

# High-Sensitivity C-Reactive Protein Potential Adjunct for Global Risk Assessment in the Primary Prevention of Cardiovascular Disease

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**Abstract**—Inflammation plays a major role in atherothrombosis, and measurement of inflammatory markers such as high-sensitivity C-reactive protein (HSCRP) may provide a novel method for detecting individuals at high risk of plaque rupture. Several large-scale prospective studies demonstrate that HSCRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women and that the addition of HSCRP to standard lipid screening may improve global risk prediction among those with high as well as low cholesterol levels. Because agents such as aspirin and statins seem to attenuate inflammatory risk, HSCRP may also have utility in targeting proven therapies for primary prevention. Inexpensive commercial assays for HSCRP are now available; they have shown variability and classification accuracy similar to that of cholesterol screening. Risk prediction algorithms using a simple quintile approach to HSCRP evaluation have been developed for outpatient use. Thus, although limitations inherent to inflammatory screening remain, available data suggest that HSCRP has the potential to play an important role as an adjunct for global risk assessment in the primary prevention of cardiovascular disease. (*Circulation*. 2001;103:1813-1818.)

**Key Words:** risk factors ■ inflammation ■ cardiovascular diseases ■ prevention ■ screening

Laboratory and experimental evidence indicate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process.<sup>1</sup> Thus, researchers have hypothesized that inflammatory markers such as high-sensitivity C-reactive protein (HSCRP) may provide an adjunctive method for global assessment of cardiovascular risk.<sup>2-4</sup> In support of this hypothesis, several large-scale prospective epidemiological studies have shown that plasma levels of HSCRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among individuals without known cardiovascular disease.<sup>4-14</sup> In addition, among patients with acute coronary ischemia,<sup>15-18a</sup> stable angina pectoris,<sup>19</sup> and a history of myocardial infarction,<sup>20</sup> levels of HSCRP have been associated with increased vascular event rates.

Based in part on these data, high-sensitivity assays for CRP have become available in standard clinical laboratories. However, clinical application of HSCRP testing will depend not only on demonstration of independent predictive value, but also on demonstration that addition of HSCRP testing to traditional screening methods improves cardiovascular risk prediction. Furthermore, application of HSCRP as a tool to assist in global risk assessment requires knowledge of population distribution of HSCRP, clinical characteristics of

HSCRP evaluation, and magnitude of risk of future coronary events that can be expected at each level of HSCRP.

### Epidemiological Evidence Supporting HSCRP Evaluation in Primary Prevention

The hypothesis that CRP testing might have prognostic usefulness for patients with acute myocardial infarction dates to the 1940s, when levels of CRP were observed to increase as part of the “acute-phase response” associated with ischemia. However, standard assays for CRP lack the sensitivity needed to determine levels of inflammation within normal range, and thus clinical utility of standard CRP evaluation for vascular risk detection is extremely limited.

More recently, with the recognition that inflammation is a critical component in determination of plaque stability<sup>1,21,22</sup> and with the availability of highly sensitive assay systems, CRP levels in the low-normal range were found to have predictive value for individuals admitted to hospital with acute coronary ischemia.<sup>15-17</sup> However, interpretation of these data are complex, given that acute ischemia itself may trigger an inflammatory response. Thus, application of HSCRP testing as a tool to improve coronary risk prediction required direct evaluation in large-scale prospective studies of apparently healthy individuals in which baseline levels of

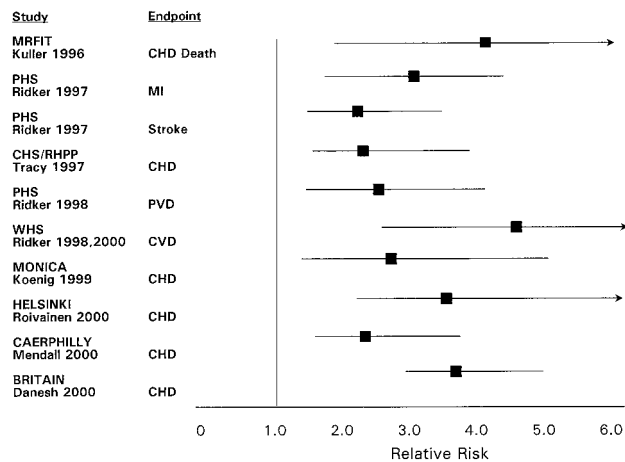
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Dr Ridker is named as a coinventor on a pending patent application filed by the Brigham and Women's Hospital on the use of markers of inflammation in coronary disease.

