Inflammation and Atherosclerosis

The Value of the High-Sensitivity C-Reactive Protein Assay as a Risk Marker

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Abstract

C-reactive protein (CRP) is a prototypic marker of inflammation. Numerous prospective studies in healthy volunteers have confirmed that high-sensitivity CRP (hsCRP) predicts cardiovascular events (CVEs), and hsCRP seems additive to an elevated total cholesterol level and a total/high-density lipoprotein cholesterol ratio in men and women in predicting risk. In smokers and people with metabolic syndrome, hsCRP levels are elevated; in elderly people, there seems to be a relationship between hsCRP and CVEs and mortality. Several properties of CRP make it proatherogenic; however, pending further studies, it should be considered as a risk marker. In people with acute coronary syndromes, hsCRP measurement may be valuable. Elevated levels in the highest quantile seem to predict greater mortality and poorer prognosis in patients with unstable angina and myocardial infarction (MI). While hsCRP is a strong independent predictor of risk of future MI, stroke, peripheral arterial disease, and vascular death, the validity of hsCRP as a risk marker needs to be assessed in all populations. Weight loss, statin drugs, aspirin, and high-dose alpha tocopherol therapy could affect hsCRP. It has its greatest validity as an adjunctive measure in the primary prevention of cardiovascular disease.

Pathobiology of Atherosclerosis

The earliest event in atherogenesis is endothelial cell dysfunction.[1] Various noxious insults, including hypertension, diabetes, dyslipidemia, smoking, and hyperhomocysteinemia, can result in endothelial cell dysfunction, which manifests primarily as a deficiency of nitric oxide and/or prostacyclin, among other aberrations. Following endothelial cell dysfunction, mononuclear cells such as monocytes and T lymphocytes attach to the endothelium, initially loosely, and thereafter, they adhere firmly to the endothelium and then migrate to the subendothelial space. Thereafter, they mature into macrophages, incorporate lipid from modified lipoproteins, eg, oxidized low-density lipoprotein (LDL) via the scavenger receptor pathway, and become foam cells, the hallmark of the fatty streak lesion. The various adhesion molecules that are involved in monocyte endothelial adhesion are summarized in ❚Table 1❚. Cell adhesion molecules are clearly present in atherosclerotic lesions, predominantly in the endothelium and intima (smooth muscle cells, monocytes, and macrophages), and are crucial to the early events of monocyte-endothelial cell adhesion.[2] Increased levels of soluble cell adhesion molecules (intercellular adhesion molecule [ICAM], vascular cell adhesion molecule [VCAM], E-selectin), which are shed from activated endothelium and/or phagocytes, are found in patients with coronary artery disease and carotid atherosclerosis.[3-5] Also, increased levels are present in people with diabetes and end-stage renal disease, both of which are identified with increased cardiovascular risk.[6,7] Results of studies suggest that some of these adhesion molecules, such as ICAM, can predict cardiovascular events,[8] while other adhesion molecules, such as VCAM-1 and E-selectin, are elevated with clinically evident atherosclerosis in cross-sectional studies.[3-5] Thus, soluble

❚Table 1❚
Cell Adhesion Molecules and Atherosclerosis

Adhesion Molecules	Tissue Distribution of Adhesion Molecules	Counterreceptors	Cellular Distribution of Counterreceptors
L-Selectin	Leukocytes	Sialylated oligosaccharide	Endothelium
P-Selectin	Megakaryocytes, platelets, endothelium	Sialylated Lewis-x–like structures	Myeloid cells, lymphocytes
E-Selectin	Cytokine-activated endothelium	Sialylated Lewis-x–like structures	Granulocytes, lymphocytes
Immunoglobulin superfamily			
ICAM-1,2	Widespread	LFA-1 (CD11a/18); MAC-1 (CD11b/18)	All leukocytes
VCAM-1	Endothelium, macrophages, dendritic cells	VLA-4 (CD49D/29)	Lymphocytes, monocytes, basophils, eosinophils

ICAM, intercellular adhesion molecule; LFA, leukocyte function-associated antigen-1; MAC, macrophage-1 antigen; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

