In Part 1 of this series we reviewed the evidence supporting an association between inflammation and cardiovascular disease. Part 2 focuses on C-reactive protein (CRP), the well-known marker of inflammation and one of the promising new markers of cardiovascular risk.

Physiologic characteristics of CRP

In 1930, CRP was identified in the plasma of patients with pneumonia and was named for its ability to bind and precipitate the C-polysaccharide of pneumococcus.\(^1\) One of the most assayed acute phase proteins, CRP is a sensitive marker of the inflammation that results from infectious disease, tissue damage, malignancy, and various non-infective ailments.\(^2\) Within hours of injury or the onset of inflammation, various cytokine mediators (interleukin-1, interleukin-6, and tumor necrosis factor-\(\alpha\)) stimulate hepatocytes to synthesize CRP, and plasma levels of CRP peak within 24-48 hours.\(^3\) CRP may also be synthesized by local inflammatory cells in the area of tissue damage, infection, etc.\(^4\) The plasma half-life of CRP is short, approximately 20 hours, yet is remarkably consistent regardless of the underlying inflammatory condition.\(^5\) This is unique for an acute phase reactant and implies that plasma levels of CRP are a direct indication of its rate of synthesis.

Despite being one of the most studied acute phase proteins, the exact physiologic role of CRP is somewhat uncertain. CRP exhibits a number of pro-inflammatory and pro-coagulant characteristics thought to be relevant to its association with cardiovascular disease. CRP binds to damaged cell membranes,\(^6\) to phosphatidylcholine,\(^7\) and to many pneumococcal polysaccharides;\(^8\) it also enhances the activity of phagocytic cells,\(^9\) modifies T-cell lymphocyte function,\(^10\) destroys leukocytes,\(^11\) activates the complement system,\(^12\) and stimulates mononuclear cells to produce tissue factor, the primary initiator of coagulation.\(^13\) In addition, although it has not been demonstrated in vivo, aggregated CRP binds LDL cholesterol in vitro,\(^14\) a characteristic that has been proposed to implicate CRP in the progression of atherosclerosis.\(^15\)

Evidence of CRP in atherosclerosis

Direct evidence of CRP’s involvement in atherosclerosis is provided by a recent immunohistochemical investigation. Studying human hearts obtained at autopsy, Zhang et al.\(^16\) reported that CRP was not present in normal coronary arteries, but appeared with progressively greater tissue-staining intensity in developing atherosclerotic plaques. CRP staining was diffuse in early-stage plaques and in thickened areas of the intima, corresponding to a point in the progression of atherosclerosis in which lipids are present as lipoproteins. Further along in atherosclerotic plaque development, CRP staining was more concentrated, corresponding with lipid release from affected lipoproteins. Finally, the most intense CRP staining was found localized in the lipid-rich cores of large atherosclerotic plaques.

CRP staining also correlated with increasing thickness of the intima, with decreasing lumen diameter, and with the presence of lipid-filled macrophages — three prominent characteristics of atherosclerosis.

There were also sections of the intima that showed no CRP reactivity, suggesting that the inflammation associated with atherosclerosis may be localized, rather than systemic. It is also possible that inflammation may repeatedly appear and disappear within the intima, perhaps coordinating with the appearance and disappearance of clinical symptoms.

Less direct evidence of CRP’s involvement in atherosclerosis is provided by two recent studies involving the angiographic evaluation of patients with coronary artery disease (CAD) and coronary heart disease (CHD). Abdelmouatitaleb et al.\(^17\) measured serum CRP and plasma fibrinogen (the precursor of the clotting protein, fibrin) and an acute phase protein) in 179 patients undergoing diagnostic coronary angiography for CAD. Compared to the control group, both CRP and fibrinogen were significantly elevated in patients with angiographically-confirmed atherosclerosis; this included patients with myocardial infarction (MI), unstable angina pectoris (UA), and stable angina pectoris (SA). CRP levels were significantly higher in patients with MI or UA than in lower-risk, SA patients. CRP levels did differ significantly between the SA group and another low-risk group, patients with normal coronary angiograms but with other evidence of cardiac dysfunction (e.g., stable valvular heart disease, atypical chest pain, or dilated cardiomyopathy). Both of these low-risk groups, however, had CRP levels significantly higher than the healthy controls, emphasizing the association between CRP and cardiovascular disease.

