Association of Fibrinogen With Quantity of Coronary Artery Calcification Measured by Electron Beam Computed Tomography

Lawrence F. Bielak, George G. Klee, Patrick F. Sheedy II, Stephen T. Turner, Robert S. Schwartz, Patricia A. Peyser

Abstract—Increased plasma fibrinogen concentration is an independent risk factor for cardiovascular disease. Fibrinogen is the main coagulation protein in plasma, a determinant of blood viscosity, and can act as a cofactor for platelet aggregation. In this study of middle-aged men and women, we examined the association between plasma fibrinogen concentration and coronary artery calcification (CAC), a marker of preclinical coronary atherosclerosis. Two hundred twenty-eight participants were selected from the community-based Epidemiology of Coronary Artery Calcification Study, in which CAC was measured noninvasively by electron beam computed tomography. One hundred fourteen participants (57 men) were selected because they had high quantities of CAC; the remaining 114 participants (57 men) were selected because they had no detectable CAC. Logistic regression models were used to investigate the association between plasma fibrinogen concentration and high quantity of CAC. In men, an increase of 1 standard deviation in fibrinogen concentration was associated with a statistically significant odds ratio of 1.6 (95% CI 1.1 to 2.5) for a high quantity of CAC. In women, the corresponding odds ratio was 2.5 (95% CI 1.6 to 4.1). Inferences from sex-specific bivariate logistic models for odds ratios adjusted individually for each coronary risk factor and C-reactive protein were similar to those from the univariate models. In women, there was also a significant interaction between fibrinogen concentration and age. According to the models, younger women with high plasma fibrinogen were more likely to have high quantities of CAC than were younger women with low plasma fibrinogen. The strength of this association was diminished in older women.

Key Words: fibrinogen ■ coronary artery calcification ■ electron beam computed tomography

Increased plasma fibrinogen concentration is an independent and modifiable risk factor for cardiovascular disease. Fibrinogen is the main coagulation protein in plasma, an important determinant of blood viscosity, and can act as a cofactor for platelet aggregation. In a recent meta-analysis of 18 prospective studies (4018 cases of coronary heart disease), fibrinogen concentration was positively and significantly associated with coronary heart disease. The association between plasma fibrinogen and coronary artery calcification (CAC), a marker of preclinical coronary atherosclerosis and predictor of coronary events, has not been previously investigated. The present study examined the association between plasma fibrinogen concentration and quantity of CAC measured noninvasively by electron beam computed tomography (EBT) in middle-aged men and women from the general population of Rochester, Minn.

Methods

Sample
Since 1991, 870 men (aged 20 to 82 years) and 868 women (aged 20 to 87 years) have been examined in the community-based Epidemiology of Coronary Artery Calcification (ECAC) Study. The 228 participants (114 men) in the present study were selected from 705 ECAC participants who were examined and underwent EBT examinations for CAC between October 1, 1996, and April 30, 1999. Men aged <40 years and women aged <50 years were excluded because prevalence of CAC is low in these groups. The 228 participants (57 men and 57 women) were selected because they had high quantities of CAC, defined as CAC at or above the 80th percentile for their sex and 10-year age group. The remaining participants (57 men and 57 women) were selected because they had no detectable CAC at EBT. Only middle-aged participants (aged <70 years) without a history of myocardial infarction, stroke, or surgery involving the coronary arteries were selected. All participants provided written informed consent, and the study protocols were approved by the Mayo Clinic Institutional Review Board.

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Board and the University of Michigan Health Sciences Institutional Review Board.

**Measures**

Coronary artery disease (CAD) risk factors, including cholesterol/HDL cholesterol, systolic blood pressure, and body mass index (BMI), were measured at the time of a physical examination that included an EBT examination for CAC and a blood sample drawn after a 12-hour overnight fast. Age and history of myocardial infarction, stroke, and cigarette use were assessed through an interview preceding the physical examination. Plasma samples, anticoagulated with EDTA at the time of blood drawing and stored at −70°C, were thawed. C-reactive protein (CRP) and fibrinogen were measured by immunoturbidimetric assays (Kamiya Biomedical Corp) on a Roche Cobas Mira. The intra-assay coefficients of variation for CRP and fibrinogen were ~6% and ~7%, respectively. Less than one half of the study participants (89 [39%] of 227) had CRP concentrations >2.4 mg/L. Therefore, CRP concentrations were dichotomized on the basis of this threshold, and elevated CRP was analyzed as a binary variable. Fibrinogen concentration was considered as a continuous variable.

