Plasma Concentration of Cross-Linked Fibrin Degradation Product (D-Dimer) and the Risk of Future Myocardial Infarction Among Apparently Healthy Men

Paul M. Ridker, MD; Charles H. Hennekens, MD; Andrew Cerskus, PhD; Meir J. Stampfer, MD

Background Plasma levels of d-dimer, the primary degradation product of cross-linked fibrin, are elevated in several acute thrombotic disorders. However, whether elevated d-dimer levels among healthy individuals are associated with future coronary thrombosis is unknown.

Methods and Results To evaluate whether levels of d-dimer are associated with the occurrence of future myocardial infarction (MI) among apparently healthy men, levels were measured in plasma samples collected at baseline from 296 participants in the Physicians’ Health Study who later developed a first MI and from an equal number of age- and smoking status-matched control subjects who remained free of vascular disease during a mean follow-up period of 60.2 months. In univariate analyses, baseline plasma concentrations of d-dimer in the upper ranges of normal were associated with elevated risks of MI. Specifically, the relative risk of future MI for individuals with baseline d-dimer concentration exceeding the 95th percentile of the control distribution was two times higher than that of individuals with lower levels (relative risk [RR], 2.02; 95% confidence interval [CI], 1.04 to 4.02; P = .04). This association persisted in multivariate analyses controlling for nonlipid cardiovascular risk factors (RR, 2.12; 95% CI, 1.05 to 4.28; P = .04) and for lipoprotein(a) (RR, 2.02; 95% CI, 1.04 to 3.94; P = .03). In contrast, this association was attenuated and no longer statistically significant in analyses that controlled for total and high-density lipoprotein cholesterol (RR, 1.74; 95% CI, 0.78 to 3.91; P = .2) or for endogenous tissue-type plasminogen activator and its primary inhibitor, plasminogen activator inhibitor type 1 (RR, 1.58; 95% CI, 0.67 to 3.77; P = .3).

Conclusions Elevated levels of d-dimer are associated with increased risks of future MI, although they do not appear to be an independent predictor when other risk factors are considered. As the presence of d-dimer in plasma reflects ongoing fibrin degradation, these data support the hypothesis that activation of the endogenous fibrinolytic system occurs many years in advance of coronary arterial occlusion. (Circulation. 1994;90:2236-2240.)

Key Words • fibrin degradation products • d-dimer • infarction • epidemiology • risk factors

In prospective epidemiological studies of atherosclerotic patients as well as healthy subjects, baseline abnormalities of fibrinogen,1-3 antithrombin III,4 coagulation factor VII,5-7 and platelet reactivity5-7 have been associated with increased risk of acute thrombotic disorders. These observations suggest that activation of the endogenous fibrinolytic and anticoagulant systems may occur in advance of arterial occlusion and that measurement of this activation may serve as a marker of risk for a variety of arterial occlusive diseases. For example, Hamsten and colleagues8-9 have demonstrated that plasma concentration of plasminogen activator inhibitor type 1 (PAI-1) is associated with risk of recurrent myocardial infarction (MI) among young individuals, and we have demonstrated that elevated levels of tissue-type plasminogen activator (TPA) are associated with elevated risks of future MI10 and stroke11 among apparently healthy men. However, because the primary function of PAI-1 and TPA is to act in concert to regulate plasminogen conversion to plasmin, their levels may be only indirect markers of fibrinolytic activation. In contrast, d-dimer is the primary degradation product of cross-linked fibrin and therefore may serve as a direct marker of ongoing fibrinolysis.12 Because plasma concentration of d-dimer reflects the presence of active fibrin degradation, measurement of d-dimer has proven clinically useful in the diagnosis of acute venous thrombosis, pulmonary embolism, and disseminated intravascular coagulation.13-17 We therefore sought to test whether elevations of baseline d-dimer concentration among apparently healthy men might serve as a marker of risk for future coronary occlusion.

