Inflammatory biomarkers in coronary artery disease

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Summary Current evidence supports that inflammation is a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption; patients with a more pronounced vascular inflammatory response have a poorer outcome.

Biomarkers are generally considered to be proteins or enzymes — measured in serum, plasma, or blood — that provide independent diagnostic and prognostic value by reflecting an underlying disease state. In the case of coronary artery disease (CAD), inflammatory biomarkers, have been extensively investigated; more evidence exists for C-reactive protein (CRP). Using high sensitivity (hs) assays, epidemiologic data demonstrate an association between hs-CRP and risk for future cardiovascular morbidity and mortality among those at high risk or with documented CAD. Moreover, a series of prospective studies provide consistent data documenting that mild elevation of baseline levels of hs-CRP among apparently healthy individuals is associated with higher long-term risk for cardiovascular events. Yet, the predictive value of hs-CRP is found to be independent of traditional cardiovascular risk factors.

Recent studies suggest that, besides CRP, other inflammatory biomarkers such as cytokines [interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1)], soluble CD40 ligand, serum amyloid A (SAA), selectins (E-selectin, P-selectin), myeloperoxidase (MPO), matrix metalloproteinases (MMPs), cellular adhesion molecules [intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1)], placental growth factor (PlGF) and A2 phospholipases may have a potential role for the prediction of risk for developing CAD and may correlate with severity of CAD.

Finally, indications suggest that the increased risk associated with inflammation may be modified with certain preventive therapies and biomarkers may help to identify the individuals who would benefit most from these interventions.

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Introduction

Pathophysiology of inflammation in atherosclerotic plaque

There is an extensive literature supporting the role of inflammation in coronary artery disease (CAD). Inflammatory cells, inflammatory proteins, and inflammatory responses from vascular cells play a pivotal role in the pathogenesis of various stages of atherosclerosis, including the initiation and progression of atheroma, plaque instability and rupture, and post-angioplasty and restenosis [1—3]. The vascular endothelium is subject to injury from numerous potential insults, such as hemodynamic forces [4], oxidative stress [5], and modified lipoproteins [6]. Although circulating leukocytes do not adhere to the normal vascular endothelium, injured endothelium expresses several classes of adhesion molecules that selectively bind to leukocytes [7]. Endothelial dysfunction is marked by the up-regulation of cellular adhesion molecules, such as vascular adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and selectins, which cooperate with chemokines and mediate increased adhesion of mononuclear and neutrophil leukocytes [8]. Following adherence to the endothelium, chemoattractant cytokines, such as monocyte chemoattractant protein-1 (MCP-1), mediate transmigration of inflammatory cells into the subendothelial space [9]. In addition to MCP-1, macrophage colony-stimulating factor (M-CSF) contributes to the differentiation of monocytes into macrophages [10]. The concurrent uptake of low-density lipoproteins (LDLs) by monocyte-derived macrophages transforms them into the lipid-laden foam cells that constitute a key element of the fatty streak, the first recognizable progenitor of the advanced atherosclerotic lesion [11—13]. Mononuclear cells release cytokines, including interleukin (IL)-1 [14] and IL-6 [15]. Cytokine release from the fatty streak recruits further inflammatory cells (macrophages, mast cells, activated T cells), resulting in further uptake and oxidation of LDLs. These cytokines also stimulate smooth muscle cell proliferation and development of a collagenous fibrous cap that covers this inflammatory mixture [14]. This fibrous cap separates the pro-coagulant contents of the atheroma core from the circulating blood; collagen produced by smooth muscle cells in the lesion provides mechanical strength and stability to the fibrous cap. The synthesis and breakdown of collagen is dynamically controlled by inflammatory signals [16,17].

There are data showing that the risk for an acute coronary event has less to do with the degree of angiographic luminal stenosis than with the underlying pathology of the atherosclerotic plaque that makes it susceptible to rupture [17]. Activated T cells secrete interferon gamma (INF-γ) that decreases smooth muscle cell production of collagen. Activated macrophages secrete matrix metalloproteinases (MMPs) that proteolytically...
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Inflammatory biomarkers in CAD. This inflammatory...
In a recent study, conducted on patients those without heredity for myocardial infarction (MI) had elevated CRP levels compared with at least one of their parents with myocardial infarction. In addition to healthy controls, the CRP levels, age, waist circumference, homocysteine, and triglycerides were predictors of intima-media thickness of the carotid artery assessed by Doppler, while CRP, age, and triglycerides were predictors of plaque formation. Further, a small case-control study found that 60 patients with chronic stable angina had twice median CRP levels compared with controls.

**The role of CRP for prognosis in CAD**

CRP levels may be useful for short-term prognosis and long-term risk assessment after a cardiovascular event. Therefore, several studies have looked at associations between CRP levels and the risk of early death or recurrent cardiac events shortly after an acute coronary syndrome (ACS).