Abdelmouatitaleb et al. found no correlation between CRP and the extent of CAD in this study. This observation, combined with the lack of a significant difference between the two lower-risk cardiac groups and the significantly elevated CRP levels in the high-risk MI and UA patients, suggested to the authors that CRP’s involvement in atherosclerosis or CAD may be limited to facilitating the onset of acute coronary episodes, rather than being involved as a causative factor. If CRP was directly involved in causing atherosclerosis, one would expect CRP levels to correlate with the severity of arterial narrowing or CAD. As this was not the case, the authors reasoned that CRP and inflammation may be primarily involved in plaque instability and rupture. This hypothesis is consistent with the immunohistochemical evidence presented by Zhang et al.\(^16\)

In another investigation involving coronary angiography, Riazi et al.\(^18\) studied 100 patients with angiographically-confirmed CHD, evaluating several markers related to inflammation: CRP, serum amyloid A, interleukin-6 (IL-6), and soluble intercellular adhesion molecule-1. CRP, serum amyloid A, and IL-6 were elevated in all confirmed CHD patients and remained statistically significant after adjusting for several established cardiovascular risk factors. Of these markers, CRP demonstrated the strongest association with CHD. The statistical significance of serum amyloid A and IL-6 was lost after adjustment for CRP, while CRP remained significant after adjustment for serum amyloid A and IL-6. Furthermore, the data provided by serum amyloid A or IL-6 did not improve CRP’s predictive ability.

As in the study of Abdelmouatitaleb et al.,\(^17\) none of these markers correlated with extent of CHD, underscoring the theory that inflammation is involved, but not causative, in atherosclerosis.

CRP in asymptomatic, apparently healthy individuals

In addition to the relatively recent studies discussed above, a wealth of evidence has existed for quite some time implicating CRP in cardiovascular disease. In 1990, Berk et al.\(^19\) reported CRP elevations in a population of UA patients. Since then, several studies have confirmed that CRP is indeed elevated in association with many coronary syndromes, often several years prior to the onset of clinical symptoms.\(^20-22\)

Several prospective studies have been concisely reviewed and indicate that baseline levels of CRP significantly correlate with risk of MI, stroke, and CHD-related mortality in individuals apparently free from cardiovascular disease.\(^23\) Two of the larger, population-based studies reported to date are the USA-based Physicians Health Study\(^24,25\) and the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Augsburg (Germany) survey.\(^26,27\) The Physicians’ Health Study, which evaluated

**Abbreviations used in this article**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
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<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CRP</td>
<td>C-Reactive Protein</td>
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<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
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<td>HDL-C</td>
<td>High-Density Lipoprotein Cholesterol</td>
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<td>IL-6</td>
<td>Interleukin-6</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<td>SA</td>
<td>Stable Angina Pectoris</td>
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<tr>
<td>TnT</td>
<td>Troponin T</td>
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<tr>
<td>UA</td>
<td>Unstable Angina Pectoris</td>
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C-Reactive Protein, Inflammation, & Cardiovascular Disease

The traditional markers of cardiovascular risk (see Sidebars: Markers of Cardiovascular Risk) as well as many novel markers, recruited approximately 22,000 apparently healthy men, aged 40 - 84, with no history of cardiovascular disease. The CRP portion of the study was included in 543 randomly assigned participants. Subjects were followed for 8 years and monitored for cardiovascular events.

CRP was a significant predictor of future peripheral vascular disease: Individuals with high baseline CRP levels eventually developed severe vascular insufficiency, those with moderate levels developed claudication, and those with low levels remained disease free. In addition, CRP was a strong indicator of future cardiovascular disease: Subjects with CRP levels stratifying them into the highest quartile had a 2-fold increase in risk of a stroke and a 3-fold increase in risk of MI. CRP's correlation with outcome was independent of other established cardiovascular risk factors.

In addition to CRP, other markers studied included total cholesterol, total cholesterol:HDL-C ratio, lipoprotein(a), fibrinogen, total plasma homocysteine, and tissue-type plasminogen activator. Figure 1 shows the efficacy of these markers for predicting MI and indicates that CRP was the best single indicator of risk. Furthermore, the predictive ability of CRP was additive to that of the total cholesterol:HDL-C ratio; this was confirmed by another report in which CRP levels > 2.1 mg/dL correlated with, and were additive to, hypercholesterolemia in predicting the occurrence of an initial coronary event in men (Figure 2).