Methods

We performed a nested case-control study using prospectively collected plasma samples from a cohort of apparently healthy men participating in the Physicians’ Health Study18 a randomized, double-blind, placebo-controlled trial of aspirin and beta-carotene in the primary prevention of cardiovascular disease and cancer. The details of the Physicians’ Health Study18 have been presented previously; in brief, 22,071 US male physicians aged 40 to 84 years and free of prior MI, stroke, transient ischemic attack, or cancer were randomly assigned in a 2×2 factorial design to one of four treatment

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groups: 325 mg aspirin on alternate days (supplied as Bufferin by Bristol Myers Products), 50 mg beta-carotene on alternate days (supplied as Lurotin by BASF), both, or neither. Six months after randomization and annually thereafter, questionnaires assessing risk factors and disease outcomes were mailed and returned by these physicians with 99.7% follow-up for morbidity and 100% for mortality.

At enrollment, all potentially eligible men were assigned to a regimen of active aspirin and placebo beta-carotene in an 18-week run-in to identify good compliers for long-term follow-up. During the run-in, all participants were asked to provide baseline blood plasma samples. Blood collection kits including EDTA Vacutainer tubes and plastic collection vials were sent to each participant along with instructions for blood drawing. Participants were asked to have blood drawn into the EDTA tubes, centrifuge the tubes, and return the plasma, accompanied by cold pack, by overnight courier. On arrival in the laboratory, specimens were placed in aliquots and stored at −80°C. Of 22,071 physicians randomized, 14,916 returned a baseline blood sample specimen (68%). No specimens were inadvertently thawed or even warmed appreciably during storage.

Hospital records were requested for all reported cases of fatal and nonfatal MI occurring after randomization. These medical records, death certificates, and autopsy reports were reviewed by an end points committee of physicians using standardized criteria to confirm or refute each reported event. The diagnosis was considered confirmed if the reported event met the World Health Organization criteria for MI, which include symptoms plus either elevations of cardiac enzyme levels or diagnostic changes on the ECG.19 For fatal MIs, we also accepted diagnoses based on autopsies and deaths confirmed by records as due to coronary heart disease (International Classification of Diseases codes 411 to 414). Silent MI discovered on routine examination was not included since it could not be assigned an accurate date of occurrence. Sudden deaths in men with no history of coronary disease were not included unless coronary disease could be confirmed as the cause of death.

Using a nested case-control design, each physician who provided an adequate baseline plasma blood sample and had a confirmed MI after randomization was matched to one control subject. Only the first MI was considered in any individual. Control subjects were randomized physicians who provided a baseline plasma sample and did not report cardiovascular disease at the time infarction occurred in the patient. Control subjects were selected at random from participants who met the matching criteria of age (±1 year), smoking habit (current, past, or never-smoker), and time since randomization by 6-month intervals.

For each patient (case) and each control subject, plasma collected and stored at baseline was thawed and assayed for d-dimer using an ELISA with TintElize d-dimer kits produced by Biopool AB. This ELISA uses two monoclonal antibodies directed against specific nonoverlapping antigenic determinants present in fragment D-dimer of cross-linked fibrin but not fragment D of non–cross-linked fibrin or fibrinogen.20 d-Dimer concentrations measured with this assay correlate extremely well (r = 0.92) with levels measured using conventional latex assays employing a monoclonal antibody directed at the cross-linking site.

All assays were performed in duplicate in the laboratory of Dr Cerskus (Biopool Canada Inc.), and investigators and laboratory personnel were blinded to case or control status. Blood specimens from patients and controls were analyzed in pairs with the position of the cases varied at random within pairs to avoid systematic bias and interassay variability. Pairs of samples were handled together and identically throughout processing and analysis. In these data, the mean within-pairs coefficient of variation for plasma d-dimer concentration, calculated using repeat values for all 296 case-control pairs, was 5.8%; the between-pairs coefficient of variation, calculated using repeat values obtained from 32 randomly placed blinded split samples, was 7.2%.

In addition to providing blood samples before randomization, participating physicians also reported baseline cardiovascular risk factors, including the matching variables of age and smoking status as well as height, weight, systolic and diastolic blood pressures, parental history of MI before age 60, presence of diabetes mellitus, and frequency of vigorous exercise. Total and high-density lipoprotein (HDL) cholesterol levels were measured enzymatically, whereas TPA antigen, PAI-1 antigen, and total lipoprotein(a) [Lp(a)] were measured by ELISA as previously described.10,11,21-23

