growth factor ⁵⁴
Monocyte chemoattractant protein-1 (MCP-1) ⁵⁵
Interleukin-6 (IL-6) ⁵⁶
Interleukin-10 (IL-10) ⁵⁷
Interleukin-8 (IL-8) ⁵⁸
Cystatin C ⁵⁹
Secretory Intercellular adhesion molecule-1 (sICAM-1)
Vascular cell adhesion molecule 1 (VCAM-1) ⁶⁰
E-selectin ⁶⁰
Human cartilage glycoprotein 39 (YKL-40) ⁶¹

phospholipase A₂ (Lp-PLA₂), given the abundance of data available to suggest their clinical utility. Similar approaches and concepts can be applied to other emerging biomarkers as they continue to be studied further.

High-sensitivity CRP

CRP is a protein produced mainly by the liver and also by adipocytes and vascular smooth muscle cells in response to a rise in interleukin-6 and tissue necrosis factor-alpha (TNF- α). It is also a well-known acute-phase reactant.^{10,11} Yeh and colleagues have provided evidence that CRP is produced in smooth muscle cells and in adipocytes of arterial blood vessels and that it contributes to vascular inflammation under the influence of interleukins and TNF- α .¹¹ CRP levels often increase substantially in response to a wide variety of biological insults, infections, inflammatory conditions and cancer.^{10,12} While CRP has multiple pro-inflammatory and pro-atherogenic properties,¹² recent studies using a Mendelian randomization approach have not supported a causal role for it in atherogenesis.¹³ However, given its consistent association with increased CHD risk, CRP remains an established 'marker of risk', (e.g. its increased levels are associated with increased CHD risk), and it may very well be a contributor to the vascular inflammatory process in coronary arteries in humans.

Multiple prospective cohort studies have established that increased hsCRP levels are associated with increased CHD risk in both genders, across a wide age range, in primary as well as secondary prevention settings, and in different ethnic groups.^{6,9,10,14} These findings have been consistent in different populations with diverse ethnic backgrounds and in diverse clinical settings, and they have predicted risk of a variety of CV outcomes, including incident AMI, stroke, sudden cardiac death, stroke, peripheral artery disease and also incident diabetes and new-onset hypertension.^{8,9,15,16}

A recent meta-analysis of individual records of 160,309 people without a known history of vascular disease from 54 long-term prospective studies showed that serum CRP concentration has continuous associations with the risk of CHD, ischemic stroke and vascular mortality.¹⁷ Risk ratios (RRs) for CHD per 1-SD higher log(e) CRP concentration, (3-fold higher) were 1.37 when adjusted for conventional risk.¹⁷ It should be noted that the predictive power of hsCRP is partially affected by conventional risk factors and obesity and is decreased after multivariate adjustments. Moreover, CRP is a non-specific marker of inflammation, and its levels rise in response to infections, autoimmune diseases and malignant processes. In the absence of

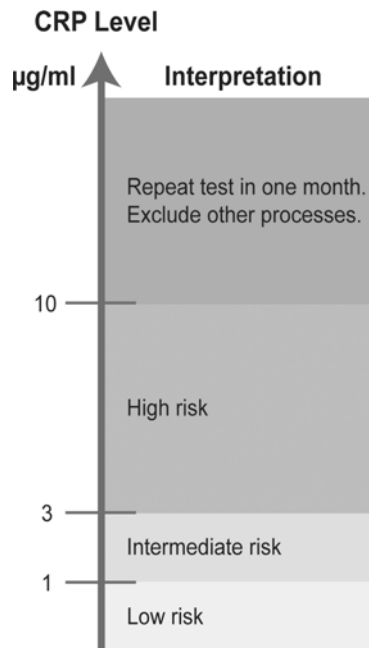


Fig. 1 Interpretation of CRP level. Reproduced with permission from Madjid *et al.*¹⁰

inflammation, hsCRP levels of <1 $\mu\text{g/ml}$ confer a lower risk for CHD, while levels above 3 $\mu\text{g/ml}$ increase the risk of CHD (Fig. 1).¹⁰ Multiple measures known to reduce CHD risk (i.e., smoking cessation, losing weight, exercise) also decrease serum CRP levels. Several medications, including aspirin and clopidogrel, and, in particular, statins, are also known to reduce serum CRP levels.^{18–21}

