

C-REACTIVE PROTEIN AND OTHER MARKERS OF INFLAMMATION IN THE PREDICTION OF CARDIOVASCULAR DISEASE IN WOMEN

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ABSTRACT

Background Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events.

Methods We conducted a prospective, nested case-control study among 28,263 apparently healthy postmenopausal women over a mean follow-up period of three years to assess the risk of cardiovascular events associated with base-line levels of markers of inflammation. The markers included high-sensitivity C-reactive protein (hs-CRP), serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule type 1 (sICAM-1). We also studied homocysteine and several lipid and lipoprotein measurements. Cardiovascular events were defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for coronary-revascularization procedures.

Results Of the 12 markers measured, hs-CRP was the strongest univariate predictor of the risk of cardiovascular events; the relative risk of events for women in the highest as compared with the lowest quartile for this marker was 4.4 (95 percent confidence interval, 2.2 to 8.9). Other markers significantly associated with the risk of cardiovascular events were serum amyloid A (relative risk for the highest as compared with the lowest quartile, 3.0), sICAM-1 (2.6), interleukin-6 (2.2), homocysteine (2.0), total cholesterol (2.4), low-density lipoprotein (LDL) cholesterol (2.4), apolipoprotein B-100 (3.4), high-density lipoprotein (HDL) cholesterol (0.3), and the ratio of total cholesterol to HDL cholesterol (3.4). Prediction models that incorporated markers of inflammation in addition to lipids were significantly better at predicting risk than models based on lipid levels alone ($P < 0.001$). The levels of hs-CRP and serum amyloid A were significant predictors of risk even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (3.4 mmol per liter), the target for primary prevention established by the National Cholesterol Education Program. In multivariate analyses, the only plasma markers that independently predicted risk were hs-CRP (relative risk for the highest as compared with the lowest quartile, 1.5; 95 percent confidence interval, 1.1 to 2.1) and the ratio of total cholesterol to HDL cholesterol (relative risk, 1.4; 95 percent confidence interval, 1.1 to 1.9).

Conclusions The addition of the measurement of C-reactive protein to screening based on lipid levels may provide an improved method of identifying women at risk for cardiovascular events. (N Engl J Med 2000;342:836-43.)

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HALF of all myocardial infarctions occur in persons in whom plasma lipid levels are normal.¹ In an effort to better identify patients at high risk for cardiovascular events, several markers of risk have been proposed for use in screening, including homocysteine and fibrinogen levels, fibrinolytic capacity, and levels of apolipoprotein A-I, apolipoprotein B-100, and Lp(a) lipoprotein. However, the clinical value of many of these markers has been limited because of inadequate standardization of assay conditions, inconsistency of prospective data, or lack of evidence of significant improvement in the prediction of risk over that afforded by standard lipid screening alone.²

With the recognition that atherosclerosis is an inflammatory process,³ several plasma markers of inflammation have also been evaluated as potential tools for prediction of the risk of coronary events. Among them are markers of systemic inflammation produced in the liver, such as high-sensitivity C-reactive protein (hs-CRP) and serum amyloid A; cytokines such as interleukin-6; and adhesion molecules such as soluble intercellular adhesion molecule type 1 (sICAM-1).⁴⁻¹¹ However, as with other proposed predictors of the risk of cardiovascular events, the prognostic value of these markers of inflammation remains uncertain. For example, a widely held clinical view is that levels of markers of inflammation vary too greatly over time to allow accurate prediction of risk. Furthermore, few prospective studies have measured all these markers of inflammation in a single group of patients, so the relative usefulness of each marker cannot be easily evaluated. In addition, data supporting the hypothesis that markers of inflammation significantly increase the predictive value of lipid screening are scant and are limited almost exclusively to data from studies of hs-CRP in middle-aged men.^{7,12} Finally, clinical application of these findings has been limited, since standardized, commercial assays for most markers of inflammation are only now being developed.

In a previous study, in which we used an experimental assay for hs-CRP, we found higher levels of this marker among healthy postmenopausal women participating in the Women's Health Study who subse-

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quently had cardiovascular events than among those who did not have such events.¹³ On the basis of that finding and in the effort to address the clinical issues outlined above, we used a commercial assay to measure hs-CRP in the same cohort and simultaneously measured plasma levels of serum amyloid A, interleukin-6, sICAM-1, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, the ratio of total cholesterol to HDL cholesterol, apolipoprotein A-I, and apolipoprotein B-100. In addition, to allow comparison with other proposed markers, we measured plasma levels of Lp(a) lipoprotein and homocysteine. We thus were able to evaluate directly the relative value of each of these 12 measurements as an independent predictor of future cardiovascular events in a large cohort of apparently healthy women. We also sought to determine whether the measurement of markers of inflammation in addition to standard screening of lipid levels might provide a clinically useful method for improving overall prediction of the risk of cardiovascular events.