Statistical Analysis

Mean values and proportions for baseline cardiovascular risk factors were computed for patients and control subjects. The significance of any difference in mean value was tested using the paired Student’s t test, whereas the significance of any difference in proportions was tested using the χ² statistic. Relative risks of future MI were calculated for men with baseline levels of d-dimer antigen exceeding the 25th, 50th, 75th, 90th, and 95th percentiles of the distribution as defined by the control population. Risk estimates were further adjusted using conditional logistic regression models that controlled for both lipid and nonlipid risk factors, TPA antigen, PAI-1 antigen, and Lp(a). Pearson correlation coefficients between plasma d-dimer level and total cholesterol, HDL cholesterol, TPA antigen, PAI-1 antigen, and Lp(a) were calculated. Partial correlations between d-dimer level and these factors were also computed after adjustment for age using the residuals from multiple linear regression. All analyses controlled for randomized treatment assignment. Confidence intervals of the risk estimates are calculated at the 95% level, and all P values are two-tailed.

Results

Table 1 displays baseline characteristics of the 296 case-control pairs. As previously reported for this cohort,21 patients had higher mean cholesterol and lower mean HDL cholesterol levels than control subjects. In addition, patients had a higher mean body mass index, exercised less frequently, and were more likely to have a parental history of MI or a personal history of diabetes. As expected, because both patients and control subjects were healthy at the time of blood collection, baseline levels of d-dimer for >98% of the sample were within normal limits, and only two values (one patient and one control) were above cutoff points clinically considered to be associated with an increased probability of acute thrombosis. Mean baseline d-dimer concentration was nonsignificantly higher among patients than control subjects (56.3 versus 48.9 ng/mL, P = .17). However, as shown in Table 2, high normal levels of d-dimer were associated with increased risks of future MI. Specifically, the relative risks associated with baseline d-dimer concentrations exceeding the 25th, 50th, 75th, 90th, and 95th percentiles of the control distribution were .99 (P = .9), 1.25 (P = .2), 1.28 (P = .2), 1.82 (P = .02), and 2.02 (P = .04). As illustrated in the Figure, nearly all of the excess risk of MI associated with baseline d-dimer concentration was among healthy men with levels ≥107 ng/mL (the 95th percentile cutoff point of the control distribution), whereas there was virtually no excess risk associated with levels <57 ng/mL (the 75th percentile cutoff point of the control distribution).

Because d-dimer is the breakdown product of cross-linked fibrin and therefore may be related to the extent
of underlying atherosclerosis and fibrinolytic activation, we assessed whether the association of D-dimer and infarction risk was affected by usual atherosclerotic and thrombotic risk factors. As shown in Table 3, controlling for nonlipid risk factors, including body mass index, diabetes, family history of MI before age 60, and exercise frequency, had minimal effect on the association between elevated D-dimer concentration and risk of MI (multivariate RR, 2.12; 95% CI, 1.05 to 4.28; \( P = .04 \)). Similarly, controlling for baseline Lp(a) concentration had no material effect on this association (RR, 2.02; 95% CI, 1.04 to 3.94; \( P = .03 \)). However, in analyses that controlled for total and HDL cholesterol, the relative risk of MI associated with D-dimer concentrations exceeding the 95th percentile of the control distribution was reduced to 1.74 and no longer statistically significant (95% CI, 0.78 to 3.91; \( P = .2 \)). In analyses adjusting for baseline TPA and PAI-1 antigen levels, the association between D-dimer and infarction risk was similarly attenuated and nonsignificant (RR, 1.58; 95% CI, 0.67 to 3.77; \( P = .3 \)).

To assess for any potential linear relations between D-dimer and other measured atherosclerotic and/or thrombotic risk factors in this study population, unadjusted and age-adjusted correlation coefficients were computed and are displayed in Table 4. As shown, no statistically significant correlations were found between baseline D-dimer concentration and total or HDL cholesterol, Lp(a), TPA, or PAI-1.

### Discussion

In univariate analysis of these prospective data, apparently healthy men with baseline plasma concentrations of D-dimer exceeding the 95th percentile of the control distribution (\( \geq 107 \) ng/mL) had twice the risk of future MI than men with lower levels. This association persisted in multivariate analyses controlling for nonlipid risk factors and for Lp(a) but was no longer statistically significant in analyses adjusting for total and HDL cholesterol or for endogenous TPA and PAI-1 antigen levels.

