C-Reactive Protein and Atherosclerosis of the Thoracic Aorta

A Population-Based Transesophageal Echocardiographic Study

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Background: An association between systemic inflammatory markers and the presence and severity of atherosclerotic plaques has not been demonstrated in a non-selected population. The purpose of this study was to examine the association of inflammatory markers with aortic atherosclerotic plaques in a sample of the general population and in a subgroup free of clinical vascular disease.

Methods: Transesophageal echocardiography was performed in 386 subjects (median age, 66 years; 53% men). We examined the association between systemic inflammatory markers and aortic atherosclerotic plaques.

Results: Aortic plaques were present in 267 subjects (69%). Plaques at least 4 and 6 mm thick and mobile debris were present in 114, 41, and 20 subjects, respectively. High-sensitivity C-reactive protein (hs-CRP) level was associated with the presence of aortic plaques, adjusting for age, sex, smoking status, and additional atherosclerosis risk factors. Among subjects with plaques, hs-CRP level was independently associated with plaques at least 6 mm thick; similar trends were observed for the associations of hs-CRP level with plaques at least 4 mm thick and mobile debris. In subjects with aortic plaques who were free of clinically apparent coronary artery or cerebrovascular disease, hs-CRP level was independently associated with plaques at least 6 mm thick.

Conclusions: Level of hs-CRP is independently associated with the presence and severity of aortic atherosclerotic plaques. These observations establish the association of systemic inflammation with anatomically defined atherosclerosis in the general population.

Arch Intern Med. 2004;164:1781-1787

Inflammation has a major role in the pathogenesis of atherosclerosis. The association of various markers of systemic inflammation with clinical cardiovascular events has been well demonstrated in numerous populations, including subjects apparently free of cardiovascular disease. This association is independent of other atherosclerosis risk factors, including plasma lipid levels, and enables cardiovascular risk to be better defined.

It has been hypothesized that the association between markers of systemic inflammation and cardiovascular events reflects the underlying atherosclerotic plaque burden, specifically the presence of unstable, rupture-prone plaques. Alternatively, these associations with atherothrombotic complications may result directly from the proinflammatory or prothrombotic (e.g., fibrinogen) effects of several acute-phase reactants.

The Stroke Prevention: Assessment of Risk in a Community (SPARC) study is a population-based study funded by the National Institutes of Health, Bethesda, Md. The study is designed to evaluate the prevalence of risk factors for stroke in the population of Olmsted County, Minnesota. Study participants underwent evaluation with multiple modalities, including transesophageal echocardiography (TEE).

The objective of our present analysis was to examine the association of systemic inflammatory markers with aortic atherosclerotic plaques in a sample of the general population and in a subgroup of subjects free of clinical vascular disease.

Methods

Study Population

The study design and initial results of the first phase of the SPARC study have been described in detail. In brief, the original study cohort consisted of 581 subjects, an age- and sex-stratified random sample of the Olmsted County population 45 years and older. Approximately 4 to 5 years after the initial evalu-
Data on clinical cardiovascular risk factors and clinical coronary artery and cerebrovascular disease were collected by interviews and abstracting of medical records (at Mayo Clinic and Olmsted Medical Center, the 2 primary health care providers in Olmsted County). Blood pressure was measured during an office interview related to the SPARC study. Two sitting blood pressure measurements were taken 5 to 10 minutes apart and averaged. Pulse pressure was defined as the difference between the mean systolic and diastolic measurements. Treatment of hypertension or hyperlipidemia was defined as the use of antihypertensive or lipid-lowering drugs, respectively (self-reported during interview).

Fasting blood samples were collected (on the day TEE was performed for 97% of participants and within 20 days of TEE for all participants). With the use of standard commercially available assays, samples were analyzed for (1) plasma lipid levels (measurements of total cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides, and apolipoprotein A-I and B levels and calculation of low-density lipoprotein [LDL] cholesterol level); (2) homocysteine level; (3) blood cell counts (including leukocyte differential count); (4) fibrinogen level; and (5) high-sensitivity C-reactive protein (hs-CRP) level, measured with a high-sensitivity latex-enhanced immunoturbidimetric assay (Kamiya Biomedical Co, Seattle, Wash).

TRANSESOPHAGEAL ECHOCARDIOGRAPHY

Transesophageal echocardiography was performed using commercially available ultrasound instruments equipped with multiple probes. Local pharyngeal anesthesia and intravenous sedation (midazolam hydrochloride and meperidine hydrochloride) were used as clinically indicated.

