Interleukin-6, Fibrin D-Dimer, and Coagulation Factors VII and XIIa in Prediction of Coronary Heart Disease

Gordon D.O. Lowe, Ann Rumley, Alex D. McMahon, Ian Ford, Denis St. J. O’Reilly, Christopher J. Packard; for the West of Scotland Coronary Prevention Study Group

Objective—Activated inflammation and activated blood coagulation are believed to increase the risk of coronary thrombosis and are related. We therefore compared plasma IL-6 (a key cytokine in the inflammatory process), fibrin D-dimer (a marker of fibrin turnover), and coagulation factors VII and XIIa (initiators of extrinsic and intrinsic blood coagulation, respectively) as predictors of coronary risk in the West of Scotland Coronary Prevention Study of pravastatin in men with hypercholesterolemia.

Methods and Results—485 men who had had a coronary event (nonfatal myocardial infarction, death from coronary heart disease, or revascularization) were matched for age and smoking status with 934 controls. Baseline IL-6 and D-dimer were strong univariate predictors of coronary risk (relative risk in the highest quintile approximately twice that in the lowest quintile) and were associated with each other and with C-reactive protein. On multivariate analyses, D-dimer retained a significant association with coronary risk (relative risk, 1.86; 95% CI, 1.24 to 2.80), whereas IL-6 (1.47; 0.95 to 2.28) and C-reactive protein (1.33; 0.85 to 2.08) did not. Neither factor VII nor factor XIIa antigens were predictors of coronary events.

Conclusions—Fibrin D-dimer may be a stronger predictor of coronary risk than inflammatory markers, perhaps through its ability to stimulate monocyte release of IL-6.

Key Words: coronary heart disease, coagulation, fibrin degradation products, inflammation

There is increasing evidence that inflammation plays a key role in rupture of atherosclerotic plaques, which precipitates thrombosis and coronary heart disease (CHD) events. Meta-analyses of prospective studies have shown that circulating levels of inflammatory markers including C-reactive protein (CRP), as well as plasma viscosity and erythrocyte sedimentation rate, are independent predictors of CHD. A key regulator of the inflammatory response is the cytokine, IL-6, which stimulates synthesis of acute phase proteins including CRP and fibrinogen (elevated levels of which increase plasma viscosity and erythrocyte sedimentation rate), as well as release of white blood cells from bone marrow into circulating blood. We have recently observed in 2 population cohorts that CRP was associated with fibrin D-dimer (a marker of increased turnover of intravascular cross-linked fibrin and an independent predictor of CHD) and that these variables interact in prediction of risk of CHD. Possibly because D-dimer and other fibrin degradation products stimulate monocyte synthesis and release of IL-6, other thrombotic variables that have been associated with CHD include factor VII and activated factor XII (XIIa), which respectively initiate the extrinsic (tissue factor) and intrinsic systems of blood coagulation.

The West of Scotland Coronary Prevention Study demonstrated that pravastatin therapy reduced the incidence of CHD events by approximately one-third in men with hypercholesterolemia. We recently reported that baseline levels of circulating inflammatory markers, including fibrinogen, white cell count, and plasma viscosity, as well as CRP (in a nested case-control comparison) were associated with increased risk of CHD events in this study. In this nested case-control study, we compared the extent to which baseline plasma antigen levels of IL-6, fibrin D-dimer, and coagulation factors VII and XIIa predicted the risk of a coronary event, in comparison to classical risk factors and other markers of inflammation. The aims of the present study were to establish whether the association of D-dimer with CHD events was attributable to IL-6 or CRP (or vice versa), or to levels of factors VII or XIIa antigens.
Methods

Study Design and Subjects

Study design and subjects have been described previously. Briefly, 6595 men aged 45 to 64 years who had low-density lipoprotein cholesterol levels between 4.5 and 6.0 mmol/L and who had no history of myocardial infarction or revascularization were randomly assigned to receive 40 mg of pravastatin or placebo daily. All major risk factors were assessed during recruitment. Plasma fibrinogen and viscosity, and full blood cell count including white cell count and hematocrit, were measured in blood anticoagulated with K$_2$ EDTA (1.5 mg/mL) collected at the third screening visit. Platelet count and hematocrit, were measured in blood anticoagulated with K$_3$ EDTA (1.5 mg/mL) collected at the third screening visit. All major risk factors were assessed during recruitment. Plasma fibrinogen and viscosity, and full blood cell count including white cell count and hematocrit, were measured in blood anticoagulated with K$_2$ EDTA (1.5 mg/mL) collected at the third screening visit. All subjects provided written informed consent. The study was approved by local ethics committees. In the present nested case-control study, we used an expanded end point comprising the frequency of the end point in patients in quintiles 2 through 5 with that in the reference quintile 1. We used multivariate conditional logistic regression models to assess the independent prognostic value of variables. Each was included as a continuous variable and in a separate analysis as a categorical variable (in which quintiles were used). We calculated relative risks and 95% confidence intervals. We assessed associations among variables in the control subjects with use of Spearman rank-correlation coefficient.