Lipoprotein-associated phospholipase A₂

Lp-PLA₂, also known as platelet activating factor-acetyl hydrolase (PAF-AH), belongs to a family of A₂ phospholipases. Lp-PLA₂ catalyzes oxidized phosphatidylcholine into oxidized non-esterified free fatty acids and lysophosphatidylcholine in the plasma, exerting a pro-inflammatory effect believed to be involved in atherogenesis.²² Lp-PLA₂ is secreted into the plasma by hematopoietic cells (primarily by macrophages) and is then transported mainly by low-density lipoprotein (LDL) (80–85%), and in lesser amounts by high-density lipoproteins (HDL) and lipoprotein (a).²² This enzyme can be measured based on its mass or enzymatic activity. Lp-PLA₂ mass and activity are correlated with age, male gender, smoking and LDL cholesterol levels²³ and are reduced significantly by statins.²⁴

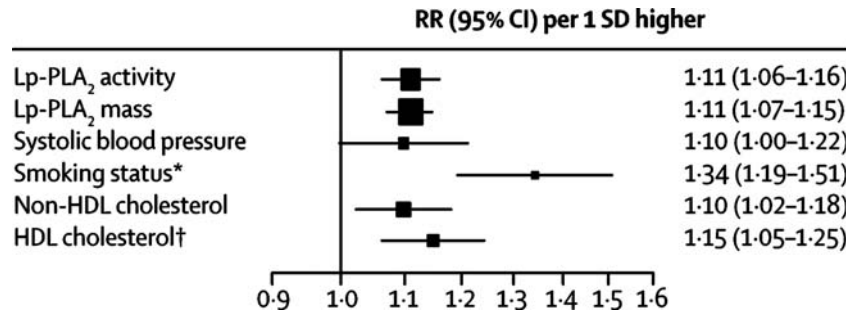


Fig. 2 Adjusted RRs for CHD per 1-SD higher baseline Lp-PLA2 activity, mass and several conventional risk factors in a meta-analysis of a common set of participants in a meta-analysis. Reproduced with permission from Thompson *et al.*²⁶

Multiple large, prospective cohort studies have shown that Lp-PLA2 is a strong, independent predictor of multiple CV outcomes.^{23,25} A large meta-analysis using individual data from 79,036 participants in 32 prospective studies showed that both Lp-PLA2 activity and mass predicted risk of CHD and vascular death (Fig. 2).²⁶ After adjustment for conventional risk factors, the relative risk for CHD was 1.10 (95% confidence interval (CI), 1.05–1.16) with Lp-PLA2 activity and 1.11 (1.07–1.16) with Lp-PLA2 mass. Both mass and activity were predictors of ischemic stroke, vascular mortality and non-vascular mortality. These associations were also observed in people with and without known vascular disease. The magnitude of risk was comparable to that of non-HDL cholesterol or systolic blood pressure.²⁶

The effect of Lp-PLA2 on CHD risk remains significant after adjustment for CRP. The Atherosclerosis Risk in Communities (ARIC) and Rancho Bernardo studies also report a statistically significant, though modest, improvement in risk discrimination as increment in the area under the curve (AUC) for Lp-PLA2.^{27,28}

Can inflammatory biomarkers improve our ability to predict CHD?

Traditional risk factors, such as those included in the Framingham risk tables, fail to predict all subjects at risk of CV events. Therefore, there is a need to find biomarkers that can identify subjects at risk of adverse CV events and improve the current risk prediction models. As a general rule, a new risk marker needs to be biologically plausible, measurable, repeatable, and show a strong and graded relationship to the disease.

