Novel Clinical Markers of Vascular Wall Inflammation

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Abstract—Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and mediate many of the stages of atheroma development from initial leukocyte recruitment to eventual rupture of the unstable atherosclerotic plaque. Elevated plasma levels of several markers of the inflammatory cascade have been shown to predict future risk of plaque rupture. These markers include P-selectin, interleukin-6, tumor necrosis factor-α, soluble intercellular adhesion molecule-1, and C-reactive protein (CRP). Produced in the liver in response to interleukin-6, CRP has emerged as the most powerful inflammatory marker of future cardiovascular risk. Initially considered an innocent bystander in the atherosclerotic process, recent evidence suggests that CRP may have direct proinflammatory effects. Numerous large-scale, prospective studies have found that elevated baseline levels of CRP are a strong independent predictor of future vascular risk. Furthermore, aspirin and statin therapy appear to be particularly effective among individuals with high CRP levels. The addition of CRP screening to traditional lipid testing has the potential to identify individuals at high risk for future cardiovascular events who may benefit from targeted preventive interventions. (Circ Res. 2001;89: 763-771.)

Key Words: inflammation ■ risk factors ■ atherosclerosis

From the initial phases of leukocyte recruitment, to eventual rupture of vulnerable atherosclerotic plaque, inflammatory mediators appear to play a key role in the pathogenesis of atherosclerosis. From the initial phases of leukocyte recruitment, to eventual rupture of vulnerable atherosclerotic plaque, inflammatory mediators appear to play a key role in the pathogenesis of atherosclerosis.1 Early atherosclerotic lesion development involves tethering and adherence of monocytes to, and subsequent transmigration through, the vascular endothelium. Differentiation of monocytes to macrophages and subsequent accumulation of lipid results in foam cell generation and fatty streak formation. Further recruitment of inflammatory cells and proliferation of smooth muscle cells lead to the development of a mature atherosclerotic plaque, with a fibrous cap separating the prothrombotic lipid pool from luminal blood flow. Fibrous cap thinning may lead to plaque rupture and precipitate the onset of an acute ischemic event.2 Accumulating evidence suggests that inflammatory processes are intimately involved in each of these stages in atherogenesis.

Inflammatory Mechanisms in Atherothrombosis

The adherence and subsequent transmigration of leukocytes across the vascular endothelium are mediated by cellular adhesion molecules (CAMs).3 The selectins are adhesion molecules that mediate the initial rolling of inflammatory cells along endothelial cells and platelets. P-selectin is stored in the α granules of platelets and the Weibel-Palade bodies of endothelial cells4 and can be rapidly redistributed to the surface of these cells after stimulation by agonists such as thrombin and ADP. E-selectin is synthesized de novo by
endothelial cells when activated by interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α). The principal source of CRP production is the liver. However, recent data show that arterial tissue can produce CRP as well as complement proteins. These products and their associated mRNA are substantially upregulated in atherosclerotic plaque, with smooth muscle cells and macrophages the main producers. This supports the concept that CRP may be an endogenous activator of complement in atheromatous lesions.

As the atheromatous plaque matures, it develops a fibrous cap and lipid core. The vulnerable plaque is characterized by a thin fibrous cap and large lipidpool. Interstitial collagen, produced by smooth muscle cells, confers tensile strength and stability to the fibrous cap. Platelet-derived growth factor, released by platelets during thrombosis, and transforming growth factor-β, increase the rate of collagen production. Conversely, macrophages, in response to stimulation by TNF-α, can produce matrix metalloproteinases (MMPs), which actively break down the collagen and other extracellular matrix proteins in the fibrous cap. T cells may also produce interferon-γ, which signals to smooth muscle cells to decrease collagen synthesis. Thus a dynamic balance is maintained between collagen synthesis and breakdown. If the equilibrium is tipped to a proinflammatory state, fibrous cap thinning and eventual rupture may result.

Rupture of the fibrous cap results in spilling of the prothrombotic lipid pool into the lumen, likely heralding an acute ischemic event. Tissue factor, overexpressed by endothelial cells and macrophages, is a key initiator of thrombosis. IL-1 and TNF-α stimulate tissue factor expression by endothelial cells, and it has recently been shown that a CD40 ligand (CD154) binding to leukocytes can stimulate tissue factor expression. Platelets express CD154, illustrating a potentially important interaction between proinflammatory and prothrombotic pathways. Moreover, it has recently been shown that leukocyte binding and migration across a carpet of platelets adherent to diseased or injured intima are dependent on the leukocyte integrin Mac-1 and platelet glycoprotein 1b. The trigger for the inflammatory response, however, remains unclear. Attention has focused on infectious sources as potential instigators. Distant infection with organisms such as Helicobacter pylori, leading to increased circulating cytokines, or persistent local infectious processes within atherosclerotic plaque by intracellular organisms such as Chlamydia pneumoniae and cytomegalovirus, could potentially be the stimulus. Although pathophysiologically appealing, data regarding the value of testing for antibodies to these and other infectious agents in prospective epidemiological studies of cardiovascular risk are inconclusive.

