Relationship Between C-Reactive Protein and Subclinical Atherosclerosis

The Dallas Heart Study

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Background—Elevated levels of C-reactive protein (CRP) are associated with increased risk for incident cardiovascular events on the basis of observations from several prospective epidemiological studies. However, less is known regarding the relationship between CRP levels and atherosclerotic burden.

Methods and Results—We measured CRP in 3373 subjects 30 to 65 years of age who were participating in the Dallas Heart Study, a multiethnic, population-based, probability sample. Electron-beam CT scans were used to measure coronary artery calcification (CAC) in 2726 of these subjects, and MRI was used to measure aortic plaque in 2393. CRP levels were associated with most traditional cardiovascular risk factors. Subjects with CAC had higher median CRP levels than those without CAC (men: median, 2.4 versus 1.8 mg/L, \(P<0.001\); women: median, 5.2 versus 3.6 mg/L, \(P<0.001\)), and there was a modest trend toward increasing CRP levels with increased CAC levels in men (\(P\) for trend \(=0.003\)) but not in women (\(P\) for trend =0.08). Male subjects with aortic plaque also had higher CRP levels than those without (median, 2.3 versus 1.8; \(P<0.001\)). In multivariate analysis adjusted for traditional cardiovascular risk factors, body mass index, and estrogen and statin medication use, the associations between CRP levels and CAC and CRP levels and aortic plaque were no longer statistically significant.

Conclusions—In a large, population-based sample, subjects with higher CRP levels had a modest increase in the prevalence of subclinical atherosclerosis, but this association was not independent of traditional cardiovascular risk factors. CRP is a poor predictor of atherosclerotic burden. (Circulation. 2006;113:38-43.)

Key Words: atherosclerosis ■ imaging ■ inflammation

Several prospective epidemiological studies have demonstrated a consistent relationship between higher C-reactive protein (CRP) levels and increased risk of cardiovascular (CV) events, including myocardial infarction, stroke, and CV death.\(^1\)\(^-\)\(^7\) Despite these robust epidemiological data, the mechanisms relating CRP to incident CV events are unclear. CRP may reflect a greater burden of atherosclerosis or alternatively may identify a high-risk atherosclerosis phenotype with active inflammation and atherosclerotic plaque that is vulnerable to rupture.\(^8\)\(^-\)\(^9\)

Angiographic studies relating CRP to atherosclerotic burden have provided conflicting results and are limited by examining only luminal atherosclerotic disease,\(^10\)\(^-\)\(^-\)\(^13\) a relatively insensitive method for assessing the net burden of atherosclerosis.\(^14\) Coronary artery calcium (CAC) determination by CT appears to provide more accurate estimates of the burden of coronary atherosclerosis,\(^15\) and aortic plaque measurements with MRI are a promising modality to quantify atherosclerosis.\(^16\) Only a few studies have examined the relationship between CRP and CAC as a measure of atherosclerotic burden and have provided inconsistent results.\(^1\)\(^-\)\(^6\)\(^,\)\(^17\)\(^-\)\(^20\) However, these studies were generally limited in size and were composed of select research populations. A detailed examination of the relationship between CRP and MRI-determined aortic plaque has not been previously reported.

Given the uncertainty regarding the relationship between CRP and atherosclerotic burden and the limitations of the published data, we sought to determine whether CRP is independently associated with CAC and aortic plaque using...
data from a large, ethnically diverse, population-based sample from Dallas County.

Methods

The Dallas Heart Study is multiethnic, population-based, probability sample of Dallas County residents 30 to 65 years of age; the study design has previously been published.21 All participants provided informed consent to participate in the study, and the protocol was approved by the Institutional Review Board of the UT Southwestern Medical Center. From an initial cohort of 6101 subjects who underwent a detailed in-home survey, 3398 also volunteered to provide blood and urine samples for laboratory testing, and 2971 subsequently underwent a detailed clinic visit, including additional data collection and cardiac imaging. Definitions of the variables have been previously described.22 The average of 5 sequential blood pressure readings was taken, and hypertension was defined as average systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medication. Diabetes was defined as a fasting glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, or the previous diagnosis of diabetes mellitus coupled with the use of any glucose-lowering medication. Estrogen use refers to the use of any oral estrogen medication. Race was determined by subject self-report.