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**Figure 1.** Prospective studies of HSCRP as a marker for future cardiovascular events among individuals without known coronary disease. For consistency across studies, risk estimates and 95% CI are calculated as comparison of top vs bottom quartile within each study population. See references 4–14.

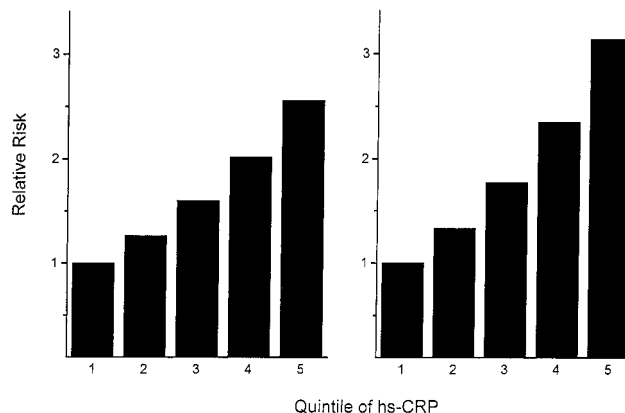
HSCRP could be related to future risk of cardiovascular events.

As shown in Figure 1, several studies from both the United States and Europe indicate that elevated levels of HSCRP among apparently healthy men and women are a strong predictor of future cardiovascular events.<sup>4–14</sup> For example, in a cohort of 22 000 middle-aged men with no clinical evidence of disease, those with baseline levels of HSCRP in the highest quartile had a 2-fold increase in risk of stroke or peripheral vascular disease and a 3-fold increase in risk of myocardial infarction.<sup>6,9</sup> These effects were independent of all other lipid and nonlipid risk factors and were present among smokers as well as nonsmokers.

Epidemiological data supporting the role of HSCRP as an biomarker for vascular risk are consistent across different study populations, including smokers enrolled in the Multiple Risk Factor Intervention Trial<sup>5</sup> and elderly patients followed in the Cardiovascular Health Study<sup>7</sup>; postmenopausal women in the Women’s Health Study<sup>4,8</sup>; and in 3 independent European cohorts, the MONICA Augsburg cohort,<sup>10</sup> the Helsinki Heart Study,<sup>11</sup> and the British Regional Practice study.<sup>13</sup> In most of these studies, effect of HSCRP on vascular risk remained highly significant after adjustment for traditional risk factors typically used in global risk-assessment programs.<sup>13</sup> Recent data also demonstrate association between HSCRP and all-cause mortality.<sup>12,14</sup>

### Risk Estimates Associated With HSCRP Evaluation

Although epidemiological studies demonstrate association between low-grade inflammation and vascular risk, application of HSCRP testing in clinical practice requires estimates of risk across a spectrum of HSCRP levels. However, distribution of HSCRP is rightward skewed such that clinical application will likely require recasting measured HSCRP levels into an ordinal system. A useful approach to this problem is to divide HSCRP values into population based quintiles. Risk estimates based on such an analysis for



**Figure 2.** Adjusted relative risks of future myocardial infarction associated with increasing quintiles of HSCRP (hs-CRP) among apparently healthy middle-aged men (left) and women (right). Risk estimates are adjusted for age, smoking status, body mass index (kg/m<sup>2</sup>), diabetes, history of hyperlipidemia, history of hypertension, exercise level, and family history of coronary disease.