The inflammatory response may be promoted at several different sites. Although many inflammatory markers are derived from the liver, including CRP, fibrinogen, and serum amyloid A, low levels may also be derived from other sources including the endothelium itself. Production of inflammatory markers is stimulated by circulating cytokines such as IL-6 and TNF-α, which in turn may also be generated from a variety of systemic sources, including adipose tissue, which is a potent source of cytokines, and inflammatory cells either in the atherosclerotic lesion in the arterial wall or elsewhere.
**Inflammation and Endothelial Dysfunction**

Several workers have sought to explore the hypothesis that impairment of endothelial function by inflammatory responses might provide a link between systemic inflammation and ischemic syndromes. In this regard, the administration of *Salmonella typhi* vaccine, used to generate a systemic inflammatory response in healthy volunteers, was associated with a temporary but profound dysfunction of arterial endothelium, as assessed by forearm blood flow response to acetylcholine and bradykinin. Furthermore, among patients with coronary artery disease, increased CRP levels have been shown to be associated with impairment of forearm endothelial vascular reactivity. Importantly, normalization of CRP levels over time was associated with a significant improvement in endothelial-dependent coronary vasodilation after 1 month of treatment.48 These data suggest that endothelial dysfunction may be an important factor in the relationship between low-grade chronic inflammation and cardiovascular disease. Interestingly, statin therapy in humans has been shown to upregulate endothelial nitric oxide synthesis47 and improve endothelial-dependent coronary vasodilation after 1 month of treatment.48

**Antinflammatory Effects of Lipid Lowering**

Lipid lowering has been found to have favorable effects on inflammatory processes within atheromatous plaque. Rabbits fed a high-cholesterol diet develop atheroma with a high number of macrophages in the lipid pool.59 These macrophages overexpress MMP-1, the rate-limiting enzyme in collagen breakdown. If the rabbits were switched to a low-cholesterol diet, the numbers of macrophages and levels of MMP-1 were dramatically reduced.

Statin therapy has also been shown to have salutary effects on plaque composition. Fluvastatin and lovastatin decrease MMP-1 expression in human vascular endothelial cells in a time- and dose-dependent manner.50 Fluvastatin and pravastatin have recently been shown to decrease MMP-1, MMP-3, and MMP-9 expression by macrophages in the intima of hyperlipidemic rabbits and to increase procollagen production by smooth muscle cells.51 Pravastatin causes favorable changes in atheromatous plaque independent of its cholesterol-lowering effects. Pravastatin-treated monkeys had better vasodilator function and fewer macrophages in the intima and media and less calcification and less neovascularization in the intima than control animals with similar changes in lipid profile caused by diet alone.52 Recent data from human carotid plaque show favorable change with pravastatin therapy, with fewer macrophages, reduced MMP-2 activity, and higher collagen content.53

Statins also cause decreased macrophage expression of soluble ICAM-1 (sICAM-1) and lipo polysaccharide-induced secretion of IL-6 and TNF-α.54–58 Simvastatin reduces monocyte expression of TNF-α and IL-1b,57 whereas atorvastatin reduces MCP-1 levels in the intima and media of hypercholesterolemic rabbits.58 This effect is related to a reduction in nuclear factor-κB activation, a transcription factor involved in the induction of other proinflammatory cytokines, such as IL-1 and TNF-α,59 and the regulation of E-selectin expression.60 Lipophilic statins have also been shown to decrease tissue factor expression and activity in cultured human monocyte-derived macrophages.61 As will be described later, in clinical studies statins have been found both to attenuate inflammatory risk and to reduce CRP levels.62–67

Statins also directly inhibit induction of major histocompatibility complex class-II (MHC-II) expression by interferon-γ and thus act as repressors of MHC-II-mediated T-cell activation.68 This effect is due to inhibition of the transactivator CITIA and is observed in several cell types including endothelial cells and macrophages. Recent studies also suggest that statins may promote vasculogenesis by mobilizing bone marrow–derived endothelial precursor cells and augmenting circulating endothelial precursor cells through stimulation of the Akt signaling pathway.69,70

**Inflammatory Markers for Clinical Risk Prediction**

Overt hyperlipidemia is present in less than half of all patients who have myocardial infarction. Given the pivotal role of inflammatory mediators in atherogenesis and the determination of plaque vulnerability, attention has focused on whether plasma levels of inflammatory markers can help predict individuals at increased risk for plaque rupture.71 Candidate markers include P-selectin, sICAM-1, IL-6, TNF-α, and CRP.