We are aware of no prior data assessing the relation between D-dimer levels and risk of future MI among

### Table 1. Baseline Characteristics of 296 Subjects Who Subsequently Developed Myocardial Infarction (Patients) and 296 Age- and Smoking Status-Matched Control Subjects Who Remained Free of Vascular Disease During the Follow-up Period

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=296)</th>
<th>Control Subjects (n=296)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.4</td>
<td>59.4</td>
<td></td>
</tr>
<tr>
<td>Smoker, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>42.4</td>
<td>42.4</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>43.7</td>
<td>43.7</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>13.9</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Lipid profile, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>220.8</td>
<td>214.0</td>
<td>.06</td>
</tr>
<tr>
<td>HDL</td>
<td>43.9</td>
<td>48.0</td>
<td>.001</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>131.2</td>
<td>127.5</td>
<td>.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81.5</td>
<td>79.0</td>
<td>.002</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.5</td>
<td>25.0</td>
<td>.06</td>
</tr>
<tr>
<td>Family history, % positive</td>
<td>16.9</td>
<td>11.9</td>
<td>.09</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>7.1</td>
<td>2.4</td>
<td>.01</td>
</tr>
<tr>
<td>Exercise frequency, % ( \geq 1/wk )</td>
<td>63.5</td>
<td>74.3</td>
<td>.01</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein.

### Table 2. Relative Risks of Future Myocardial Infarction at Prespecified Cutoff Points for D-Dimer Concentration

<table>
<thead>
<tr>
<th>Cutoff Point, %</th>
<th>D-Dimer Level, ng/mL</th>
<th>Control Subjects (n=296)</th>
<th>Patients (n=296)</th>
<th>RR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25th</td>
<td>( \geq 26 )</td>
<td>220</td>
<td>218</td>
<td>0.99</td>
<td>0.68-1.43</td>
<td>.9</td>
</tr>
<tr>
<td>50th</td>
<td>( \geq 38 )</td>
<td>147</td>
<td>161</td>
<td>1.25</td>
<td>0.90-1.74</td>
<td>.2</td>
</tr>
<tr>
<td>75th</td>
<td>( \geq 57 )</td>
<td>73</td>
<td>86</td>
<td>1.28</td>
<td>0.89-1.86</td>
<td>.2</td>
</tr>
<tr>
<td>90th</td>
<td>( \geq 81 )</td>
<td>29</td>
<td>49</td>
<td>1.82</td>
<td>1.11-2.99</td>
<td>.02</td>
</tr>
<tr>
<td>95th</td>
<td>( \geq 107 )</td>
<td>14</td>
<td>28</td>
<td>2.02</td>
<td>1.04-4.02</td>
<td>.04</td>
</tr>
</tbody>
</table>

RR indicates relative risk; CI, confidence interval.

Cutoff points are defined by the distribution of the control values.

All models matched for age and smoking status and controlled for randomized treatment assignment.
TABLE 3. Relative Risks of Myocardial Infarction Associated With Baseline D-Dimer Antigen Concentrations Exceeding the 95th Percentile of the Control Distribution After Adjustment for Other Potential Atherosclerotic and/or Thrombotic Markers of Cardiovascular Risk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer</td>
<td>2.02</td>
<td>1.04-4.02</td>
<td>.04</td>
</tr>
<tr>
<td>D-Dimer + nonlipid risk factors*</td>
<td>2.12</td>
<td>1.05-4.28</td>
<td>.04</td>
</tr>
<tr>
<td>D-Dimer + total + HDL cholesterol</td>
<td>1.74</td>
<td>0.78-3.91</td>
<td>.2</td>
</tr>
<tr>
<td>D-Dimer + lipoprotein(a)</td>
<td>2.02</td>
<td>1.04-3.94</td>
<td>.03</td>
</tr>
<tr>
<td>D-Dimer + TPA + PAI-1</td>
<td>1.58</td>
<td>0.67-3.77</td>
<td>.3</td>
</tr>
</tbody>
</table>

RR indicates relative risk; CI, confidence interval; HDL, high-density lipoprotein; TPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; and MI, myocardial infarction.

*Nonlipid risk factors: diabetes (yes/no), diastolic blood pressure (mm Hg), body mass index (kg/m²), and family history of MI < age 60 (yes/no).

