High-Sensitivity C-Reactive Protein: A Novel and Promising Marker of Coronary Heart Disease

NADER RIFAİ1,2,4* and PAUL M. RIDKER2,3,5

Background: Coronary heart disease remains the leading cause of morbidity and mortality in the industrialized world. Clinical and laboratory studies have shown that inflammation plays a major role in the initiation, progression, and destabilization of atherosclerotic plaques. C-Reactive protein (CRP), an acute phase reactant that reflects low-grade systemic inflammation, has been studied in a variety of cardiovascular diseases.

Approach: Findings from prospective clinical trials were examined to determine the prognostic utility of CRP in acute coronary syndromes, and observations from epidemiological studies were reviewed to determine the ability of CRP to predict future first coronary events. The analytical considerations of CRP measurement in these clinical applications were also examined.

Content: In patients with established coronary disease, CRP has been shown to predict adverse clinical events. In addition, prospective studies have consistently shown that CRP is a strong predictor of future coronary events in apparently healthy men and women. The relative risk associated with CRP is independent of other cardiovascular disease risk factors. High-sensitivity CRP (hs-CRP) assays are needed for risk assessment of cardiovascular disease. Such assays are currently available but may require further standardization because patients’ results will be interpreted using population-based cutpoints. Preventive therapies to attenuate coronary risk in individuals with increased hs-CRP concentrations include aspirin and statin-type drugs.

Summary: hs-CRP has prognostic utility in patients with acute coronary syndromes and is a strong independent predictor of future coronary events in apparently healthy subjects.

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Coronary heart disease (CHD)6 is the major cause of death in the developed world. Atherosclerosis, the underlying cause of most CHD, is a process that starts early in life and progresses slowly and silently for decades. The clinical manifestation usually occurs in the form of myocardial infarction (MI), stroke, angina, or sudden death between ages 50 and 60 years in men and between 60 and 70 years in women. Cholesterol screening has been used as a tool to identify individuals who are at increased risk of developing future coronary events. Although this approach has been useful, it fails to identify almost one-half of the 1.3 million individuals who develop MI in the US each year who have either normal or only moderately increased serum cholesterol concentrations.

Laboratory and clinical evidence has demonstrated that atherosclerosis is not simply a disease of lipid deposits. Rather, systemic inflammation also plays a pivotal role in atherothrombotic inception and progression (1–3).

Mononuclear cells, macrophages, and T lymphocytes are prominent in atheromatous plaques in the arterial wall (4–7). Furthermore, the shoulder region of a plaque, the most vulnerable site for rupture in acute coronary syndromes, is heavily infiltrated with inflammatory cells (8–10). Cytokines, which cause the de novo hepatic production of acute phase reactants such as C-reactive protein (CRP) (11), have been shown to increase in acute coronary syndromes even in the absence of myocardial necrosis (12). Therefore, CRP has been examined as a surrogate marker of other inflammatory mediators such as interleukin-6 and tumor necrosis factor-α to better understand the inflammatory component of atherosclero-
sis (13, 14). Current knowledge, however, suggests that the CRP concentration might reflect the vulnerability of the atherosclerotic lesion and the likelihood of a plaque to rupture (2, 3, 15). This acute phase reactant has been studied over the last several years in a wide variety of atherosclerotic diseases (12, 16–20). Its prognostic utility in acute coronary syndromes (12, 16–20) and its ability to predict future coronary events in apparently healthy men and women (21–30) have been demonstrated. The development of high-sensitivity CRP (hs-CRP) assays has been instrumental in exploration of the role of this acute phase reactant in predicting first cardiovascular events. Prospective studies have consistently demonstrated a positive association between hs-CRP and future coronary events. For hs-CRP to make the transition from clinical research to the routine clinical setting, however, several important issues must be satisfactorily addressed: (a) the availability of population-based cutpoints for interpretation and risk assessment; (b) the existence of potential therapeutic modalities; and (c) the reliability of the analytical systems used for measurement.