In a substudy of TIMI 11A trial, CRP was identified as an independent predictor of mortality at 14 days in ACS, including those with a negative troponin level. In another large trial, the Fragmin during InStability in Coronary Artery Disease (FRISC) study, a randomized trial of low-molecular weight heparin in unstable coronary syndromes, CRP levels were independent predictors of cardiac death. Mortality was 5.7% among patients with CRP levels <2 mg/L, 7.8% among patients with CRP levels 2–10 mg/L, and 16.5% among patients with CRP levels >10 mg/L. In the Global Use of Strategies to open Occluded arteries IV (GUSTO-IV) substudy, although hs-CRP elevation during the acute stage of unstable CAD was associated with an increased 30-day mortality independent of troponin levels, there was no association with an increased risk of non-fatal recurrent ischemic events. Therefore, it appears that after ACS, CRP is a better predictor of death than nonfatal ischemic events.

Similarly, the association between CRP and a long-term risk assessment in patients with stable CAD or after an ACS has been efficiently investigated. In the prospective European Concerted Action on Thrombosis and Disabilities Study (ECAT), it was found that among 2121 patients with angina, each standard deviation increase in baseline hs-CRP was associated with a 45% increase in the relative risk (RR) of nonfatal myocardial infarction or sudden cardiac death over 2 years of follow-up. In addition, recent data also confirm that CRP is a strong independent predictor of mortality among ACS patients who are treated with early revascularization. In a prospective study of patients who underwent early invasive therapy after non-ST
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CRP and MI
It has been shown that CRP levels are associated with the size of the infarct [59]. In addition, it was suggested that in patients with acute MI, CRP levels correlate with the presence of plaque rupture, as assessed by intravascular ultrasound [60]. Furthermore, an increased temperature at unstable coronary plaques, evaluated by the invasive thermogenetic catheter, has been shown to be related to CRP levels [61]. Consequently, it was suggested that CRP in the acute phase of MI could be correlated with risk assessment. However, a prospective study of 1360 patients with unstable or stable angina pectoris or acute MI found that adjusted hazard ratios for death/acute MI for CRP levels above the first tertile showed a significant risk of 1.8 with stable angina, 2.7 for unstable angina, and only 1.0 for acute MI [62]. On the contrary, in stable post-MI patients, elevated hs-CRP predicted a significantly higher risk for recurrent nonfatal MI or fatal coronary events (75% higher in the highest versus lowest quintile of hs-CRP), suggesting that CRP is not only a marker for the extent of myocardial damage [63]. Based on these data, one could suppose that CRP levels are predictive of short- and long-term cardiac events in patients with stable and unstable angina, but may not be predictive in the acute stage of MI. Therefore, the measurements should be delayed until the acute phase reaction is over and levels have returned to baseline.

Nevertheless, the results are not homogenous even in the case of stable MI. The Thrombogenic Factors and Recurrent Coronary Events (THROMBO) study was a multicenter investigation of 1045 post-MI patients that measured CRP levels 2 months after the event and again at 2 years. Although CRP levels were found to be associated with recurrent coronary events, in multivariable analyses, CRP was not proved to be an independent marker for recurrent coronary events [64].

CRP and primary prevention
Several prospective studies in apparently healthy individuals have shown that elevated hs-CRP levels are correlated with higher risk for future cardiovascular morbidity and mortality. The Multiple Risk Factor Interventional Trial (MRFIT), a nested case-control study, found that increased CRP levels predicted increased risk of cardiovascular disease in middle-aged men, but this relationship was statistically significant only for smokers [65]. The Physicians’ Health Study (PHS), a prospective, nested, case-control study of men who did not have prior history of cardiovascular disease and had low rates of cigarette use, showed that those with highest baseline levels of hs-CRP had two times the risk of future stroke, three times the risk of future MI, and four times the risk to develop severe peripheral arterial disease. Cardiovascular risks were not influenced by smoking status, and were independent of lipid or other cardiovascular factors [66]. Data from European Monitoring Trends and Determinants of Cardiovascular Disease (MONICA)-Augsburg prospective study offer consistent observations regarding the prognostic capacity of hs-CRP among individuals without clinical evidence of CAD. This study followed 936 healthy, middle-aged men over 8 years and noted a 19% increase in risk for future nonfatal or fatal coronary events for each standard deviation increase in baseline hs-CRP after adjustment for multiple risk factors, including smoking status [67].

Recently, in the prospective Prevention of Renal and Vascular Endstage Disease (PREVEND) study, 8139 individuals without previous documented coronary artery disease were followed for the incidence of coronary angiography and coronary events from 1997 to 2003. hs-CRP levels were found to be associated with angiographic characteristics and clinical consequences of plaque instability during follow-up [68].

The Women’s Health Study (WHS), a prospective nested case-control study of postmenopausal women showed that hs-CRP was the most powerful predictor of cardiovascular risk compared with other inflammatory markers, baseline lipid levels, and homocysteine. Women who developed cardiovascular events had higher baseline levels of CRP than control subjects; yet, those in the highest quartile of CRP had a relative risk of 4.4 of any cardiovascular event compared with those in the lowest quartile. A subgroup analysis of women with LDL levels of <130 mg/dL, a group traditionally considered to be low risk, found that those who had elevated baseline CRP were at higher risk for future events. A follow-up study of the entire cohort showed that CRP levels were stronger predictors of cardiovascular events than LDL cholesterol [69,70].

