C-Reactive Protein: An Inflammatory Biomarker in Clinical Practice

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ABSTRACT

The importance of inflammation in the etiology of vascular events is becoming more obvious, as a result of both clinical and laboratory studies. C-Reactive Protein (CRP), a non-specific indicator of inflammation, has emerged as a useful parameter for assessing individual risk of cardiovascular disease and acute events. When added to conventional measurements such as cholesterol fractions (LDL and HDL), CRP enhances their discriminatory power.

INTRODUCTION

Although most patients who manifest heart disease have one or more cardiovascular risk factors, some who lack any of these risk factors may still be vulnerable. Data from a 26 year follow-up of patients in the original Framingham Heart Study demonstrate that perhaps 35% of coronary heart disease occurs in those with total cholesterols of <200 mg/dl. Clearly, there is more to the story than just cholesterol.

THEORETICAL BASIS

For decades pathologists have observed an inflammatory infiltrate at the site of the culprit plaque in patients who die from myocardial infarction, but in early studies it wasn't clear whether the infiltrate developed before or after the infarction. A clue to resolving this dilemma came from epidemiologic data that indicated an increase in the rate of vascular events in patients recovering from acute illness and surgery, both of which are known to evoke an inflammatory response. Subsequent reports confirmed the presence of elevated inflammatory markers in such patients—suggesting that inflammation indeed preceded the vascular events.[1]

Additional evidence supporting the role of inflammation in vascular events arose from studies of HMG Co-A reductase inhibitors (statins). These drugs reduce primary and secondary cardiovascular events out of all proportion to the degree they lower lipids.[2] One explanation for their effects may lie in the association between cholesterol production and the activation of Rho-associated kinase, which shares the same metabolic pathway. Activation of Rho-kinase appears to play a key adverse role in cardiovascular disease by down-regulating endothelial nitric oxide synthase, as well as by promoting thrombosis and vasospasm, inflammatory cell migration, and oxidative stress. Fasudil, a specific inhibitor of Rho-kinase, has been effective in treating cerebral and coronary vasospasm, angina, pulmonary hypertension and heart failure.[3] Similarly, HMG Co-A reductase inhibitors, which act early in the synthetic pathway, prevent activation of Rho-kinase, which may account for their multi-faceted effects.

C-REACTIVE PROTEIN

C-Reactive Protein was first identified bound to the C-polysaccharide of the pneumococcal cell wall (hence it's name), and is a fundamental component of the immune response. CRP is a non-specific marker of inflammation, and assays for its measurement have been available for decades. The structure of this pentameric molecule is remarkably stable over time, allowing measurement of many thousands of stored serum samples in large data bases. These measurements form the basis for the initial observations tying inflammatory markers to clinical outcomes.

Levels of traditional, or "non high sensitivity" CRP, vary significantly in diseases such as rheumatoid arthritis, but the assay is not sufficiently sensitive at the low range. "High sensitivity" CRP (hs-CRP) uses a different assay that is able to detect these low-range differences, and has thus become the main focus of research in vascular inflammation.

Most CRP is produced in the liver, though it is also made by vascular endothelium. Increased CRP levels are associated with increased body-mass index, advanced age, hypertension, insulin resistance, diabetes, tobacco use, chronic kidney disease, decreased left ventricular function, extensive atherosclerosis, active infection, and depression.

Conditioned athletes have lower CRP levels, and the degree of reduction correlates with the intensity of

training. Given the strong inverse association between cardiovascular events and physical activity, CRP reduction has been postulated as a possible mechanism for this beneficial effect.

Obese patients have higher CRP levels and higher cardiovascular risk. Adipocytes produce large basal quantities of interleukin-6, a potent stimulus of CRP production.[4] In a study of healthy obese women placed on a restricted calorie diet, the degree of CRP reduction correlated with the degree of weight loss.[5]

EPIDEMIOLOGIC STUDIES

Substantial data now link elevated levels of C-reactive protein (CRP) with vascular events.

The Physician's Health Study followed almost 22,000 healthy men for 8-10 years. For men in the quartile with the highest CRP, the risk of myocardial infarction (MI) was 3× that of men in the lowest quartile ($p < 0.001$), and the risk of stroke was twice as great ($p = 0.02$). The higher relative risk was constant over time during the 10 year follow-up period, indicating that high CRPs were probably not related to pre-clinical or sub-clinical ischemia.