**hs-CRP as a Prognostic Indicator in Acute Coronary Syndromes**

Several studies have demonstrated that hs-CRP, measured at either presentation or discharge, may have prognostic value in patients with acute coronary syndromes. Some reports have also examined the risk stratification of patients by hs-CRP alone or in combination with cardiac troponins.

Liuzzo et al. (12) showed that in 31 patients with severe unstable angina and no evidence of myocardial necrosis, as documented by the absence of increased cardiac troponin T, hs-CRP concentrations >3 mg/L at admission were associated with an increased incidence of recurrent angina, coronary revascularization, MI, and cardiovascular death. The same group later demonstrated that hs-CRP >3 mg/L at discharge in 53 unstable angina patients was associated with increased readmission for recurrent instability and MI (16). In a similar study of unstable angina, Ferreiros et al. (18) concluded that the prognostic value of hs-CRP measured at discharge was better than that determined at admission in predicting adverse outcome at 90 days. Furthermore, hs-CRP was the strongest independent predictor of adverse events in multivariate analysis. Data from the Thrombolysis In Myocardial Infarction (TIMI) IIa (TIMI 11A), a study of unstable angina and non-Q-wave MI, showed that markedly increased hs-CRP (15.5 mg/L) at presentation in 437 patients was a good predictor of 14-day mortality in that population (19). Furthermore, hs-CRP helped to identify those patients with negative cardiac troponin T (qualitative rapid bedside method with cutoff of <0.2 μg/L) who were at increased risk of mortality (19). Morrow et al. (19) concluded from that study that a strategy for risk stratification using both cardiac troponin T and hs-CRP should be considered. Similar conclusions were reported in a follow-up report by the same group using serum amyloid A, another acute phase reactant, instead of hs-CRP (31). A recent report by de Winter et al. (17) showed that hs-CRP concentrations >5 mg/L at admission in 150 patients with non-ST-elevation acute coronary syndromes were associated with an increased incidence of major cardiac events within 6 months, regardless of cardiac troponin I values.

**hs-CRP as a Predictor of Future Coronary Events**

Over the last 6 years, several prospective studies have demonstrated that hs-CRP is a predictor of future cardiovascular morbidity and mortality among individuals with known cardiovascular disease. Data from the European Concerted Action on Thrombosis and Disabilities (ECAT) Angina Pectoris Study Group, a study of 2121 men and women with stable and unstable angina, demonstrated that each standard deviation increase in hs-CRP was associated with a 45% increase in the relative risk (RR) of nonfatal MI or sudden cardiac death [95% confidence interval (95% CI), 1.15–1.83] (20). Similarly, in the Cholesterol and Recurrent Events (CARE) trial, hs-CRP was a predictor of recurrent coronary events in men and women who had already suffered a MI (32). Those with hs-CRP concentrations in the highest quintile had an 80% higher chance of developing another coronary event within the 5-year study period (RR = 1.77; 95% CI, 1.1–2.9). Therefore, hs-CRP has the potential to be used in the stratification of patients into high- and low-risk groups.