METHODS

Study Participants

We designed a prospective, nested case-control study involving participants in the Women's Health Study, an ongoing trial of aspirin and vitamin E for primary prevention among postmenopausal women with no history of cardiovascular disease or cancer.¹⁴ Blood samples were collected in tubes containing EDTA at base line from 28,263 women (71 percent of the Women's Health Study participants) and stored in liquid nitrogen until the time of analysis.

For this analysis, case subjects were study participants from whom a base-line blood sample was obtained who subsequently had a cardiovascular event (defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or a coronary-revascularization procedure) during a mean follow-up period of three years. Myocardial infarction was classified as confirmed if symptoms met the criteria of the World Health Organization¹⁵ and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic changes. Stroke was classified as confirmed if the patient had a new neurologic deficit that lasted more than 24 hours. Computed tomographic scans or magnetic resonance images were available for the majority of women in whom stroke occurred. Performance of revascularization procedures was confirmed by review of hospital records. Death from coronary heart disease was confirmed by review of the autopsy report, the death certificate, medical records, or information from family members regarding the circumstances of death.

For each woman who had a confirmed cardiovascular event during follow-up, two control subjects of the same age (within one year) and smoking status (former smoker, current smoker, or non-smoker) were selected from among the remaining study participants from whom a base-line blood sample had been obtained and who remained free of reported cardiovascular disease during follow-up. With use of these criteria, 122 case subjects and 244 control subjects were selected.

Procedures

Base-line plasma samples from each woman with an event and each control subject were thawed and assayed for hs-CRP, serum amyloid A, and Lp(a) lipoprotein with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Del.). Apolipoprotein A-I and apolipoprotein B-100 were simultaneously measured with this device by immunoassay. Total cholesterol, HDL cholesterol, and directly obtained LDL choles-

terol levels were measured on a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis) with reagents from Roche Diagnostics and Genzyme (Cambridge, Mass.). Plasma levels of sICAM-1 and interleukin-6 were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis), and the total plasma homocysteine level was measured with an IMx homocysteine assay (Abbott Laboratories, Abbott Park, Ill.) as previously reported.¹⁶ Samples were handled in identical and in blinded fashion throughout the study. Samples were analyzed in triplicate and in random order so as to reduce systematic bias and interassay variation.

Statistical Analysis

Means and proportions for risk factors for cardiovascular events at base line were calculated for women who had cardiovascular events during follow-up and those who did not. The significance of differences in means between the two groups was assessed with Student's t-test, and the significance of differences in proportions was tested with use of the chi-square statistic. Analysis of trends was used to test for associations between increasing levels of each plasma variable and the risk of future cardiovascular events, after the sample was divided into quartiles according to the distribution of control values for that marker. Adjusted risk estimates were obtained with use of logistic-regression models that, in addition to accounting for the variables used for matching (age and smoking status), adjusted for random assignment to aspirin or vitamin E in the Women's Health Study; several risk factors for cardiovascular events, including a history of hypertension, body-mass index, a history of diabetes, and a parental history of myocardial infarction before the age of 60 years; and other measured plasma markers.

We evaluated the combined role of lipid levels and markers of inflammation as predictors of the risk of future cardiovascular events in a series of analyses in which we explored the sensitivity and robustness of our findings from a clinical perspective. First, we used the likelihood-ratio test to determine whether logistic-regression models that included measurements of lipid variables and markers of inflammation provided a significantly better fit than did logistic-regression models limited to lipid measurements alone. Second, to estimate the clinical relevance of these effects, we computed the area under receiver-operating-characteristic curves for prediction models based on lipid measurements alone and for models based on measurements of both lipid levels and markers of inflammation. Third, we divided the study participants into nine groups according to low, medium, and high levels of total cholesterol and low, medium, and high levels of each marker of inflammation. In these analyses, logistic regression was used to evaluate simultaneously the risk of future cardiovascular events in each of the nine groups; the group of women in the lowest third for total cholesterol and in the lowest third for the respective marker of inflammation was considered the reference group. Finally, to address the clinical need for improved assessment of risk among persons with cholesterol levels currently considered safe, we performed a subgroup analysis of study participants with LDL cholesterol levels of less than 130 mg per deciliter (3.4 mmol per liter), the target level for the primary prevention of coronary heart disease according to the current guidelines of the National Cholesterol Education Program.¹⁷

All P values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance. All confidence intervals were calculated at the 95 percent level.