Cardiac electron-beam CT (EBCT) scanning was performed on 2726 subjects with CRP measures using an Imatron C-150XP EBCT scanner with 3-mm slices; duplicate scans were performed within 1 to 2 minutes. CAC scores were recorded as the average of the 2 scans using the Agatston method.23 A score >10 was selected as a data-derived threshold to define the presence of calcium to maximize the signal-to-noise ratio and reproducibility.24 Details of the EBCT imaging techniques have been reported previously.25 Cardiovascular MRI, including determination of aortic plaque, was completed in 2393 subjects with CRP measures (2239 with CAC scores) using a 1.5-T whole-body MRI system (Intera, Philips Medical Systems). Six transverse slices of the infrarenal abdominal aorta were obtained with a free-breathing, ECG-gated, T2-weighted turbo spin-echo (black-blood) sequence. Images were analyzed with the Magnetic Resonance Analytical Software Systems cardiac analysis software package (version 4.2 beta, Medis Medical Imaging Systems Inc) by personnel blinded to all participant data. Atherosclerotic plaque in the aortic wall was characterized as areas of hyperintense signal, luminal protrusion, and focal wall thickening, as previously described, and was categorized as present or absent.16

High-Sensitivity CRP Assay

Blood samples were obtained on 3398 subjects after an overnight fast in EDTA tubes and were stored for ≤4 hours at 4°C before processing. Plasma aliquots were frozen at −80°C until assays were performed. High-sensitivity CRP measurements were performed on 3373 available thawed specimens with the Roche/Hitachi 912 System, Tina-quant C-Reactive Protein (latex) assay (Roche Diagnostics), a latex-enhanced immunoturbidimetric method.25 The minimal detectable range of this assay is 0.1 mg/L, and the upper limit of the assay is 20 mg/L. The 220 subjects (6.5%) with CRP values >20 mg/L were treated as having CRP values of 20 mg/L. The interassay coefficient of variance was 5.0% at a CRP concentration of 4.3 mg/L and 3.2% at a concentration of 13.2 mg/L. Clinical validation of this assay has been described previously.5

Statistical Analysis

Categorical data are reported as proportions and continuous data as mean values with standard deviations. Subjects were divided into quartiles based on levels of CRP. Baseline demographic variables and CV risk factors were compared across CRP quartiles with the χ² trend test for categorical variables and the test for trend across ordered groups for continuous variables. CAC scores were categorized using two previously described classification schemes:4,26: (1) ≤10=no calcium and >10=detectable calcium and (2) ≤10=no calcium, >10 to 100=mild, >100 to 400=moderate, and >400=severe. Associations between CRP values and CAC and aortic plaque subgroups were analyzed with the Wilcoxon rank-sum test for 2-group comparisons and the test for trend across ordered groups for 4-group comparisons. Adjusted odds ratios (ORs) for CAC >10 and prevalent aortic plaque were determined from multivariate logistic regression models that included CRP levels and traditional CV risk factors as covariates.26 Ordinal logistic regression models were also used to assess the combined odds of different CAC thresholds (>10, >100, >400);27 and the proportional odds assumption of this model was confirmed with the Score test. This study had 97% power to detect a 1-mg/L difference in mean CRP levels between subjects with and without detectable CAC at a 2-sided significance level of 0.05 and 99% power to detect such a difference between those with and without aortic plaque.

Results

Baseline characteristics according to CRP quartiles are presented in Table 1. CRP levels were associated with most traditional cardiovascular risk factors, including diabetes, hypertension, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and family history of myocardial infarction, as well as oral estrogen use and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) use, but not with smoking or aspirin use. A strong positive association was also observed between CRP levels and both female sex and body mass index (BMI); the mean BMIs of subjects in the lowest and highest quartiles of CRP were 26.5 and 35.7 kg/m², respectively.