Aspirin was beneficial in preventing MI mainly in patients with the highest CRPs, causing a significant (55.7%, $p = 0.02\%$) reduction in relative risk of MI in the highest CRP quartile, but no significant reduction in the lowest quartile (13.9%, $p = 0.77$).

Eleven different atherothrombotic markers were evaluated for their ability to predict the development of peripheral arterial disease. As expected, the best lipid predictor was the total cholesterol:HDL ratio, whereas CRP proved to be the strongest non-lipid predictor when the highest quartile was compared to the lowest, with a 2.8 fold increase in relative risk. The addition of CRP to standard lipid measurements increased their ability to portend risk ($P < 0.001$).[6]

The Women's Health Study followed 28,263 mostly postmenopausal healthy women for 3 years after baseline CRP measurements. Twelve lipid and non-lipid markers were analyzed in relation to the incidence of death from coronary heart disease, non-fatal myocardial infarction, stroke, and the need for coronary revascularization.[7] The risk of events for each marker was compared between the highest and lowest quartiles. Of the 12 markers, CRP was the strongest predictor of coronary events by univariate analysis. The quartile with the highest CRP had 4.4 × the risk of the lowest quartile ($p < 0.001$). Multivariate analysis that adjusted for age, body-mass index, smoking, diabetes, hypertension, and premature family history of CHD, showed that only CRP and the total cholesterol: HDL-cholesterol ratio predicted cardiovascular events ($p = 0.02$).

Further, there were consistent increases in cardiovascular events with rising levels of hs-CRP. Subgroup analysis of low-risk women with no history of hypertension, hyperlipidemia, tobacco use, diabetes, or a family history of vascular disease, demonstrated a consistent increase in relative risk as CRP rose. These subgroups are probably the lowest risk populations we encounter, and yet the trends were still statistically significant across each clinical subset. Because CRP measurements demonstrate a correlation with relative risk even in these patients who presumably lack bulky atherosclerotic plaque, the findings suggest that CRP has a direct influence on plaque stability.

Though the Women's Health Study revealed no correlation between levels of LDL-cholesterol and levels of CRP, a comparison was made between the relative impact of LDL-cholesterol and CRP on cardiovascular risk. Not surprisingly, the best outcomes were seen in the group with the lowest levels of both, and the poorest outcomes were seen in those with the highest levels of both. But the two other groups provided an unexpected surprise: the group with *high LDL* and *low CRP* had better outcomes than the group with *low LDL* but *high CRP*. This finding suggests that CRP has a greater influence than LDL does on cardiovascular risk.

Both the Physician's Health Study and the Women's Study were large trials conducted in North America, and their results have been confirmed by numerous European trials including the MONICA-Augsburg Cohort Study, and the Helsinki Heart Study.

IMPROVED RISK ASSESSMENT

Traditional screening methods for assessing cardiovascular risk include LDL-cholesterol, total cholesterol:HDL ratio, cardiac stress testing, and electron-beam calcium score. If CRP is also measured with any of these standard risk assessment tools, CRP either adds complementary benefit or is itself actually superior.

The Framingham Risk Score utilizes standard risk factors to categorize the risk of developing clinically apparent coronary heart disease within a 10 year follow-up period. A likelihood of <10% is defined as *low* risk, 10-20% is *intermediate*, and >20% is *high* risk.

The Women's Health Study confirmed the benefit of adding CRP assessment across the full range of Framingham risk. Data from the MONICA cohort, a population-based study of 3,435 healthy middle-aged German men, also demonstrated a benefit of adding CRP measurement to those with intermediate Framingham scores.[8] Additive predictive value was also seen in the South Bay Heart Watch study of 967 patients without diabetes, when CRP measurement was added to their risk assessment based on their coronary calcium scores.[9]

Measurement of C-reactive protein at discharge was compared to later stress testing in 139 consecutive patients who presented with chest pain and subsequently ruled out for myocardial infarction.[10] The primary endpoint was all-cause mortality and/or acute myocardial infarction within 90 days of discharge. Elevated CRP level was shown to be a more sensitive and specific predictor for the primary endpoint; positive and negative predictive values were also superior A Kaplan-Meier plot of freedom from events demonstrates highly significant differences in outcomes between high and low CRP patients, whereas there was no significant difference when risk was determined by stress test.