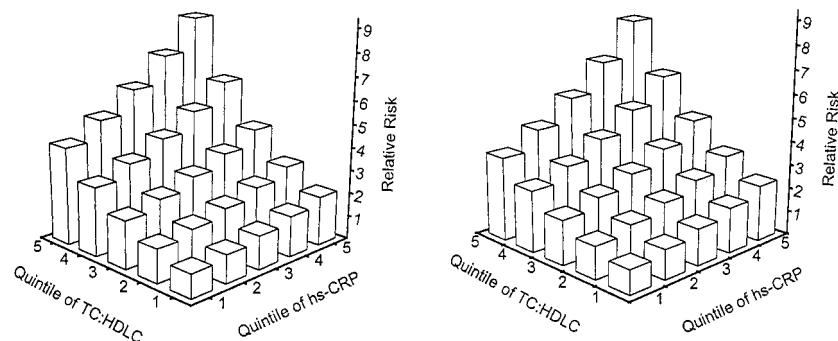
apparently healthy American men and women are shown in Figure 2.<sup>4,6</sup> Overall, for each quintile increase in HSCRP, the adjusted relative risk of suffering a future cardiovascular event increased 26% for men (95% CI 11% to 44%; *P*<0.005) and 33% for women (95% CI 13% to 56%; *P*<0.001). In addition to being stratified by gender, data presented in Figure 2 are adjusted for age, smoking status, family history of premature coronary disease, diabetes, hypertension, hyperlipidemia, exercise level, and body-mass index, the major determinants of risk evaluated in global cardiovascular prediction algorithms such as that developed from the Framingham Heart Study.

Application of this quintile approach to HSCRP testing requires knowledge of the population distribution of HSCRP. The Table presents a representative population distribution of HSCRP based on analysis of >5000 Americans without known cardiovascular disease. In this survey, median HSCRP level was 0.16 mg/dL and ranges of HSCRP for those with lowest (quintile 1) to highest (quintile 5) vascular risk were 0.01 to 0.069, 0.07 to 0.11, 0.12 to 0.19, 0.20 to 0.38, and >0.38 mg/dL. As risk estimates appear to be linear across the spectrum of inflammation, these sequential quintiles can be considered in clinical terms to represent individuals with low, mild, moderate, high, and highest relative risks, respectively, of future cardiovascular disease.

### Distribution of HSCRP Among Apparently Healthy American Men and Women

Quintile	Range, mg/dL	Risk Estimate
1	0.01–0.07	Low
2	0.07–0.11	Mild
3	0.12–0.19	Moderate
4	0.20–0.38	High
5	0.38–1.50	Highest

Data derived by Dade-Behring assay for HSCRP.



**Figure 3.** Interactive effects of HSCRP (hs-CRP) and lipid testing in men (left) and women (right). In these analyses, HSCRP quintile cut points are those described in Table 1. In clinical practice, recommended quintile cut points for total cholesterol:HDL cholesterol ratio (TC:HDL) are <3.5, 3.5 to 4.3, 4.4 to 5.0, 5.1 to 6.1, and >6.1 for men and <3.1, 3.1 to 3.6, 3.7 to 4.3, 4.4 to 5.2, and >5.2 for women. These latter data derive from the NHANES surveys (Harvey Kaufman, MD, personal communication, 2001).

**Potential Additive Value of HSCRP In Global Risk Assessment**

In current strategies of global risk assessment, lipid testing is the only blood test routinely recommended. However, HSCRP evaluation may have the potential to improve cardiovascular risk prediction models when used as an adjunct to this approach.<sup>4,10,23</sup> For example, in the Women’s Health Study, area under the receiver-operator curve associated with HSCRP testing in combination with total and HDL cholesterol evaluation was significantly greater than that associated with lipid evaluation alone ( $P<0.001$ ).<sup>4</sup>

Although ROC characteristics are useful for interpreting test sensitivity and specificity, these data can be more easily understood by examining estimates of relative risk associated with combined lipid and HSCRP testing.<sup>4,6,23</sup> Such an analysis for middle-aged men is presented in Figure 3, left, with the quintile approach outlined above. As shown, men with levels of both HSCRP and the total cholesterol:HDL cholesterol ratio in the top quintile represent a very-high-risk group compared with men with levels of both parameters in the lowest quintile. However, as also shown, increasing quintiles of HSCRP have additive predictive value at all lipid levels, including those typically associated with low to moderate risk. A similar quintile-based analysis of combined HSCRP and lipid testing for women is provided in Figure 3, right.

