**Inflammation and Atherosclerosis**

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

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**Key Words:** Atherosclerosis; Inflammation; C-reactive protein; Risk marker

**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. 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. 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. Also, increased levels are present in people with diabetes and end-stage renal disease, both of which are identified with increased cardiovascular risk. Results of studies suggest that some of these adhesion molecules, such as ICAM, can predict cardiovascular events, while other adhesion molecules, such as VCAM-1 and E-selectin, are elevated with clinically evident atherosclerosis in cross-sectional studies. Thus, soluble
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. CRP is characterized by a cyclic pentameric structure, displays radial symmetry, and has a molecular mass of 23,048 d. 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. 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. These studies are summarized in Table 2. The various studies include the Multiple Risk Factor Intervention Trial; PHS, Physicians Health Study; WHS, Women’s Health Study.

<table>
<thead>
<tr>
<th>Study/Reference (year)</th>
<th>End Point</th>
<th>Relative Risk (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRFIT/Kuller et al28 (1996)</td>
<td>CAD death</td>
<td>2.8 (1.4-5.4)</td>
</tr>
<tr>
<td>PHS/Ridker et al34 (1997)</td>
<td>MI</td>
<td>2.9 (1.8-4.6)</td>
</tr>
<tr>
<td>CHS/RHPP/Tracy et al32 (1997)</td>
<td>Stroke</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>PHS/Ridker et al32 (1998)</td>
<td>MI</td>
<td>2.7 (1.04-6.81)</td>
</tr>
<tr>
<td>WHS/Ridker et al34 (2000)</td>
<td>PVD</td>
<td>4.1 (1.2-6.6)</td>
</tr>
<tr>
<td>MONICA/Koenig et al37 (1999)</td>
<td>CAD</td>
<td>4.4 (2.2-8.9)</td>
</tr>
<tr>
<td>Helsinki/Roivainen et al30 (2000)</td>
<td>CAD</td>
<td>1.7 (1.3-2.2)</td>
</tr>
<tr>
<td>Caerphilly/Mendall et al38 (1996)</td>
<td>CAD</td>
<td>3.6 (1.9-6.6)</td>
</tr>
<tr>
<td>Britain/Danesh et al39 (2000)</td>
<td>CAD</td>
<td>2.1 (1.2-3.6)</td>
</tr>
<tr>
<td>AFCAPS/TexCAPS/Ridker et al35 (2001)</td>
<td>Coronary events</td>
<td>2.1 (1.4-3.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7 (P = .01)</td>
</tr>
</tbody>
</table>
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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 al19 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 underscores the relationship between inflammation and the metabolic syndrome is the demonstration by Pickup et al18 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 cardiovascular risk factors such as lipoprotein(a), homocysteine, and fibrinogen, it seems to be superior. 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). 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, 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. 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. Furthermore, a recent study demonstrated that people with high CRP levels had impaired endothelial vasoreactivity. 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. 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. 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. To determine variability and clas-

sification accuracy, Ockene et al42 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 predi-
tion. 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 accom-
plished 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. 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. 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 al47
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 reduc-
tion in hsCRP while demonstrating a profound reduction in
thromboxane B2. 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). In this study, Ridker et al48 showed a
median 17% reduction in hsCRP levels in the group that
received pravastatin (40 mg/d). Thus, it appears that prava-
statin, 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
al49 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 al49 failed to show a significant
correlation with regard to reduction of the LDL cholesterol
level and hsCRP reduction, in agreement with the Choles-
terol and Recurrent Events study, suggesting that the effects
of statins might be pleiotropic. Recently, Horne et al50
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, prava-
statin, 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.

There also is evidence to show that high-dose alpha
tocopherol therapy is anti-inflammatory. 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
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\(^\text{12}\) 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, “macroinflammation,” 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, especially 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 prognostic value in acute coronary syndromes requires further study. Inflammation clearly represents a new target for both treatment and prevention of acute myocardial infarction. 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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