Among subjects who underwent EBCT or aortic MRI scanning, 21.3% had CAC scores >10, and 40.2% had detectable aortic plaque. These 2 measures of subclinical atherosclerosis were concordant in 66% of the subjects who underwent both scans. In univariable analyses, CRP levels were higher in those with detectable CAC than in those without for both men and women (men: median, 2.4 versus 1.8 mg/L, P<0.001; women: median, 5.2 versus 3.6 mg/L, P<0.001). There was also a modest trend toward increasing CRP levels with increased CAC burden in men (P for trend=0.003) but not in women (P=0.08), with considerable overlap in the CRP distributions of these groups (Figures 1 and 2). Men with aortic plaque had higher CRP levels than those without (median, 2.3 versus 1.8; P<0.001), but there was no significant relationship between aortic plaque and CRP in women (median, 3.9 versus 3.6; P=0.2) (Figures 1 and 2).

After multivariate adjustment for traditional CV risk factors, race, estrogen and statin use, and BMI through logistic regression analysis, CRP levels were no longer independently associated with the presence of CAC (CAC >10) in the overall cohort, in men, or in women (Table 2). Using an ordinal logistic regression model that combined the 3 CAC thresholds (CAC >10, >100, and >400) did not alter these results, nor did stratified analyses of women by estrogen use (Table 2). CRP levels also were not independently associated with the presence of aortic plaque after multivariable adjustment (Table 3). Excluding subjects with high CRP values above the detection threshold (>20 mg/L) did not significantly affect these results.

Discussion

In a large, population-based study, CRP levels were only modestly associated with atherosclerotic burden as assessed
by either EBCT measurements of coronary calcium or novel MRI measurements of aortic plaque. These relationships were no longer significant in men and women after adjustment for other CV risk factors. Our findings provide a better understanding of the relationship between CRP and cardiovascular disease, suggesting that CRP is a poor predictor of atherosclerotic plaque burden.

Only a few previous studies have examined the relationship between CRP and atherosclerosis using CAC scanning as the measure of atherosclerotic burden. Select research populations consisting of postmenopausal women, male military recruits, and hypertensive siblings were used in 3 of these smaller studies, which revealed no relationship between CRP levels and CAC, even in univariable analyses. In contrast, the Framingham Heart Study, which is the only population-based study published to date evaluating these associations, demonstrated a positive correlation between CRP and CAC scores in men and women (Spearman’s ρ=0.35 and 0.34, respectively). After adjustment for age, Framingham risk score, and BMI, the correlation was attenuated but remained significant in men (r=0.19, P<0.05), but it was no longer significant in women. The largest study reported to date examining the relationship between CRP and CAC consisted of 914 subjects with a family history of premature coronary artery disease. In univariable analyses, greater CRP levels were associated with higher mean CAC scores in women but not in men. In multivariate analyses, there was no independent relationship between CRP and CAC after adjustment for age, traditional cardiovascular risk factors, and BMI.

### Table 1: Baseline Characteristics According to CRP Levels

<table>
<thead>
<tr>
<th>CRP Quartile</th>
<th>CRP Quartile 2</th>
<th>CRP Quartile 3</th>
<th>CRP Quartile 4</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;1.2 mg/L)</td>
<td>(&gt;1.2–2.9 mg/L)</td>
<td>(&gt;2.9–7.1 mg/L)</td>
<td>(&gt;7.1 mg/L)</td>
<td></td>
</tr>
<tr>
<td>(n=869)</td>
<td>(n=836)</td>
<td>(n=820)</td>
<td>(n=848)</td>
<td></td>
</tr>
</tbody>
</table>