It appears that among healthy men and women, elevated CRP levels predict risk of a first cardiovascular event independently of other factors. Based on PHS [66] and WHS [69], the adjusted relative risk of a future cardiovascular event increases 26% for men and 33% for women overall for each quartile
increase in hs-CRP levels [71]. Therefore, data suggest that hs-CRP levels add to risk prediction based on lipid parameters, and may be useful for identifying patients at risk for future cardiovascular events who would have been classified as low risk.

Finally, a recent study showed that hs-CRP may be a useful marker in screening the children who are at risk of CAD in adulthood. The levels of hs-CRP were measured in 51 children (11.79 ± 3.14 years) with risk factors for CAD (hypercholesterolemia, hypertension, obesity, low HDL-cholesterol, and familial history of CAD). The results were compared with 26 children (12.98 ± 2.59 years) without any risk factors. The children with risk factors had significantly higher levels of hs-CRP compared to the control group (P < 0.01). The concentrations of CRP were significantly increased in children with three or more risk factors [72]. Early identification of the children with risk factors and interventions for obesity, harmful habits, and lifestyle in childhood might decrease the incidence of coronary heart disease in adulthood.

**CRP and preventive therapies**

Apart from providing global risk prediction, CRP screening may also provide a method of targeting preventive interventions. In vitro and in vivo studies show that CRP is not only an indicator of inflammation, but also contributes to plaque development, plaque instability, and thrombus formation [73,74]. Therefore, the relationship between certain cardioprotective medications, such as statin and aspirin therapy, and CRP levels has been examined.

Studies have shown that statin therapy lowers CRP levels independently of lipid levels, supporting the possibility that statins have anti-inflammatory effects. Data from the Cholesterol And Recurrent Events (CARE) study, a randomized trial of pravastatin in post-MI patients, revealed that although the risk of recurrent coronary events was reduced by therapy with pravastatin among individuals with and without evidence of inflammation, the relative risk reduction was greater for those with consistent evidence of inflammation (54% versus 25%) even though the lipid profiles were similar in both groups [63]. This information suggests that statin therapy may be particularly effective among patients with elevated CRP levels. PRavastatin Or atorVastatin Evaluation and Infection Therapy (PROVE-IT) trial demonstrated that intensive statin therapy that lowered hs-CRP levels to a mean of less than 2 mg/L resulted in a reduction risk for recurrent MI or fatal coronary event, irrespective of the degree of LDL lowering [75].

In the PRavastatin Inflammation CRP Evaluation (PRINCE) study, pravastatin reduced hs-CRP levels in subjects without prior history of cardiovascular disease [76]. A further analysis of the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) has extended the observation that statin therapy directly lowers CRP levels as primary prevention. Subjects with average levels of total cholesterol and below-average levels of HDL-cholesterol were divided into groups according to median levels of LDL cholesterol and CRP. Those with elevated LDL (above median value) were at increased risk for future cardiovascular events and benefited substantially from randomization to lovastatin, irrespective of CRP level. Those with low LDL and low CRP (below median values) were at low risk and derived little benefit from lovastatin therapy. Those with low LDL (below median value) but high CRP (above median value) were at increased risk and derived substantial benefit from lovastatin therapy [77]. Recently, the results from the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) were reported [78]. JUPITER, was a randomized, double-blind, placebo-controlled, multicenter trial which assigned 17802 apparently healthy men and women with low LDL levels (less than 130 mg/dL) but hs-CRP of 2 mg/L or higher to receive rosuvastatin 20 mg daily or placebo, in order to assess the incidence of a first major cardiovascular event. The subjects were followed up for a median of 1.9 years (maximum, 5.0). Rosuvastatin reduced LDL cholesterol levels by 50% and hs-CRP by 37%. The rates of the primary end point were 0.77 and 1.36 per 100 person-years of follow-up in the rosuvastatin and placebo groups, respectively (hazard ratio for rosuvastatin, 0.56; 95% confidence interval [CI], 0.46–0.69; P < 0.00001), with corresponding rates of 0.17 and 0.37 for MI (hazard ratio, 0.46; 95% CI, 0.30–0.70; P = 0.0002), 0.18 and 0.34 for stroke (hazard ratio, 0.52; 95% CI, 0.34–0.79; P = 0.002), 0.41 and 0.77 for revascularization or unstable angina (hazard ratio, 0.53; 95% CI, 0.40–0.70; P < 0.00001), and 0.45 and 0.85 for the combined end point of MI, stroke, or death from cardiovascular causes (hazard ratio, 0.53; 95% CI, 0.40–0.69; P < 0.00001). The authors concluded that rosuvastatin significantly reduced the incidence of major cardiovascular events in apparently healthy persons without hyperlipidemia but with elevated hs-CRP levels [78]. These studies provide evidence for the non-lipid effects of statins and suggest that testing for CRP may identify many subjects, who are not eligible for statin therapy based on LDL levels, but are at high risk for future cardiovascular events and might benefit from statin therapy. Therefore, CRP levels may provide a method for targeting
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Table 1  Factors affecting serum CRP levels.