HSCRP testing may also have potential prognostic value among “low-risk” subgroups as determined by traditional methods of global risk detection. Among postmenopausal women, HSCRP levels are a strong predictor of subsequent cardiovascular risk among nonsmokers, as well as among those without hypertension, diabetes, or a family history of myocardial infarction.<sup>8</sup> Moreover, in an analysis of women with LDL levels below 130 mg/dL (current target for lipid reduction set by National Cholesterol Education Program guidelines for primary prevention) those with elevated levels of HSCRP still had markedly elevated risks of future myocardial infarction, stroke, and coronary revascularization, even after adjustment for other traditional risk factors.<sup>4</sup>

Further support for potential utility of HSCRP testing as an adjunct in global risk assessment is provided in a recent meta-analysis of 14 population-based cohorts adjusted for smoking and most major vascular risk factors.<sup>13</sup> In that analysis, which in aggregate included 2557 cases with a mean follow-up of 8 years, individuals with baseline HSCRP levels in the top third of the distribution had a 2-fold increase in risk of future vascular events (95%CI 1.5 to 2.3;  $P<0.001$ ).

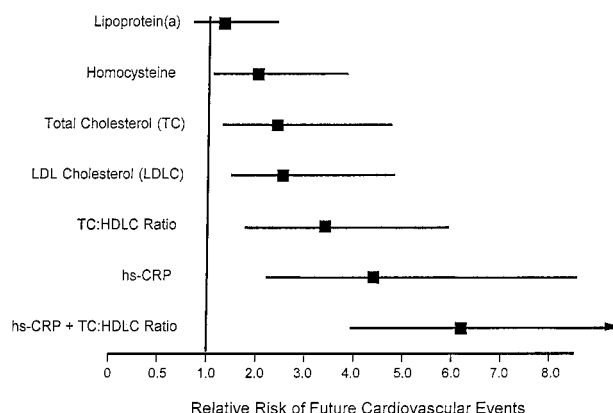
Importantly, no evidence was seen of heterogeneity among these studies, which indicates broad consistency in predictive value of HSCRP across different population groups.

**Direct Comparisons of HSCRP With Other Novel Markers of Vascular Risk**

Testing for homocysteine and lipoprotein(a), both of which are involved in atherothrombosis, have been recommended for certain high-risk groups. For example, homocysteine evaluation is recommended among those with impaired methionine metabolism due to renal failure or hypothyroidism, whereas lipoprotein(a) assessment has been recommended for those with premature atherosclerosis in the absence of other risk factors.<sup>24,25</sup>

Three large-scale prospective studies have compared directly the relative efficacy of homocysteine screening to HSCRP evaluation.<sup>4–6,26–29</sup> In each study, magnitude of risk prediction associated with HSCRP levels in the top quintile was greater than that associated with similar elevations of homocysteine.

In 1 prospective cohort of women, levels of homocysteine, lipoprotein(a), several inflammatory parameters including HSCRP, and a full lipid panel were simultaneously measured as markers of subsequent vascular risk.<sup>4</sup> Figure 4 shows univariate relative risk of future cardiovascular events in that cohort for women in the top versus bottom quartile for each



**Figure 4.** Direct comparison of magnitude of relative risk of future cardiovascular events associated with HSCRP (hs-CRP), cholesterol levels, lipoprotein(a), and homocysteine among apparently healthy women. For consistency, relative risks and 95% CI are shown for individuals in the top vs bottom quartile for each factor.

of these parameters. As shown, HSCRP was the single strongest predictor of risk (RR 4.4 for the highest versus lowest quartile). In multivariate analysis, only HSCRP level and total:HDL cholesterol ratio proved to have independent predictive value once age, smoking status, obesity, hypertension, family history, and diabetes also were accounted for.