Perhaps of greater clinical importance is the demonstration that hs-CRP concentrations predict first MI and stroke. To date, 10 prospective studies, 6 in the US and 4 in Europe, have consistently shown that hs-CRP is a powerful predictor of future first coronary event in apparently healthy men and women (Fig. 1). Findings from the Multiple Risk Factors Intervention Trial (MRFIT) demonstrated a positive association between hs-CRP and CHD mortality in men followed over a 17-year period (RR = 2.8; 95% CI, 1.4–5.4) (22). This relationship, however, was evident only among smokers. A similar association between hs-CRP and future coronary events was noted in the Cardiovascular Health Study and Rural Health Promotion Project, which included men and women over 65 years of age with subclinical cardiovascular disease (26). The Physicians’ Health Study (PHS) demonstrated similar positive association between hs-CRP and future coronary events in apparently healthy men (23). Unlike the observation in MRFIT, however, this association was evident in both smokers and nonsmokers. This study showed that those in the highest quartile of hs-CRP had a twofold higher risk of future stroke (RR = 1.9; 95% CI, 1.1–3.3), threefold higher risk of future MI (RR = 2.9; 95% CI, 1.8–4.6), and fourfold higher risk of future peripheral vascular disease (PVD; RR = 4.1; 95% CI, 1.2–6.0) (23, 28). The RR were stable over a long period of time (≥6 years) and independent of other CHD risk factors. The European MONICA (Monitoring Trends
and Determinants in Cardiovascular Disease) Augsburg study showed that an increase of one standard deviation in the log-transformed value of hs-CRP was associated with a 50% increase in coronary risk and that subjects with hs-CRP concentrations in the highest quintile had a 2.6-fold higher risk of developing future coronary events (21). A recent report from the Helsinki Heart Study confirmed these observations and demonstrated that those in the highest quartile of hs-CRP had a more than threefold higher risk of future MI or cardiac death (RR 5 3.56; 95% CI, 1.93–6.57) (27).

Two reports from the Women’s Health Study (WHS) showed that hs-CRP is a strong predictor of future cardiovascular events in women (RR = 4.4; 95% CI, 2.2–8.9) (24, 25). In stratified analyses, hs-CRP continued to be a strong predictor of future cardiovascular events even among subgroups of women with no history of hyperlipidemia, hypertension, smoking, diabetes, or family history of CHD (25). The hs-CRP concentrations seen in these postmenopausal women were somewhat higher than those reported previously in men. Although no difference in hs-CRP values was noted between premenopausal women and age-matched males, recent reports showed that hormone replacement therapy (estrogen alone or estrogen and progestin) is associated with increased hs-CRP concentrations (33, 34). These findings suggest that the increased hs-CRP seen in the WHS subjects may reflect the influence of hormone replacement therapy rather than the effect of gender.

Predictive Value of hs-CRP and Other Biochemical Markers for CHD Risk

The RR estimates derived from most of the above-mentioned prospective studies were independent of other recognized cardiovascular risk factors. Data from both the PHS (35) and WHS (24) showed that the predictive value of hs-CRP was significantly higher than that associated with traditional biochemical CHD risk markers [total cholesterol (TC), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C)] or novel markers [lipoprotein(a), homocysteine, apolipoproteins A1 and B]. In women, for example, the univariate RR of future cardiovascular events presented in Fig. 2 demonstrate that hs-CRP was the single strongest predictor of risk (RR = 4.4; 95% CI, 1.3–4.6). Furthermore, in a multivariate analysis that accounted for other CHD risk factors (obesity, hypertension, diabetes, family history), only hs-CRP and the ratio of TC to HDL-C had independent predictive value. In the same study of postmenopausal women (24), hs-CRP was shown to predict risk among those with LDL-C values <1300 mg/L, a concentration deemed “desirable” by the current National Cholesterol Education Program guidelines for primary prevention. In this subgroup (mean LDL-C, 1040 mg/L), the RRs of future MI, stroke, and coronary revascularization in the lowest to the highest quartiles of hs-CRP were 1.0, 2.4, 2.9, and 4.1, respectively (95% CI for the 4th vs 1st quartile, 1.7–11.3).

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<td>MRFIT Kuller 1996</td>
<td>CHD Death</td>
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<td>PHS Ridker 1997</td>
<td>MI</td>
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Fig. 1. Prospective studies of hs-CRP as a risk factor for future cardiovascular disease in populations of apparently healthy men and women. RR estimates (■) and 95% CIs (lines) are computed for those in the top compared with the bottom quartile. Data from Refs. (21–30).
After adjustment for other CHD risk factors and concentration of HDL-C, the RR associated with hs-CRP remained highly significant (RR = 3.1; 95% CI, 1.1–8.3) and increased ~39% with each increasing quartile of hs-CRP. This study thus demonstrated that hs-CRP can identify individuals at increased risk of developing future coronary events who otherwise would be missed if only lipid measurements were used. Other examined markers of inflammation, e.g., serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule-1, showed consistent association but a slightly weaker RR of future coronary events.