To gain utility in clinical settings, a marker needs to have a widely available and accurate assay with acceptable variability. Ideally, though not necessarily, a candidate biomarker might be used as a target for therapy to reduce CHD risk as well, especially if there is considerable evidence that such a biomarker contributes to inflammation, atherosclerosis and/or instability of atherosclerotic plaques.

While some studies suggest that CRP can add a modest improvement to risk prediction models,²⁹ others have failed to show a significant improvement to risk prediction models (e.g., area under receiver-operating characteristic curve) after adjusting for conventional risk factors.^{14,30} It should be noted that inflammatory biomarkers may have different values in subjects with different levels of CHD risk. While CRP might not improve the risk prediction in low- and high-risk groups, it has been suggested to be useful in helping reclassify subjects who fall into the intermediate-risk category.^{14,31} However, this concept has been found to be inconsistent in some studies.³⁰

Ridker *et al.* have developed and validated a new prediction model (the Reynolds Risk Score) that—by incorporating CRP—can reclassify intermediate-risk men and women into higher or lower risk groups, hence improving global CV risk prediction.^{31,32}

A recent scientific statement from the American Heart Association and the American College of Cardiology recommends that in asymptomatic intermediate-risk men 50 years of age or younger or women 60 years of age or younger, measurement of CRP may be reasonable for CV risk assessment (Level of Evidence: B).¹⁶ However, in low- or high-risk subjects, CRP measurement might not be helpful.¹⁶

Folsom *et al.* have studied the impact of 19 inflammatory markers in predicting incident CHD beyond traditional risk factors in 15,792 adults.²⁸ The traditional risk factor model showed CHD prediction with an AUC of about 0.8. Adding Lp-PLA2 led to a statistically significant, but modest, incremental increase in the AUC (Fig. 3).²⁸ Other markers that increased the AUC were vitamin B6, IL-6 and soluble thrombomodulin, but CRP did not significantly increase the AUC.²⁸ A similar study also demonstrated a modest increase in AUC by addition of Lp-PLA2.³³

It should be noted that evaluating the predictive ability of a new biomarker may go beyond using the AUC. Pencina *et al.* have extensively discussed this issue and proposed elaborate measures to assess improvement in the performance of prediction models accomplished by adding emerging biomarkers.³⁴ These measures, (i.e. net reclassification improvement and integrated discrimination improvement), add great value to related studies, and they should be considered in future studies.³⁴

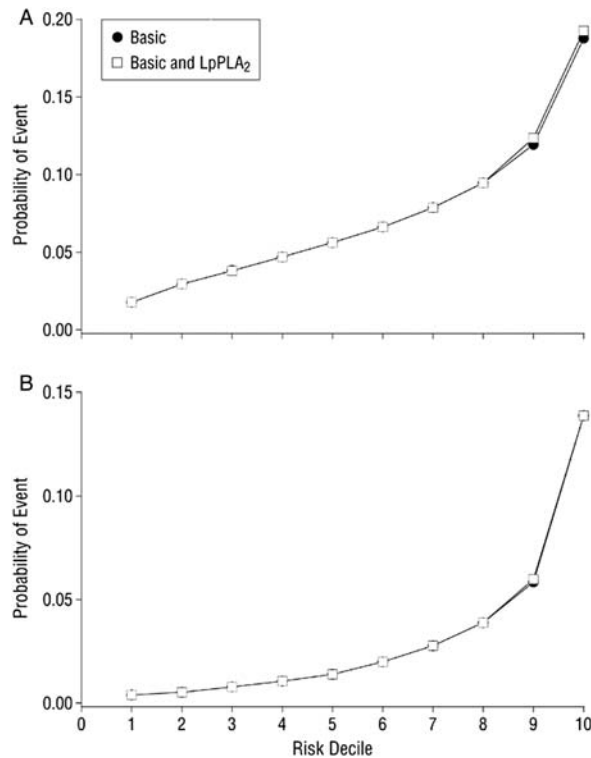


Fig. 3 Predicted 5-year risk of an incident CHD event, comparing the basic model with the basic model plus LpPLA₂, for men (A) and women (B) in the Atherosclerosis Risk in Communities Study. Reproduced with permission from Folsom *et al.*²⁸