Previous studies have shown that EDTA plasma gives higher values of fibrinogen than does citrated plasma, most likely because of the dilutive effect of collecting blood in aqueous sodium citrate.11 To understand these differences, we conducted a calibration study by collecting paired EDTA plasma and citrated plasma samples from 35 additional clinical patients who had clottable fibrinogen measured in citrated plasma by a thrombin clotting rate assay on an MDA-180 instrument (Organon Teknika). The normal reference range for this assay is 1.75 to 3.50 mg/L. For these 35 patients, EDTA plasma samples were frozen at −70°C. These samples were then thawed, and fibrinogen was measured with the immunoturbidimetric assay used in the present study. In the calibration study, there was a strong correlation in fibrinogen levels measured by the 2 methods (r = 0.97).

Immunologic fibrinogen measured in EDTA plasma was ~46% higher than clottable fibrinogen measured in citrated plasma (data not shown).

The quantity of CAC was measured with an Imatron C-150 EBT scanner (Imatron Inc). A scan run consisted of 40 contiguous 3-mm-thick tomographic slices, from the root of the aorta to the apex of the heart. Scan time was 100 ms per tomogram. All images were triggered at end diastole during 2 to 4 breath-holdings with the use of ECG gating.

Tomograms were scored by a radiological technologist using an automated scoring system.12 CAC was defined as a hyperattenuating focus within 5 mm of the arterial midline, at least 4 adjacent pixels of the maximum likelihood of the logistic regression model with the just the intercept was taken to approximate a χ² distribution with 1 df.

Bivariate logistic regression models were then used to investigate the association of plasma fibrinogen concentration with a high quantity of CAC, after adjusting for each CAD risk factor. The likelihood ratio test was used to compare the model with each risk factor and fibrinogen (model 2) to a model with just the risk factor (model 1). Next, the significance of interaction terms between each CAD risk factor and fibrinogen was assessed by using the likelihood ratio test to compare the model with the interaction term with the model without the interaction term (model 2).

Finally, multiple logistic regression models were fit in which the association of fibrinogen with a high quantity of CAC was evaluated after adjusting for all CAD risk factors simultaneously along with any interaction terms that were found to be significant in the bivariate analyses. The likelihood ratio test was used to compare the full model with all CAD risk factors, plasma fibrinogen, and any interaction terms with a reduced model with just the CAD risk factors.

Odds ratios and 95% CIs for having a high quantity of CAC for a 1–standard deviation (1-SD) increase in quantitative variables or change in status for elevated CRP or smoking history were calculated.

**Results**

One woman was excluded from all analyses because she had no data for cholesterol. The final study group included 114 men and 113 women. Among men, 34 were aged 40 to 49 years (10 with a high quantity of CAC), 42 were aged 50 to 59 years (21 with a high quantity of CAC), and 38 were aged 60 to 69 years (26 with a high quantity of CAC.) Among women, 59 were aged 50 to 59 years (30 with a high quantity of CAC), and 54 were aged 60 to 69 years (27 with a high quantity of CAC).

**Associations With High Quantities of CAC in Men**

The mean ± SD CAC score among men with high quantities of CAC was 965.7 ± 800.9 (range 40.5 to 3036.7, Table 1). Compared with men with no detectable CAC, men with high quantities of CAC had significantly higher mean age, BMI, systolic blood pressure, history of cigarette smoking, and plasma fibrinogen concentration (Table 1). On the basis of the univariate logistic regression model, a 1-SD increase in plasma fibrinogen concentration was associated with an odds ratio of 1.6 (95% CI 1.1 to 2.5) for a high quantity of CAC (model 1 in Table 2).