RESULTS

The base-line characteristics of the women who subsequently had cardiovascular events (case subjects) and those who remained free of reported cardiovascular disease (controls) are shown in Table 1. As expected, women who had cardiovascular events were heavier at base line than those who remained free of cardiovascular disease and were more likely to have hypertension, diabetes, or a parental history of prema-

TABLE 1. BASE-LINE CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS.*

| CHARACTERISTIC | WOMEN WITH CARDIOVASCULAR EVENTS (N=122) | WOMEN FREE OF CARDIOVASCULAR EVENTS (N=244) | P VALUE† |
|--|---|--|-------------|
| Mean age (yr) | 59.3 | 59.3 | — |
| Mean body-mass index‡ | 27.1 | 26.0 | 0.04 |
| History of hypertension (%) | 55.5 | 31.3 | 0.001 |
| History of diabetes (%) | 9.8 | 2.1 | 0.001 |
| Parental history of myocardial infarction before 60 yr (%) | 21.3 | 12.7 | 0.04 |
| Smoking status (%) | | | — |
| Former smoker | 29.5 | 29.5 | |
| Current smoker | 27.9 | 27.9 | |
| Nonsmoker | 42.6 | 42.6 | |
| Frequency of exercise (%) | | | 0.9 |
| >3 times/wk | 6.6 | 8.2 | |
| 1–3 times/wk | 27.9 | 27.1 | |
| <1 time/wk | 21.3 | 20.1 | |
| Rarely or never | 44.3 | 44.5 | |
| Frequency of alcohol consumption (%) | | | 0.6 |
| Daily | 12.3 | 8.2 | |
| Weekly | 27.9 | 31.2 | |
| Monthly | 14.8 | 13.9 | |
| Rarely or never | 45.1 | 46.7 | |
| Current use of hormone-replacement therapy (%) | 44.3 | 41.0 | 0.1 |

*Because of rounding, not all percentages total 100.

†P values were not calculated for variables used in matching of case and control subjects, since the distribution of these variables was identical in the two groups.

‡The body-mass index is the weight in kilograms divided by the square of the height in meters.

ture myocardial infarction (before the age of 60 years). The frequency of exercise, the frequency of alcohol consumption, and rate of use of hormone-replacement therapy were similar in the two groups. Because of matching, the women who had cardiovascular events and the control subjects were virtually identical with respect to mean age and smoking status.

Base-line plasma levels of the inflammation markers hs-CRP ($P<0.001$), serum amyloid A ($P=0.003$), sICAM-1 ($P=0.03$), and interleukin-6 ($P=0.003$) were higher among the women who subsequently had cardiovascular events than among those who did not (Table 2). Similarly, base-line plasma levels of total cholesterol ($P=0.01$), LDL cholesterol ($P=0.003$), apolipoprotein B-100 ($P<0.001$), and homocysteine ($P=0.02$) and the ratio of total cholesterol to HDL cholesterol ($P<0.001$) were significantly higher among women with subsequent events than those without such events, whereas levels of HDL cholesterol were significantly lower among women with subsequent events ($P<0.001$). Base-line levels of Lp(a) lipoprotein were somewhat higher and levels of apolipoprotein A-I somewhat lower among the women with events

TABLE 2. BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | WOMEN WITH CARDIOVASCULAR EVENTS | WOMEN FREE OF CARDIOVASCULAR EVENTS | P VALUE |
|--|--|---|---------|
| High-sensitivity C-reactive protein (mg/dl) | | | <0.001 |
| Median | 0.42 | 0.28 | |
| Interquartile range | 0.21–0.83 | 0.11–0.55 | |
| Serum amyloid A (mg/dl) | | | 0.003 |
| Median | 0.63 | 0.52 | |
| Interquartile range | 0.45–1.01 | 0.35–0.78 | |
| Soluble intercellular adhesion molecule type 1 (ng/ml) | 349.7±121.3 | 321.3±107.4 | 0.03 |
| Interleukin-6 (pg/ml) | | | 0.003 |
| Median | 1.65 | 1.30 | |
| Interquartile range | 1.14–2.62 | 1.00–2.03 | |
| Total cholesterol (mg/dl) | 230.5±41.2 | 219.2±37.5 | 0.01 |
| LDL cholesterol (mg/dl) | 132.2±34.6 | 121.5±30.2 | 0.003 |
| HDL cholesterol (mg/dl) | 45.4±14.6 | 51.1±15.4 | <0.001 |
| Apolipoprotein A-I (mg/dl) | 163.8±40.3 | 168.5±36.1 | 0.3 |
| Apolipoprotein B-100 (mg/dl) | 128.5±31.0 | 115.0±26.7 | <0.001 |
| Lp(a) lipoprotein (mg/liter) | | | 0.3 |
| Median | 79 | 74 | |
| Interquartile range | 34–247 | 29–203 | |
| Ratio of total cholesterol to HDL cholesterol | 5.5±1.9 | 4.6±1.4 | <0.001 |
| Homocysteine (μmol/liter) | 14.1±8.0 | 12.4±5.8 | 0.02 |

*Plus-minus values are means ±SD. For normally distributed variables, P values were computed with t-tests; for non-normally distributed variables, P values were computed with the Wilcoxon rank-sum test for the difference in medians. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

than among control subjects, but these differences were not significant.