Multiple distinct pathways and molecular mechanisms are involved in atherogenesis. Therefore, a multimarker approach using several biomarkers, each representing a different pathway, might offer additional predictive power. Only a few studies have evaluated such an approach.^{28,35} A study of 3,209 participants in the Framingham Heart Study measured 10 biomarkers (CRP, B-type natriuretic peptide, N-terminal pro-atrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen-activator inhibitor type 1, homocysteine and the urinary albumin-to-creatinine ratio) in the Framingham Heart study. During a median follow-up of 2.7 years, the addition of multimarker scores to conventional risk factors resulted in only small enhancement in risk classification.³⁵ A similar approach to using multiple markers in Malmo Diet and Cancer and Cardiovascular Health Study showed that the addition of more novel biomarkers to conventional risk factors led to small increments in the discrimination power to identify high-risk subjects.^{36,37}

Can inflammatory markers be used as targets for therapy?

Multiple inflammatory biomarkers are associated with an increase risk in CHD risk, and intensive research is ongoing to determine if they can be targets of therapy in order to reduce CHD risk. While it has been shown that several health lifestyle measures, such as low-fat diet, exercise, weight reduction and smoking cessation, can reduce these markers, specific inhibitors of these markers are of great interest. Statin drugs are known to reduce the levels of inflammatory biomarkers, such as CRP, and CHD risk, and it has been suggested that at least part of the cardioprotective effect of these drugs could be due to their pleiotropic, anti-inflammatory effects. The Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial was designed to determine whether treatment with a statin might benefit subjects with elevated CRP levels, but without hyperlipidemia.¹⁸ This randomized, double-blind, placebo-controlled trial used rosuvastatin (20 mg/day) vs. placebo in 17,802 men and women free of clinical CHD or diabetes who had levels of LDL cholesterol <130 mg/dl and CRPs > 2 mg/l. After a median follow-up of 1.9 years, the study was terminated early, as rosuvastatin caused a significant reduction ((hazard ratio (HR): 0.56 (95% CI, 0.46–0.69)) in the combined primary end point of MI, stroke, arterial revascularization, hospitalization for unstable angina or CV death (Fig. 4).¹⁸ The observed reductions in outcomes were consistent in all subgroups, regardless of sex, age, race or Framingham risk group.¹⁸ There was a 65% reduction in vascular events in rosuvastatin-treated subjects who achieved levels of LDL cholesterol < 70 mg/dl and hsCRP < 2 mg/l vs. a 33% reduction in those who achieved one or neither target ($P < 0.0001$). The risk reduction was even lower (=79%) in those who achieved levels of LDL cholesterol <70 mg/dl and hs-CRP < 1 mg/l (Fig. 5).³⁸ These findings are in line with those from previous studies. In Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) study, 3745 patients with ACS were randomized to receive either 80 mg of atorvastatin or 40 mg of pravastatin per day.³⁹ The risk of recurrent MI or coronary death was significantly and equally reduced in those who achieved LDL cholesterol levels <70 mg/dl or CRP levels <2 mg/l after statin therapy.³⁹ Interestingly, subjects who achieved LDL cholesterol levels <70 mg/dl and CRP levels of <1 mg/l after statin therapy had the lowest rate of recurrent events (Fig. 6). Moreover, subjects who achieved lower CRP levels after therapy had fewer recurrent events, regardless of the achieved level of LDL cholesterol.³⁹ Nissen *et al.* simultaneously reported the Reversal of