❚Table 2❚
Prospective Studies of High-Sensitivity C-Reactive Protein as a Marker of Cardiovascular Risk

Study/Reference (year)	End Point	Relative Risk (95% Confidence Interval)
MRFIT/Kuller et al[28] (1996)	CAD death	2.8 (1.4-5.4)
PHS/Ridker et al[44] (1997)	MI	2.9 (1.8-4.6)
	Stroke	1.9 (1.1-3.3)
CHS/RHPP/Tracy et al[24] (1997)	MI	2.7 (1.04-6.81)
PHS/Ridker et al[15] (1998)	PVD	4.1 (1.2-6.0)
WHS/Ridker et al[14] (2000)	CVD	4.4 (2.2-8.9)
MONICA/Koenig et al[27] (1999)	CAD	1.7 (1.3-2.2)
Helsinki/Roivainen et al[30] (2000)	CAD	3.6 (1.9-6.6)
Caerphilly/Mendall et al[58] (1996)	CAD	2.1 (1.2-3.6)
Britain/Danesh et al[59] (2000)	CAD	2.1 (1.4-3.3)
AFCAPS/TexCAPS/Ridker et al[35] (2001)	Coronary events	1.7 (P = .01)

AFCAPS/TexCAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; CAD, coronary artery disease; CHS/RHPP, cardiovascular health study and the rural health promotion project; MI, myocardial infarction; MONICA, Monitoring Trends and Determinants in Cardiovascular Disease (study); MRFIT, Multiple Risk Factor Intervention Trial; PHS, Physicians Health Study; WHS, Women's Health Study.

cell adhesion molecules might emerge in the future as novel markers for atherogenesis. However, much work is needed to standardize these assays before introduction into the clinical laboratory.

Following the early fatty streak lesion, smooth muscle cells migrate into the intima and form the fibrous cap. It is believed that the lipid-laden macrophage releases matrix metalloproteinases, which cause a rent in the endothelium. Also, the lipid-laden macrophages are enriched in tissue factor. When tissue factor is released and comes in contact with the circulating blood, it results in thrombus formation and acute coronary syndromes (unstable angina and myocardial infarction). Thus, endothelial cell dysfunction, inflammation, smooth muscle cell proliferation, and thrombus formation culminate in acute coronary syndromes.

Inflammation and Atherosclerosis

With regard to inflammation, the prototypic marker is C-reactive protein (CRP), a member of the pentraxin family.[9] CRP is characterized by a cyclic pentameric structure, displays radial symmetry, and has a molecular mass of 23,048 d.[10] CRP binds phosphoesters in the presence of calcium, and its synthesis in the liver is triggered by various proinflammatory cytokines derived from monocyte-macrophages or adipose tissue. The general consensus is that proinflammatory risk factors such as oxidized LDL and infectious agents such as *Chlamydia pneumoniae* trigger a proinflammatory response.[11] The proinflammatory response results in the increased secretion of interleukin (IL)-1 beta and tumor necrosis factor alpha (TNF-alpha), which then results in the release of the messenger cytokine IL-6. IL-6, following engagement of its receptor on the liver, results in the secretion and release of CRP and serum amyloid A.

C-Reactive Protein and Cardiovascular Risk

Numerous studies have confirmed that high-sensitivity CRP (hsCRP) in healthy volunteers predicts cardiovascular events.[12] These studies are summarized in ❚Table 2❚. The various studies include the Multiple Risk Factor Intervention

Trial, the Physicians Health Study, the Cardiovascular Health Study, the Women's Health Study, the Monitoring Trends and Determinants in Cardiovascular Disease study, the European Concerted Action on Thrombosis study, the Helsinki Heart Study, the Caerphilly Study, the Britain Study, and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). The consistency of these findings in these different populations is impressive. In men and women, hsCRP seems to be an additive risk factor for coronary artery disease to an elevated total cholesterol level (>75th percentile) and a total cholesterol/high-density lipoprotein ratio.[13,14] Also, in a recent report from the Women's Health Study,[14] the novel observation was made that despite these apparently healthy postmenopausal women having LDL cholesterol levels below 130 mg/dL (3.36 mmol/L), hsCRP continued to predict an approximately 3-fold increased risk for cardiovascular events in women in the fourth quartile compared with those in the first quartile. In addition, hsCRP has been shown to be a predictor for peripheral vascular disease.[15]