Table 3 shows the relative risks of cardiovascular events according to the quartile of each marker of inflammation or lipid measured in plasma. Measurements of hs-CRP, serum amyloid A, sICAM-1, and interleukin-6 were predictive of the risk of future cardiovascular events. Of the 12 measures, the level of hs-CRP was the most powerful predictor of risk in the univariate analysis (relative risk for women in the highest quartile as compared with the lowest quartile, 4.4; 95 percent confidence interval, 2.2 to 8.9; $P<0.001$). Of the lipid variables, the ratio of total cholesterol to HDL cholesterol (relative risk, 3.4; $P=0.001$) and the apolipoprotein B-100 level (relative risk, 3.4; $P=0.001$) were the most powerful predictors of risk. Nonsignificant trends were observed for apolipoprotein A-I and Lp(a) lipoprotein. As reported previously,¹⁶ increasing levels of homocysteine were also associated with increased risk.

Levels of several markers of inflammation were highly correlated. For example, the correlation coefficient for the relation between hs-CRP and serum

TABLE 3. RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | QUARTILE OF PLASMA LEVEL | | | | P VALUE FOR TREND |
|--|--------------------------|---------------|---------------|---------------|-------------------|
| | 1 | 2 | 3 | 4 | |
| High-sensitivity C-reactive protein | | | | | |
| Median — mg/dl | 0.06 | 0.19 | 0.38 | 0.85 | |
| Relative risk (95% CI) | 1.0 | 2.1 (1.0–4.5) | 2.1 (1.0–4.4) | 4.4 (2.2–8.9) | <0.001 |
| Serum amyloid A | | | | | |
| Median — mg/dl | 0.25 | 0.43 | 0.62 | 1.17 | |
| Relative risk (95% CI) | 1.0 | 1.8 (0.9–3.6) | 1.9 (0.9–3.8) | 3.0 (1.5–6.0) | 0.002 |
| Soluble intercellular adhesion molecule type 1 | | | | | |
| Median — ng/ml | 228.7 | 273.9 | 319.1 | 439.3 | |
| Relative risk (95% CI) | 1.0 | 1.5 (0.7–3.1) | 2.0 (1.0–4.1) | 2.6 (1.3–5.1) | 0.004 |
| Interleukin-6 | | | | | |
| Median — pg/ml | 0.82 | 1.15 | 1.58 | 2.70 | |
| Relative risk (95% CI) | 1.0 | 1.3 (0.6–2.7) | 1.4 (0.7–2.8) | 2.2 (1.1–4.3) | 0.02 |
| Total cholesterol | | | | | |
| Median — mg/dl | 176 | 206 | 224 | 267 | |
| Relative risk (95% CI) | 1.0 | 1.2 (0.6–2.3) | 1.7 (0.9–3.3) | 2.4 (1.3–4.7) | 0.003 |
| LDL cholesterol | | | | | |
| Median — mg/dl | 88.4 | 108.9 | 127.4 | 156.6 | |
| Relative risk (95% CI) | 1.0 | 0.9 (0.4–1.9) | 1.7 (0.9–3.3) | 2.4 (1.3–4.6) | 0.001 |
| HDL cholesterol | | | | | |
| Median — mg/dl | 34.5 | 44.5 | 54.9 | 68.5 | |
| Relative risk (95% CI) | 1.0 | 0.5 (0.3–0.8) | 0.5 (0.2–0.8) | 0.3 (0.2–0.6) | 0.001 |
| Apolipoprotein A-I | | | | | |
| Median — mg/dl | 127 | 152 | 176 | 212 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.4–1.4) | 0.4 (0.2–0.8) | 0.8 (0.4–1.4) | 0.1 |
| Apolipoprotein B-100 | | | | | |
| Median — mg/dl | 86 | 104 | 121 | 149 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.5–2.3) | 1.6 (0.8–3.3) | 3.4 (1.8–6.8) | <0.001 |
| Lp(a) lipoprotein | | | | | |
| Median — mg/liter | 16 | 55 | 107 | 329 | |
| Relative risk (95% CI) | 1.0 | 1.0 (0.5–1.9) | 1.1 (0.6–2.1) | 1.3 (0.7–2.4) | 0.4 |
| Ratio of total cholesterol to HDL cholesterol | | | | | |
| Median | 3.06 | 4.00 | 4.80 | 6.34 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.3–1.5) | 1.7 (0.9–3.4) | 3.4 (1.8–5.9) | <0.001 |
| Homocysteine | | | | | |
| Median — μ mol/liter | 8.2 | 10.3 | 12.1 | 15.7 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.6–2.2) | 1.1 (0.5–2.1) | 2.0 (1.1–3.8) | 0.02 |

*P values were calculated by logistic-regression analyses. In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, LDL low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

amyloid A was 0.81 (P<0.001). In contrast, correlations between markers of inflammation and lipid measures were low; less than 10 percent of the variance in any marker of inflammation was explained by any of the lipid measures.

