Plasma Fibrin D-Dimer Levels and Risk of Stable Coronary Artery Disease
Results of a Large Case-Control Study

Wolfgang Koenig, Dietrich Rothenbacher, Albrecht Hoffmeister, Martin Griesshammer, Hermann Brenner

Abstract—Increased levels of fibrin D-dimer are indicative of a hypercoagulable state, as found in acute coronary syndromes. Few well-controlled studies have assessed D-dimers in patients with stable coronary artery disease (CAD). We measured levels of D-dimers (in ng/mL by enzyme-linked immunosorbent assay) in 312 patients with angiographically proved CAD and stable angina pectoris and in 477 age- and sex-matched healthy blood donors. Demographic characteristics were assessed by a standardized questionnaire, and a complete lipid profile was performed for all subjects. In addition, a variety of other markers of hemostasis and inflammation were measured. The distribution of D-dimer levels was skewed to the right, and plasma median levels were higher in cases than in controls (median: 11.2 vs 2.8 ng/mL; \(P<0.001\)). In controls, correlations of D-dimer were found with fibrinogen, plasma viscosity, and interleukin-6. In logistic regression analysis, the age- and sex-adjusted odds ratio (OR) for the presence of CAD was 2.6 (95% confidence interval [CI], 1.9 to 3.5) when the highest quartile of the D-dimer distribution was compared with the combined lower 3 quartiles. The OR did not change appreciably after controlling for nonlipid risk factors (OR, 2.7; 95% CI, 1.9 to 3.9) and remained significant after further adjustment for other hemostatic parameters (OR, 2.4; 95% CI, 1.7 to 3.3) and markers of inflammation (OR, 2.1; 95% CI, 1.5 to 2.9). Plasma D-dimer levels are strongly and independently associated with the presence of CAD in patients with stable angina pectoris. These results support the concept of a contribution of intravascular fibrin to atherothrombogenesis. (Arterioscler Thromb Vasc Biol. 2001;21: 1701-1705.)

Key Words: D-dimer ■ hemostasis ■ inflammation ■ coronary artery disease ■ case-control study

The acute coronary syndrome is characterized by a ruptured, vulnerable plaque and subsequent intraluminal thrombus formation.\(^1\) Besides local hypercoagulability, a number of studies have shown a profound systemic imbalance of the hemostatic system with a shift to increased procoagulation and decreased fibrinolysis. Yet the hemostatic system not only seems to play an important role during the acute event but may also be important in the initiation and progression of atherosclerosis. Various hemostatic proteins like fibrinogen,\(^2\) plasminogen activator inhibitor-1 (PAI-1),\(^3\) and von Willebrand factor (vWF)\(^4\) have been found to be independently associated with future coronary events in apparently healthy subjects and in patients with manifest atherosclerosis.

Recently, fibrin D-dimer, the degradation product of cross-linked fibrin, has gained increasing interest for several reasons. First, it can be considered as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system. Second, in contrast to several other markers of hemostasis, D-dimer assays are more stable and more practical to measure and therefore may be more suitable for routine clinical and epidemiological purposes.\(^5\)

Thus, the main aim of the present study was to investigate the association between D-dimer and angiographically determined coronary artery disease (CAD) in patients with stable angina and in controls, taking into account its potential relationship with various other hemostatic and inflammatory variables that have been related to atherosclerotic disease. In control subjects, we additionally assessed the determinants of plasma D-dimers, and in cases, its association with the severity and extent of CAD as measured by 3 different angiographic scores.

Methods

Patients and Controls

Patients and controls were recruited between October 1996 and November 1997. Participation was voluntary, and written informed
consent was obtained from each subject. The study was approved by the ethics committee of the University of Ulm. The case group was admitted to the Department of Cardiology at the University of Ulm Medical Center for elective coronary angiography. A total of 312 patients of German nationality aged 40 to 68 years with clinically stable, angiographically confirmed CAD (>50% diameter stenosis of at least 1 major coronary artery), diagnosed only within the previous 2 years to reduce the likelihood of survival bias, were included. Patients with acute ischemic syndromes within the previous 4 weeks and those on anticoagulant therapy, with acute infectious diseases, or with evidence of malignant diseases, possibly associated with an acute-phase reaction, were excluded.