DOES CRP CAUSE ATHEROSCLEROSIS?

CRP was initially felt to be just a marker for vascular inflammation, but a growing body of data demonstrates a more active role. The enzyme which results in the beneficial release of nitric oxide (a coronary vasodilator) is down-regulated by CRP; adhesion molecules which initiate atherosclerosis are up-regulated; and a deleterious release of endothelin-1 (a coronary vasoconstrictor) occurs. CRP has also been shown to increase endothelial cell expression of plasminogen activator inhibitor-1, a prothrombotic compound.

Elevated CRP levels were recently shown to be a strong independent predictor of rapid progression of disease in patients with known CAD.[11] Most agree that one of the important steps in the transition from a 'stable' atherosclerotic plaque to an active, unstable lesion involves degradation of the protective fibrous cap, mediated by the enzyme metalloproteinase. Increased activity of this enzyme has been correlated with rising CRP levels.

Monocytes respond to CRP by increasing not only the production of inflammatory cytokines, but also expression of tissue factor, and chemotaxis, all of which are proatherothrombotic.

Elegant work from Willa Hsueh's lab at UC-Davis has demonstrated CRP's ability to up-regulate the gene associated with apoptosis of human vascular smooth muscle cells (GADD153), which results in nuclear-programmed death of vascular smooth muscle.[12]

CRP MODULATION

Various interventions have been associated with lower CRP levels. Non-pharmacologic measures include weight loss, fish oils, vitamin E, red wine, a low-fat diet, the Mediterranean diet, and LDL-cholesterol apheresis. Drugs that lower CRP include statins, angiotensin-2 receptor blockers, rosiglitazone, and ezetimibe when added to statin therapy.

Statins potently lower CRP levels seen in patients with either stable symptoms or acute coronary syndromes, and this observation may, in part, explain their efficacy in reduction of primary and secondary vascular event rates.

The VALMARC trial recently demonstrated that blockade of angiotensin-2 lowers CRP levels independent of the degree that blood pressure is lowered, which suggests that activation of renin-angiotensin has an inflammatory component.[13]

The FLUVACS trial assessed the effect of flu vaccine on subsequent cardiovascular mortality in a randomized study of patients who presented with acute coronary syndrome and had subsequent percutaneous intervention.[14] In the next 12 months, 23% of the unvaccinated patients either died or developed recurrent acute coronary syndromes, compared with 11% of the patients randomized to receive flu vaccine. Thus, prevention of influenza reduced major adverse cardiovascular events by 50%, perhaps by blocking the associated inflammatory response.

The effect of aspirin on CRP levels was examined in two small prospective trials which found no significant CRP

reductions in patients receiving daily doses from 40 to 325 mg. Nonetheless, in the Physician's Health Study, aspirin 325 mg every other day reduced the risk of MI, and the greatest reduction was seen in those with the highest initial CRPs.

CRP IN CLINICAL PRACTICE

Despite the absence of prospective data, a series of retrospective analyses provide compelling reasons to include CRP measurements in decisions about the clinical management of patients.

- The TXCAPS/AFCAPS trial, a primary prevention trial of lovastatin in the general population, demonstrated that statin therapy was equally effective in reducing the incidence of coronary events whether patients had elevated lipid levels, or low lipid levels accompanied by high CRP levels.[15]
- The PROVE IT-TIMI 22 trial examined the impact of moderate or intensive lipid lowering in 3,745 patients with acute coronary syndrome.[16] A retrospective analysis demonstrated benefit for both lipid lowering and CRP reduction.[17] When LDL-cholesterol fell to <70mg/dl, clinical events (MI or cardiac death) fell from 4 to 2.7 per 100 patient-years (p = 0.008). If CRP fell to <2mg/L, events fell in a similar manner from 3.9 to 2.8 per 100 patient-years regardless of the level of achieved LDL. The lowest event rate (1.9 per 100 patient-years) occurred when LDL was lowered to <70 mg/dL and CRP fell to <2 mg/L.
- The REVERSAL trial assessed stable patients with angiographically proven CAD using intra-coronary ultrasound at baseline and after 18 months of moderate or intensive lipid lowering.[18] After adjustment for the degree of lipid reduction, CRP independently and significantly correlated with the rate of CAD progression.
- PROVE IT and REVERSAL used atorvastatin and pravastatin as agents for intensive and moderate lipid lowering respectively. Both these trials demonstrated benefit when LDL fell below 70 mg/dL and/or CRP fell to <2 mg/L, regardless of the drug utilized.