Bar graph shows relative risks of future myocardial infarction associated with baseline plasma concentration of cross-linked fibrin degradation product, measured as d-dimer. A statistically significant increase in risk is present for individuals with baseline d-dimer levels exceeding 107 ng/mL, the 95th percentile cutoff point of the control distribution.

otherwise healthy men. However, the current data are consistent with recent work demonstrating an association between increased levels of d-dimer and cardiovascular risk among patients with known atherosclerosis. In that study, the relative risk of coronary events among patients with symptomatic peripheral arterial disease was 4.4 times higher for subjects with d-dimer levels in the highest quintile (≥179 ng/mL) than for subjects with levels in the lowest quintile (<65 ng/mL). Although the higher relative risk in that study may indicate that patients with prevalent arteriosclerotic disease are more likely to have elevated fibrin turnover than asymptomatic individuals, it is also possible that this difference from our results is simply due to chance since the 95% CI of the risk estimates for each study broadly overlap each other (1.1 to 4.0 versus 1.3 to 19.0). Thus, while confirming that d-dimer levels are associated with increased risks of coronary disease, the present study also indicates that this effect is present even among asymptomatic individuals.

In multivariate analyses, the relation between d-dimer and risk of MI was attenuated and no longer statistically significant after controlling for total and HDL cholesterol or for TPA and PAI-1 antigens. This finding suggests that d-dimer levels may be related to factors directly involved in atherosclerotic progression and endogenous fibrinolysis. We have previously shown in this cohort that elevated levels of TPA antigen, the primary mediator of intravascular fibrinolysis, are associated with increased risks of future MI, as are total and HDL cholesterol. Thus, as would be expected when controlling for factors in the same potential causal pathway, adjusting for these variables would, as we observed, reduce the association of d-dimer and infarction risk to nonsignificance. In contrast, nonlipid risk factors and Lp(a) may operate through mechanisms unrelated to d-dimer, so it is not surprising that controlling for these factors had minimal effect on the relation between d-dimer and infarction risk. Although we were unable in simple and age-adjusted correlations to find a significant relation between d-dimer level and other measured plasma factors, this may simply reflect the fact that any true association between d-dimer and these other markers is nonlinear, like the observed association between d-dimer and MI risk (Figure).

The finding in these data of a positive association between baseline d-dimer concentration and risk of a first MI supports the hypothesis that activation of the endogenous fibrinolytic system occurs several years in advance of coronary occlusion. In addition, these data are potentially important in understanding the pathophysiology of thrombotic occlusion since the presence of d-dimer implies ongoing formation and degradation of fibrin and thus may help to explain the mechanism by which elevated levels of fibrinogen appear to be a marker of risk for cardiovascular events. Future work will be required to identify the source of the
cross-linked fibrin degradation products in these apparently healthy men. In this regard, it is possible that the elevated levels of D-dimer found in our study may reflect increased fibrin turnover among individuals with elevated levels of circulating fibrin. On the other hand, since D-dimer is present in arterial thrombi and fibrin is a major component of atherosclerotic plaque, it is also possible that the elevations of D-dimer found in our study are a consequence of important preclinical atherosclerosis.

Potential limitations of the present study merit consideration. For example, although elevations of D-dimer have been reported among patients with acute MI,28 the prospective design of our study rules out the possibility that increased concentrations of D-dimer are a result of infarction and markedly reduces the possibility of bias in the selection of control subjects. Altered D-dimer concentrations due to the long storage period before assay also appear to be an unlikely source of bias as the mean (48.9 ng/mL) and median (37.0 ng/mL) D-dimer levels among our control subjects are consistent with prior studies of D-dimer among healthy populations.15-17,29 In addition, because patient and control samples were stored for the same amount of time and handled identically throughout the study, systematic bias based on case status is improbable. Finally, it is important to point out that random misclassification cannot explain our findings as the presence of this effect would, if anything, lead to an underestimation of the true association.

In sum, these data indicate that baseline plasma D-dimer levels at the upper end of the normal distribution are associated with increased risks of future MI among apparently healthy men. This finding is consistent with a major role for fibrinogen and fibrin in the pathogenesis of atherosclerosis and supports the hypothesis that baseline assessment of hemostatic and thrombotic parameters may serve as markers of risk for future MI.30

Acknowledgments

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