The 3 segments of the thoracic aorta (ascending aorta, aortic arch, and descending thoracic aorta) were visualized in short- and long-axis views with high-frequency (7-MHz) ultrasonographic imaging. Atherosclerotic plaques were defined as irregular intimal thickening (>2 mm thick) with increased echogenicity. The presence of plaques of any degree in any location within the thoracic aorta was determined. Among subjects with plaques, maximal plaque thickness and the presence of mobile debris in any aortic segment were determined.

In this analysis, plaque thickness was arbitrarily dichotomized at 4 mm and 6 mm (detected in approximately 10% of the study population).

STATISTICAL ANALYSIS

We summarized continuous data as medians and interquartile (25-75 percentile) ranges and categorical data as percentages. For continuous data, groups were compared by the unpaired t test (normal data) or Wilcoxon rank sum test (nonnormal data).

RESULTS

Aortic plaques of any degree were present in 267 subjects (69% of the study population). Plaques were uncommon in the ascending aorta (23 subjects [6%]), but common in the aortic arch (244 subjects [63%]) and descending thoracic aorta (211 subjects [55%]). Plaques at least 4 mm thick, plaques at least 6 mm thick, and mobile debris (in any aortic segment) were present in 114 (30%), 41 (11%), and 20 subjects (5%), respectively. Plaque thickness was significantly greater in subjects with mobile debris than in those without debris (median plaque thickness, 6 vs 3 mm, respectively; P < .001).

For categorical data, groups were compared by the χ² test or Fisher exact test, as appropriate. We calculated Spearman correlation coefficients to examine the correlations between continuous variables.

We performed 2 separate series of multivariate analyses. The first series of analyses compared subjects with aortic plaques (of any degree) with subjects without plaques. The second series of analyses, done in the subgroup of subjects with aortic plaques, compared subjects with more severe forms of plaques (defined by plaque thickness or presence of mobile debris) with subjects with plaques of lesser severity. We used logistic regression to assess the impact of age, sex, clinical and laboratory atherosclerosis risk factors, treatment variables, and inflammatory markers on the odds of aortic plaques (proportion with any aortic plaques divided by proportion without plaques). Among subjects with plaques, we assessed the odds of plaques of at least 4 mm (proportion with plaques ≥4 mm divided by proportion with plaques <4 mm), plaques of at least 6 mm (proportion with plaques ≥6 mm divided by proportion with plaques <6 mm), and mobile debris (proportion with mobile debris divided by proportion without debris). Variables with skewed distributions were logarithmically transformed to normalize the data before assessing them in the logistic regression models.

Initially, with the use of stepwise logistic regression, atherosclerosis risk factor models were developed for each plaque variable. Clinical and laboratory atherosclerosis risk factors, summarized in Table 1 (not including clinical coronary artery and cerebrovascular disease variables), competed for entry into the models. All stepwise logistic modeling was adjusted for age. Sex was forced into models with a large number of subjects with the plaque variables (models of any aortic plaques and plaques ≥4 mm). Smoking was forced into all models because of its association with increased levels of systemic inflammatory markers. Smoking was modeled as current and past (vs never) for models with a large number of subjects with the plaque variables and as ever vs never for models with a small number of subjects with the plaque variables (models of plaques ≥6 mm and mobile debris). The P value to enter and leave the stepwise models was .05. When the list of independent variables was finalized, all 2-way interactions of the variables were analyzed for each of the models.

Subsequently, we used these logistic regression models to examine separately the associations of each individual inflammatory variable with each plaque variable, adjusting for age, smoking, and additional noninflammatory risk factors included in the stepwise models. These associations were assessed in the total study population and, subsequently, in the subgroup of subjects without clinically evident coronary artery disease (previous myocardial infarction, angina pectoris, coronary artery bypass graft surgery, or percutaneous coronary angioplasty) or cerebrovascular disease (previous ischemic stroke, transient ischemic attack, or carotid endarterectomy).
The clinical and laboratory atherosclerosis risk factors in subjects with various degrees of aortic plaques are presented in Table 1. Results of the stepwise multivariate logistic modeling assessing the impact of noninflammatory atherosclerosis risk factors on the odds of aortic plaques are summarized in Table 2. Age was strongly associated with the presence of aortic plaques (of any degree) and, within the subgroup with plaques, was associated with thick plaques (≥4 and ≥6 mm thick) and mobile debris. Smoking and higher pulse pressure were associated with plaques of any degree and of at least 4 mm; higher body mass index was associated with plaques of at least 4 mm and with mobile debris.