<table>
<thead>
<tr>
<th>Increased levels</th>
<th>Decreased levels</th>
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</thead>
<tbody>
<tr>
<td>Increased blood pressure</td>
<td>Moderate alcohol consumption</td>
</tr>
<tr>
<td>Increased body mass index</td>
<td>Increased activity/endurance exercise</td>
</tr>
<tr>
<td>Metabolic syndrome/diabetes mellitus</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Low HDL/high triglycerides</td>
<td>Medications; statins—fibrates—niacin</td>
</tr>
<tr>
<td>Estrogen/progestogen use</td>
<td>Aspirin</td>
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<tr>
<td>Chronic infections (gingivitis, bronchitis)</td>
<td>? ACE inhibitors?</td>
</tr>
<tr>
<td>Chronic inflammation (rheumatoid arthritis)</td>
<td></td>
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Modified from Pearson et al. [47].

Cytokines

Cytokines include a number of pleiotropic proteins that have been extensively implicated in the process of inflammation. Among the main cytokines are IL-1, IL-6, IL-10, tumor necrosis factor alpha (TNF-α), and MCP-1 [9,14,15]. IL-1 and IL-6 drive production of reactant proteins, including CRP. IL-6 may increase plaque instability driving expression of matrix metalloproteinases, TNF-α, and MCP-1 [80].

Elevated IL-6 levels in healthy men correlated with increased risk for future MI independently of hs-CRP [81]. Recently, a case-control study of 294 patients with clinically stable ACS (group I) and clinically stable angina pectoris (group II), showed that median IL-6 levels were significantly higher in group I than in group II (P < 0.05) [82]. In the Fragmin and/or early Revascularization during InStability in Coronary artery disease (FRISC-II) study, elevated IL-6 (>5 ng/L) was associated with higher 6- and 12-month mortality, independent of troponin and hs-CRP [83]. In this study patients with high IL-6 had a greater response to an invasive versus conservative strategy than patients with low IL-6 levels. These data suggest that elevated IL-6 levels may identify patients with more severe events, who would benefit from more aggressive treatment. However, the application of IL-6 as a biomarker is limited by large circadian variations and lack of confirmatory studies.

It has been shown that plasma concentrations of IL-1β are elevated in patients with hypercholesterolemia [84]. It seems that statin therapy may lower IL-1β levels in these patients [85]. However, further data from clinical studies are needed for assessment of the relationship between IL-1β and atherosclerotic clinical events.

TNF-α has been implicated in myocardial dysfunction and remodelling after acute coronary events [86]. In the CARE study, recurrent coronary events after a MI were associated with higher TNF-α levels compared with controls [87].

MCP-1, as already mentioned, is a chemokine that helps recruit monocytes into the arterial intima and activates these cells to promote atherosclerosis [9,88]. As assessed by coronary angiogram, measurements of MCP-1 in the coronary blood of patients with unstable angina demonstrated an association between MCP-1 levels and the extent of coronary atherosclerosis [89]. In the Orbofiban in Patients with Unstable coronary Syndromes (OPUS)-TIMI 16 trial, MCP-1 levels above the 75th percentile (238 pg/mL) were associated with

if patients with only elevated CRP levels should be treated [47].

statin therapy in primary and secondary prevention.

In the PHS, use of aspirin was associated with a statistically significant (55.7%) risk reduction for future MI among men who had hs-CRP levels in the highest quartile and with a nonsignificant (13.9%) reduction among those who had hs-CRP levels in the lowest quartile [66]. However, only few data are available regarding the direct effect of aspirin on CRP levels. A small randomized trial of low-dose aspirin in healthy volunteers, found that aspirin had no detectable effect on CRP [79].

Finally, the CDC/AHA statement contains recommendations for the use of CRP in the diagnosis and management of cardiovascular disease [47]. The authors recommend two separate CRP measurements, preferably 2 weeks apart. Yet, the CDC/AHA statement suggests use of hs-CRP for risk assessment in patients who are at intermediate risk for cardiovascular events (10—20% 10-year risk of coronary event) and classifies hs-CRP levels of <1.0 mg/L as low risk, 1—3 mg/L as average risk, and >3.0 mg/L as high risk. A CRP level greater than 10 mg/L indicates the presence of a significant acute phase response; usually indicates a noncardiovascular source of inflammation, and further assessment is required to determine the cause [47]. (Table 1 indicates certain patient characteristics and conditions that have been associated with increased or decreased CRP levels.) Therefore, the authors indicate the measurement of CRP for identifying patients without known CAD who may be at a higher risk than estimated by traditional risk factors. However, they suggest that further data from prospective clinical trials are needed to determine
an increased risk of death or MI after 10 months, even after adjustment for traditional risk factors [90]. However, although MCP-1 is a promising biomarker, further research is needed to evaluate its clinical utility.

IL-10 mediates anti-atherogenic pathways. In the c7E3 AntiPlatelet Therapy in Unstable REfractory angina (CAPTURE) study, patients with elevated IL-10 levels had a decreased risk of death or nonfatal MI. Yet, those with elevated CRP and IL-10 were at lower risk than those with elevated CRP only [91]. These data suggest that IL-10 may be protective against proinflammatory mediators in ACS.

