Additive Value of Immunoassay-Measured Fibrinogen and High-Sensitivity C-Reactive Protein Levels for Predicting Incident Cardiovascular Events

Samia Mora, MD, MHS; Nader Rifai, PhD; Julie E. Buring, ScD; Paul M Ridker, MD, MPH

Background—Current guidelines suggest measuring high-sensitivity C-reactive protein (hs-CRP) as an aid to coronary risk assessment in adults without cardiovascular disease (CVD). Whether other inflammatory biomarkers, such as fibrinogen, add further prognostic information is uncertain.

Methods and Results—In a prospective study of 27,742 initially healthy middle-aged women, the associations of baseline immunoassay fibrinogen and hs-CRP measurements with incident CVD were examined over a 10-year follow-up period. Compared with women in the bottom biomarker quintile, age-adjusted hazard ratios (95% confidence intervals [CIs]) for incident CVD for quintiles 2 to 5 of fibrinogen were 1.10 (0.86 to 1.41), 1.30 (1.03 to 1.65), 1.46 (1.16 to 1.85), and 2.43 (1.95 to 3.02); for hs-CRP they were 1.48 (1.06 to 2.05), 1.70 (1.24 to 2.33), 2.20 (1.63 to 2.96), and 3.24 (2.43 to 4.31). After further adjustment for established risk factors, both biomarkers remained associated (P for trend ≤0.001) with incident CVD (hazard ratio, 1.35; 95% CI, 1.07 to 1.71 for top fibrinogen quintile; and hazard ratio, 1.68; 95% CI, 1.22 to 2.29 for top hs-CRP quintile compared with the bottom quintiles). Further adjustment for the other biomarker resulted in hazard ratios of 1.23 and 1.56 (P for trend=0.02 and 0.002), respectively. Although fibrinogen correlated positively with hs-CRP (r_s=0.41, P<0.001), the highest CVD risk was associated with elevated levels of both fibrinogen and hs-CRP: age-adjusted hazard ratio of 3.45 (95% CI, 2.60 to 4.57) for women with fibrinogen >393 mg/dL and hs-CRP >3 mg/L compared with <329 mg/dL and <1 mg/L, respectively.

Conclusions—In this cohort of initially healthy women, baseline levels of fibrinogen measured with a high-quality immunoassay provided additive value to hs-CRP and traditional risk factors in predicting incident CVD. (Circulation. 2006;114:381-387.)

Key Words: acute-phase proteins • C-reactive protein • fibrinogen • inflammation • women

Fibrinogen is a circulating glycoprotein that has long been known to be a nonspecific acute-phase reactant in addition to its important role as a clotting factor.1,2 Numerous studies have related fibrinogen levels to established cardiovascular risk factors, cardiovascular disease (CVD), and mortality.3–12 A recent meta-analysis from the Fibrinogen Studies Collaboration found moderate to strong associations with cardiovascular outcomes in a comprehensive analysis of 154,211 asymptomatic individuals from 31 prospective studies.13

Despite the evidence linking fibrinogen with CVD, recent guidelines from an expert panel from the Centers for Disease Control and Prevention/American Heart Association recommended against measuring fibrinogen or other acute-phase reactants, at the same time that they favored the use of high-sensitivity C-reactive protein (hs-CRP), as an aid for coronary risk assessment in the primary prevention of CVD.14 The main reasons cited against using fibrinogen are related to concerns about assay precision and accuracy due to the existence of a variety of methods (functional and mass-based assays) used for its measurement, resulting in substantial analytical variation and limiting efforts at assay standardization.14,15 Even if these assay considerations were to be overcome, it is unclear that measuring fibrinogen would provide additive value beyond that conferred by hs-CRP, given the positive correlation between inflammatory biomarkers.16

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Therefore, this study was conducted to determine whether baseline fibrinogen levels, alone and in combination with hs-CRP, predict incident CVD in an asymptomatic cohort of women with the use of a reliable mass-based fibrinogen immunoassay that can be standardized and for which a World Health Organization calibrator is available.\textsuperscript{17} We hypothesized that the combined measurement of fibrinogen, by this high-quality immunoassay, and hs-CRP may have additive value for predicting CVD, potentially reflecting different pathophysiological aspects of atherothrombosis, namely, prothrombotic and proinflammatory pathways.