Given their role in the initial stages of atherogenesis, researchers have investigated the value of CAMs for prediction of subsequent vascular risk among healthy subjects. After activation by cytokines, CAMs are shed from the surface of endothelial cells and leukocytes and plasma levels of circulating CAMs can be measured. Although the pathogenic role of these circulating CAMs remains unclear, these molecules may serve as markers of endothelial activation and vascular inflammation.

Soluble P-selectin has been shown to be an independent predictor of future cardiovascular risk in a large-scale prospective study of 28,263 apparently healthy women enrolled in the Women’s Health Study (WHS).72 Overall mean levels of P-selectin were significantly higher at baseline among women who subsequently experienced cardiovascular events compared with those who did not. The risk of future cardiovascular events among women in the highest quartile of P-selectin levels was 2.2 times higher than those in the lowest quartile (P=0.01), an effect that was independent of traditional risk factors.

In a prospective study among 14,916 healthy men enrolled in the Physicians’ Health Study (PHS), baseline levels of sICAM-1 have also been shown to be independent predictors of future cardiovascular risk.73 Baseline levels were higher among men who subsequently developed myocardial infarction than those who did not, and the adjusted relative risk for those with the highest quartile of baseline sICAM-1 levels compared with those in the lowest quartile was 1.8 (P=0.03). The risk of myocardial infarction associated with raised concentrations of sICAM-1 seemed to increase with length of follow-up, an effect potentially consistent with the early role of ICAM-1 in the atherosclerotic process.

The Atherosclerosis Risk in Communities (ARIC) study also found that sICAM-1 was an independent predictor of...
coronary heart disease. However, in both the PHS and the ARIC study, baseline plasma levels of VCAM-1 were not associated with an increase in cardiovascular risk. These data suggest that there may be important distinctions between the roles of different CAMs in athrogenesis. Indeed, in human atheroma, ICAM-1 is highly expressed by both endothelial cells and macrophages whereas VCAM-1 is found in fewer than one third of lesions and its expression is predominantly restricted to endothelial cells and occasional spindle cells.

IL-6 and TNF-α occupy a central role in the amplification of the inflammatory cascade. Evidence in support of the role of inflammatory processes in acute coronary syndromes comes from data showing that increased levels of IL-6 and IL-1 receptor antagonist at 48 hours after admission are associated with a complicated in-hospital course. Furthermore, elevations of TNF-α in the stable phase after myocardial infarction were associated with an increased risk of recurrent coronary events. Specifically, those with baseline levels in excess of the 95th percentile of the control distribution had a 2.7-fold increase in risk (P = 0.004). The plasma half-life of TNF-α is short, a factor that may limit its potential clinical utility as a screening tool.

In a further analysis from the PHS, baseline levels of IL-6 were higher among apparently healthy men who subsequently had a myocardial infarction than among those who did not. The risk of future myocardial infarction increased with increasing quartiles of baseline IL-6 concentration such that the men in the highest quartile at entry had a relative risk 2.3 times higher than those in the lowest quartile (P = 0.005). CRP was the strongest correlate of IL-6 in these data (r = 0.43; P < 0.001), an observation consistent with the finding that IL-6 is the main stimulus for hepatic production of CRP. Nevertheless, the relationship of IL-6 with subsequent risk remained after adjustment for CRP.

Of all the plasma markers of vascular inflammation, CRP has been the most extensively investigated in clinical studies. Baseline levels of CRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral vascular disease, and stroke and vascular death among healthy individuals without known vascular disease. Furthermore, levels of CRP have been found to predict future risk among patients with stable and unstable angina, in the chronic phase after myocardial infarction, and among patients undergoing revascularization procedures.