The control group consisted of 477 subjects who were occasional blood donors at the local Red Cross center serving the University hospitals of Ulm. All controls had no history of definite or suspected CAD and did not report infections or surgery within the previous 4 weeks. Participation rate was 78% in eligible patients and 84% in eligible controls. Frequency matching for age and sex was performed, and a case-control ratio of 1:1.5 was intended. The sample size was sufficient to detect an odds ratio (OR) of 1.5-fold or larger for an association of D-dimer (top quartile vs combined 3 lower quartiles) and CAD with 80% power at the 5% level of significance.

All subjects underwent standardized interviews conducted by trained interviewers. Participants were asked about their medical history, including specific questions related to physician-diagnosed hypertension, diabetes, and gastroduodenal disease. Furthermore, current medication, sociodemographic data, and lifestyle habits (including smoking and alcohol consumption) were recorded.

**Laboratory Methods**

Venous blood was drawn in the morning under standardized conditions, and a complete blood count was done (Coulter STKS chamber, Coulter Co.). Within 30 minutes, the remaining blood was centrifuged at 3000g for 10 minutes, immediately divided into aliquots, and frozen at -70°C until analysis. For determination of D-dimer levels, the Dimertest Gold EIA (Agen Biomedical Ltd; distributed by Hemachrom Diagnostics) was used. This assay uses DD-3B6 as a monoclonal antibody that recognizes a specific epitope on the cross-linked y-polypeptide chains in the D domain of fibrin molecule. Other parameters also determined by ELISA were as follows: interleukin-6 (IL-6; Quantikine, R&D Systems), PAI-1 activity (Immuno), and vWF (Hemochrom). In addition, C-reactive protein (CRP) determinations were done by an immunoradiometric assay (range, 0.05 to 10 mg/L) calibrated with the World Health Organization reference standard 85/506. Fibrinogen was measured by immunonephelometry (Dade Behring) and according to the Clauss method. Serum amyloid A (SAA) was also determined by immunonephelometry (Dade Behring), and measurement of plasma viscosity was done in a Harkness Coulter viscometer (Coulter Electronics). Finally, total homocysteine was determined by high-performance liquid chromatography. Interassay coefficients of variation were 7.2% for D-dimer, 7% for IL-6, 12% for CRP, 4.9% for SAA, 5% for fibrinogen, 11% for PAI-1, 15.8% for vWF, 7.4% for total homocysteine, and 2% for plasma viscosity. Total and HDL cholesterol concentrations were determined by routine enzymatic methods. Lipoprotein (a) (Lp(a)) and apoproteins were determined by immunoturbidimetry on a Wako R-30 automated analyzer. All laboratory analyses were done in a blinded fashion.

**Angiographic Evaluation**

Coronary angiography was performed among cases by the Judkins method. Three different scores were used to evaluate the angiographic severity and extent of CAD: (1) the number of stenosed (>50% of luminal diameter) or occluded vessels (1- to 3-vessel disease); (2) the quantitative extent score (1 to 15 segments) according to the guidelines of the American Heart Association; and (3) qualitative and quantitative evaluation by the Gensini score. Scoring of all coronary angiograms was done by a single observer who was blinded to clinical and laboratory data. The intraclass correlation coefficient for intrarater reliability was 1.0 (1- to 3-vessel disease score), 0.79 (tertiles of the extent score), and 0.85 (tertiles of the Gensini score).