Methods

Study Population

Study participants were enrolled in the Women’s Health Study, a recently completed randomized, double-blinded, placebo-controlled clinical trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer in US female healthcare professionals.\textsuperscript{18–20} Eligible participants were apparently healthy women, aged 45 years or older, who were free of self-reported CVD or cancer at study entry (1992–1995), with follow-up for incident CVD through February 2005. At the time of enrollment, participants gave written informed consent, completed questionnaires on demographics, medical history, medications, and lifestyle factors, and were asked to provide a blood sample. In total, 27,742 women with both fibrinogen and hs-CRP baseline measurements constituted the study population for this analysis. The study was approved by the institutional review boards of the Brigham and Women’s Hospital (Boston, Mass). The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Baseline Plasma Measurements

EDTA blood samples were obtained at the time of enrollment and stored in vapor phase liquid nitrogen (\textasciitilde170°C). Samples were thawed and analyzed in a core laboratory certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program. Fibrinogen was measured with reagents from Genzyme Corporation (Cambridge, Mass) and Roche Diagnostics (Indianapolis, Ind) with the use of a Hitachi 917 autoanalyzer.

TABLE 1. Baseline Characteristics of Participants According to Quintiles of Fibrinogen

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quintiles of Fibrinogen, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=27,742)</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.7±7.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>25.1</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>11.6</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.8</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>54.3</td>
</tr>
<tr>
<td>Postmenopausal hormone use, %</td>
<td>43.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.9±5.0</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>212±42</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>124±34</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>53.8±15.0</td>
</tr>
<tr>
<td>hs-CRP, median (IQR), mg/L</td>
<td>2.01 (0.80–4.37)</td>
</tr>
</tbody>
</table>

Values shown for continuous variables are mean±SD unless otherwise indicated. IQR is 25th to 75th percentile. ANOVA \(P\) for trend \(<0.001\) for continuous variables expressed as means. \(P\) values for categorical variables obtained from \(\chi^2\) tests were \(<0.001\). Value from the nonparametric Cuzick’s extension to the Wilcoxon rank sum test for trend comparing median hs-CRP levels across quintiles of fibrinogen was \(<0.001\).
of high or low fibrinogen (greater than or less than or equal to top tertile among women not taking hormone therapy) and high or low hs-CRP (>3 or ≤3 mg/L) as well as based on 9 categories of fibrinogen tertiles and hs-CRP clinical cut points (<1, 1 to 3, and >3 mg/L). We also repeated this analysis of the joint association of hs-CRP and fibrinogen using hs-CRP tertiles instead of clinical cut points with essentially unchanged results, and hence these results were not shown.

Finally, statistical tests for interaction between fibrinogen tertiles and hs-CRP categories were obtained from age-adjusted Cox regression models. On an a priori basis, we also tested for interaction between fibrinogen and hs-CRP categories with each of smoking, obesity, diabetes, and postmenopausal state because these variables may predispose individuals to an inflammatory state. All reported probability values were 2-tailed, with <0.05 considered significant.

Results

The study participants were middle-aged women at baseline (Table 1), with median (interquartile range [IQR]) fibrinogen levels of 351 (IQR, 308 to 403) mg/dL and median hs-CRP levels of 2.01 (IQR, 0.80 to 4.37) mg/L. Baseline prevalence of cardiovascular risk factors was positively associated with levels of 2.01 (IQR, 0.80 to 4.37) mg/L. Baseline prevalence of fibrinogen (1.49; 95% CI, 1.19 to 1.87 for top versus bottom quintile) and hs-CRP (1.83; 95% CI, 1.35 to 2.47 for top versus bottom quintile) were both associated with incident cardiovascular events (CVD), with P<0.001 for both variables, obtained from log-rank tests of significance.