### Assay Characteristics of HSCRP Tests

Standard clinical assays for CRP typically have a lower detection limit of 3 to 8 mg/L. Thus, these assays lack sensitivity within the low-normal range and cannot be used effectively for vascular risk prediction. In recognition of this limitation, initial epidemiological studies used research-based assays designed to determine CRP levels with excellent fidelity and reproducibility across the normal range.<sup>30–31</sup> Several such “high-sensitivity” or “ultra-sensitive” assays for CRP are now commercially available or in development, and formal standardization programs have been undertaken to ensure comparability across HSCRP assays.<sup>32–34</sup>

Clinical studies demonstrate that results with 1 commercial HSCRP assay (Dade Behring Inc) correlate well with HSCRP levels on the basis of early research assays.<sup>32</sup> In several large-scale prospective studies, this assay has been shown to reproduce predictive value of HSCRP testing for both peripheral arterial disease<sup>32</sup> and for myocardial infarction and stroke.<sup>4</sup> At this time, several other HSCRP assays are in clinical development and appear to have acceptable test characteristics.<sup>34</sup> In the low normal range needed for vascular risk detection, the variability and classification accuracy of HSCRP is similar to that of total cholesterol.<sup>34a</sup>

HSCRP levels increase with acute infection and trauma.<sup>35</sup> Thus, testing should be avoided within a 2- to 3-week window in patients who have had an upper respiratory infection or other acute illness. Individuals with clinically apparent inflammatory conditions such as rheumatoid arthritis or lupus are likely to have elevations of HSCRP well into the clinical range; HSCRP evaluation for the purpose of vascular risk prediction may be of limited value in such patients. However, for most individuals, HSCRP levels appear to be stable over long periods of time.<sup>36</sup> These latter data support the possibility that enhanced inflammatory response and, hence, increased propensity to plaque rupture may involve important genetic determinants.

In an ongoing survey of several thousand American men and women, <2% of all HSCRP values have been >1.5 mg/dL, a level considered to be indicative of a clinically relevant inflammatory condition. In such cases, the HSCRP measure should be repeated to exclude possibility of recent infection. If a second clinically elevated level is observed, evaluation for a previously unsuspected inflammatory condition may be warranted.

In contrast to results for cytokines such as IL-6, no circadian variation appears to exist for HSCRP.<sup>37</sup> Thus, clinical testing for HSCRP can be accomplished without regard for time of day.

### Management of Patients With Elevated Levels of HSCRP

No specific therapy has been evaluated for its ability to reduce HSCRP, nor does any direct evidence indicate that

reduction of HSCRP necessarily will result in reduced risk of cardiovascular events. However, data derived from randomized clinical trials of acetylsalicylic acid (aspirin)<sup>6</sup> and statin therapy<sup>20</sup> suggest that attributable risk reductions achieved by these agents are greater in the presence of elevated HSCRP levels. For example, in a randomized trial of aspirin, attributable risk reduction for this agent was 56% among those with baseline levels of HSCRP in the upper quartile but was sequentially smaller as levels of HSCRP declined.<sup>6</sup>

Similarly, in the Cholesterol and Recurrent Events (CARE) trial, patients with evidence of ongoing inflammation as detected by high levels of HSCRP as well as a second marker of inflammation, serum amyloid A, appeared to have a greater relative risk reduction in subsequent coronary events attributable to pravastatin than did those without a detectable inflammatory response.<sup>20</sup> In that trial, mean HSCRP levels decreased nearly 40% during a 5-year period among those allocated to pravastatin versus placebo, an effect not related to pravastatin-induced changes in LDL cholesterol.<sup>36</sup>

Postmenopausal hormone replacement therapy has been shown in cross-sectional<sup>38,39</sup> and intervention studies<sup>40,41</sup> to increase levels of HSCRP, an effect that may not be present for specific estrogen receptor modulators or for transdermal estrogen preparations. Although the mechanism of this effect is uncertain, these data may help explain the potential increase in vascular risk associated with initiation of hormone replacement therapy observed in the Heart Estrogen/progestin Replacement Study.<sup>42</sup> Ongoing research will be needed to determine whether net benefit or hazard of hormone replacement therapy in postmenopausal women can be predicted on the basis of HSCRP evaluation.