Statistical Analysis

Baseline characteristics in cases and controls were compared by 2-sample $t$ tests. The distributions of IL-6, D-dimer, CRP, and triglyceride levels were markedly skewed and were therefore log-transformed. We established quintile ranges according to the values in the control subjects and obtained risk ratios by comparing the frequency of the end point in patients in quintiles 2 through 5 with that in the reference quintile 1. We used multivariate conditional logistic regression models to assess the independent prognostic value of variables. Each was included as a continuous variable and in a separate analysis as a categorical variable (in which quintiles were used). We calculated relative risks and 95% confidence intervals. We assessed associations among variables in the control subjects with use of Spearman rank-correlation coefficient.
TABLE 2. Associations Between Blood Variables and Coronary Risk Factors in Control Subjects

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>IL-6</th>
<th>D-Dimer</th>
<th>Factor VII</th>
<th>Factor XIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>917</td>
<td>923</td>
<td>928</td>
<td>934</td>
</tr>
<tr>
<td>Age</td>
<td>0.19**</td>
<td>0.24**</td>
<td>0.12**</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.07</td>
<td>−0.07</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.11**</td>
<td>0.04</td>
<td>0.10*</td>
<td>0.15**</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.06</td>
<td>−0.08</td>
<td>0.16**</td>
<td>0.21**</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.12**</td>
<td>−0.04</td>
<td>−0.02</td>
<td>−0.01</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.05</td>
<td>−0.04</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Triglyceride (log)</td>
<td>0.14**</td>
<td>−0.12**</td>
<td>0.24**</td>
<td>0.26**</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.10*</td>
<td>−0.02</td>
<td>0.06</td>
<td>0.10*</td>
</tr>
<tr>
<td>White cell count</td>
<td>0.34**</td>
<td>0.07</td>
<td>0.09</td>
<td>0.10*</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.03</td>
<td>−0.01</td>
<td>−0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>0.36**</td>
<td>0.14**</td>
<td>0.16**</td>
<td>0.22**</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.39**</td>
<td>0.16**</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.58**</td>
<td>0.12**</td>
<td>0.05</td>
<td>0.13**</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>0.21**</td>
<td>0.07</td>
<td>0.14**</td>
<td></td>
</tr>
<tr>
<td>D-dimer</td>
<td>−0.04</td>
<td>−0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VII</td>
<td>0.12**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given as Spearman rank correlation coefficients. *P<0.01; **P<0.001

the control group are shown in Table 2. Relationships were similar in the case group (data not shown). Correlations with classical risk factors were generally weak, with the strongest being with triglyceride for all 4 variables (versus for D-dimer), and with systolic blood pressure and total cholesterol for factor VII and factor XIIa. IL-6 showed an inverse correlation with HDL cholesterol. All 4 variables correlated with plasma viscosity; all except factor VII correlated with CRP; and D-dimer and IL-6 correlated with fibrinogen. IL-6 correlated strongly with CRP (r = 0.58) and with fibrinogen, viscosity, and white cell count (r = 0.34 to 0.39), but not with platelet count. D-dimer correlated with IL-6 (r = 0.21). Current cigarette smoking was associated with higher IL-6 (geometric mean 2.03 versus 1.74 pg/mL; P = 0.002). Neither diabetes nor angina showed significant associations with levels of D-dimer, IL-6, or factors VII or XIIa.