Similar to the Physicians' Health Study but involving a European cohort, the MONICA survey 14 -16 analyzed the association of several risk factors with the development of an acute coronary event over an 8-year period in 4,000 initially healthy men and women with no history of cardiovascular disease. The CRP portion of the study involved 936 men, aged 45 - 64 years. The results obtained were very similar to those from the Physicians' Health Study as subjects with CRP values in the highest quintile had a 2.6-fold increase in risk of a future coronary event. Of note, CRP levels were twice as high in smokers as in non-smokers, but ex-smokers had levels similar to those of individuals who had never smoked. CRP was also twice as high in obese individuals vs. the non-obese. CRP correlated significantly with age, obesity, smoking, and diabetes, but multivariate analyses indicated that CRP was independent of these and other risk factors.

CRP also returned strong results in four other prospective studies, these involving high-risk, asymptomatic populations. Tracy et al. 32 and Harris et al. 44 demonstrated a correlation between elevated CRP and increased risk for a coronary event in aged (>65 years) populations, some with evidence of atherosclerosis. Ridker et al. 45 also found an association between CRP and cardiac risk in an older population: post-menopausal women. Koller LH 46 studied a high-risk population of smokers and reported a significant correlation between CRP levels and CHD-related mortality. In each of these studies, the correlation between CRP and outcome was strong, and when CRP levels were stratified, the CRP values corresponded to approximately a 2- to 3-fold increase in risk of a future coronary event.

CRP in symptomatic SA and UA patients
Not only has CRP demonstrated clinical utility in assessing the cardiac risk of asymptomatic, apparently healthy individuals, but it also has proven useful in predicting outcome in several symptomatic coronary syndromes (Table 1).

In a large, prospective study involving over 2,100 symptomatic patients with either SA or UA, Haverkate et al. 47 reported that patients with CRP > 3.6 mg/dL had approximately a 2-fold increase in risk of an acute cardiac event within 2 years. Of note in this study, similar CRP levels were found between the UA and SA patient groups. This is in contrast with the report by Abdelmouttaleb et al., 48 in which CRP levels were lower in SA patients than in UA patients. Abdelmouttaleb et al. suggested that the discrepant results may stem from the different patient populations in the two studies. Specifically, the study of Haverkate et al. involved out-patients; therefore, it was possible that the acute symptoms of the UA patients may have subsided by the time of assay, providing time for their CRP levels to decrease to those of the SA patients.

In a study involving patients with UA or non-Q-wave MI, de Winter et al. 49 measured CRP and cardiac troponin I (Tnl) in 150 subjects. CRP elevations were defined as > 5 mg/L and elevated Tnl as > 0.4 µg/L. As shown in Figure 3, the incidence of a major cardiac event — cardiac death, recurrent MI, or recurrent UA — within the 6 month follow-up period was highest (42%) in patients with both CRP and Tnl elevations. CRP was an independent marker of risk in the total study population: 23% of patients with elevated CRP suffered a major event, regardless of Tnl status. CRP remained a significant predictor of risk even in patients without Tnl elevations: 11% of those patients with elevated CRP and normal Tnl had a cardiac event, whereas none of the patients with normal levels of both CRP and Tnl had an event.

Kaplan-Meier survival analysis showed that event-free survival was significantly lower in patients with both CRP and Tnl elevated than in those with either or none elevated. Multivariate analyses involving age, gender, history of infarction, hypertension, aspirin use, and diabetes showed that the predictive value of each of these factors improved significantly when CRP, Tnl, or both were included, thus indicating independent and additive prognostic value for both CRP and Tnl.

In a conflicting report, Toss et al. 50 found that elevated CRP in the absence of elevated cardiac troponin T (TnT) was not predictive of outcome in UA. de Winter et al. 49 suggested, however, that the results of the Toss study may have been confounded by the inordinate large proportion of patients with elevated TnT.

The results of the de Winter study are consistent, however, with those of two other studies. Morrow et al. 51 found CRP and TnT values to have independent, additive, and significant predictive value in predicting 14-day mortality in acute coronary syndromes. In addition, Liuzzo et al. 52 found that CRP elevations in the absence of TnT elevations were associated with a poor outcome in UA.