Dyslipidemia was significantly associated with the presence and severity of aortic plaques. Lipid-lowering treatment was associated more strongly with the presence of plaques (of any degree) than the actual measurements of lipid levels. Among subjects with plaques, lower HDL cholesterol and apolipoprotein A-I (an HDL-associated apolipoprotein) levels were the lipid measures most strongly associated with thick plaques (≥6 and ≥4 mm thick, respectively), and total cholesterol level was the lipid measure most strongly associated with mobile debris. There were no significant interactions between the final variables in all the risk factor models presented in Table 2.

The values of inflammatory variables in subjects with various degrees of aortic plaques are shown in Table 3. Table 4 summarizes the associations between these variables and aortic plaques, adjusting for the risk factors in the respective multivariate logistic models presented in Table 2. The distribution of hs-CRP concentration in the population is shown in Figure 1. Because of its skewed distribution, hs-CRP values were logarithmically transformed, achieving near-normal distribution, and odds ratios (ORs) were estimated per 2-fold increase in hs-CRP concentration (Table 4 and Figure 2). In the total population, hs-CRP level was independently associated with the presence of aortic plaques (OR, 1.25 per 2-fold increase in hs-CRP level), adjusting for age, sex, smok-
ing status, and additional atherosclerosis risk factors defined in the risk factor model. Among the subgroup with plaques, hs-CRP level was independently associated with thick (≥6 mm) plaques (OR, 1.36 per 2-fold increase in hs-CRP level), adjusting for age, smoking, and additional atherosclerosis risk factors. These associations remained significant (P = .04 for any plaques and P = .03 for plaques ≥6 mm) after additional adjustment for body mass index, which is a major determinant of hs-CRP concentration.13 Similar trends, which did not reach statistical significance, were noted for the associations of hs-CRP level with plaques at least 4 mm thick and mobile debris among the subgroup with plaques.

Two hundred ninety-seven subjects (77%) were free of clinical coronary artery and cerebrovascular disease. The associations between hs-CRP level and aortic plaques in this subgroup are presented in Figure 2. In the subgroup of apparently healthy subjects who had aortic plaques, the odds of plaques at least 6 mm thick were almost 2-fold greater per 2-fold increase in hs-CRP level (OR, 1.92 per 2-fold increase in hs-CRP level; 95% confidence interval, 1.26-2.93; P = .003), adjusting for age, smoking, and additional atherosclerosis risk factors.

Most of the correlations of blood cell counts and fibrinogen with hs-CRP level were statistically significant, albeit weak (all correlation coefficients <0.4). None of these additional inflammatory variables were independently associated with the presence or severity of aortic plaques after adjusting for the risk factors in the respective multivariate logistic models (Table 4).
Our population-based study demonstrates the association of plasma hs-CRP level, predominantly within the reference range, with aortic atherosclerotic plaques. High-sensitivity CRP level was associated with the presence of aortic plaques and, among subjects with atherosclerosis, it was associated with more severe plaques. These associations were independent of age, smoking, and additional atherosclerosis risk factors (including lipid levels) and were apparent in the total study population, a sample of the general population, and the subgroup free of clinical cardiovascular disease. We did not detect any association between other markers of inflammation, such as leukocyte count or fibrinogen level, and aortic plaques, a finding concordant with clinical studies that have shown a stronger association of hs-CRP level with cardiovascular events, compared with other inflammatory markers.

Although the association between systemic inflammatory markers and clinical cardiovascular events is well established in multiple populations, current data on the association of these markers with atherosclerotic plaques (ie, anatomically defined atherosclerosis) in nonselected populations are less conclusive. In healthy middle-aged women, ultrasonographically measured carotid artery intima-medial thickness was associated with hs-CRP levels, although this association was weak and confined to ever-smokers. No association between hs-CRP level and carotid artery intima-medial thickness was noted in a sample of apparently healthy elderly subjects participating in the Cardiovascular Health Study or in subjects participating in a large cross-sectional family-based study. Recently, an association between hs-CRP level and coronary artery calcification, a surrogate of coronary artery atherosclerosis, has been demonstrated in the Framingham Offspring Study, but this association was of borderline significance in women (after adjusting for body mass index). Two other studies failed to demonstrate an association between hs-CRP level and coronary calcification in asymptomatic postmenopausal women and in healthy young men.