**Statistical Analysis**

Demographic and clinical characteristics in patients and controls were compared in a descriptive way. Levels of markers of hemostasis and inflammation are reported as arithmetic means (±1SD) except for CRP, SAA, PAI-1, and IL-6, for which the geometric means and medians are given owing to their highly skewed distributions. Categorical variables are reported as percentages. Levels of D-dimer were compared by the Kruskal-Wallis test. Spearman rank correlation coefficients were calculated between D-dimer levels and a variety of markers of hemostasis, inflammation, and classic cardiovascular risk factors. Unconditional logistic regression was used to assess the independent association of elevated D-dimer levels (top quartile vs combined 3 lower quartiles) with CAD. In a basic model, only the matching variables age (years) and sex were controlled for (model 1). Other models additionally controlled for nonlipid risk factors like body mass index (BMI, kg/m²), number of pack-years smoked, history of hypertension, history of diabetes, alcohol consumption (g/d), and years of formal school education (model 2); lipid risk factors (total cholesterol [mmol/L]; HDL cholesterol [mmol/L]; and apo A1, A2, B, C, and E (model 3); Lp(a) (model 4); hemostatic factors (fibrinogen, PAI-1, vWF, and plasma viscosity) (model 5); markers of inflammation (CRP, SAA, IL-6, and leukocyte count) (model 6); and all of the above factors (model 7).

To assess the association of the severity of CAD according to different coronary scores with D-dimer levels, we performed a test for trend after adjustment for age and sex. A 2-tailed P value <0.05 was considered statistically significant. All computations were done with SAS software.

**Results**

**Study Population**

Table 1 shows the demographic and laboratory characteristics of the study population. This table shows that mean BMI and the proportion of subjects with less school education were somewhat higher in cases than in controls. There were only small differences in the lipid profile between groups with the exception of HDL cholesterol and apo A, which were considerably higher in controls compared with cases. Approximately two thirds of the patients (62%) had a history of myocardial infarction within the previous 2 years. By coronary angiography, 48% had single-vessel disease, 34% had double-vessel disease, and 18% had triple-vessel disease.

**Distribution of D-Dimer Levels**

Plasma levels of D-dimer were positively skewed to the right and are therefore given as median, interquartile range, and total range. They were statistically significantly higher in cases than in controls (median, 11.2 ng/mL; interquartile range, 0 to 28.9 ng/mL, range, 0 to 309.2 ng/mL vs 2.8 ng/mL; interquartile range, 0 to 15.1 ng/mL; range, 0 to 579.3 ng/mL; P<0.001). They were also higher in females compared with males (cases: 18.2 vs 10.4 ng/mL, P=0.12; controls: 7.0 vs 1.9 ng/mL; P=0.04). Other markers of hemostasis and inflammation were also consistently higher in cases than in controls.

**Correlation Between D-Dimer Levels and Hemostatic, Inflammatory, and Conventional Risk Variables (Controls Only)**

Plasma D-dimer levels were positively and significantly correlated with fibrinogen (Clauss method and nephelometry), plasma viscosity, and IL-6 (Table 2). Spearman rank correlation coefficients ranged between 0.12 (IL-6) and 0.25 for fibrinogen (Clauss method and nephelometry), plasma viscosity, and IL-6 (Table 2). Spearman rank correlation coefficients ranged between 0.12 (IL-6) and 0.25 for fibrinogen.
In logistic regression analysis (Table 3), the age- and sex-adjusted OR for the presence of CAD was 2.6 (95% confidence interval [CI], 1.9 to 3.5) when the highest quartile of the D-dimer distribution was compared with the combined lower 3 quartiles. Because we were specifically interested in the potential independent contribution of D-dimers to the risk of CAD, we defined several pathophysiological meaningful clusters of variables, carried out adjustments for these sets of variables separately, and included all of them in the final model. The OR did not change appreciably after controlling for nonlipid risk factors (OR, 2.7; 95% CI, 1.9 to 3.8). Because Lp(a) may act in a prothrombogenic manner through several potential pathways, we separately adjusted for this variable. However, there was no appreciable effect seen on the risk estimate (OR, 2.6; 95% CI, 1.9 to 3.6). Neither additional adjustment for total homocysteine (data not shown) nor controlling for cardiovascular active compounds (aspirin, β-adrenoceptor blockers, angiotensin-converting enzyme inhibitors, lipid-lowering drugs, and diuretics) altered the results appreciably (OR, 2.3; 95% CI, 1.2 to 4.5). Results were similar in those with a history of previous myocardial infarction compared with those without (data not shown).