To determine the independent predictive value of each of the 12 measures, we performed a series of logistic-regression analyses that simultaneously controlled for increasing quartiles of hs-CRP, serum amyloid A, sICAM-1, interleukin-6, homocysteine, and Lp(a) lipoprotein and the ratio of total cholesterol to HDL cholesterol (because of colinearity with this ratio, levels of apolipoprotein A-I, apolipoprotein B-100, and LDL cholesterol were not included in these analyses). As shown in Table 4, only the level

of hs-CRP and the ratio of total cholesterol to HDL cholesterol were found to be independent predictors of risk in models in which women were matched for smoking status and age or in models that included further adjustments for body-mass index, hypertension, diabetes, and parental history of premature coronary artery disease. In similar models that were limited to markers of inflammation, hs-CRP remained an independent predictor of the risk of future cardiovascular events. In contrast, the beta coefficients associated with serum amyloid A, sICAM-1, and interleukin-6 decreased substantially and were no longer statistically significant in analyses that included control for the quartile of hs-CRP.

To explore whether any of the markers of inflam-

TABLE 4. ADJUSTED RELATIVE RISK OF CARDIOVASCULAR EVENTS ASSOCIATED WITH AN INCREASE OF ONE QUARTILE IN THE CONCENTRATION OF EACH PLASMA MARKER.*

| VARIABLE | ADJUSTED FOR OTHER PLASMA MARKERS | | ADJUSTED FOR OTHER PLASMA MARKERS AND RISK FACTORS† | |
|--|-----------------------------------|---------|---|---------|
| | RELATIVE RISK (95% CI) | P VALUE | RELATIVE RISK (95% CI) | P VALUE |
| High-sensitivity C-reactive protein | 1.4 (1.1–1.9) | 0.02 | 1.5 (1.1–2.1) | 0.02 |
| Serum amyloid A | 1.1 (0.8–1.4) | 0.5 | 1.1 (0.8–1.6) | 0.4 |
| Soluble intercellular adhesion molecule type 1 | 1.1 (0.9–1.4) | 0.4 | 1.1 (0.8–1.4) | 0.6 |
| Interleukin-6 | 0.9 (0.7–1.2) | 0.6 | 0.8 (0.6–1.1) | 0.2 |
| Homocysteine | 1.1 (0.9–1.4) | 0.2 | 1.1 (0.8–1.4) | 0.6 |
| Lp(a) lipoprotein | 1.1 (0.9–1.3) | 0.6 | 1.0 (0.8–1.2) | 0.8 |
| Ratio of total cholesterol to HDL cholesterol | 1.4 (1.1–1.7) | 0.01 | 1.4 (1.1–1.9) | 0.02 |

*In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, and HDL high-density lipoprotein.

†These models were adjusted for the following additional risk factors: body-mass index (the weight in kilograms divided by the square of the height in meters), a history of hypertension, a history of diabetes, and a parental history of myocardial infarction.

mation added to the predictive value of lipid-based screening, several additional analyses were performed. First, we computed the relative risk of cardiovascular events in analyses in which study participants were stratified into nine groups according to total cholesterol level as well as each marker of inflammation. As shown in Figure 1, for each marker of inflammation included in this analysis, the risk of cardiovascular events was lowest among women with low total cholesterol levels and low levels of the marker in question. In contrast, the risk tended to be highest among women with high total cholesterol levels and high levels of a marker of inflammation. However, even among the women with low total cholesterol levels, the risk of cardiovascular events was significantly higher among those with high levels of hs-CRP and serum amyloid A than among those with low levels of these markers (Fig. 1). These associations were also evident, but to a lesser extent, for interleukin-6 and sICAM-1. In all of the analyses, these additive effects were robust with respect to the choice of cutoff point and the choice of the lipid variable analyzed. For example, the addition of hs-CRP to lipid screening produced a significant and additive predictive effect when regression analyses were based on cutoff points for quartiles (rather than cutoff points for the division of the study group into thirds) and on analysis of the ratio of total cholesterol to HDL cholesterol (rather than on total cholesterol alone).

Second, likelihood-ratio tests were used to compare the fit of predictive models that were based on measurement of a marker of inflammation in combi-

nation with lipids to the fit of models based on lipid measurements alone. In these analyses, each of the markers of inflammation significantly improved the usefulness of lipid screening in predicting risk. For example, models including both hs-CRP and total cholesterol were significantly better in the prediction of the risk of cardiovascular events than were models including only total cholesterol ($P < 0.001$). Likewise, models involving both hs-CRP and the ratio of total cholesterol to HDL cholesterol allowed significantly better prediction of risk than did models based solely on this lipid ratio alone ($P < 0.001$). Similar additive effects were seen for serum amyloid A, sICAM-1, and interleukin-6 when these markers were added to models based on total cholesterol or the ratio of total cholesterol to HDL cholesterol alone ($P < 0.01$ for all comparisons).