The predictive value of hs-CRP in men and women increased considerably when evaluated in models that included lipid values. Data from the PHS demonstrated that, compared with those with TC and hs-CRP below the 75th percentile, those with increased TC alone had a 2.3-fold increase in risk (95% CI, 1.5–3.7), whereas those with increased hs-CRP alone had a 1.5-fold increase in risk (95% CI, 0.9–2.4) (35). In contrast, the risk of developing coronary events increased 5-fold (95% CI, 2.5–9.8) among those with high concentrations of both TC and hs-CRP. Therefore, the joint effects of both risk factors are greater than the product of the individual effects of each risk factor considered alone. Furthermore, when the study participants were stratified according to quintile of hs-CRP and quintile of TC:HDL-C ratio, the RR of first coronary event in those in the highest quintiles of both hs-CRP and TC:HDL-C ratio was approximately ninefold higher than that of men in the lowest quintiles of these analytes. Data from the WHS demonstrated similar findings such that women in the highest quintile of both hs-CRP and TC:HDL-C ratio had a RR more than eightfold higher than that of women in the lowest quintiles (Fig. 3). In all of these analyses, risk prediction models that incorporated lipids were significantly better (P <0.001) than those based on hs-CRP alone (24).

**Interpretation of hs-CRP Values**

For the purpose of assessing risk of future first coronary events, hs-CRP concentration should be interpreted using cut points established by prospective clinical studies. Each patient will be classified into a quintile of risk, depending on the hs-CRP concentration. Therefore, the reporting of hs-CRP results focuses on the quintile of risk and not on the actual mass concentration.

The within-person biologic variability of hs-CRP is low over a long period of time (36). Laboratory measurements on paired samples obtained from 236 subjects at baseline and 5 years later showed that an individual’s log-normalized hs-CRP concentrations are highly correlated (r = 0.60). Somewhat comparable correlation coefficients were noted for TC (r = 0.37), LDL-C (r = 0.32), HDL-C (r = 0.74), and triglycerides (r = 0.49) over the 5-year follow-up period. This finding lends further support to the fact that hs-CRP is a good and biologically stable predictor of future MI despite the fact that it is an acute phase reactant, providing that the patient is not suffering from an active infection or using a drug that affects hs-CRP concentration. hs-CRP values >15 mg/L (~99th percentile of the general population) indicate an active inflammation; patients should be advised to have a repeat measurement in 2–3 weeks or after the infection is resolved.

As indicated earlier, models containing both hs-CRP and TC or the TC:HDL-C ratio were better able to predict future first coronary events than those containing hs-CRP alone. The RRs of future first coronary events for men and women as well as lipid concentrations were computed in quintiles from the PHS and WHS databases, respectively.
and are presented in Fig. 3. Because the computed RRs did not vary significantly between men and women, a single risk assessment algorithm is suggested for both genders (Table 1) (37). The hs-CRP concentrations were derived from ongoing population-based surveys. It is important to note that it is not necessary in this case to report the actual hs-CRP concentration to the clinician but only the patient’s RR. The clinical laboratory should play an active role in the interpretation and implementation of this clinical application. Providing incomplete information or just the actual hs-CRP concentration will only frustrate and prevent the clinician from correctly interpreting the data and managing the patient.

Potential Preventive Therapies

Although no specific therapies have been developed to decrease hs-CRP and there is no direct evidence that risk of future cardiovascular events is diminished by reducing hs-CRP, studies have shown that aspirin (23) and pravastatin (32) are effective in decreasing the incidence of future coronary events in those with increased hs-CRP concentration. These studies suggest that the two examined drugs possess antiinflammatory characteristics.