D-Dimer and the Severity and Extent of CAD (Cases Only) In 305 of 312 patients with angiographically determined CAD, the severity of CAD was evaluated by 3 different coronary scores (Table 4). No association between D-dimer plasma levels and any of the 3 scores, representing severity and extent of CAD, was found.

### TABLE 2. Spearman Rank Correlation Coefficients Between Plasma D-Dimer Levels and Conventional Risk Factors and Markers of Hemostasis and Inflammation in Controls (n=477)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>0.005</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.009</td>
<td>0.84</td>
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<tr>
<td>Triglycerides, log</td>
<td>0.007</td>
<td>0.86</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>Apo A1</td>
<td>0.02</td>
<td>0.72</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Lp(a), log</td>
<td>-0.004</td>
<td>0.93</td>
</tr>
<tr>
<td>Fibrinogen (Clauss)</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Fibrinogen (nephelometric)</td>
<td>0.25</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>0.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>PAI-1 activity</td>
<td>-0.009</td>
<td>0.85</td>
</tr>
<tr>
<td>vWF</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>CRP, log</td>
<td>0.04</td>
<td>0.35</td>
</tr>
<tr>
<td>SAA, log</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>IL-6, log</td>
<td>0.12</td>
<td>0.007</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Total homocysteine, log</td>
<td>0.06</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### TABLE 3. Odds Ratios of Coronary Artery Disease Associated With D-Dimer Levels Exceeding the Third Quartile (15.1 ng/mL) in Controls After Various Adjustments*

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Additionally Adjusted for</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age and sex</td>
<td>2.6</td>
<td>1.9–3.5</td>
</tr>
<tr>
<td>2</td>
<td>Nonlipid risk factors†</td>
<td>2.7</td>
<td>1.9–3.9</td>
</tr>
<tr>
<td>3</td>
<td>Lipid risk factors‡</td>
<td>2.7</td>
<td>1.9–3.8</td>
</tr>
<tr>
<td>4</td>
<td>Lp(a)</td>
<td>2.6</td>
<td>1.9–3.6</td>
</tr>
<tr>
<td>5</td>
<td>Fibrinogen, PAI-1 activity, vWF</td>
<td>2.4</td>
<td>1.7–3.3</td>
</tr>
<tr>
<td>6</td>
<td>CRP, SAA, IL-6, leukocytes</td>
<td>2.1</td>
<td>1.5–2.9</td>
</tr>
<tr>
<td>7</td>
<td>Fully adjusted model</td>
<td>2.4</td>
<td>1.6–3.6</td>
</tr>
</tbody>
</table>

*All models were adjusted for age and sex (matching variables).
†BMI, smoking status, alcohol intake, school education years, hypertension, and diabetes.
‡Total and HDL cholesterol; apo A1, B, and E.
D-Dimer and Left Ventricular Function
(Cases Only)
There was no correlation between D-dimer levels and angiographically determined left ventricular ejection fraction. However, the number of patients with an ejection fraction <50% was rather small.

Discussion
In this study, patients with CAD as determined by angiography and stable angina pectoris had elevated plasma D-dimer levels compared with those of healthy blood donors. These data are consistent with several studies that have reported elevated levels of D-dimers not only in symptomatic patients with atherosclerotic manifestations in various vascular beds but also in subjects with subclinical disease. Correlations with other conventional risk factors, including a broad lipid profile, were negligible. These results are partly in contrast with data obtained within the Edinburgh Artery Study, but overall in that study, only 25% of the variation in fibrin D-dimer levels could be explained by known risk factors. Correlations with other hemostatic proteins in our study were found only for fibrinogen. Interestingly, positive correlations were seen with several markers of inflammation, with the highest correlation coefficients found for plasma viscosity and IL-6, which has not been reported before.