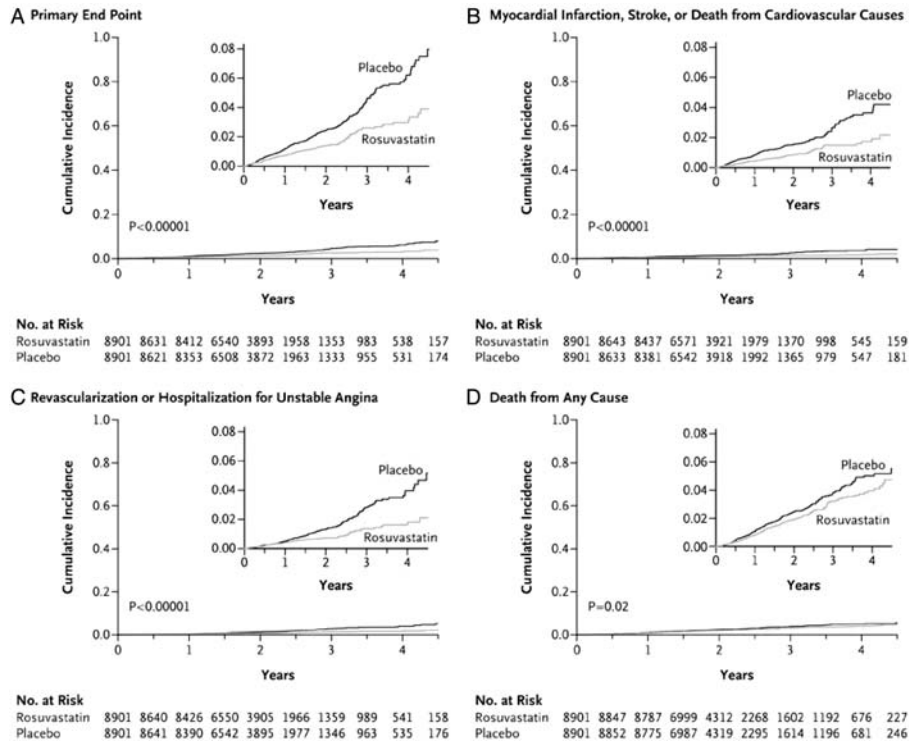


Fig. 4 Cumulative incidence of CV events according to study group. (A) The cumulative incidence of the primary end point (non-fatal myocardial infarction, non-fatal stroke, arterial revascularization, hospitalization for unstable angina or confirmed death from CV causes). The HR for rosuvastatin, when compared with placebo, was 0.56 (95% CI, 0.46–0.69; $P < 0.00001$). (B) The cumulative incidence of non-fatal myocardial infarction, non-fatal stroke or death from CV causes, for which the HR in the rosuvastatin group was 0.53 (95% CI, 0.40–0.69; $P < 0.00001$). (C) The cumulative incidence of arterial revascularization or hospitalization for unstable angina, for which the HR in the rosuvastatin group was 0.53 (95% CI, 0.40–0.70; $P < 0.00001$). (D) The cumulative incidence of death from any cause, for which the HR in the rosuvastatin group was 0.80 (95% CI, 0.67–0.97; $P = 0.02$). In each panel, the inset shows the same data on an enlarged y axis and on a condensed x-axis. Reproduced with permission from Ridker *et al.*¹⁸

Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial in which they randomized 502 patients with coronary artery disease to moderate (40 mg of pravastatin per day) vs. intensive (80 mg of atorvastatin per day) treatment.⁴⁰ Patients' coronary arteries were studied with intravascular ultrasonography at baseline and after 18 months. After adjustment for the reduction in lipid parameters, the decrease in CRP levels was significantly correlated with the rate of progression of atherosclerotic lesions. Patients with greater than median reductions in both LDL cholesterol and CRP had significantly slower rates of atherosclerosis progression (Fig. 7).⁴⁰