Among apparently healthy men in the PHS with increased hs-CRP (>2.1 mg/L), aspirin use decreased the risk of future MI by almost 60% (23). In contrast, aspirin use was associated with a much smaller, although statistically significant, 14% decrease in future MI among men with low hs-CRP (<0.55 mg/L). Although the magnitude of reduction in future risk of MI depended on the concentration of hs-CRP, it is important to note that all subjects benefited from aspirin use. These findings suggest that aspirin was acting not only as an antiplatelet agent but also as an antiinflammatory drug.

Similar findings were also noted with pravastatin use in the CARE study (32). As indicated earlier, CARE is a prospective study of men and women with average lipid concentrations who have suffered an MI. Participants

| Table 1. RR estimates for future coronary events in men and women associated with quintiles of hs-CRP and TC:HDL-C ratio. a |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Quintile of TC:HDL-C ratio | Men 1 (<0.7) | Men 2 (0.7–1.1) | Men 3 (1.2–1.9) | Men 4 (2.0–3.8) | Men 5 (3.9–15.0) | Women 1 (<3.4) | Women 2 (3.4–4.1) | Women 3 (4.2–4.7) | Women 4 (4.8–5.5) | Women 5 (>5.5) |
| 1 | 1 | 1.2 | 1.4 | 1.7 | 2.2 |
| 2 | 1.4 | 1.7 | 2.1 | 2.5 | 3 |
| 3 | 2 | 2.5 | 2.9 | 3.5 | 4.2 |
| 4 | 2.9 | 3.5 | 4.2 | 5.1 | 6 |
| 5 | 4.2 | 5 | 6 | 7.2 | 8.7 |

a RR estimates and TC:HDL-C ratio were derived from the PHS (23) and the WHS (24) databases. hs-CRP concentrations were derived from ongoing population-based surveys.
were randomized between 40 mg of pravastatin per day and placebo and followed for 5 years (38). Study participants with high hs-CRP (>9.9 mg/L or 90th percentile) at baseline experienced a reduction of 54% in the incidence of recurrent coronary events compared with a reduction of 25% in those with low hs-CRP (<9.9 mg/L or 90th percentile), although baseline lipid values were almost identical in the two groups. Moreover, during the 5-year follow-up, pravastatin lowered mean hs-CRP by almost 40%. This represented a 22% difference at 5 years in median hs-CRP between the pravastatin and placebo groups. Furthermore, the magnitude of change in hs-CRP appeared to be unrelated to that of LDL-C in both the pravastatin and placebo groups. These findings suggest that pravastatin may have antinflammatory characteristics that are independent from its lipid-lowering property. Clinical trials are currently ongoing to further explore the interaction between pravastatin, aspirin, and the inflammatory response in primary and secondary prevention settings.

Interrelationships with Other CHD Risk Factors
Several CHD risk factors appear to modulate the inflammatory response and affect hs-CRP concentration. Obesity, for example, is directly associated with increased hs-CRP concentrations, an intriguing observation considering that interleukin-6, the primary stimulant of the de novo hepatic synthesis of CRP, is secreted by adipose tissue (39, 40). Therefore, the attenuation of the inflammatory response may represent a mechanism by which diet and weight loss reduce cardiovascular risk. Cigarette smoking has also been shown to increase the concentration of several inflammatory markers, including hs-CRP, interleukin-6, and soluble intercellular adhesion molecule-1. Increases of both interleukin-6 (41) and soluble intercellular adhesion molecule-1 (42) were shown to be associated with increased risk of future first coronary events in both men and women. Smoking cessation decreases these markers. Diabetic patients are reported to have increased hs-CRP values (43); in this regard, links between hs-CRP and the insulin resistance syndrome have also been reported (44). In addition, experimental findings suggest that increased blood pressure promotes endothelial expression of cytokines and inflammatory activation (6, 45, 46). These observations suggest that perhaps better control of diabetes and hypertension may attenuate the contribution of the inflammatory response to overall cardiovascular risk. Finally, physical exercise has been shown to have a beneficial effect in terms of reducing the concentration of several inflammatory markers (47, 48). Taken together, the available evidence thus supports the hypothesis that hs-CRP concentrations correlate with endothelial dysfunction (49).