Obesity is associated directly with increased plasma levels of HSCRP, an observation consistent with findings that adipocytes secrete interleukin-6, a primary hepatic stimulant for CRP production.<sup>43,44</sup> Indeed, interleukin-6 levels as well as levels of tumor necrosis factor- $\alpha$  have been found to predict risk of first and recurrent coronary events.<sup>4,45,46</sup> Thus, attenuation of the inflammatory response may represent a mechanism by which diet and weight loss reduce vascular risk. Effects of low levels of exercise on coronary risk have recently been demonstrated, which is an intriguing issue given that exercise also reduces several inflammatory markers.<sup>47</sup> Diabetic patients have increased levels of HSCRP,<sup>48</sup> which suggests a role for systemic inflammation in diabetogenesis and the insulin resistance syndrome.<sup>44,49</sup> Smokers have elevated levels of HSCRP, interleukin-6, and soluble intercellular adhesion molecule type-1, and smoking cessation may lead to reductions in these parameters. Finally, growth hormone replacement reduces levels of several inflammatory markers, including HSCRP, which is of interest because growth hormone-deficient adults have increased cardiovascular mortality.<sup>50</sup>

Ongoing clinical studies will help to address remaining areas of controversy regarding use of inflammatory markers such as HSCRP in coronary risk prediction. At this time, available data indicate that HSCRP testing may increase the yield of programs designed to detect high-risk patients for subsequent coronary occlusion, particularly in the setting of primary prevention. Thus, HSCRP may be of assistance in



global risk-assessment programs designed to better target intervention efforts, including smoking cessation, weight loss, diet, and exercise.<sup>4</sup> Potential utility of HSCRP testing as a means to improve cost-to-benefit ratio of statin therapy is also under evaluation, given that data from the AFCAPS/TexCAPS trial of lovastatin and from the WOSCOPS trial of pravastatin indicate that these agents reduce risk among populations free of clinical coronary disease.<sup>51,52</sup> The possibility that HSCRP may provide an adjunctive method to target statin therapy in primary prevention by reducing the number needed to treat is promising but requires direct testing.

### Limitations of HSCRP Evaluation

Several limitations of HSCRP evaluation require consideration. Inflammatory markers are nonspecific, increase with acute infection or trauma, and have been shown to predict total mortality as well as cardiovascular events. The need to avoid HSCRP evaluation during times of infection or trauma and among individuals with known systemic inflammatory conditions thus may limit clinical utility. However, these effects have tended to lead to underestimation of the true predictive value of HSCRP in epidemiological studies. The utility of HSCRP testing across different ethnic groups also is uncertain. On the other hand, although cost effectiveness of HSCRP testing has not been formally evaluated, testing for HSCRP is inexpensive and likely to prove cost effective, particularly when compared with techniques such as electron-beam calcium scanning or magnetic resonance imaging.

Finally, the consistency of data concerning HSCRP in primary prevention does not imply that screening for HSCRP among postinfarction patients will have clinical utility. After acute ischemia, levels of CRP can rise substantially such that determining an individual's underlying basal level is difficult, an effect that may result in misclassification. In addition, measures of ventricular function and infarct size are likely to have far greater predictive value among individuals who have recently suffered acute infarction. Thus, rather than generalizing results from primary prevention, carefully controlled studies of postinfarction patients that include information about ventricular function and other important prognostic factors are needed to determine whether HSCRP evaluation has utility in secondary prevention.

### Summary

Inflammation plays a major role in atherothrombosis, and measurement of inflammatory markers such as HSCRP may provide a novel method for detecting individuals at high risk of plaque rupture. Several large-scale prospective studies demonstrate that HSCRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women. Recent data describing CRP within atheromatous plaque,<sup>53</sup> as a correlate of endothelial dysfunction,<sup>54</sup> and as having a direct role in cell adhesion molecular expression<sup>55</sup> raise the possibility that CRP may also be a potential target for therapy.