An AHA/CDC Scientific Statement concluded 'it is reasonable to measure hs-CRP as an adjuct to the major risk factors to further assess absolute risk for coronary disease.' Those with levels <1 mg/L are felt to be at low risk for developing subsequent CV events, whereas a level >3mg/L conveys a 3-4 fold increase in relative risk over the subsequent decade. Levels greater then 10 mg/L should be repeated as they may be related to a more serious but subclinical condition. A recent report indicates that the risk of a CV event rises in a linear fashion with CRP levels above 3 mg/L.[19] This suggests regardless of cause, elevated CRP poses increased relative risk and the greater the rise, the greater the risk.

SUMMARY

It is clear that the inflammatory response is an important part of our immune defense system, but the ideal response is a calibrated one that is adequate to protect the host without becoming exaggerated and predisposing to a stroke or MI.

Data indicate that patients without manifest vascular disease who have a CRP >3 mg/L have a 3-4 fold increased relative risk of MI. In patients with other risk factors for CAD, such an elevation provides a 3-fold increased risk of death, and in those admitted with chest pain, CRP is predictive of those who rule in for acute coronary syndrome.[20]

In patients with established heart disease, lowering CRP to <2 mg/L results in a statistically significant reduction in growth of plaque and a decrease in clinical events. Though these observations come from observational retrospective analyses, if they are confirmed, measurement of hs-CRP may well be recommended in future versions of the ATP Guidelines. (National Cholesterol Education Program – Adult Treatment Panel)

Much of what we do in clinical medicine involves treatment based on the patient's individual risk, and assessment of that risk is therefore fundamental. We reassure low risk patients, and help them to stay that way, while high risk patients are candidates for aggressive intervention.

For a marker of risk to be valid, it must have:

- a plausible biological mechanism
- applicability to both genders
- ability to enhance our current estimation of risk
- applicability to populations in widespread geographic locales

In the case of CRP, not only are these criteria met, but the risk factor can be modified.

Intriguing data demonstrate an association among periodontal disease, elevated CRP, and acute myocardial infarction.[21] Dental hygiene and flu vaccination should also be included under the umbrella of preventive cardiovascular care. We await ongoing clinical trials that will provide an evidence-based approach for the optimal treatment of patients with isolated, unexplained elevations of CRP.

Taken together, data from TXCAPS/AFCAPS, PROVE-IT, and REVERSAL, although retrospective, allow inclusion of hs-CRP measurements in clinical management decisions. In patients with established coronary disease and a 'borderline' LDL level, an hs-CRP level below 1 mg/L tempers enthusiasm for additional treatment, whereas a level >3mg/L provides incentive for more intensive intervention. Weight loss and exercise have the most potent effect on CRP, but it can be difficult to convince patients of the need for more intensive lifestyle interventions. It is often helpful to explain to patients that this inflammatory marker indicates whether 'the fuse is lit.'

In addition to proper diet, exercise, and fish oils, statins lower LDL-cholesterol and markers of inflammation, all of which benefit the population at risk. The annual cost of statin therapy in the United States is estimated to be $12 billion. The 'lower is better' axiom suggested by many trials, added to the emerging data on the utility of inflammatory markers, may increase this cost several fold as broadened guidelines and treatment goals are introduced.

It is reasonable to ask if we can afford such an expense as a society. In that regard, the Lancaster Intelligencer Journal of March 11, 2004 reported that Americans spend $31 billion annually on dog treats. The reader can decide if better patient outcomes are worth the added expense to expand therapy with statins.

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