In bivariate logistic regression models, plasma fibrinogen concentration remained significantly associated with the probability of a high quantity of CAC after considering the effects of BMI, cholesterol/HDL cholesterol, history of cigarette smoking, systolic blood pressure, or elevated CRP (model 2 versus model 1, Table 2). In the age-adjusted model, plasma fibrinogen concentration was marginally associated with the probability of a high quantity of CAC (P = 0.0704). In all bivariate models (data not shown), a 1-SD increase in plasma fibrinogen concentration was associated with similar odds ratios that varied from 1.5 in the model adjusted for age to 1.7 in the model adjusted for systolic blood pressure. There were no significant interaction terms between fibrinogen level and any CAD risk factors.

After adjusting for all CAD risk factors in a multiple logistic regression model, plasma fibrinogen concentration was no longer a significant predictor of a high quantity of CAC. The odds ratio for plasma fibrinogen was 1.3 (95% CI
TABLE 1. Risk Factor Status of Study Participants by Quantity of CAC

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High CAC* (n=57)</td>
<td>No CAC</td>
<td>P†</td>
<td>High CAC* (n=56)</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.6±7.4</td>
<td>53.8±7.6</td>
<td>0.0009</td>
<td>60.5±5.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.0±6.3</td>
<td>28.4±3.6</td>
<td>0.0063</td>
<td>32.5±8.2</td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol</td>
<td>5.2±1.6</td>
<td>5.0±1.3</td>
<td>0.3740</td>
<td>4.5±1.9</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128.5±18.0</td>
<td>121.2±16.9</td>
<td>0.0262</td>
<td>133.2±15.2</td>
</tr>
<tr>
<td>Fibrinogen, μmol/L</td>
<td>4.3±0.7</td>
<td>4.0±0.6</td>
<td>0.0131</td>
<td>5.3±0.9</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.6±7.4</td>
<td>53.8±7.6</td>
<td>0.0009</td>
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<td>0.0131</td>
<td>5.3±0.9</td>
</tr>
<tr>
<td>History of cigarette smoking, %</td>
<td>79.0</td>
<td>59.7</td>
<td>0.026</td>
<td>59.7</td>
</tr>
<tr>
<td>Elevated CRP, %</td>
<td>29.8</td>
<td>19.3</td>
<td>0.192</td>
<td>50.1</td>
</tr>
</tbody>
</table>

Values are mean±SD or percentages.

*High CAC is defined as a calcium score at or above the 80th percentile for one's sex and 10-year age group.
†Based on t test for equality of means or χ² test for equality of percent between participants with high quantity of CAC and no CAC.
‡Estimated clottable fibrinogen concentration was obtained from the equation from the calibration study (estimated clottable fibrinogen=immunologic fibrinogen/1.4666). Because these values were estimated, no statistical tests were performed.
§Elevated CRP is defined as that >2.4 mg/L.

0.8 to 2.2, P=0.2198 for full versus reduced model). In the full model, age was the only significant predictor of a high quantity of CAC (P<0.05), whereas BMI (P=0.0636) and history of smoking (P=0.0704) were marginally significant (data not shown).

Associations With High Quantity of CAC in Women

The mean±SD CAC score among women with a high quantity of CAC was 442.4±681.4 (range 2.1 to 3349.4, Table 1). Compared with women with no detectable CAC, women with a high quantity of CAC had significantly higher BMI, systolic blood pressure, history of cigarette smoking, and plasma fibrinogen concentration (Table 1). On the basis of the univariate logistic regression model, a 1-SD increase in plasma fibrinogen concentration was associated with an odds ratio of 2.5 (95% CI 1.6 to 4.1) for having a high quantity of CAC (model 1 in Table 2).

In bivariate analyses, plasma fibrinogen concentration remained significantly associated with the probability of a high quantity of CAC after considering the effects of each CAD risk factor (model 2 versus model 1, Table 2). In all bivariate models (data not shown), a 1-SD increase in plasma fibrinogen concentration was associated with similar odds ratios that varied from 2.1 in the model adjusted for BMI to 2.6 in the model adjusted for age. Additionally, there was a significant interaction term between plasma fibrinogen concentration and age. The predicted probability of a high quantity of CAC for women with high and low plasma fibrinogen concentrations (mean±1 SD) across the age range of women observed in the present study (50 to 69 years) is plotted in the Figure. On the basis of the model, younger women with high levels of plasma fibrinogen were much more likely to have a high quantity of CAC than were younger women with low levels of plasma fibrinogen. At