Given that inexpensive commercial assays for HSCRP are now available, clinicians will need to gain knowledge regarding population distribution of HSCRP, magnitude of vascular

risk that can be expected at each level of HSCRP, and utility of preventive strategies that attenuate inflammatory risk. Although limitations inherent to inflammatory screening remain, available data suggest that HSCRP has the potential to play an important role as an adjunct to global risk assessment in primary prevention of cardiovascular disease.

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### References

- Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
- Ridker PM. Evaluating novel cardiovascular risk factors: can we better predict heart attacks? *Ann Intern Med.* 1999;130:933–937.
- Lagrand WK, Visser CA, Hermens WT, et al. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation.* 1999;100:96–102.
- Ridker PM, Hennekens CH, Buring JE, et al. C reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342:836–843.
- Kuller LH, Tracy RP, Shaten J, et al, for the MRFIT Research Group. Relationship of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol.* 1996;144:537–547.
- Ridker PM, Cushman M, Stampfer MJ, et al. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997;336:973–979.
- Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol.* 1997;17:1121–1127.
- Ridker PM, Buring JE, Shih J, et al. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation.* 1998;98:731–733.
- Ridker PM, Cushman M, Stampfer MJ, et al. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation.* 1998;97:425–428.
- Koenig W, Sund M, Froelich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (MONITORING trends and determinants in Cardiovascular disease) Augsburg Cohort Study, 1984 to 1992. *Circulation.* 1999;99:237–242.
- Roivainen M, Viik-Kajander M, Palosuo T, et al. Infections, inflammation, and the risk of coronary heart disease. *Circulation.* 2000;101: 252–257.
- Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med.* 1999;106:506–512.
- Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ.* 2000;321:199–204.
- Mendall MA, Strachan DP, Butland BK, et al. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J.* 2000;21:1584–1590.
- Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med.* 1994;331:417–424.
- Morrow D, Rifai N, Antman E, et al. C-reactive protein is a potent predictor of mortality independently and in combination with troponin T in acute coronary syndromes. *J Am Coll Cardiol.* 1998;31:1460–1465.
- Biasucci LM, Liuzzo G, Grillo RL, et al. Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. *Circulation.* 1999;99:855–860.
- Toss H, Lindahl B, Siegbahn A, et al, for the FRISC Study Group. Prognostic influence of fibrinogen and C-reactive protein levels in unstable coronary artery disease. *Circulation.* 1997;96:4204–4210.
- Lindahl B, Toss H, Siegbahn A, et al, for the FRISC Study Group. Markers of myocardial damage and inflammation in relation to long-term