C-Reactive Protein and the Metabolic Syndrome

The metabolic syndrome seems to be one of the major risk factors for cardiovascular disease, and numerous studies have confirmed that CRP levels are elevated in patients with the metabolic syndrome.[16-19] Festa et al,[16] in a report of the Insulin Resistance and Atherosclerosis Study, showed that hsCRP was positively correlated with body mass index, waist circumference, blood pressure, and levels of triglycerides, cholesterol, LDL-cholesterol, plasma glucose, and fasting insulin, and inversely correlated with the high-density lipoprotein cholesterol level and the insulin sensitivity index. This has been confirmed by numerous groups,[17-19] and there seems to be a clear relationship between a number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, hypertension) and increasing hsCRP levels.[17,18] It is believed that the proinflammatory cytokines in the metabolic syndrome derive from adipose tissue.[19] This area has created so much interest that Yudkin et al[19] have argued that IL-6 might be the major trigger culminating in the various manifestations of the metabolic syndrome, including insulin resistance, hypertension, dyslipidemia, endothelial dysfunction, and a procoagulant state and that the IL-6 levels could be driven by psychosocial stress, smoking, central obesity, and genotypic programming. Additional evidence underscoring the relationship between inflammation and the metabolic syndrome is the demonstration by Pickup et al[18] that both IL-6 and hsCRP are elevated in people with diabetes with features of the metabolic syndrome. We confirmed this.[20] Furthermore, in diabetes there is clear evidence of inflammation, such as increased activity of nuclear factor–kappa B, in addition to an increased monocyte release of proinflammatory cytokines such as IL-1 beta, TNF-alpha, and IL-6.[7,21]

C-Reactive Protein and Aging

There also seems to be a relationship between hsCRP and cardiovascular events and mortality in elderly people. In the Leiden 85 Plus Study (age, >85 years), baseline CRP levels were 2-fold higher in subjects who died of stroke during the first 5 years of follow-up than in control subjects.[22] Since levels of hsCRP were similar in fatal stroke vs noncardiovascular death, hsCRP was a strong nonspecific risk factor for mortality in this study. In the IOWA 65 Plus Rural Health Study in people older than 65 years, higher IL-6 levels and higher hsCRP levels were associated with a 2.6-fold increased risk for all-cause mortality.[23] In the Cardiovascular Health Study in people older than 65 years, in women but not men, the mean hsCRP was higher for case subjects with incident cardiovascular events compared with control subjects, with an odds ratio of 2.7.[24] In the Helsinki Aging Study of people older than 75 years, baseline hsCRP levels significantly predicted both total mortality and cardiovascular mortality.[25]

C-Reactive Protein and Smoking

Smoking is an established risk factor for coronary artery disease. CRP levels also are increased in smokers, and this confers greater cardiovascular risk. This is evident from several trials such as the European Concerted Action on Thrombosis trial, the Monitoring Trends and Determinants in Cardiovascular Disease study, and the Multiple Risk Factor Intervention Trial.[26-28] In the Cardiovascular Health Study,[29] CRP levels strongly correlated with smoking status. In the Physicians Health Study, the increased cardiovascular risk attributable to increased CRP remained significant even when adjusted for smoking status.[28] In the Helsinki Heart Study,[30] smokers with an hsCRP level in the highest quartile had a risk of 8.6, while smokers with a low hsCRP level had a cardiovascular disease relative risk of 1.6. Smokers also have activated proinflammatory cytokine networks, including increased concentrations of IL-1, IL-6, and TNF.[31] Since it is well known that these proinflammatory cytokines stimulate CRP production from the liver, it is possible that this may be the mechanism to explain the increased levels of CRP associated with cigarette smoking.