Third, as a measure of clinical usefulness, we computed the area under the receiver-operating-characteristic curve associated with risk-prediction models based on lipid screening alone and compared it with those based on a combination of lipids and markers of inflammation. In these analyses, the use of hs-CRP levels in addition to total cholesterol increased the area under the receiver-operating-characteristic curve from 0.59 to 0.66 ($P < 0.001$) and in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.68 ($P < 0.001$). Similar effects were observed for analyses that included serum amyloid A, sICAM-1, and interleukin-6: the addition of these markers to screening based on total cholesterol increased the area under the curve from 0.59 to 0.63, 0.63, and 0.64, respectively ($P < 0.003$ for all three comparisons). Use of the serum amyloid A level in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.67 ($P = 0.007$); the use of sICAM-1 in addition to this ratio led to a smaller change (area under the curve, 0.65; $P = 0.01$), as did the use of interleukin-6 (area under the curve, 0.65; $P = 0.01$).

Finally, to address the clinical observation that many persons with “safe” lipid levels nonetheless have cardiovascular events, we performed a subgroup analysis limited to women whose levels of LDL cholesterol were less than 130 mg per deciliter, the target level currently recommended for primary prevention of coronary heart disease by the National Cholesterol Education Program.¹⁷ In this analysis, women with increased base-line levels of hs-CRP, serum amyloid A, interleukin-6, or sICAM-1 were found to be at increased risk for future cardiovascular events. This effect was strongest for hs-CRP and serum amyloid A. In this subgroup, the relative risks of cardiovascular events for women in the lowest to the highest quartiles of hs-CRP were 1.0, 2.4, 2.9, and 4.1 (95 percent confidence interval for women in the highest as compared with the lowest quartile, 1.7 to 11.3; $P = 0.002$; P for trend across quartiles, 0.005). After adjustment