To make things more confused, in a recent study, 158 consecutive patients with angiographically identified stable CAD were enrolled. Plasma levels of the following 10 cytokines were measured: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-γ. The multivariate analysis using multi-vessel disease, diabetes, and the levels of all 10 cytokines and hs-CRP showed that the only independent predictor of cardiovascular events was IL-8 (RR, 2.98; 95% CI, 1.64–7.24; \( P = 0.0001 \)), during a 7-year follow-up period [92]. These data, in fact, suggest that the role of cytokines in the prediction of cardiovascular risk is still controversial, or, at least, we do not know yet, which one is better as a prognostic biomarker and probably a therapeutic target in CAD.

**Soluble CD40 ligand**

Soluble CD40 ligand (sCD40L) is a proinflammatory marker that has been shown to promote atherosclerosis and plaque instability [93]. Healthy women with high levels of sCD40L have been shown to be at increased risk for cardiovascular events [94]. In the CAPTURE trial, patients with elevated sCD40L (>5 μg/L) were at increased 6-month risk of death or nonfatal MI. Treatment of these patients with abciximab before coronary angioplasty reduced the risk. This benefit was observed in patients with and without troponin elevation, and no benefit of abciximab was observed in patients with elevated troponin values but low sCD40L values [95]. The investigators of the CAPTURE trial concluded that sCD40L may be an independent risk marker of cardiovascular events and a marker for determining benefit from therapy with glycoprotein IIb/IIIa inhibitors. Similarly, a substudy of the OPUS-TIMI 16 trial showed that patients with sCD40L levels above the median value were at an increased risk for death or recurrent MI and that sCD40L provided prognostic ability independent of troponin I and CRP [96].

Finally, a recent study of 96 patients with ST-segment elevation myocardial infarction showed that serum ratio of sCD40L/IL-10 was a better independent predictor of in-hospital adverse events than individual sCD40L and IL-10 measurements in these patients [97].

**Serum amyloid A**

Serum amyloid A (SAA), like CRP, is an acute phase protein. In a substudy of TIMI 11A, elevated SAA levels predicted increased risk of 14-day mortality in patients with ACS [98]. In the Women’s Ischemia Syndrome Evaluation (WISE) study of women referred for coronary angiography because of suspected ischemia, elevated SAA values were correlated with angiographic severity of CAD and 3-year risk for cardiovascular events [99].

However, the results were not similar in the Thrombogenic Factors and Recurrent Coronary Events study; the measurement of SAA levels, 2 months after MI did not show significant association between SAA levels and risk of recurrent cardiovascular events over 2 years [64].

**Adhesion molecules**

Cell adhesion molecules (CAMs) and their counter receptors of the β2-integrin family, mainly Mac-1, play a pivotal role in the interactions between leukocytes, platelets, and vascular endothelium [8]. Adherence of circulating leukocytes to the endothelium and their transmigration into the arterial wall is an early step of atherosclerosis [8]. Soluble CAMs (sCAMs: sICAM-1, sVCAM-1 and E- and P-selectins) are shed from cell surfaces and reflect cellular activation [100]. Soluble CAMs have been studied in the early diagnosis of ACS and in risk stratification in CAD.

Hollander et al. considered that markers of platelet aggregation may detect ACS earlier than cardiac markers as plaque rupture/platelet aggregation precedes myocardial ischemia. However, although theoretically attractive, creatinine kinase MB fraction had a higher specificity for detection of acute MI, ACS, and serious cardiac events, upon emergency department arrival, than both soluble and membrane-bound P-selectin [101].

Mulvihill et al. measured CAMs in patients with ACS (unstable angina and non-Q MI) at presentation and then after 3, 6, and 12 months. CAMs levels increased within 10h of chest pain onset and remained elevated for up to 6 months, suggesting that the inflammatory stimulus triggering expression of CAMs is long sustained, returning gradually toward control values [102].
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In the WHS, elevated P-selectin levels in healthy women were associated with increased cardiovascular risk [103]. However, the role of CAMs in risk stratification in patients with CAD is not established as the results on predicting cardiovascular risk are rather confusing.

In a prospective study, among patients with stable angiographically documented CAD, CAMs (sICAM-1, sVCAM-1, and E-selectin) were measured, and follow-up information on cardiovascular events was obtained for a mean of 2.7 years. Although all CAMs were higher in patients with future death, in a model that simultaneously controlled for all inflammatory and soluble adhesion markers determined, only sVCAM-1 remained independently significant for future fatal cardiovascular events, with a 2.8-fold increase in risk. Especially sVCAM-1 added to the predictive value of classic risk factors and hs-CRP in determining the risk of death from cardiovascular causes [104].