TABLE 2. Results From Univariate and Bivariate Logistic Regression to Predict High Quantity of CAC

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
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<th>Women</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Model 1 vs Model 2</td>
<td>Model 1 vs Model 1 (Additional Contribution of Plasma Fibrinogen Concentration)</td>
<td>Model 1 vs Model 1 (Additional Contribution of Plasma Fibrinogen Concentration)</td>
<td></td>
</tr>
<tr>
<td>Odds Ratio†</td>
<td>95% CI</td>
<td></td>
<td>Odds Ratio†</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, y</td>
<td>1.9</td>
<td>1.3–2.9</td>
<td>0.0009</td>
<td>1.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1.8</td>
<td>1.1–2.8</td>
<td>0.0061</td>
<td>2.1</td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol</td>
<td>1.2</td>
<td>0.8–1.7</td>
<td>0.3684</td>
<td>1.4</td>
</tr>
<tr>
<td>History of cigarette smoking, %</td>
<td>2.5</td>
<td>1.1–5.8</td>
<td>0.0246</td>
<td>2.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>1.6</td>
<td>1.0–2.4</td>
<td>0.0238</td>
<td>1.5</td>
</tr>
<tr>
<td>Elevated CRP‡</td>
<td>1.3</td>
<td>0.9–1.9</td>
<td>0.1900</td>
<td>0.8</td>
</tr>
<tr>
<td>Fibrinogen, μmol/L</td>
<td>1.6</td>
<td>1.1–2.5</td>
<td>0.0126</td>
<td>2.5</td>
</tr>
</tbody>
</table>

A high quantity of CAC is defined as a calcium score at or above the 80th percentile for one’s sex and 10-year age group.

*Value of P for test statistic for χ² likelihood ratio test comparing the full model with a reduced model.
†Odds ratio from univariate logistic regression model (model 1) for a 1-SD increase in quantitative variables or change in status for a dichotomous variable.
‡Elevated CRP is defined as that >2.4 mg/L.
older ages, the difference in probability of a high quantity of CAC between women with high or low levels of plasma fibrinogen decreased (see Figure).

After adjustments were made for all CAD risk factors in a multivariate logistic regression model, plasma fibrinogen concentration and an interaction term between plasma fibrinogen concentration and age were the only significant predictors of a high quantity of CAC ($P<0.0001$ for full versus reduced model). In the full model, a history of cigarette smoking ($P=0.0554$) was a marginally significant predictor of a high quantity of CAC (data not shown).

**Discussion**

CAC has previously been shown to be a marker of preclinical coronary atherosclerosis and predicts coronary events in symptomatic and asymptomatic individuals.

High plasma fibrinogen levels were also found to be associated with increased coronary heart disease risk in healthy and high-risk individuals. In a meta-analysis of 22 studies, Maresca et al found an estimated odds ratio of 1.99 (95% CI 1.85 to 2.13) for the risk of cardiovascular disease for high versus low plasma fibrinogen concentration. In a meta-analysis of 18 prospective studies, Danesh et al found an estimated relative risk of 1.8 (95% CI 1.6 to 2.0) for coronary heart disease between the top and bottom thirds of plasma fibrinogen concentration. In the present study, we found a similar strength of association of plasma fibrinogen concentration with a high quantity of CAC (Table 2).

Some caution should be used in interpreting our results because participants were selected for the present study on the basis of the results of their EBT examinations. Thus, we cannot exclude the possibility that the observed elevated fibrinogen concentration was a result, rather than a cause, of the preclinical atherosclerosis as measured with EBT. Although elevated fibrinogen concentration may reflect inflammatory activity associated with atherosclerosis, fibrinogen has also been shown to stimulate smooth muscle migration and proliferation, promote platelet aggregation, and contribute to blood viscosity and thrombi, and it is a component of atherosclerotic plaques.

In the present study, after adjusting for another marker of inflammation (elevated CRP), plasma fibrinogen remained significantly associated with a high quantity of CAC. Therefore, these findings provide further evidence for direct mechanisms by which fibrinogen may contribute to the atherosclerotic process and coronary events.

Additionally, although CAC almost always indicates the presence of atherosclerosis, soft plaques with no or very low levels of CAC that are undetectable by EBT may exist. Therefore, some participants who did not have detectable CAC in the present study may actually have atherosclerosis.