Table 3 shows the risk ratios for incident CHD by quintiles of each thrombotic variable, with and without adjustment for age, treatment group, and conventional CHD risk factors. D-dimer showed a significant association, with the risk ratio in the top quintile being 2.00 (95% CI, 1.37 to 2.92; P = 0.0003). After adjustment, this risk ratio was 2.00 (95% CI, 1.37 to 2.92; P = 0.0001). IL-6 showed a significant association on univariate analysis (risk ratio in top quintile 2.05; 95% CI, 1.43 to 2.96), which was reduced on multivariate analysis to 1.64 (95% CI, 1.11 to 2.40). No significant associations were observed for factors VII or XIIa. After

**TABLE 3. Risk Ratios (95% CI) for Incident CHD by Quintiles of Interleukin-6 and Thrombotic Variables**

<table>
<thead>
<tr>
<th>Quintiles</th>
<th>IL-6</th>
<th>Factor VII</th>
<th>Factor XIIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>1396</td>
<td>≤1.11</td>
<td>1.11–1.29</td>
</tr>
<tr>
<td>Risk ratio</td>
<td>1</td>
<td>1.18 (0.80–1.72)</td>
<td>1.31 (0.90–1.91)</td>
</tr>
<tr>
<td>Adjusted risk ratio*</td>
<td>1395</td>
<td>1</td>
<td>1.13 (0.76–1.68)</td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>1403</td>
<td>&lt;37</td>
<td>37–51</td>
</tr>
<tr>
<td>Risk ratio</td>
<td>1</td>
<td>1.20 (0.82–1.78)</td>
<td>1.91 (1.32–2.75)</td>
</tr>
<tr>
<td>Adjusted risk ratio*</td>
<td>1402</td>
<td>1</td>
<td>1.11 (0.74–1.67)</td>
</tr>
<tr>
<td>Risk ratio</td>
<td>1</td>
<td>1.20 (0.85–1.68)</td>
<td>1.08 (0.76–1.53)</td>
</tr>
<tr>
<td>Adjusted risk ratio*</td>
<td>1410</td>
<td>1</td>
<td>1.22 (0.85–1.76)</td>
</tr>
<tr>
<td>Factor XIIa (ng/ml)</td>
<td>1419</td>
<td>≤1.55</td>
<td>1.55–1.99</td>
</tr>
<tr>
<td>Risk ratio</td>
<td>1</td>
<td>0.76 (0.53–1.08)</td>
<td>0.98 (0.70–1.39)</td>
</tr>
<tr>
<td>Adjusted risk ratio*</td>
<td>1418</td>
<td>1</td>
<td>0.81 (0.55–1.18)</td>
</tr>
</tbody>
</table>

*Adjusted for age, treatment group, diabetes, hypertension, angina, body mass index, systolic blood pressure, cholesterol (total, LDL, and HDL), and triglyceride.
exclusion of age, treatment group, and CHD risk factors (as in TABLE 3); additional adjustments then performed for the two other variables.

### Discussion

We showed that plasma levels of IL-6, a key regulator of the inflammatory response, were predictors of coronary risk in middle-aged men with hypercholesterolemia on multivariate analyses including conventional CHD risk factors. These findings are consistent with those in recent reports from the US Physicians Study,23 from a study of the older population,24 and from the Women’s Health Initiative Study.25 Plasma IL-6 showed significant correlations with several markers of the inflammatory response, which we have previously reported to be coronary risk predictors in the West of Scotland Coronary Prevention Study: fibrinogen,26 white cell count,27 plasma viscosity,28 and CRP.21 These findings confirm and extend those of smaller previous studies of random samples of men and women11,12 and support the hypothesis that IL-6 plays a key role in the associations between inflammatory markers and risk of CHD.11,12 The causality of this potential role awaits testing, for example, in randomized trials of IL-6 antagonists. We have previously reported that plasma IL-6 levels in this study (as in others) were poorly related to IL-6 to 174G→C gene polymorphisms, and that this polymorphism was not associated with corresponding increases in cardiovascular risk.29 Hence the association of plasma IL-6 with incident CHD may result from other genetic determinants of IL-6, or from environmental determinants (for example, its origin from inflammatory arterial plaques).14

We also showed that plasma levels of fibrin D-dimer, a marker of fibrin turnover, were predictors of coronary risk. This finding is consistent with those of a meta-analysis of prospective epidemiological studies13 and a further recent study,27 and extend this association for men with hypercholesterolemia. We recently observed that D-dimer was associated with CRP in 2 population cohorts13,14 and postulated that inflammation and fibrin turnover were linked, possibly through effects of fibrin degradation products on inflammation, including monocyte secretion of IL-614,15,28. In the present study, we confirmed that D-dimer was associated with CRP14,29 and also that D-dimer was also associated with IL-6.30 Together with experimental evidence that D-dimer induces the synthesis and release of biologically active IL-6 from monocytes in vitro,15 these findings support the hypothesis that fibrin degradation products containing D-dimer promote vascular inflammation, partly through release of...
IL-6. We have recently shown that warfarin, which normalizes increased D-dimer levels in subjects with atrial fibrillation, also lowers plasma levels of IL-6 and CRP.  