With regard to risk prediction among patients with acute coronary syndromes, patients presenting with unstable angina who have elevated blood levels of CRP (≥ 3 mg/L) have been found to have higher rate of death, acute myocardial infarction, and need for revascularization procedures compared with patients with CRP levels <3 mg/L. Patients with acute myocardial infarction show a rise in CRP within 6 hours of symptom onset, suggesting that the rise in CRP may be secondary to an underlying proinflammatory state, rather than due to myocardial necrosis. This concept is supported by the finding that patients with coronary vasospasm have persistently normal CRP levels, despite frequent episodes of ST-segment elevation. Moreover, in both short-term and long term studies, CRP has had prognostic value even among patients with acute coronary syndromes with no discernible evidence of myocardial necrosis as evidenced by normal troponin levels. Further evidence in support of a role for inflammatory processes in unstable coronary syndromes comes from data showing that increased levels of IL-1 receptor antagonist and IL-6 at 48 hours after admission are associated with a complicated hospital course. However, given that patients with acute coronary syndromes have already declared themselves to be at increased risk, CRP testing may have greatest clinical utility in the primary prevention setting, where it may be used to guide targeted preventive interventions.

Data in support of a role for CRP for cardiovascular risk prediction among apparently healthy individuals are robust and remarkably consistent across several European and US cohorts (Figure 1). A recent analysis from the WHS sought to compare the risk associated with baseline levels of CRP with other inflammatory and lipid markers of risk. Incident cardiovascular events included death from coronary heart disease, nonfatal myocardial infarction, stroke, and need for coronary revascularization over a mean follow-up of 3 years. Baseline levels of CRP, serum amyloid A (SAA), IL-6, and sICAM-1 were significantly elevated at baseline among the women who subsequently developed cardiovascular events compared with those who did not. Similarly, levels of total cholesterol, LDL cholesterol, and the ratio of total cholesterol to HDL cholesterol (TC:HDL ratio) were significantly higher among patients than control subjects. As shown in Figure 2, of all the inflammatory and lipid markers, CRP was the single most powerful predictor of cardiovascular risk (relative risk for highest compared with lowest quartile = 4.4; P < 0.001). Multivariate analyses, matched for age and smoking and adjusted for other cardiovascular risk factors, found that only CRP and TC:HDL ratio were independent predictors of future cardiovascular risk. Of both clinical and pathological interest, the addition of CRP, IL-6, SAA, or

Figure 1. Prospective studies of CRP as a marker of future cardiovascular risk among individuals without known coronary disease. Risk estimates and 95% confidence intervals are calculated as comparison of top versus bottom quartile within each study group. See References 59 through 69. CRP indicates C-reactive protein; CHD, coronary heart disease; MI, myocardial infarction; PVD, peripheral vascular disease; and CVD, cardiovascular disease. Adapted from Ridker.
ICAM-1 testing to lipid testing significantly improved upon risk prediction based on lipid profile alone (Figure 3). Furthermore, even women with low cholesterol levels were found to be at increased risk if CRP or other inflammatory biomarker levels were high. In a subgroup analysis performed on women with LDL <130 mg/dL, women with increased levels of markers of inflammation were found to be at increased risk for subsequent cardiovascular events, an effect that was strongest for CRP (relative risk for highest compared with lowest quartile = 4.1, \( P = 0.002 \)). Similar data have recently been reported for men at risk of developing peripheral arterial disease.\(^{99}\)

These data suggest that the combination of CRP testing with traditional lipid screening may significantly improve cardiovascular risk prediction, particularly when LDL is low. This might indicate a group among whom aggressive primary prevention therapies should be targeted, such as weight loss, exercise, and smoking cessation. In this regard, CRP levels are known to be higher among patients with several traditional risk factors. Obesity is associated with elevated CRP levels,\(^{102}\) an observation consistent with the finding that adipocytes secrete IL-6,\(^{103}\) the main hepatic stimulus for CRP production. Diabetic patients also have elevated CRP levels,\(^{104}\) which may suggest a role for inflammatory processes in the pathogenesis of diabetes; in this regard, very recent data indicate that IL-6 and CRP levels are elevated at baseline among apparently healthy individuals destined to develop type II diabetes mellitus.\(^{105}\) Smokers have also been shown to have elevated levels of CRP, IL-6, and sICAM-1.