In a prospective observational study, among patients presenting acutely with ACS (unstable angina and non-Q MI) raised concentrations of sVCAM-1 and CRP — but not sICAM-1, E-selectin, and P-selectin — were predictive of an increased major cardiovascular event within 6 months after presentation. The sensitivity of CRP >3 mg/L and sVCAM-1 >780 ng/mL for predicting future events was >90% [105]. Similarly, patients with ACS presented with increased levels of sVCAM-1 compared with patients with stable angina or healthy individuals; moreover, those with in-hospital adverse coronary events had elevated sVCAM-1 levels independently of CRP levels [106]. In patients with unstable angina undergoing coronary stenting, CRP and sICAM-1 — but not sVCAM-1 — proved useful for identifying those at higher risk of a cardiac event; yet, it was suggested that CRP may play a direct role in promoting the inflammatory component of atherosclerosis by inducing significant expression of sICAM-1 [107]. However, a prospective study of patients presenting to the emergency department with chest pain, failed to reveal any association between sICAM-1 and the risk of a serious cardiovascular event during hospital admission [108]. To make things more confused, Hillis et al. studied 126 consecutive patients presenting with clinical myocardial ischemia. In this study, only P-selectin and cardiac troponin I — but not sICAM-1, sVCAM-1, or E-selectin — were significantly higher among patients who had a serious cardiac event during the index admission or the subsequent 3 months. Moreover, both remained independently predictive in a multivariable regression equation [109].

Mac-1 expression on the surface of polymorphonuclear leucocytes is up-regulated after balloon angioplasty and coronary stenting. The maximum up-regulation of Mac-1, which is observed 48 h after PCI activation, is an early and robust predictor of late lumen loss [3,27]. Therefore, assessing the expression levels of integrin Mac-1 after coronary stenting may prove helpful in predicting risk of restenosis.

In conclusion, the utility of CAMs in predicting the outcome of individual patients seems to be limited. More prospective studies are needed for the study of secondary prevention. The counter receptor of CAMs, integrin Mac-1, may prove useful to predict restenosis after PCI.

Myeloperoxidase

Myeloperoxidase (MPO) is a heme protein produced by activated neutrophils, monocytes, and tissue macrophages, and catalyzes the modification of LDL which is a critical step in atherogenesis [110].

Several studies have suggested that there is an association between MPO levels and CAD [110]. In a prospective study of patients presenting to the emergency department with chest pain, elevated MPO levels identified patients with undetectable troponin T (TnT) levels who were at increased risk of MI during their hospital stay or after discharge [111]. According to this information, MPO may be useful for early risk stratification of patients with chest pain and non-ST elevation MI. In another well-designed prospective study among patients with ACS, those with elevated MPO levels had a statistically significant increase in death or nonfatal cardiovascular events at 72 h, 30 days, and 6 months [112]; moreover, MPO levels were independent of TnT, CRP, and sCD40L levels, suggesting that MPO is an independent predictor of risk in CAD.

Finally, in the prospective European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study, serum MPO levels were associated with the future risk of coronary artery disease, in apparently healthy individuals [113].

Matrix metalloproteinases

Matrix metalloproteinases are endoproteases that are regulators of the extracellular matrix. They are localized at the shoulder of the plaque, have collagenase and/or gelatinase activity, and participate in vascular remodelling and plaque instability [114,115].

There are not many data for the association between MMPs and the prognosis of cardiovascular disease. Peripheral blood levels of MMP-1, MMP-2, and MMP-9 are elevated in patients with ACS [116]. Yet, in patients with either stable or unstable CAD,
those with elevated MMP-9 levels were at increased risk for future cardiovascular death [117]. A recent study enrolling 909 patients with acute MI, 466 patients with stable angina, and 1023 healthy older control subjects, showed that circulating levels of MMP-2 and MMP-9 were independently associated with the development of an acute MI rather than stable angina, as the initial clinical presentation of coronary artery disease [118]. In another study, elevations in MMP-1 at 7 and 14 days after ACS were negatively correlated with left ventricular ejection fraction [119]. However, the application of MMPs as biomarkers is limited because of the slow elevation of MMP levels after ACS and, in fact, lack of significant clinical studies, as yet.

**Placental growth factor**

Placental growth factor (PIGF) is a member of a family of platelet-derived proteins that function as chemoattractants for monocytes and is involved in the regulation of vascular endothelial growth [120]. PIGF appears to be stable in the circulation and may prove a strong candidate as a biomarker for plaque instability, myocardial ischemia, and prognosis for patients with ACS.

In the CAPTURE study, patients presenting to the emergency department with ACS had elevated PIGF levels compared with patients with noncardiac chest pain or stable angina. Between patients with unstable angina and NSTEMI there was no difference in PIGF concentrations. PIGF levels >27.0 ng/L were associated with increased risk of death or nonfatal MI at 72 h. Moreover, PIGF provided prognostic value independently of the levels of CRP, sCD40L, and cardiac TnT and identified a group of patients without elevated sCD40L or cardiac TnT that were at increased 30-day risk for a cardiovascular event [121].

**A2 phospholipases**

Lipoprotein-associated phospholipase A(2) [Lp-PLA(2)], a member of the phospholipase superfamily, seems to be a highly active enzyme in the circulation; it is also known as platelet-activating factor acetylhydrolase. Lp-PLA(2) participates in the oxidative modification of LDL by cleaving oxidized phosphatidylcholines, generating lysophosphatidylcholine and oxidized free fatty acids [122]. Recent reports indicate that Lp-PLA(2) has a distinct role in atherogenesis. Seminal findings support further the potentially damaging role that in situ release of LDL-associated oxidative products by Lp-PLA(2) may have in the formation of arterial wall lesions [123]. Local production of Lp-PLA(2) and lysophosphatidylcholine, the active product of Lp-PLA(2), in the coronary circulation were associated with early coronary atherosclerosis and endothelial dysfunction in humans [124].