| Age, y*      | 42.8 (8.7)     | 44.0 (8.2)     | 45.5 (9.7)     | 46.3 (9.4)  | <0.0001 |
| Female sex, %| 44.1           | 46.7           | 60.5           | 72.6        | <0.0001 |
| Black race, %| 45.8           | 46.1           | 54.4           | 61.8        | <0.0001 |
| Diabetes, %  | 5.6            | 9.1            | 11.8           | 22.1        | <0.0001 |
| Smoking, %   | 29.9           | 28.9           | 29.9           | 29.9        | 0.9     |
| Hypertension, %| 21.6           | 32.9           | 40.6           | 47.0        | <0.0001 |
| Total cholesterol, mg/dL* | 173.4 (35.7)  | 181.8 (38.3)  | 185.6 (39.4)  | 182.9 (44.5) | <0.0001 |
| Triglycerides, mg/dL* | 109.2 (87.8)  | 126.5 (102.6) | 130.5 (101.8) | 136.6 (133.3) | <0.0001 |
| HDL, mg/dL*  | 52.8 (15.9)    | 49.6 (15.5)    | 48.7 (14.3)    | 48.5 (13.3)  | <0.0001 |
| Family history of MI, % | 30.0           | 31.0           | 34.8           | 38.7        | <0.0001 |
| BMI, kg/m²*  | 26.5 (5.0)     | 29.4 (5.7)     | 31.7 (6.9)     | 35.7 (8.1)  | <0.0001 |
| Statin use, %| 5.3            | 5.4            | 7.3            | 8.0         | 0.01     |
| Estrogen use, %| 2.9            | 7.4            | 12.7           | 17.1        | <0.0001 |
| Aspirin use, %| 8.3            | 8.0            | 9.2            | 10.3        | 0.1      |
| CAC score*   | 59.1 (432.6)   | 53.2 (236.1)   | 63.5 (296.3)   | 65.7 (278.3) | 0.6      |
| Aortic plaque, %| 34.7           | 40.3           | 44.8           | 41.7        | 0.004    |

*MI indicates myocardial infarction.

*Data are presented as mean and SD.

**Figure 1.** Median CRP levels for CAC and aortic plaque categories in men. Data are shown as median (25th, 75th percentiles).

**Figure 2.** Median CRP levels for CAC and aortic plaque categories in women. Data are shown as median (25th, 75th percentile).
The discrepancy between these previous studies is likely due to differences in sample size and prevalence of CAC, selection of study populations, and analysis techniques. Thus, our study helps to provide a definitive answer to this important question by using a multiethnic, population-based sample that is several-fold larger than the previous studies. Similar to the Framingham study,\textsuperscript{20} we observed a modest association between levels of CRP and CAC in unadjusted analyses (OR for detectable CAC, 1.04 to 1.06 for 1-mg/L increase in CRP), which was stronger for men than for women. However, these associations were no longer present when adjustment was made for potential confounders, the most important of which include age, smoking, diabetes, and BMI. Our null findings using a single CAC threshold of 10 were confirmed with ordinal logistic regression that modeled the combined probability of 3 different CAC thresholds as the outcome.\textsuperscript{27}

Our findings demonstrate that CRP levels do not independently reflect atherosclerotic burden as measured with EBCT. However, because not all atherosclerosis is calcified, CRP levels could be related to noncalcified, “soft” plaque.\textsuperscript{15} To address this important issue, we used a novel measure of atherosclerotic burden that does not involve calcification: aortic plaque assessment by MRI.\textsuperscript{16,28–30} MRI measurement of aortic plaque correlates closely to measures determined by transesophageal echocardiography\textsuperscript{28} and with histopathological determinations of aortic plaque area in animal models.\textsuperscript{29,31} Aortic plaque assessed by MRI is also related to the extent of coronary artery disease.\textsuperscript{30} Our study is the largest to date using this technique, with an overall prevalence of detectable abdominal aortic plaque of 40%, which is nearly identical to the prevalence of 39% reported in another population-based cohort study of 318 subjects.\textsuperscript{16} Only 1 previous study has examined the relationship between CRP and MRI-derived aortic plaque.\textsuperscript{30} The investigators reported an increase in the extent of plaque with increasing CRP levels in crude analysis but did not provide any multivariate adjustment for other cardiovascular risk factors. Although modestly increased CRP levels were observed in male subjects with aortic plaque in our study, this relationship was no longer evident after adjustment for other risk factors. The observed lack of an independent relationship between CRP levels and non–calcium-based measures of atherosclerotic burden has also been seen in studies of carotid intima-medial thickness.\textsuperscript{32–34} Thus, our null findings relating CRP to atherosclerotic burden are not limited to “hard,” calcified atheroma.