Analytical Considerations in the Measurement of hs-CRP
Historically, CRP has been measured in clinical laboratories by immunoturbidimetric and immunonephelometric assays designed to detect active inflammation and infection. The dynamic range of these assays spans from 3 mg/L (~90th percentile of the general population) to well over 200 mg/L. Such traditional assays, however, do not have appropriate sensitivity in the range required for the determination of cardiovascular risk in apparently healthy men and women.

To achieve the desired limit of quantification, manufacturers and investigators have continued to use immunochemical techniques in their attempts to measure hs-CRP, but with modifications to increase the detectable signal. Several approaches have been used, including the labeling of anti-CRP antibodies with either an enzyme (ELISA) or a fluorescent compound, and attaching the antibodies, either monoclonal or polyclonal, to polystyrene beads (50–55). The latter approach was popular among manufacturers because it enabled the adaptation to commonly used automated analyzers in clinical chemistry laboratories. Currently, hs-CRP concentrations as low as 0.15 mg/L (<2.5th percentile of the general population) can be reliably measured. It is important to note, however, that not all hs-CRP assays possess a similar sensitivity or lower limit of quantification (56). For practical considerations, it is advisable to have a single CRP assay in the clinical laboratory that is capable of measuring low and high concentrations. However, if that is impossible, clinicians should be aware of the availability of two different CRP assays and request hs-CRP for cardiovascular risk prediction purpose.

Because the hs-CRP value of an individual patient is interpreted in the context of cutpoints established by prospective clinical studies, standardization of hs-CRP assays is crucial. Poor agreement among methods will lead to misclassification and mismanagement of patients. Recent reports have indicated that the measurement of low hs-CRP concentrations is not consistent among various methods, suggesting that standardization efforts are needed (51, 56). In one case where a significant bias was noted between two methods (51), the manufacturers of both reagent systems claimed to have their calibrators traceable to the WHO reference materials. Unfortunately, this is not an unusual occurrence. Although manufacturers attempt to standardize their assays using the WHO calibrators, they often fail to follow the appropriate value transfer protocol from the reference materials to their own calibrators (57). Invariably, this leads to suboptimal standardization.

An in-house hs-CRP ELISA method (52), utilizing polyclonal antibodies from Calbiochem, was used in MRFIT, the Cardiovascular Health Study, and the Rural Health Promotion Project as well as in the early work from the PHS. The analytical performance and clinical efficacy of the ELISA assay were compared with those of an automated and commercially available latex-enhanced method (Dade Behring) (51) used at present in several prospective studies, including the PHS, WHS, Women’s Health Initiative, Nurses’ Health Study, Health Profes-
hs-CRP measurement at the lower concentration range may improve the cost-to-benefit ratio in statin therapy is currently under development or are under development and are expected to be commercially available soon.

CONCLUSION

hs-CRP is a very promising novel biochemical marker for the prediction of future first or recurrent coronary events. American and European prospective studies have been highly consistent regarding the ability of hs-CRP to predict future CHD risk in both men and women. Potential preventive therapeutic modalities to attenuate coronary risk in those with increased hs-CRP concentrations have been suggested. The potential use of hs-CRP as a means to improve the cost-to-benefit ratio in statin therapy is currently under investigation. Although standardization of hs-CRP measurement at the lower concentration range among various methods should be addressed, a robust and Food and Drug Administration-approved method is currently available. Several other sensitive assays are under development and are expected to be commercially available soon.

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