In a Cox model that additionally adjusted fibrinogen for hs-CRP, the hazard ratio comparing the top and bottom quintiles of fibrinogen was somewhat attenuated to 1.23 (95% CI, 0.97 to 1.57), but the trend across quintiles remained significant (P for trend=0.02) (Table 2). Similarly, in a Cox model that adjusted hs-CRP for fibrinogen, the hazard ratio comparing the top and bottom quintiles of hs-CRP was also mildly attenuated to 1.56 (95% CI, 1.13 to 2.16), but the trend across quintiles remained significant (P for trend=0.002).

In joint analyses of fibrinogen and hs-CRP with incident CVD according to 4 prespecified groups of high and low fibrinogen or hs-CRP (Figure 2), the highest CVD event rates were significantly associated with high levels of both fibrinogen and hs-CRP, and the lowest rates were significantly associated with low levels of both biomarkers (P log-rank <0.001). Of note, women with high fibrinogen but low hs-CRP levels had similar event rates during follow-up compared with women who had low fibrinogen but high hs-CRP levels, with virtual overlap of the 2 event rate curves.
We then divided the participants into 9 categories on the basis of fibrinogen tertiles and hs-CRP clinical cut points (<1, 1 to 3, and >3 mg/L) according to guidelines. With the use of the referent group of women with the lowest fibrinogen and hs-CRP levels (fibrinogen <329 mg/dL and hs-CRP <1 mg/L), the age-adjusted hazard ratios for women with fibrinogen >393 mg/dL and hs-CRP >3 mg/L was 3.45 (95% CI, 2.60 to 4.57) (Figure 3). In comparison, when examined separately, the age-adjusted hazard ratios for high versus low levels of fibrinogen and hs-CRP, with the use of the above cut points, were 1.93 (95% CI, 1.63 to 2.29) and 2.27 (95% CI, 1.89 to 2.73), respectively. Participants with either top values for fibrinogen but bottom or intermediate values for hs-CRP, or top values for hs-CRP but bottom or intermediate values for fibrinogen, were at increased risk for CVD compared with women with bottom values for both biomarkers. When we used tertile cut points to define elevated levels of hs-CRP instead of clinical cut points, the results were essentially unchanged.

There was no evidence of multiplicative interaction between fibrinogen tertiles and hs-CRP categories for the outcome of incident CVD (P for interaction=0.24). In addition, there was no interaction between fibrinogen tertiles or hs-CRP categories with smoking, obesity, diabetes, or postmenopausal state. The association of fibrinogen and hs-CRP with incident total CVD was similar to that observed in analyses of the components of the composite end point.

### TABLE 2. Association of Fibrinogen With Incident CVD

<table>
<thead>
<tr>
<th>Quintiles of Fibrinogen, mg/dL</th>
<th>1 ≤303 (Bottom)</th>
<th>2 &gt;303 to 341</th>
<th>3 &gt;341 to 379</th>
<th>4 &gt;379 to 428</th>
<th>5 &gt;428 (Top)</th>
<th>P for Linear Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.10 (0.86–1.41)</td>
<td>1.30 (1.03–1.65)</td>
<td>1.46 (1.16–1.85)</td>
<td>2.43 (1.95–3.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus risk factors*</td>
<td>1.00</td>
<td>0.94 (0.73–1.21)</td>
<td>1.05 (0.82–1.33)</td>
<td>1.02 (0.80–1.30)</td>
<td>1.35 (1.07–1.71)</td>
<td>0.001</td>
</tr>
<tr>
<td>Plus hs-CRP†</td>
<td>1.00</td>
<td>0.92 (0.72–1.19)</td>
<td>1.01 (0.79–1.28)</td>
<td>0.96 (0.75–1.22)</td>
<td>1.23 (0.97–1.57)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are hazard ratio (95% CI).
*Obtained from Cox proportional hazard regression models that adjusted for age, smoking status, systolic blood pressure, total and HDL cholesterol, diabetes mellitus, hormone use, and body mass index. The reference category for hazard ratios was the lowest quintile of each biomarker. P values were obtained from models with the use of the median value in each quintile and testing for linear trend.
†Adjusted for all the above variables plus fibrinogen.