C-Reactive Protein and Other Evolving Risk Factors

When hsCRP is compared with other evolving cardio-vascular risk factors such as lipoprotein(a), homocysteine, and fibrinogen, it seems to be superior.[32] With regard to lipoprotein(a), standardized methods to measure it are not available, and the results of prospective studies have not been consistent. Furthermore, there is no evidence to show that there is added benefit to lowering lipoprotein(a).[33] Assays for homocysteine are not well standardized, and the results of prospective studies in primary prevention have been equivocal; presently, no additional benefit is gained from measuring homocysteine and the lipid profile. With respect to fibrinogen, most researchers do not use the "gold standard" method of von Clauss,[34] and, thus, method standardization is urgently needed. Consistent prospective data are available for fibrinogen, and measurement of the fibrinogen level in addition to the lipid profile has been shown to substantially improve cardiovascular risk prediction. However, benefits of lowering the fibrinogen level are yet to be established. Of all the evolving risk factors, only hsCRP has an assay standardized to World Health Organization (WHO) reference material, has consistent data with regard to at least 11 prospective studies, and is additive to the lipid profile in predicting risk.

The cost-effectiveness of measuring hsCRP was demonstrated in a recent primary prevention study (AFCAPS/TexCAPS) with lovastatin therapy.[35] In this study, the number needed to treat in patients with LDL cholesterol levels more than the median level was 42 (relative risk for acute coronary events, 0.53; $P = .001$), while the number needed to treat in subjects with LDL cholesterol levels less than the median of 149 mg/dL (3.85 mmol/L) and a CRP level more than the median of 1.6 mg/L was 48 (relative risk, 0.58; $P = .04$). If the number needed to treat is used to estimate effect of therapy, measurement of hsCRP provides an additional method for targeting therapy, especially if lipid levels are average. Thus, for a new test to be introduced into a clinical laboratory, it needs to be shown that it provides additional information to the present repertoire of tests and also is cost-effective. The hsCRP assay seems to fulfill these criteria.

While at this point it is safe to state that hsCRP is clearly a risk marker, data are evolving to suggest that CRP also might be proatherogenic. To date it has been shown that CRP induces the production of inflammatory cytokines in monocytes, promotes tissue factor expression in monocytes, is chemotactic for monocytes, and induces shedding of cell adhesion molecules. Also, it is present in the foam cells in atherosclerotic lesions and colocalizes with activated fragments of the complement system.[8,36-38] Furthermore, a recent
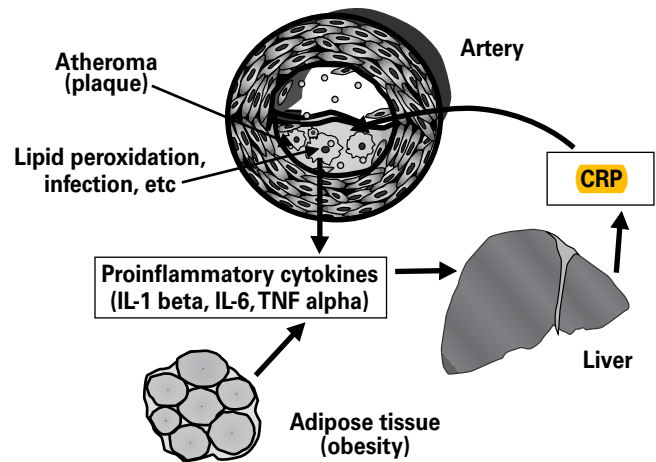
❙Figure 1❙ Schema depicting the role of highly sensitive C-reactive protein (CRP) in inflammation and atherosclerosis. IL, interleukin; TNF, tumor necrosis factor.

study demonstrated that people with high CRP levels had impaired endothelial vasoreactivity.[39] A schema depicting the factors that trigger the proinflammatory response and CRP is shown in ❙Figure 1❙.