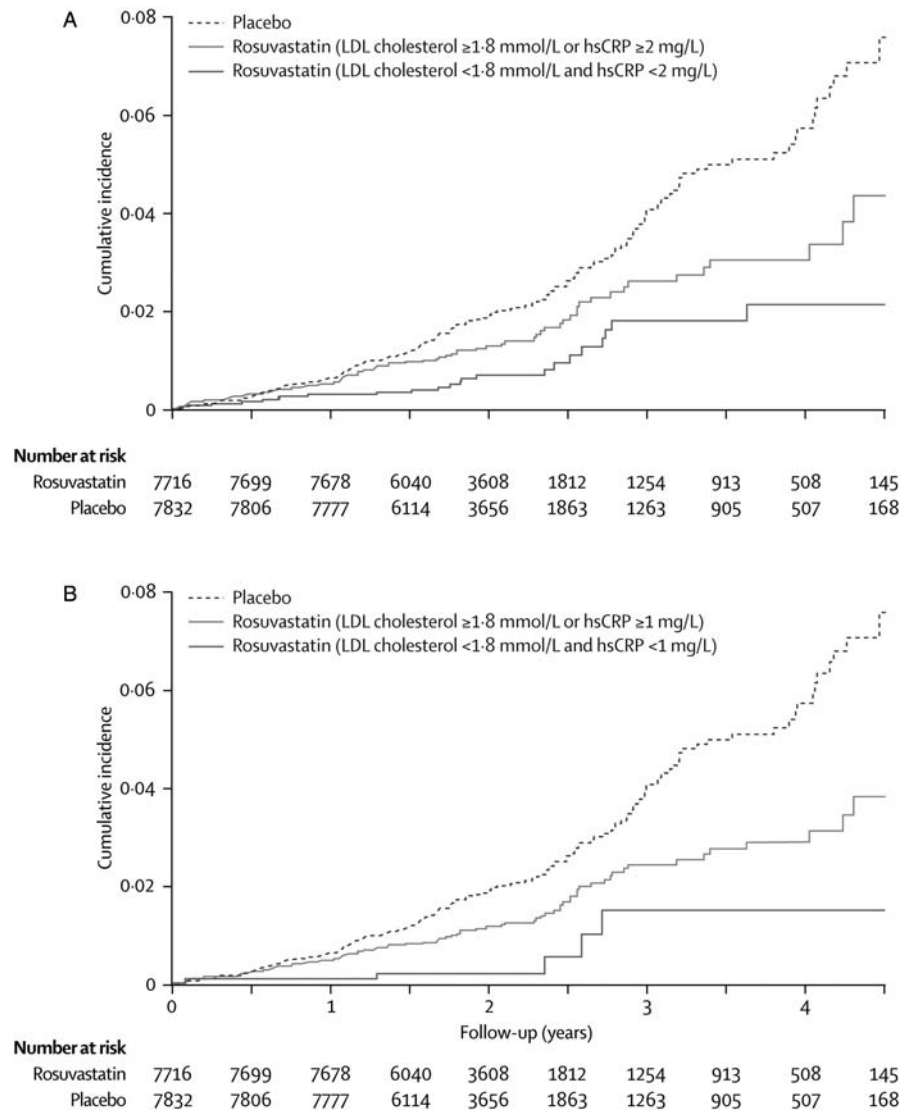


Fig. 5 Cumulative incidence of CV events in JUPITER in the placebo and rosuvastatin groups according to whether or not reductions in both LDL cholesterol and hsCRP were achieved. **(A)** Analysis using targets of LDL cholesterol < 1.8 mmol/l and hsCRP < 2 mg/l. **(B)** Analysis using targets of LDL cholesterol < 1.8 mmol/l and hsCRP < 1 mg/l. Reproduced with permission from Ridker et al.³⁸