Thrombosis and inflammation play an important role not only in the pathophysiology of acute ischemic syndromes but also in the process of atherogenesis, especially in the progression of disease. Based on these considerations, a variety of epidemiological and clinical studies have been carried out to investigate new biochemical markers for their ability to improve risk prediction for future cardiovascular events. In the vast majority of these studies, however, only 1 of these markers has been tested. Although several of them have been shown to predict risk independently of conventional risk factors, the question remains which marker should be preferred in the routine clinical setting, or which cluster of variables reflecting different pathophysiological aspects of atherothrombosis should be used. Besides analytical and technical considerations, the main reason in favor or against a given marker consists in its independence of other markers reflecting the same pathophysiological pathway. Thus, the main aim of the present study was to investigate the association between D-dimer and CAD, taking into account its potential relationship with various other hemostatic and inflammatory variables that have been related to atherosclerotic disease.

The association that we found between D-dimer levels and CAD was strong and at least on the order of that found for conventional risk factors, and it decreased only slightly after controlling for a large variety of potential confounders in multivariable analysis. It is important to note that the association was essentially independent of other prothrombotic variables like fibrinogen, PAI-1 activity, vWF, total homocysteine, and Lp(a) and was only slightly reduced after controlling for several markers of inflammation. Furthermore, major cardiovascular compounds used in this population did not affect the association between D-dimer levels and CAD.

Elevated D-dimer levels have been found to predict the risk of future coronary events independently of conventional risk factors in initially healthy, middle-aged male and female subjects, in elderly men and women, as well as in patients with known peripheral arterial occlusive disease and after myocardial infarction. In the Edinburgh Artery Study, a positive association with stroke was also found. Only in the Physicians’ Health Study was the association between D-dimer and risk of myocardial infarction no longer significant after controlling for either total and HDL cholesterol or markers of the fibrinolytic system. In a formal meta-analysis of prospective studies, an OR of 1.7 (95% CI, 1.3 to 2.2) was discovered when individuals with baseline D-dimer values in the top third versus those in the bottom third were compared, with no relevant differences between population-based cohorts and patients with preexisting vascular disease.

Similar to our results obtained in a case-control design, Lowe et al reported no significant confounding of the association between D-dimer and the future risk of myocardial infarction by either traditional nonlipid or lipid risk factors. Only the introduction of markers of inflammation decreased the association. The strongest decrease in the OR in our analyses, though still moderate in magnitude, was also seen after controlling for markers of inflammation. However, the association between D-dimers and CAD remained significant. D-dimer may also be involved in pathophysiological pathways mediating inflammation, because it is known to represent a measure of extracellular fibrin turnover, for example, in various inflammatory states. Alternatively, the reduction in the OR seen in this study may be a random event, because in the fully adjusted model, it was essentially unchanged compared with age-adjusted analysis.

Finally, no association was observed between the severity or extent of atherosclerosis of the coronary tree and D-dimer levels in patients. In 1 study, a weak correlation was seen, which seemed to be due to the inclusion of controls without significant CAD. In another study, the severity of peripheral atherosclerosis was significantly related to D-dimer levels. Such a finding is conceivable because peripheral arterial disease usually involves much larger atheromatous beds and
results in higher D-dimer levels and greater variability compared with CAD.

Our study has several limitations that need to be addressed. First, its case-control design did not allow causal inferences to be made but only hypotheses to be generated. Second, asymptomatic CAD in control subjects cannot be ruled out because no electrocardiogram or angiogram was available; however, the prevalence of CAD in an asymptomatic, middle-aged population appears to be low.28 Third, blood donors tend to be healthier than population-based controls. However, we tried to minimize this potential bias by performing multivariable adjustments. The present study has also several strengths. We investigated a homogeneous group of patients with exclusively chronic stable CAD. Furthermore, we measured a variety of biomarkers reflecting the hemostatic system and inflammation, which enabled us to carefully analyze the relationship between D-dimers and these other emerging markers of CAD risk.

Conclusions

D-dimers can be regarded as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system. D-dimer levels seem to be essentially independent of other cardiovascular risk factors, which suggests that they might add relevant information in addition to lipid variables and other classic risk factors. In contrast to several other markers of hemostasis, D-dimer assays are more stable and more practical to measure and therefore, may be more suitable from a technical point of view for epidemiological purposes.29 However, among several other reasons, a lack of standardization still represents 1 major problem that must be solved before its introduction into the clinical routine can be recommended to help improve risk prediction in atherothrombotic diseases.

Acknowledgments

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References


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