Measurement of C-Reactive Protein

Historically, CRP, measured as a marker of active inflammation and infection, has a dynamic range (4-200 mg/L). During an acute inflammatory condition, CRP levels are increased as much as 1,000-fold.[40] However, the assays, usually immunoturbidimetric or immunonephelometric, do not have the appropriate sensitivity in the range required to determine cardiovascular risk. While most methods have been standardized to WHO reference material, this standard has a CRP concentration in the acute phase reactant range (>4 mg/L). The hsCRP assays use diluted WHO reference material as calibrators. At present, no secondary reference materials in the coronary artery disease risk range are available. However, the Centers for Disease Control and Prevention is in the process of developing reference material in the hsCRP range using an isotope dilution mass spectrometry method.

Because the hsCRP level of an individual patient is interpreted according to the risk profile established by prospective clinical studies, it is important that the measurement be standardized, especially since recent reports show that measurement of low hsCRP levels is not consistent among the various methods available.[41] The Dade Behring latex-enhanced method to detect hsCRP has been approved by the Food and Drug Administration in the risk assessment of cardiovascular disease. For most individuals, hsCRP

levels seem to be stable over long periods. It is important, if the hsCRP assay is going to be used for cardiovascular risk assessment, that one is cognizant of the fact that the test should be performed only 2 to 3 weeks after resolution of an acute inflammatory process (eg, trauma, infection), since CRP remains elevated for at least 14 days after an acute inflammatory condition.[40] To determine variability and classification accuracy, Ockene et al[42] examined 113 people who were scheduled to undergo 5 measurements each of hsCRP and total cholesterol at quarterly intervals over a 1-year period. When classified into quartiles, 63% of the first and second measurements of hsCRP were in agreement compared with 60% for total cholesterol measurements. Also, correct classification into the different quartiles (<0.5, 0.5-0.99, 1-1.99, and ≥2 mg/L) was obtained in 237 of the 374 pairs (64% accuracy) across the 1-year study period. Thus, in the low normal range needed for cardiovascular risk prediction, the variability and accuracy of classification of hsCRP is similar to that for total cholesterol. The predictive value of hsCRP is greatly improved if 2 measurements are made about 1 month apart and the lowest of these values is used to determine the appropriate cardiovascular risk prediction. If hsCRP values are more than 10 mg/L, repeated measurement is suggested to avoid misclassification owing to a clinically silent infection. It also is important to note that unlike the cytokines such as IL-6, no circadian variation seems to exist for hsCRP, and, thus, testing can be accomplished without regard to the time of day.

Modulation of Inflammation

Therapies that have been shown to lower hsCRP include weight loss, aspirin, statin drugs, and alpha tocopherol. A recent study reported that weight loss in obese, healthy women on a very low fat diet for 12 weeks resulted in a 26% reduction in hsCRP.[43] However, hsCRP levels were not normalized. In the Physicians' Health Study, it was suggested that the greatest benefit with aspirin was obtained in the group that had the highest quartile of hsCRP compared with the lowest quartile of hsCRP.[44] This benefit could be ascribed to an antiplatelet and an anti-inflammatory effect. Results of studies that have looked at the effect of aspirin directly on hsCRP levels specifically have been conflicting. Ikonomidis et al,[45] in a study of 40 patients with angina pectoris using 300 mg/d of aspirin for 21 days, convincingly showed a reduction in hsCRP levels. However, Feng et al,[46] who used 325 mg/d or 81 mg/d in 32 healthy subjects studied for 7 days, saw no significant effect. Feldman et al[47] administered 81 mg of aspirin every day, 81 mg every third day, and 325 mg every third day for 31 days in an apparently normal cohort comprising 30 men and 27 women. The

groups receiving aspirin failed to show a significant reduction in hsCRP, while demonstrating a profound reduction in thromboxane B_2. Thus, it seems that low-dose aspirin's major effect in preventing cardiovascular events is through its antiplatelet effect. Further studies are urgently needed to establish the role of higher doses of aspirin (325 mg/d) on hsCRP levels.