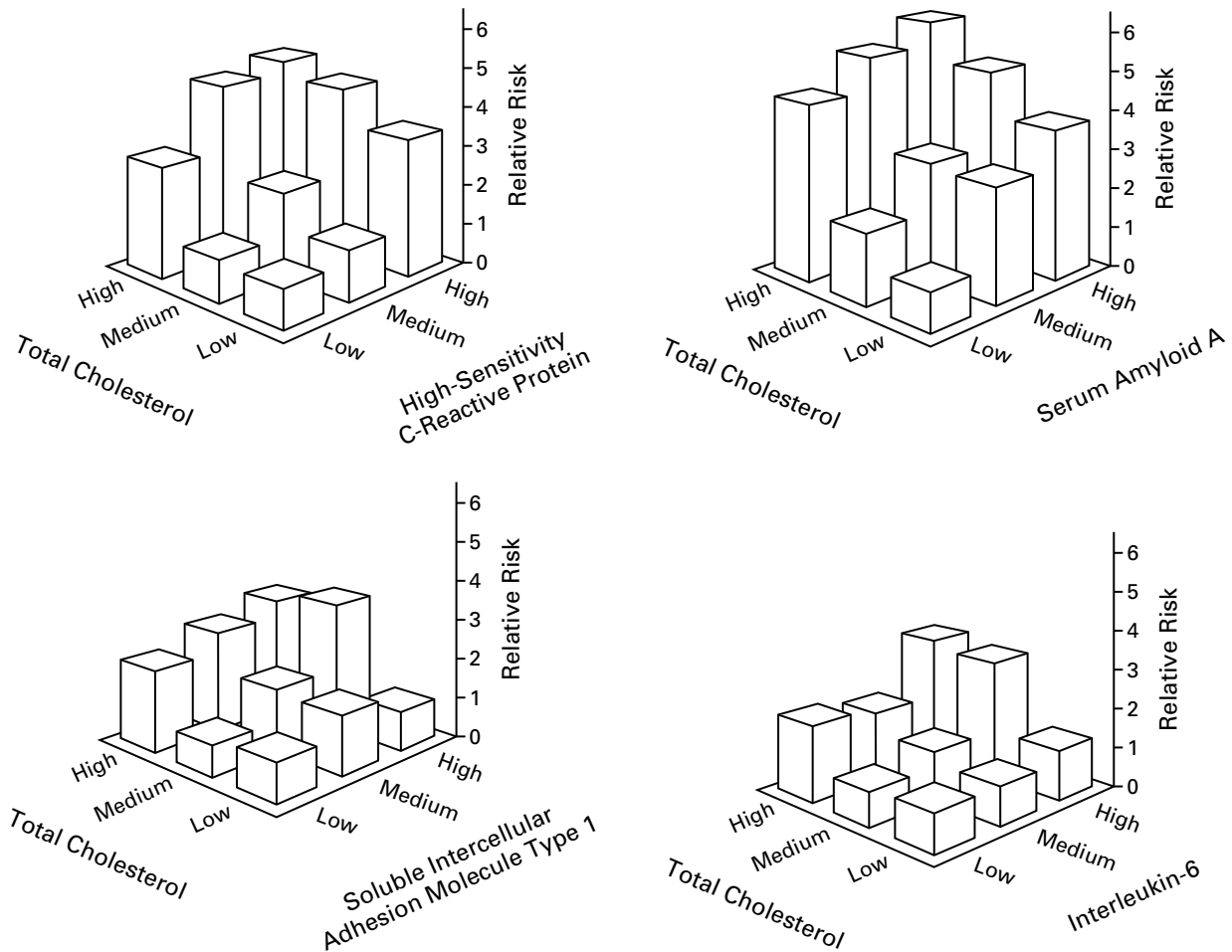


Figure 1. Relative Risk of Cardiovascular Events among Apparently Healthy Postmenopausal Women According to Base-Line Levels of Total Cholesterol and Markers of Inflammation. Each marker of inflammation improved risk-prediction models based on lipid testing alone, an effect that was strongest for hs-CRP and serum amyloid A.

for body-mass index, the presence or absence of hypertension, diabetes, or a parental history of premature myocardial infarction, and the level of HDL cholesterol, the increased risk for women in the highest quartile of hs-CRP at base line remained statistically significant (relative risk, 3.1; 95 percent confidence interval, 1.1 to 8.3; $P=0.03$). Thus, even among women with “safe” levels of LDL cholesterol, the adjusted relative risk of cardiovascular events increased approximately 39 percent with each increasing quartile for hs-CRP (95 percent confidence interval, 13 to 89 percent; $P=0.03$). The mean LDL cholesterol level in this subgroup analysis was 104 mg per deciliter (2.7 mmol per liter).

DISCUSSION

In this prospective study of apparently healthy postmenopausal women, four markers of inflammation — hs-CRP, serum amyloid A, interleukin-6, and

ICAM-1 — were found to be significant predictors of the risk of future cardiovascular events. In addition, measurement of these markers increased the predictive value of models based only on standard lipid screening. Of the 12 plasma measures evaluated in this study, hs-CRP was the most significant predictor of the risk of cardiovascular events; when measured with a widely available, standardized commercial assay,¹⁸ this marker distinguished between women at high risk and those at low risk, even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (mean, 104 mg per deciliter), the target considered safe in the current guidelines of the National Cholesterol Education Program.¹⁷

The results of the current study have several important implications. First, the findings confirm that in women, markers of inflammation are important predictors of the risk of cardiovascular events. Previous data on this issue have been derived largely from

studies of middle-aged men.⁴⁻¹¹ Thus, from a pathophysiologic perspective, the current data support the hypothesis that atherosclerosis is, in part, an inflammatory disease.³

Second, because we used a commercially available assay to measure plasma hs-CRP,¹⁸ our results provide clinically relevant confirmation of previous findings in this cohort, which were obtained with use of an experimental assay.¹³ The commercial assay is inexpensive and can be used with standard hospital and outpatient laboratory equipment; thus, screening for this predictor of cardiovascular risk would be practical in many clinical settings.

Third, we believe the current results have public health implications both in terms of the prediction of the risk of cardiovascular events and in terms of the use of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase for primary prevention. Although the results of large-scale randomized trials have indicated that HMG-CoA reductase inhibition is effective even among persons at low-to-moderate risk as defined by standard lipid screening,^{19,20} the large number of patients who would need to be treated and the high cost of this approach have limited the clinical application of those findings. Thus, our observation that measurement of markers of inflammation such as hs-CRP can significantly improve models for the prediction of cardiovascular risk may lead to better clinical identification of patients who might benefit from primary prevention and for whom the cost-to-benefit ratio for long-term use of statins would be improved. This issue is particularly intriguing because recent data from the Cholesterol and Recurrent Events trial indicate that long-term therapy with pravastatin significantly lowers plasma levels of hs-CRP²¹ and that the efficacy of pravastatin in lowering the rate of cardiovascular events is greatest in those with increased levels of hs-CRP.²² As in the current findings, which indicate that hs-CRP is a potent predictor of risk regardless of the LDL cholesterol level, data from the Cholesterol and Recurrent Events trial indicate that use of pravastatin resulted in decreased levels of hs-CRP in a manner largely independent of LDL cholesterol.²¹