Recently, an association between hs-CRP level and atherosclerotic plaques (demonstrated directly with ultrasonography), in addition to its association with intima-medial thickness, has been observed in 2 population-based studies. High-sensitivity CRP level was associated with the extent (presence of plaques in multiple carotid segments) but not the severity of carotid atherosclerosis in the Rotterdam Study, but the role of dyslipidemia as
a possible confounder in the association between hs-CRP level and carotid plaques was not examined in that study. High-sensitivity CRP level was associated with relatively mild carotid atherosclerosis in the Framingham Offspring Study, an association that was not significant in men after multivariate adjustment. To the best of our knowledge, our study is the first to demonstrate an association between hs-CRP level and the presence and complexity of atherosclerotic plaques in a population-based setting, an association that was independent of other risk factors for aortic atherosclerosis (including lipid risk factors). With high-resolution real-time TEE imaging, the full spectrum of aortic atherosclerotic disease was demonstrable, from minor plaques to protruding plaques and mobile debris. Previous studies were limited by their measurements of carotid intima-media thickness (an early preplaque atherosclerosis-related lesion), relatively mild atherosclerotic lesions, or coronary calcification (an indirect surrogate of coronary plaques).

The role of dyslipidemia as a risk factor for aortic atherosclerosis has not been well established. In our present study, treatment with lipid-lowering drugs was strongly associated with the presence of aortic plaques, a finding consistent with a confounding effect of lipid-lowering therapy. Statins were the major drugs used as lipid-lowering therapy in our study population (88% of treated subjects). Thus, it is likely that lipid-lowering therapy was a marker of high LDL cholesterol level before therapy, and the association of lipid-lowering treatment may be a surrogate of the association between increased LDL cholesterol levels and aortic plaques. Total cholesterol level, which is quantitatively determined primarily by LDL cholesterol level, was independently associated with mobile atherosclerotic debris, the echocardiographic hallmark of ruptured aortic plaques with superimposed thrombosis, suggesting that high LDL cholesterol levels may also predispose to aortic plaque rupture. Interestingly, levels of HDL cholesterol and apolipoprotein A-I, an HDL-associated apolipoprotein, were negatively associated with thick (protruding) aortic plaques, which are associated with clinical cardiovascular events. Together these observations support the results of clinical trials demonstrating a decrease in cerebrovascular events with both statins and therapy to raise HDL cholesterol levels (gemfibrozil). Recent studies have shown that statins decrease hs-CRP levels and suggest that some of the beneficial clinical effects of statin therapy may be related to its anti-inflammatory effect. Although our study was not designed to address this issue, we did not detect any interaction among lipid-lowering therapy (primarily statins), hs-CRP levels, and their association with the presence of aortic plaques, suggesting that lipid-lowering therapy did not substantially affect our study results.

Complex aortic plaques are associated with cerebral and peripheral thromboembolic events and are high-risk markers of nonembolic cardiovascular disease. Our data support the hypothesis that systemic inflammatory markers are markers of severe atherosclerosis and are presumably associated with cardiovascular events through their association with underlying high-risk plaques. Additional direct proinflammatory or prothrombotic effects of acute-phase reactants on atherosclerotic plaques cannot be excluded by our results.

CONCLUSIONS

Our study demonstrates that hs-CRP level is significantly associated with the presence and severity of atherosclerotic plaques in the general population, an association that is independent of other risk factors. These observations provide a missing anatomical link for the apparent association between systemic inflammatory markers and clinical cardiovascular events.

Accepted for publication September 30, 2003.
This study was supported in part by research grant NS-06663 from the National Institute of Neurological Disorders and Stroke, Bethesda, Md.

This study was presented at the 73rd Scientific Sessions of the American Heart Association; November 13, 2000; New Orleans, La; and published as an abstract in Agmon Y, Khandheria BK, Meissner I, et al. Serum C-reactive protein levels are associated with the presence and severity of atherosclerosis of the thoracic aorta: a population-based transesophageal echocardiographic study [abstract]. Circulation. 2000;102(suppl 2):II-43.

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REFERENCES

6. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36-44.
12. Rohde LE, Hennekens CH, Ridker PM. Survey of C-reactive protein and cardio-

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