### Discussion

In this prospective study of 27,742 initially healthy women, we found significant associations for higher levels of immunoassay-measured fibrinogen and hs-CRP, alone and in combination, with incident CVD over a 10-year follow-up period. Despite the positive correlation between fibrinogen and hs-CRP, high levels of the 2 biomarkers together were associated with the highest CVD risk. The predictive value of fibrinogen was similar in magnitude and additive to that of hs-CRP, with a joint effect that was greater than the individual effect of either biomarker separately, without evidence of multiplicative interaction, with a 3-fold increased risk associated with having a fibrinogen level >393 mg/dL together with an hs-CRP level >3 mg/L compared with levels <329 mg/dL and <1 mg/L, respectively.

A number of epidemiological studies have examined the association of fibrinogen with CVD, but few have directly compared fibrinogen with hs-CRP or examined their joint association. In the Fibrinogen Studies Collaboration meta-analysis, adjustment for established risk factors and for CRP measurements in the subgroup of participants that had such measurements did not change the significant association found for fibrinogen with incident coronary events. The additive value of fibrinogen and hs-CRP that we found in our study, when fibrinogen was measured with a reliable and high-quality assay, suggests a complementary role for the 2 biomarkers in risk predic-

### TABLE 3. Association of hs-CRP With Incident CVD

<table>
<thead>
<tr>
<th>Quintiles of hs-CRP, mg/L</th>
<th>1 ≤0.49 (Bottom)</th>
<th>2 &gt;0.49 to 1.08</th>
<th>3 &gt;1.08 to 2.08</th>
<th>4 &gt;2.08 to 4.19</th>
<th>5 &gt;4.19 (Top)</th>
<th>P for Linear Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.48 (1.06–2.05)</td>
<td>1.70 (1.24–2.33)</td>
<td>2.20 (1.63–2.96)</td>
<td>3.24 (2.43–4.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus risk factors*</td>
<td>1.00</td>
<td>1.22 (0.87–1.71)</td>
<td>1.24 (0.90–1.72)</td>
<td>1.40 (1.02–1.91)</td>
<td>1.68 (1.22–2.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus fibrinogen†</td>
<td>1.00</td>
<td>1.19 (0.85–1.67)</td>
<td>1.21 (0.87–1.67)</td>
<td>1.34 (0.98–1.84)</td>
<td>1.56 (1.13–2.16)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are hazard ratio (95% CI).
*Obtained from Cox proportional hazard regression models that adjusted for age, smoking status, systolic blood pressure, total and HDL cholesterol, diabetes mellitus, hormone use, and body mass index. The reference category for hazard ratios was the lowest quintile of each biomarker. P values were obtained from models with the use of the median value in each quintile and testing for linear trend.
†Adjusted for all the above variables plus fibrinogen.
tion that is not captured with standard risk factors or either biomarker individually. That the magnitude of predictive value in the women participants in this study was greater for hs-CRP than for fibrinogen is consistent with prior studies in men.9,28

In addition to its role as an inflammatory biomarker, fibrinogen is the predominant coagulation factor in blood plasma and plays an important role in platelet aggregation, fibrin formation, and plasma viscosity.29,30 Our findings suggest that plasma fibrinogen levels may reflect both an inflammatory and a prothrombotic state because the risk associated with higher fibrinogen levels was only partially accounted for by higher hs-CRP levels. Attenuation of the risk associated with fibrinogen after adjustment for hs-CRP, and vice versa, may be explained by potential confounding, or possible mediation, of some of the effect of fibrinogen via inflammation. It is also possible that fibrinogen and hs-CRP may represent different aspects of an underlying inflammatory process with both biomarkers contributing CVD risk information.