Investigators measured Lp-PLA(2) in 3766 patients with stable coronary artery disease, that were followed for a median of 4.8 years for adverse cardiovascular events. In these patients, an elevated level of Lp-PLA(2) was a significant predictor of nonfatal adverse cardiovascular outcomes independent of traditional clinical risk factors and hs-CRP [125]. Similarly, in another study of 2513 patients with and 719 patients without angiographically confirmed CAD, Lp-PLA(2) predicted risk for 5-year cardiac mortality independently from established risk factors and hs-CRP concentrations [126].

A nested case-control analysis of the WHS, the ability of Lp-PLA(2) to predict the risk of cardiovascular events was not statistically significant after adjustment for traditional risk factors [127]. However, in a higher risk population of men with hyperlipidemia, in the West of Scotland Coronary Prevention Study (WOSCOPS), increased Lp-PLA(2) levels predicted risk for cardiovascular events independently of other inflammatory markers, such as CRP. A 60% statistically significant increase in risk between the highest and the lowest quintile of Lp-PLA(2) was found [128]. Yet, in the Atherosclerosis Risk in Communities (ARIC) study, Lp-PLA(2) was independently associated with CAD, in patients who had LDL levels <130 mg/dL [129]. This finding suggests that Lp-PLA(2) may have a prognostic role, similar to CRP, in identifying high-risk patients who may benefit from statin therapy and are not targeted on the basis of LDL levels. Finally, a recent study found that Lp-PLA(2) levels were associated with the extent of angiographic CAD, but they were not independently predictive after adjustment for CRP, lipid status, and other traditional risk factors [130]. In the more general setting of population studies, however, it is clear that Lp-PLA(2) is a positive risk factor for CAD and measurements of its mass may contribute to the prediction of coronary heart disease risk, especially in individuals with low LDL cholesterol levels.

Besides Lp-PLA(2), secretory type-II phospholipase A(2) [sPLA(2)-II], is also a member of the phospholipase superfamily, appearing to be an important inflammatory mediator and biomarker of cardiovascular disease [131]. An analysis of patients from the Global Registry of Acute Coronary Events (GRACE) study showed that elevated sPLA(2)-II levels were associated with increased risk of death or MI, independent of other risk factors [132].

Local activity of sPLA(2)-II in the atherosclerotic plaque seems to facilitate an inflammatory
response to induce plaque instability or rupture. In a recent study, Nijmeijer et al. studied histologically the presence of sPLA(2)-II in culprit lesions in the coronary arteries of patients with AMI or stable or unstable angina, using directed coronary atherectomy. Extracellular sPLA(2)-II was more abundantly present in atherosclerotic culprit lesions that had led to AMI, than in patients with stable or unstable angina [133]. This suggests a role for extracellular sPLA(2)-II in the development of complications of atherosclerotic lesions in coronary arteries.

In humans, the weight of evidence suggests that Lp-PLA(2) and sPLA(2)-II are positive risk factors for coronary heart disease — an observation commensurate with their position in the direct pathological sequence leading from formation of oxidized LDL in the artery wall to cellular dysfunction and formation of lesions.

**Myeloid-related protein 8/14 complex**

Myeloid-related protein 8/14 complex (MRP8/14), also termed calprotectin, is a heterodimer of two calcium binding proteins (S100A8 and S100A9, also referred to as MRP8 and MRP14, or calgranulin A and B) involved in calcium-dependent signaling, cell differentiation, cell cycle progression, and cytoskeleton—membrane interactions [134]. MRP8 and MRP14 are mainly expressed in monocytes and neutrophils [135]. Upon phagocyte activation, MRP8 and MRP14 form the MRP8/14 complex, which translocates to the cytoskeleton and plasma membrane, where it is secreted [136]. Therefore, complex MRP8/14 constitutes a marker of phagocyte activation, which is involved in plaque destabilization [137]. Elevated serum levels of MRP8/14 are a useful biomarker of disease activity in inflammatory disorders, such as rheumatoid arthritis and Crohn’s disease [138].

Healy et al. found increased expression of platelet CD69 and MRP14 in STEMI patients at the mRNA level in patients presenting to the cardiac catheterization laboratory with STEMI than patients with stable CAD [139]. This led the investigators to quantify plasma protein levels of the most abundant form of MRP14, the MRP8/14. Plasma levels of MRP8/14 heterodimer were higher in STEMI patients (17.0 g/mL versus 8.0 g/mL, P < 0.001) [139]. Yet, healthy women in the validation study [140], who subsequently developed cardiovascular events during follow-up, had higher median MRP8/14 levels at baseline than women who remained free of disease (controls, P < 0.001). In addition, in matched-pair analysis that accounted for age and smoking status, the risk of a first cardiovascular event increased significantly with each increasing quartile of baseline concentration of MRP8/14 (P trend <0.001), such that the women in the highest versus lowest quartile had 3.8-fold elevation in risk (P <0.001). Risks were independent of traditional cardiovascular risk factors and CRP.