CRP in refractory UA
In UA, symptoms often repeatedly appear and subside, regardless of medical intervention, a phenomenon that may be related to plaque instability, to the rapid appearance and disappearance of occlusive thromboses, and/or to the presence or absence of myocardial ischemia. These acute episodes of UA may be resistant to treatment with conventional drugs. In patients with refractory UA, levels of CRP were consistently elevated, independent of whether the patient was stabilized or could not be stabilized. In addition, in patients without myocardial necrosis, as determined by levels of cardiac troponin T, CRP, fibrinogen, and erythrocyte sedimentation rate (ESR) were higher in refractory UA patients than in those who could be stabilized. In addition, patients without myocardial necrosis and with low levels of cardiac troponin T, CRP, fibrinogen, and ESR were also significantly higher in the refractory UA patients than in the stabilized UA patients. Multivariate analyses adjusting for established cardiovascular risk factors indicated...
Table 2. CRP Quartiles and Probability of a Future Cardiac Event in Low-Risk, Post-MI Patients.

<table>
<thead>
<tr>
<th>CRP Quartile</th>
<th>Range (mg/dL)</th>
<th>Probability of a Cardiac Event</th>
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<tr>
<td>1</td>
<td>&lt; 0.45</td>
<td>6%</td>
</tr>
<tr>
<td>2</td>
<td>0.45 - 0.93</td>
<td>12%</td>
</tr>
<tr>
<td>3</td>
<td>0.93 - 2.55</td>
<td>31%</td>
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C-Reactive Protein, Inflammation, & Cardiovascular Disease

Presently, in the USA the National Cholesterol Education Program recommends stratifying men and women at risk for cardiovascular disease based on the total number of risk factors present in an individual.66 These traditional risk factors include:

- **Family history of coronary heart disease**
- **Cigarette smoking**
- **Hypertension**
- **Hypercholesterolemia** (high total cholesterol)
- **Low levels of high-density lipoprotein cholesterol** (Age: men > 45 years, women > 55 years)
- **Diabetes mellitus**
- **Obesity**, as determined by body mass index, may also be included

Myocardial necrosis does indeed produce an acute phase response,66 but many studies indicate that this is not always so: several investigations reported no CRP elevations in patients with UA, SA, and unstable CAD.67,68 Presumably the patients in these studies had some degree of myocardial ischemia, but the presence of an acute coronary syndrome does not always invoke an inflammatory response. Luzzo et al.69 showed that severe myocardial ischemia in variant angina without evidence of CAD did not result in an increase in CRP. Likewise, CRP elevations were not present in UA patients undergoing percutaneous trans-luminal coronary angioplasty70 or in patients with an activated coagulation system.67

These studies suggest that CRP may be involved in UA, SA, and CAD only to the extent of the underlying atherosclerosis. This is consistent with the immunohistochemical analysis of necrotic debris in plaques from patients with atherosclerotic cardiovascular disease (Zhang et al.71) and angiographic studies (Abdelmoula et al.72 and Rifai et al.73) studies discussed in the body of this article in which CRP is thought to be more involved in plaque rupture than in causing atherosclerosis.
cardiac troponin and CRP. In addition to damaged myocardium, these patient have evidence of an acute inflammatory response, which may further plaque instability and increase the likelihood of life-threatening occlusive thromboses. CRP also provides predictive data additive to that provided by cholesterol measurements in determining the risk of an acute coronary event. Furthermore, throughout each these studies, CRP demonstrates independent, predictive value: Adjustment for established cardiovascular risk factors such as age, smoking, hypercholesterolemia, age, and obesity, does not reduce the statistical significance of CRP’s predictive ability.

Although the underlying mechanisms may be unclear at present, the association CRP has with atherosclerosis and cardiovascular risk is vivid. There is even evidence that CRP levels may reflect damage in the myocardium, these patient have evidence of an acute coronary event. Furthermore, throughout each these studies, CRP demonstrates independent, predictive value: Adjustment for established cardiovascular risk factors such as age, smoking, hypercholesterolemia, age, and obesity, does not reduce the statistical significance of CRP’s predictive ability.

References

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