Accumulating data suggest that the benefits of preventive measures appear to be greatest among individuals with elevated CRP levels. In a large randomized study of aspirin for the primary prevention of cardiovascular events among men, the magnitude of benefit of aspirin in preventing myocardial infarction was directly related to baseline levels of CRP; specifically, the risk reduction for aspirin was 56% \(( \text{RR} = 0.42, P = 0.02 \) among those with baseline levels of CRP in the highest quartile, while there was a small, nonsignificant reduction (14%, \( P = 0.8 \)) among those with CRP levels in the lowest quartile.\(^{92}\) This finding raises the possibility that aspirin may prevent ischemic events through clinically important antiinflammatory as well as antiplatelet effects. In a recent study of patients presenting with acute coronary syndromes, CRP was a strong predictor of future risk among those who were not pretreated with aspirin but was not a strong predictor among those who received pretreatment with aspirin.\(^{106}\) Whether or not aspirin therapy reduces CRP is controversial.\(^{107,108}\)

**Figure 2.** Direct comparison of relative risk of future cardiovascular events associated with levels of lipid and inflammatory risk factors in the Women’s Health Study.\(^{79}\) Relative risks and 95% confidence intervals are shown for women in the top versus the bottom quartile for each factor. IL-6 indicates interleukin-6; TC, total cholesterol; LDLC, low-density lipoprotein cholesterol; sICAM-1, soluble intercellular adhesion molecule-1; SAA, serum amyloid A; Apo B, apolipoprotein B-100; HDLC, high-density lipoprotein cholesterol; and CRP, C-reactive protein. Adapted from Ridker et al.\(^{79}\)

**Figure 3.** Relative risks of cardiovascular events among apparently healthy women according to baseline levels of total cholesterol and markers of inflammation. Reprinted with permission from Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. \textit{N Engl J Med.} 2000;342:836–843. Copyright © 2000 Massachusetts Medical Society. All rights reserved.
Clinical Studies of the Antiinflammatory Effects of Statins

Several studies have shown that statin therapy lowers CRP levels independent of its lipid-lowering effects. A report from the Cholesterol and Recurrent Events (CARE) study was the first to suggest that the benefit of statins for the prevention of cardiovascular events might be greatest among patients with evidence of persistent inflammation. In the CARE trial, patients with prior history of myocardial infarction were randomized to receive either pravastatin (40 mg) or placebo. Patients with a persistent inflammatory response, as evidenced by elevated levels of both CRP and SAA, were at increased risk for recurrent events. Moreover, the proportion of recurrent events prevented by pravastatin was 54% among those with evidence of persistent inflammation compared with 25% among those without persistent inflammation. These data suggest that statin therapy may be particularly effective among individuals with elevated CRP levels, a finding consistent with the potent antiinflammatory properties of statins.

A recent analysis from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), a randomized trial of lovastatin for the primary prevention of cardiovascular events, sought to test this hypothesis in the primary prevention setting. Individuals were divided into 4 groups based on median LDL and CRP levels. Individuals with low LDL (<149 mg/dL) and low CRP (<0.16 mg/dL) levels were at low risk for future cardiovascular events and showed no benefit with lovastatin therapy. Individuals with high LDL (≥149 mg/dL), irrespective of CRP levels, were at a more than 2-fold increased risk for future events and derived substantial benefit from lovastatin therapy (relative risk 0.53; 95% confidence interval 0.37 to 0.77). However, the most interesting data pertained to individuals with low LDL (<149 mg/dL) but high CRP (≥0.16 mg/dL) levels. These individuals were at high risk for future events, with a more than 2-fold increased risk compared with those with low LDL and low CRP levels. Furthermore, the benefits of lovastatin therapy were substantial in this group, with a risk reduction (relative risk 0.58; 95% confidence interval 0.34 to 0.98) similar to that seen for individuals with overt hyperlipidemia. These data, although hypothesis generating, suggest that CRP testing may be used to target statin therapy among individuals without overt hyperlipidemia. CRP, a hepatic acute-phase reactant produced in response to IL-6, appears to be the strongest predictor of future vascular risk in a variety of clinical settings. CRP, a hepatic acute-phase reactant produced in response to IL-6, appears to be the strongest predictor of future vascular risk in a variety of clinical settings. CRP screening may potentially be used to identify individuals at risk for plaque rupture. Elevated levels of P-selectin, sICAM-1, IL-6, TNF-α, and CRP have been shown to predict future vascular risk in a variety of clinical settings. CRP, a hepatic acute-phase reactant produced in response to IL-6, appears to be the strongest predictor of future cardiovascular risk. Furthermore, the addition of CRP testing to lipid testing may improve upon lipid-based testing alone. Individuals with LDL levels below current treatment guidelines may be at substantially increased risk if CRP levels are elevated.

Recent evidence suggests that statin therapy may be particularly effective among individuals with elevated CRP levels, a finding consistent with the numerous antiinflammatory actions of statins. CRP screening may potentially be used to target statin therapy for the primary prevention of cardiac events among individuals without overt hyperlipidemia.

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References


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