To illustrate the potential ability of MRP8/14 to add prognostic value to lipid- or CRP-based screening, Healy et al. computed the relative risk of cardiovascular events after study participants were stratified into nine groups according to tertiles of MRP8/14 and tertiles of total cholesterol (TC):HDL or CRP. Women with low TC:HDL or CRP and low levels of MRP8/14 had the lowest risk. In contrast, women with high TC:HDL or CRP and high levels of MRP8/14 had the highest RR [139]. Importantly, even among women with low or intermediate TC:HDL or CRP levels, the risk of cardiovascular events was greater among those with high than with lower levels of MRP8/14 [139].

Altwegg et al. suggested that MRP8/14 that is expressed by monocytes and neutrophils, which are activated in plaque destabilization, might be elevated in ACS. Therefore, they compared circulating levels of MRP8/14 in patients with ACS, stable CAD, or normal coronary arteries. Systemic levels of MRP8/14 were markedly elevated [15.1 (12.1—21.8) mg/L, P = 0.001] in ACS when compared with stable CAD [4.6 (3.5—7.1) mg/L] or normals [4.8 (4.0—6.3) mg/L]. In addition, MRP8/14 was increased prior to necrosis markers such as myoglobin, CK-MB, and troponin, while using a cut-off level of 8 mg/L, MRP8/14 identified ACS presenting within 3 h from symptom onset [141].

In conclusion, the data suggest that MRP8/14 heterodimer can independently predict risk of future cardiovascular events and may add prognostic information to that conveyed by standard risk factors and CRP. Yet, the occurrence of elevated MRP8/14 in the systemic circulation prior to markers of myocardial necrosis is advantageous and makes it a prime candidate for the detection and management of ACS. Obviously, further studies are required to elucidate the full prognostic potential of MRP8/14 in healthy patients, as the only trial in primary prevention was conducted in apparently healthy postmenopausal women followed up in the WHS [140], as well as the diagnostic and prognostic implications in patients with chest pain.

**Conclusions**

Advances in understanding the pathobiology of atherosclerosis have implicated inflammation as a central contributor to the initiation and progression
of atherosclerotic vascular disease. Inflammatory biomarkers may have prognostic value for future cardiovascular risk among those at high risk or with documented cardiovascular disease. They also may be useful for identifying apparently healthy individuals, without known CAD, who may be at a higher risk than estimated by traditional risk factors. Finally, they might help to identify the subjects who are not eligible for preventive therapies based on traditional risk factors, but are at high risk for future cardiovascular events and might benefit most from these interventions.

However, until now, the data are conflicting about which biomarker is more suitable for diagnosis or prognosis of CAD. Probably, a combination of biomarkers may prove appropriate for our target.

Key points

- The role of inflammation in atherosclerotic plaque is crucial.
- High sensitivity CRP is the most extensively studied biomarker of evolving coronary atherosclerosis. Its measurement has been proposed as an adjunct to established risk factors to assess the risk for CAD.
- A cut-off level of hs-CRP of 2 (1—3) mg/L seems to discriminate high- from low-risk patients with stable or unstable coronary disease (ACS) or even apparently healthy individuals for short- and long-term prognosis of cardiovascular events. However, the measurement of hs-CRP should be delayed in the acute stage of MI until levels have returned to baseline.
- Intensive statin therapy lowers lipid and CRP levels. The significant lessening of hs-CRP value after an ACS reduces the risk for recurrent MI or fatal coronary event, irrespective of the degree of LDL lowering.
- Statins (lovastatin or rosuvastatin) reduce the incidence of major cardiovascular events even in apparently healthy persons without hyperlipidemia but with elevated hs-CRP levels. Once more, a target value of hs-CRP of less than 2 mg/L is suggested with preventive therapies.
- Cytokines (IL-1, IL-1β, IL-6, IL-8, TNF-α, and MCP-1), lipoprotein-associated phospholipase A(2) [Lp-Pla(2)], GM-CSF, sCD40L, serum amyloid A, soluble CAMs (sICAM-1, sVCAM-1, E- and P-selectins), and their counter receptors (primarily Mac-1), myeloperoxidase, matrix metalloproteinases (mainly MMP-9) and placental growth factor (PIGF) have all been implicated in the progression of plaque instability and have been considered to add prognostic information to that conveyed by standard risk factors and CRP in primary and secondary prevention of future cardiovascular events or even restenosis (Mac-1) after PCI.
- Complex MRP8/14 heterodimer, constituting a marker of phagocyte activation, seems to add prognostic value to lipid-based screening of future CAD events. Yet, using a cut-off level of 8 mg/L, MRP8/14 identifies ACS presenting within 3h from symptom onset, prior to markers of myocardial necrosis.
- The predictive value of biomarkers is additive and beyond that of CRP suggesting the need for a "multimarker approach" in assessing cardiovascular risk.

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Inflammatory biomarkers in coronary artery disease


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