Another modality that could have an effect on hsCRP is the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) drugs. The Cholesterol and Recurrent Events study showed that the greatest benefit was achieved in the group with the highest hsCRP (54% reduction in cardiovascular events compared with 25% in the lowest quartile for hsCRP).[48] In this study, Ridker et al[48] showed a median 17% reduction in hsCRP levels in the group that received pravastatin (40 mg/d). Thus, it appears that pravastatin, in addition to having a beneficial effect on the lipid profile, reduces hsCRP in these patients and has its greatest benefit in patients with the highest hsCRP levels. Jialal et al[49] recently showed in a randomized, double-blind crossover study in patients with combined hyperlipidemia (LDL >130 mg/dL [>3.36 mmol/L], triglycerides, 200-600 mg/dL [2.26-6.77 mmol/L]) that 6 weeks of therapy with simvastatin (20 mg/d), atorvastatin (10 mg/d), or pravastatin (40 mg/d) resulted in a significant reduction in hsCRP levels. In that study, Jialal et al[49] failed to show a significant correlation with regard to reduction of the LDL cholesterol level and hsCRP reduction, in agreement with the Cholesterol and Recurrent Events study, suggesting that the effects of statins might be pleiotropic. Recently, Horne et al[50] followed up 985 patients with severe coronary artery disease (>70% stenosis). The patients were followed up for 3 years, and mortality was documented in 11% of the cohort. While in this study lipids did not predict survival, subjects receiving statin therapy, simvastatin, atorvastatin, pravastatin, lovastatin, or fluvastatin, had a decreased hazards ratio of 0.049 ($P < .05$). This benefit could be attributed to an elimination of increased mortality across increasing hsCRP tertiles in this study.[50]

There also is evidence to show that high-dose alpha tocopherol therapy is anti-inflammatory.[51,52] At least 2 studies have shown that supplementation with high-dose vitamin E (alpha tocopherol), 800 IU/d or more, is beneficial in lowering hsCRP in subjects with type 2 diabetes and in healthy control subjects.[20,53]

Other patients in whom it is of considerable interest to measure hsCRP are those with acute coronary syndromes.[54-57] It seems that elevated levels of hsCRP in the highest quantile seem to predict greater mortality and poor prognosis in patients with unstable angina and myocardial infarction.[54]

The high sensitivity CRP assay that is approved by the Food and Drug Administration is a double monoclonal

■Table 3■
High-Sensitivity C-Reactive Protein (hsCRP) and
Cardiovascular Risk

hsCRP (mg/L)	Cardiovascular Risk
<1.1	Low or mild
1.2-1.9	Moderate
>2.0	High

sandwich microparticle enzyme immunoassay referenced to
the WHO standard that has a sensitivity of 0.2 mg/L. A
simpler way to assess cardiovascular risk based on the data
of Rifai and Ridker[12] for at least 5,000 individuals without
clinical atherosclerosis is shown in ■Table 3■. However, the
levels conferring risk for women and the elderly might have
to be modified as more data emerge. A value of more than
10 mg/L suggests an acute inflammatory process, "macroin-
flammation," and should suggest that sampling be deferred
for at least 2 weeks.

Conclusion

Thus, hsCRP is a strong independent predictor of risk
of future myocardial infarction, stroke, peripheral arterial
disease, and vascular death among people without known
cardiovascular disease. In addition, among patients with
acute coronary ischemia, stable angina, and myocardial
infarction, levels of hsCRP have been associated with
increased vascular event rates. In conclusion, hsCRP
seems to be an adjunct to lipid screening in the detection
of people at high risk for coronary artery disease, espe-
cially in primary prevention. It seems to have a clear role
in stratifying people into risk groups. Also, measurement
of hsCRP would prove to be a better target for statin,
aspirin, and alpha tocopherol therapy compared with the
lipid profile alone, especially in smokers, the elderly, and
people with the metabolic syndrome. The potential prog-
nostic value in acute coronary syndromes requires further
study. Inflammation clearly represents a new target for
both treatment and prevention of acute myocardial infarc-
tion. Finally, studies need to be undertaken in minority
populations to establish the validity of hsCRP as a risk
marker in all populations.

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