- mortality in unstable coronary artery disease. *N Engl J Med.* 2000;343:1139–1147.
19. Haverkate F, Thompson SG, Pyke SDM, et al. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet.* 1997;349:462–466.
  20. Ridker PM, Rifai N, Pfeffer MA, et al, for the Cholesterol And Recurrent Events (CARE) Investigators. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. *Circulation.* 1998;98:839–844.
  21. Libby P. Molecular bases of the acute coronary syndromes. *Circulation.* 1995;91:2844–2850.
  22. Maseri A. Inflammation, atherosclerosis, and ischemic events: exploring the hidden side of the moon. *N Engl J Med.* 1997;336:1014–1016. Editorial.
  23. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation.* 1998;97:2007–2011.
  24. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association: ACC/AHA Scientific Advisory. *Circulation.* 1999;99:178–182.
  25. Welch GN, Loscalzo J. Mechanisms of disease: homocysteine and atherothrombosis. *N Engl J Med.* 1998;338:1042–1050.
  26. Ridker PM, Manson JE, Buring JE, et al. Homocysteine and risk of cardiovascular disease among postmenopausal women. *JAMA.* 1999;281:1817–1821.
  27. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA.* 1992;268:877–881.
  28. Evans RW, Shaten J, Hempel JD, et al. Homocysteine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. *Arterioscler Thromb Vasc Biol.* 1997;17:1947–1953.
  29. Chasen-Taber L, Selhub J, Rosenberg IH, et al. A prospective study of folate and vitamin B6 and risk of myocardial infarction in US physicians. *J Am Coll Nutr.* 1996;15:136–143.
  30. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy adults: implications for reference interval and epidemiologic methods. *Clin Chem.* 1997;43:52–58.
  31. Ladue TB, Weiner DL, Sipe JD, et al. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. *Clin Chem.* 1989;35:745–753.
  32. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem.* 1999;45:2136–2141.
  33. Roberts WL, Sedrick R, Moulton L, et al. Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. *Clin Chem.* 2000;46:461–468.
  34. Roberts WL, Moulton L, Law TC, et al. Evaluation of nine automated high sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. *Clin Chem.* 2001;47:418–423.
  - 34a. Ockene IS, Matthews CD, Rifai N, et al. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. *Clin Chem.* 2001;47:444–450.
  35. Pepys MG. The acute phase response and C-reactive protein. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. *Oxford Textbook of Medicine.* 3rd ed. Oxford, England: Oxford University Press; 1995:1527–1533.
  36. Ridker PM, Rifai N, Pfeffer M, et al. Long-term effects of pravastatin on plasma concentration of C-reactive protein. *Circulation.* 1999;100:230–235.
  37. Ewart HKM, Ridker PM, Rifai N, et al. Absence of diurnal variation of C-reactive protein levels in healthy human subjects. *Clin Chem.* 2001;47:426–430.
  38. Cushman M, Meilhan EN, Psaty BM, et al. Hormone replacement therapy, inflammation, and hemostasis in elderly women. *Arterioscler Thromb Vasc Biol.* 1999;19:893–899.
  39. Ridker PM, Hennekens CH, Rifai N, et al. Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation.* 1999;100:713–716.
  40. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation.* 1999;100:717–722.
  41. van Baal WM, Kenemans P, van der Mooren MJ, et al. Increased C-reactive protein levels during short-term hormone replacement therapy in healthy postmenopausal women. *Thromb Haemost.* 1998;81:925–928.
  42. Hulley S, Grady D, Bush T, et al, for the Heart and Estrogen/progestin Replacement Study (HERS) Research group. *JAMA.* 1998;280:605–613.
  43. Visser M, Bouter LM, McQuillen GM, et al. Elevated C-reactive protein levels in overweight and obese adults. *JAMA.* 1999;282:2131–2135.
  44. Yudkin JS, Stehouwer CDA, Emeis JJ, et al. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* 1999;19:972–978.
  45. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation.* 2000;101:1767–1772.
  46. Ridker PM, Rifai N, Pfeffer M, et al, for the Cholesterol And Recurrent Events (CARE) Investigators. Elevation of tumor necrosis factor- $\alpha$  and increased risk of recurrent coronary events after myocardial infarction. *Circulation.* 2000;101:2149–2153.
  47. Smith JK, Dykes R, Douglas JE, et al. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA.* 1999;281:1722–1727.
  48. Ford CS. Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care.* 1999;22:1971–1977.
  49. Festa A, D'Agostino R, Howard G, et al. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation.* 2000;102:42–47.
  50. Seshimo G, Biller BMK, Llevadot J, et al. Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency. *Ann Intern Med.* 2000;133:111–122.
  51. Downs JR, Clearfield M, Weis S, et al, for the AFCAPS/TexCAPS Research Group. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *JAMA.* 1998;279:1615–1622.
  52. Shepard J, Cobb SM, Ford I, et al, for the West Of Scotland Coronary Prevention Study group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med.* 1995;333:1301–1307.
  53. Torzewski M, Rist C, Mortensen RF, et al. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol.* 2000;20:2094–2098.
  54. Fichtlscherer S, Rosenberger G, Walter DH, et al. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation.* 2000;102:1000–1006.
  55. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation.* 2000;102:2165–2168.