A post-hoc subgroup analysis of the Heart Protection Study (HPS), which randomized 20,536 men and women (age: 40–80 years) at high risk for vascular events to simvastatin 40 mg vs. placebo, did not show any difference in the composite endpoint of coronary death, MI, stroke or revascularization based on different categories of baseline CRP levels.⁴¹ It should be noted that unlike the JUPITER study, HPS did

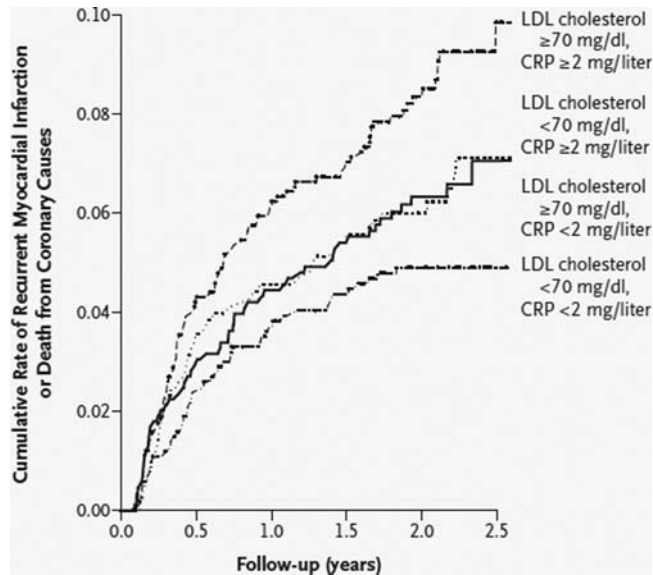


Fig. 6 Cumulative incidence of recurrent myocardial infarction or death from coronary causes, according to the achieved levels of both LDL cholesterol and CRP. The median value of each marker is included for the sake of completeness, since no patient had the exact median value of either marker. Reproduced with permission from Ridker *et al.*³⁹

not randomize subjects based on their baseline CRP levels. Additional, well-designed randomized trials are needed to explore various aspects of the role of CRP as a target for therapy.

Statins are known to decrease the levels of other inflammatory markers as well. As an example, multiple studies have demonstrated that statin use can decrease Lp-PLA2 levels. Almost 80% of Lp-PLA2 activity is associated with LDL in the circulation, and all statins are capable of reducing Lp-PLA2 levels.^{22,23} However, specific inhibitors of Lp-PLA2 are under investigation. Darapladib is an oral Lp-PLA2 inhibitor that has been studied extensively in animals and is currently in phase 3 studies in human. In a study in diabetic and hypercholesterolemic pigs, an oral inhibitor of Lp-PLA2 (e.g., darapladib) significantly reduced plasma and lesion Lp-PLA2 activity and decreased expression of multiple genes associated with macrophage and T lymphocyte functions in coronary arteries. Moreover, darapladib reduced the plaque and necrotic core area.⁴² In subjects with stable CHD and CHD risk-equivalent patients on atorvastatin, darapladib reduced Lp-PLA2 activity and also CRP and IL-6 levels.⁴³ In the IBIS-II trial, patients with CAD were randomized to receive 160 mg darapladib or placebo. After 12 months, the necrotic core volume was significantly increased in the placebo group, whereas darapladib limited necrotic core expansion.⁴⁴