As expected, IL-6 levels correlated strongly with plasma levels of CRP ($r=0.58$). A similar association was observed in the Physicians’ Health Study cohort ($r=0.43$). In the latter study, IL-6 showed a significant relationship with risk of myocardial infarction after adjustment for CRP. In the present, larger study (485 cases and 934 controls, compared with 202 cases and 202 controls in the Physicians Health Study), inclusion of the other inflammatory variable reduced the associations of IL-6 and CRP with CHD risk to nonsignificance, probably because of their higher covariance in the present study. Plasma fibrin D-dimer levels correlated with both IL-6 and CRP levels in the present study; however, the association of D-dimer with coronary events remained highly significant after adjustment for IL-6 and CRP. By contrast to the report of Cushman et al, who reported independent associations of D-dimer and CRP with risk of CHD, the associations of IL-6 or CRP with coronary events became nonsignificant after adjustment for D-dimer. One possible explanation is that D-dimer is one causal factor in elevation of IL-6 and, hence, CRP. Another possible explanation is that D-dimer may have lower assay variability (within-person and laboratory) than IL-6 and CRP. Comparative data from repeat assays of these 3 variables within cohort studies are few at present; however, recent data suggest that D-dimer may have lower variability than CRP.  

Increased fibrin turnover (as measured by plasma D-dimer levels) and risk of arterial thrombosis could be promoted by increased levels of coagulation factors. Factor VII plays a key role in initiation of blood coagulation through the extrinsic (tissue factor) pathway. In prospective studies, factor VII activity has shown inconsistent associations with coronary risk, possibly because of variation in methodology. Assays of activated factor VII (factor VIIa) or factor VII antigen may be less dependent on methodology. In the present study, factor VII antigen showed no association with coronary risk.  

Factor Xlla plays a key role in initiation of blood coagulation, fibrinolysis, and other biological systems through intrinsic (surface-activated) pathways. We did not confirm in the present study a recent report that factor Xlla was associated with risk of coronary events in another prospective study. We have recently reported that although plasma Xlla levels in the present study were strongly related to factor Xlla 46C>T gene polymorphisms, the TT genotype of this polymorphism was associated with increased coronary risk only in the pravastatin-treated group, and that this genotype was associated with lower plasma Xlla levels (possibly because of decreased intrinsic fibrinolysis). Several previous studies have suggested that plasma levels of factor VII, factor XII, or Xlla are related to plasma levels of cholesterol and triglyceride. The present study also associated these variables with cholesterol and triglyceride (Table 2).  

Although baseline plasma samples had been stored at $-70^\circ$C for 11 ($\pm$1) years before assay of these thrombotic variables, baseline levels (Table 1) were comparable to those in other studies of middle-aged men for factor VII antigen, fibrin D-dimer, factor Xlla, and IL-6. Correlations of these variables with age and CHD risk factors (Table 2) were also consistent with those of other studies. Finally, the relationships of plasma factor IL-6 and Xlla levels to their relevant genotypes in the present study were very similar to those reported in other studies. For all these reasons, we believe that storage of samples in the present study did not affect the validity of our analyses or conclusions.  

A potential limitation of the present study is that its setting was a trial of statin therapy for men with hyperlipidemia. It is therefore possible that pravastatin therapy may have affected the relationships between the baseline variables studied and the risk of CHD. However, separate analyses of the pravastatin and placebo groups showed no significant differences between these treatment groups for these relationships. Further studies (and collaborative meta-analyses, such as have been recently performed for fibrinogen) are required to establish the independent associations of D-dimer, CRP, and IL-6 with risk of CHD events.  

In conclusion, this study from a trial of statin therapy for men with hyperlipidemia confirmed a previous report that plasma IL-6 levels were predictive of coronary events in men without previous myocardial infarction, observed that neither IL-6 nor CRP (which were highly correlated) were independent coronary risk predictors after adjustment for D-dimer, and observed that fibrin D-dimer was an independent predictor of coronary risk, including adjustment for IL-6 and CRP levels. Factor VII and factor Xlla antigens were not associated with coronary risk. Markers of fibrin turnover and of the inflammatory response merit further joint study in assessment of coronary risk. In particular, the role of fibrin D-dimer in promoting systemic inflammatory responses through stimulating monocyte release of IL-6 requires further study.  

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References  


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