Only participants with high quantities of CAC or participants with no detectable CAC were selected for inclusion in the present study. This study design was useful for a preliminary exploration of the association of fibrinogen with CAC because few stored specimens were required to be thawed and measured. Given the significant findings, future studies should be conducted in a study group with a full range of quantity of CAC to determine whether there is a dose-response effect between plasma fibrinogen concentrations and the quantity of CAC.

The fibrinogen assays used in many studies measure clottable fibrinogen with citrated plasma. In the present study, we only used stored EDTA plasma. Previous studies have shown that EDTA plasma gives higher values than citrated plasma, most likely because of the dilutive effect of collecting blood in aqueous sodium citrate. In our calibration study among 35 clinical patients, immunologic fibrinogen measured in EDTA plasma was $\approx 46\%$ higher than clottable fibrinogen measured in citrated plasma. The mean±SD estimated clottable fibrinogen concentration in the study group was $3.1\pm 0.65$ μmol/L, which is within the normal reference range (1.75 to 3.50 μmol/L) for clottable fibrinogen measured in citrated plasma. Consistent with other studies, mean fibrinogen concentration was higher among women than men (Table 1).

The reasons for finding an age-dependent association of plasma fibrinogen concentration with a high quantity of CAC in women is not entirely clear. Elevated fibrinogen concentration may be associated with early atherosclerosis, as evidenced by the strong association found in younger women who tend to have few conventional CAD risk factors. Among older women, risk factors other than fibrinogen concentration may be more closely associated with a high quantity of CAC. With increasing age, the change in menopause status and the combined effects of increases in BMI, increases in blood pressure, and an adverse lipid profile may also dilute the fibrinogen effects observed in younger women. Also, there may have been an unknown amount of survival bias in the present study because individuals with a history of myocardial infarction, stroke, or surgery involving the coronary arteries were not eligible to participate. Compared with the asymptomatic women eligible for the study, these ineligible symptomatic women are expected to be older and to have higher plasma fibrinogen concentrations and higher quantities of CAC.

In the present study, bivariate logistic regression models allowed us to examine the association of fibrinogen with a high quantity of CAC, after adjusting individually for each CAD risk factor. Inferences from these models are important from a biological perspective. However, the associations in the presence of all CAD risk factors are important to consider when evaluating the clinical utility of a new marker of CAD. Our findings suggest value in using fibrinogen concentration to further stratify risks in women. The association of plasma fibrinogen with a high quantity of CAC remained statistically significant after adjusting for all CAD risk factors.
risk factors simultaneously in a multivariate logistic regression model. This is consistent with previous studies in which the prediction of cardiovascular disease was improved by the addition of fibrinogen to models containing major cardiovascular disease risk factors.14,18

Among men, the association between fibrinogen and a high quantity of CAC failed to reach statistical significance in a multivariate logistic regression model; however, the strength of the association was similar to that found in the univariate and bivariate logistic regression models. Given that the strength of the association was not as large in men as in women, it would be useful to conduct another study in a larger group to determine whether fibrinogen levels are useful to predict which men are at higher risk for subclinical atherosclerosis.

Almost 25% of the patients with premature cardiovascular disease do not have any of the conventional CAD risk factors, and the presence of a high quantity of CAC at EBT has stimulated the search for other risk factors.19 EBT has been shown to identify individuals with preclinical atherosclerosis who would be considered to be at low risk for coronary events on the basis of conventional CAD risk factors.20 Thus, scanning for CAC with EBT may help uncover novel risk factors for preclinical disease and facilitate the development of new therapies for the prevention of CAD events.9

Fibrinogen is inexpensive to measure (the cost is comparable to the cost of a typical lipid profile) and is modifiable by the use of agents such as bezafibrate.21 Presently, there is a lack of clinical trials to demonstrate the effect of lowering fibrinogen with such agents on clinical end points. EBT has been shown to be useful for the measurement of the progression of CAC over time.22,23 In one study,23 lipiddropping agents were shown to decrease the progression of CAC in a group followed for 1 year. Future studies should investigate the effects of fibrinogen-lowering agents on the progression of CAC as measured with EBT in middle-aged asymptomatic individuals.

Acknowledgments

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References

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