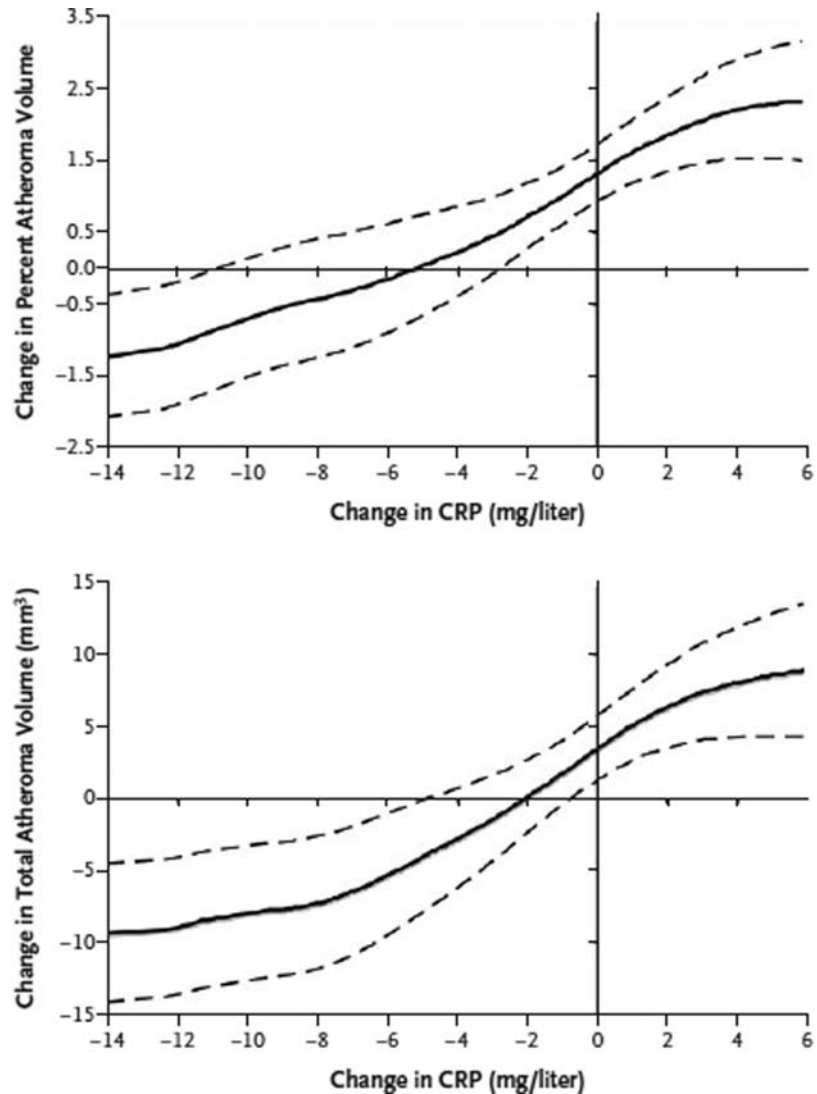


Fig. 7 Locally weighted smoothed scatterplots showing the relationship between the changes in CRP levels and the rate of progression of atherosclerosis in the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial. In each plot, the solid line represents the point estimates, and the upper and lower lines denote the 95% CIs. Reproduced with permission from Nissen *et al.*⁴⁰

Future areas of research in biomarkers

The role of biomarkers in predicting CHD risk is still evolving. While CRP was the first to be studied in this particular field, there is a need to focus on other plaque-specific biomarkers. The effects of most biomarkers found to date are generally modest, and we need to identify

new biomarkers with more robust predictive power independent of conventional risk factors. We also need to evaluate each of their potential roles in contributing to inflammation and atherogenesis in preclinical and clinical studies.

With the emergence of more novel biomarkers and the development of new diagnostic tests and the ability to simultaneously measure multiple markers, the multimarker approach might prove to be of value in future studies. Large collaborative studies pooling individual data from well-conducted prospective studies can offer valuable information. New technical developments may enable the measurement of multiple markers in reference labs as points of care during a clinic visit. Knowledge of biomarker levels may also help motivate patients to further improve their lifestyles and adhere better to their pharmacological and life style treatments.

As mentioned before, specific inhibitors of biomarkers can be tested for preventing CV events. Currently, there is a relative paucity of medications that can be used safely and effectively as primarily anti-inflammatory agents in CHD. The discovery of such medications and their administration orally, intravenously or perhaps directly into vulnerable atherosclerotic plaques, is an area of great importance in potentially preventing myocardial infarctions and their complications.

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