**Clinical Implications**

The present findings suggest that the relationship between higher CRP levels and incident cardiovascular events may reflect the composition, morphology, and stability of plaque rather than overall atherosclerotic burden.\textsuperscript{15} Because CAC scores are associated with risk for subsequent cardiovascular events\textsuperscript{36,37} and provide a measure of disease processes distinct from CRP, these 2 measures may be complementary rather than competitive for risk prediction.\textsuperscript{38}

### TABLE 2. Multivariate Analysis of CRP Levels and CAC

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Logistic Model, OR (95% CI)* for CAC &gt;10</th>
<th>OR (95% CI)* for CAC Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>Adjusted†</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Entire cohort (n=2726)</td>
<td>1.04 (1.02–1.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men (n=1224)</td>
<td>1.06 (1.04–1.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>All (n=1502)</td>
<td>1.06 (1.03–1.08)</td>
</tr>
<tr>
<td></td>
<td>Nonusers of estrogen (n=1212)</td>
<td>1.06 (1.03–1.09)</td>
</tr>
<tr>
<td></td>
<td>Estrogen users (n=290)</td>
<td>1.04 (0.99–1.09)</td>
</tr>
</tbody>
</table>

*OR and 95% CI for 1-mg/L increase in CRP.
†Adjusted for age, race, hypertension, diabetes, total cholesterol, HDL cholesterol, statin use, estrogen use, aspirin use, smoking, family history of myocardial infarction, and BMI.

### TABLE 3. Multivariate Analysis of CRP Levels and Aortic Plaque

<table>
<thead>
<tr>
<th>OR (95% CI)* for Aortic Plaque</th>
<th>Unadjusted</th>
<th>Adjusted†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire cohort (n=2393)</td>
<td>1.01 (1.0–1.03)</td>
<td>0.1</td>
<td>1.00 (0.98–1.02)</td>
</tr>
<tr>
<td>Men (n=1087)</td>
<td>1.05 (1.02–1.08)</td>
<td>&lt;0.001</td>
<td>1.03 (1.0–1.06)</td>
</tr>
<tr>
<td>Women</td>
<td>All (n=1306)</td>
<td>1.00 (0.98–1.02)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Nonusers of estrogen (n=1072)</td>
<td>0.99 (0.97–1.02)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Estrogen users (n=234)</td>
<td>1.02 (0.98–1.06)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*OR and 95% CI for 1-mg/L increase in CRP.
†Adjusted for age, race, hypertension, diabetes, total cholesterol, HDL cholesterol, statin use, estrogen use, aspirin use, smoking, family history of myocardial infarction, and BMI.
Study Limitations
Despite the large sample size and unselected nature of this cohort, the present study has several limitations. Although medication use was carefully documented before phlebotomy, we are unable to fully account for potential treatment effects of newly initiated medication use such as statins before the imaging studies. In addition, there was limited power to assess the relationship between CRP and atherosclerosis in certain subgroups such as women with oral estrogen use.

Conclusions
In a large, ethnically diverse, population-based sample, subjects with higher CRP levels had a modestly increased prevalence of subclinical atherosclerosis, but this association was not independent of traditional CV risk factors. CRP is a poor predictor of atherosclerotic burden.

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Disclosures
Dr de Lemos has received research grant support from Roche Diagnostics. The other authors report no conflicts.

References
Several prospective epidemiological studies have demonstrated a consistent relationship between higher CRP levels and increased risk of cardiovascular (CV) events. Despite these robust epidemiological data, the mechanisms relating CRP to incident CV events are unclear. CRP may reflect a greater burden of atherosclerosis or alternatively may identify a high-risk atherosclerosis phenotype with active inflammation and atherosclerotic plaque that is vulnerable to rupture. Only a few studies have examined the relationship between CRP and CAC as a measure of atherosclerotic burden and have provided inconsistent results. Also, there were no previous detailed examinations of the relationship between CRP and MRI measures of abdominal aortic plaque. Using a large, multiethnic population-based sample, we observed that both men and women with detectable CAC had higher CRP levels than those without and a modest trend toward increasing CRP levels with increasing CAC levels in men. The presence of abdominal aortic plaque was also associated with higher CRP levels in men but not in women. In multivariate analysis adjusted for traditional CV risk factors, BMI, and estrogen and statin medication use, the associations between CRP levels and CAC and CRP levels and aortic plaque were no longer statistically significant. The present findings suggest that the relationship between higher CRP levels and incident CV events may reflect the composition, morphology, and stability of plaque rather than overall atherosclerotic burden. CAC and CRP measures may also be complementary for CV risk prediction.
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