Risk factors are known to act in concert in the development of CVD.31 It is possible that factors related to adiposity and insulin resistance, such as abdominal obesity, may also be contributing to the increased CVD risk associated with fibrinogen or hs-CRP, as suggested in our data with the attenuation of the hazard ratios after adjustment for body mass index and diabetes. Adipose tissue, particularly visceral adipose tissue, is known to be metabolically active and is associated with both a prothrombotic and a proinflammatory state, both of which may be reflected in higher plasma fibrinogen and hs-CRP levels.32–34

Potential limitations to our study include the single measurements of both fibrinogen and hs-CRP, such that we were unable to examine the value of repeated measurements of the biomarkers. However, a single measurement is likely to underestimate the magnitude of the association between the biomarkers and CVD. Although we adjusted for established cardiovascular risk factors as well as examined models that additionally adjusted for alcohol use, physical activity, and education, we cannot exclude the possibility that potential confounding by unmeasured factors may explain part of the additive value of fibrinogen for CVD risk prediction. Our study population was limited to women who were healthcare professionals and mostly white. Although our study design was prospective, there is no published randomized clinical trial to date examining the clinical value of inflammatory biomarkers in tailoring therapy in a primary prevention population, although such a study is ongoing.35

Strengths of our study include the reliable measurement of both fibrinogen and hs-CRP levels with high accuracy in a core laboratory. Currently available commercial assays for fibrinogen encompass a wide variety of assays, including functional clotting assays that are difficult to standardize and demonstrate substantial variability.36 In comparison, the mass-based immunoassay that was used in this study can be standardized with available calibrators from the World Health Organization.17 Another strength of this study is the well-characterized risk factor profile of the participants that allowed us to control for potential confounding. In addition, the large size and 10-year duration of follow-up allowed for the separate as well as joint examination of both biomarkers with respect to incident events.

In summary, immunoassay-measured fibrinogen and hs-CRP both contributed CVD risk information that was additive to the risk associated with the established risk factors and the other biomarker. Although both fibrinogen and hs-CRP were positively correlated, their combined effect provided CVD risk information that was greater than...
that provided by either biomarker separately, potentially reflecting different pathophysiological processes in the development of atherothrombotic events. Future studies are needed to further evaluate whether the use of a mass-based assay, similar to the immunoassay used in this study, may complement the use of hs-CRP and established risk factors for identifying high-risk individuals in other populations.

Acknowledgments

The authors thank the investigators, staff, and participants of the Women’s Health Study for their valuable contributions.

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Disclosures

Dr Ridker is listed as a coinventor on patents held by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in CVD. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Guidelines suggest measuring high-sensitivity C-reactive protein (hs-CRP) as an aid to coronary risk assessment in the primary prevention of cardiovascular disease (CVD). Whether other inflammatory biomarkers, such as fibrinogen, may add further prognostic information is uncertain. The main limitation for using fibrinogen is assay imprecision and inaccuracy, with substantial analytical variation among different assays. We sought to determine whether baseline measurement of fibrinogen, with the use of a high-quality mass-based immunoassay for which international standards are available, may have additive value to hs-CRP and risk factors for predicting CVD. We conducted a prospective study of 27,742 initially healthy women participants in the Women’s Health Study with baseline immunoassay fibrinogen and hs-CRP measurements who were followed up for a 10-year period. Despite the positive correlation between fibrinogen and hs-CRP, high levels of the 2 biomarkers together were associated with the highest CVD risk. The predictive value of fibrinogen was similar in magnitude and additive to that of hs-CRP, with a joint effect that was greater than the individual effect of either biomarker separately, with and without adjustment for traditional risk factors. There was a 3-fold higher age-adjusted risk associated with having fibrinogen \(\geq 393\) mg/dL together with hs-CRP \(\geq 3\) mg/L compared with levels \(< 329\) mg/dL and \(< 1\) mg/L, respectively. Thus, in this cohort of initially healthy women, baseline levels of fibrinogen measured with a high-quality immunoassay provided additive value to hs-CRP and traditional risk factors in predicting incident CVD. Future studies are needed to further evaluate the prognostic value of immunoassay fibrinogen in other populations.
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