Several limitations of these analyses merit consideration. First, our cohort comprised apparently healthy postmenopausal women, and thus the results may not apply to younger women, who may also be at increased risk for cardiovascular events. Second, we measured each marker of inflammation at study entry and thus could not evaluate the effects of changes in the levels of these markers over time. However, follow-up studies have found that levels of hs-CRP are stable over long periods, as long as measurements are not made within two to three weeks of an acute infection.^{21,23} Moreover, with respect to the current results, variation over time in levels of these markers and regression dilution bias would tend, if anything, to lead

to an underestimation of net effects. Finally, although base-line levels of several markers of inflammation were greater than normal among women at risk for future cardiovascular events, the mechanisms underlying these elevations remain uncertain. In this study, we did not find significant associations between cardiovascular risk and titers of IgG antibodies against *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, or cytomegalovirus or between titers of these antibodies and plasma levels of hs-CRP.²⁴ On the other hand, markers of inflammation, including hs-CRP, interleukin-6, and interleukin-1-receptor antagonist,²⁵⁻³¹ have proved to have predictive value among persons with unstable angina or acute coronary syndromes. Thus, it is also possible that the inflammation that we detected in apparently healthy women who were at risk for future cardiovascular events may be an indirect marker of an enhanced cytokine response to a variety of inflammatory stimuli that ultimately prove critical at the time of acute plaque rupture.³²

In conclusion, in this prospective evaluation of 12 plasma variables, hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events. As in previous population-based epidemiologic studies, half of all cardiovascular events in our cohort occurred among women without overt hyperlipidemia. Thus, these data raise the possibility that the addition of hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events, who would thus be candidates for primary-prevention interventions such as the use of HMG-CoA reductase inhibitors.

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Drs. Ridker and Hennekens are named as coinventors on a pending patent application filed by Brigham and Women's Hospital on the use of markers of inflammation in coronary artery disease.

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CORRECTION

C-Reactive Protein in the Prediction of Cardiovascular Disease

To the Editor: Ridker et al. provided a stimulating article on inflammatory markers and cardiovascular disease in women (March 23 issue).¹ Not surprisingly, the popular press picked up on the article and gave their findings prominent coverage. It is not clear that Ridker et al. wanted this to happen. Their findings are cast in terms of relative risk only, not in terms of traditional predictive value; it is the latter that is more relevant to the practicing physician.² That is, we learn that subjects in the highest quartile for high-sensitivity C-reactive protein (hs-CRP), relative to those in the lowest quartile, had a 4.4-fold risk of cardiovascular events. However, the overall risk was just 0.4 percent (122 events in 28,263 subjects over a period of three years). We suspect that the positive predictive value (the proportion of all subjects with "elevated" levels of hs-CRP who had cardiac events) in this population was low.

We were unable to calculate the conventional predictive values from the data supplied in the article. It would be instructive if the authors provided the predictive values so that readers could determine whether this new test is genuinely ready for "prime-time" screening.

Even though this test performed better than measurements of conventional lipid markers such as low-density lipoprotein (LDL) cholesterol in this population (at least in terms of relative risk), there are other relevant data about LDL cholesterol that are lacking for hs-CRP. For example, we know that lowering LDL cholesterol levels has beneficial effects,³ we have effective methods to lower LDL cholesterol levels, and we have data on the cost effectiveness of such strategies.⁴

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The authors reply:

To the Editor: In our study of inflammatory and lipid markers we used a matched, nested case-control analysis that allowed direct comparison of the magnitude of risk associated with various cardiovascular risk factors after age and smoking status were taken into account. Of the 12 factors evaluated — which included LDL cholesterol, high-density lipoprotein (HDL) cholesterol, Lp(a) lipoprotein, and homocysteine — hs-CRP was the strongest predictor of future cardiovascular events. Moreover, hs-CRP levels were predictive of the risk of cardiovascular events among study participants with low levels of LDL cholesterol; these data underscore the importance of the inflammatory process in atherothrombosis.

Our matched, nested case-control study was designed to maximize biologic validity. It is not, however, conducive to calculating absolute risks. We thus concur with Horowitz and Beckwith that generalizing our results to other populations must be done with caution and that studies addressing absolute risks are needed. We further concur that the reduction of lipid levels remains a fundamentally important method to reduce cardiovascular risk. At the same time, since half of all heart attacks and strokes occur among apparently healthy men and women without overt hyperlipidemia, we believe it important for clinicians to consider emerging biologic data that go beyond the use of cholesterol screening as the sole method of assessing cardiovascular risk. With regard to hs-CRP, several large-scale studies in the United States^{1,2,3} and Europe^{4,5} have now demonstrated the potential importance of this inflammatory marker in the detection of cardiovascular risk.

Finally, we wish to correct an error in the last sentence of the Results section of our abstract. As described in the text and in Table 4 of our article, our multivariate analysis was performed on a per-quartile basis. Thus, this sentence should read, "In multivariate analyses, the only plasma markers that independently predicted risk were hs-CRP (increase in relative risk per quartile, 1.5; 95 percent confidence interval, 1.1 to 2.1) and the ratio of total cholesterol to HDL cholesterol (increase in relative risk per quartile, 1.4; 95 percent confidence interval, 1.1 to 1.9)."

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