Fibrinogen and Factor VII in the Prediction of Coronary Risk
Results From the PROCAM Study in Healthy Men

Jürgen Heinrich, Leopold Balleisen, Helmut Schulte, Gerd Assmann, Jürgen van de Loo

Abstract  Coronary thrombosis is regarded as the final occlusive event in the progress of coronary heart disease (CHD). Disturbances of the hemostatic system may favor this process and thus may indicate increased risk of myocardial infarction. Coagulation and lipid factors were measured in 2116 healthy male participants of the Prospective Cardiovascular Münster (PROCAM) study. After 6 years of follow-up, 82 coronary events (9 sudden cardiac deaths and 14 fatal and 59 nonfatal myocardial infarctions) were observed. The mean plasma fibrinogen levels of the event and nonevent groups differed by 0.23 g/L (2.88 [SD, 0.68] versus 2.63 [SD, 0.63] g/L, respectively; P=.001). The incidence of coronary events in the upper tertile of the plasma fibrinogen distribution was 2.4-fold higher than in the lower tertile. By multiple logistic function analysis, plasma fibrinogen was found to be an independent risk indicator for CHD (P<.05). Individuals in the high serum low-density lipoprotein (LDL) cholesterol tertile who also showed high plasma fibrinogen concentrations had a 6.1-fold increase in coronary risk. Unexpectedly, individuals with low plasma fibrinogen had a low incidence of coronary events even when serum LDL cholesterol was high. The mean factor VIIc activities in the event and nonevent groups did not differ significantly (112.3% [SD, 19.9] versus 108.4% [SD, 21.6]; P=.09). There was, however, a trend toward higher factor VIIc values when only fatal events were taken into account. Thus, higher levels of plasma fibrinogen markedly increased the predictive power of high serum LDL cholesterol. Low plasma fibrinogen is associated with low coronary risk even when LDL is raised. (Arterioscler Thromb. 1994;14:54-59.)

Key Words  • myocardial infarction • coronary heart disease • fibrinogen • factor VII • LDL cholesterol • smoking • prospective study

Most cases of acute myocardial infarction (MI) and cardiac death appear to be associated with the occurrence of occlusive coronary thrombi. One reason favoring or even precipitating thrombus formation might be a thrombogenic state in the patient's blood. Long-term epidemiological studies in healthy persons report increased levels of plasma fibrinogen and factor VIIc in those individuals who develop a coronary event. This might suggest an increase in coagulation activity. To evaluate the possible role of the hemostatic system, plasma fibrinogen and factor VIIc were measured in the Prospective Cardiovascular Münster (PROCAM) study. Since that time, plasma fibrinogen and factor VIIc have been determined in more than 10 000 persons who had not suffered from MI or stroke at the time of entry. The results from 2116 men who completed an observation period of 6 years are reported here.

Methods

Study Design
In the PROCAM study, apparently healthy employees of Westphalian companies were examined deliberately for cardiovascular risk factors and were then kept under observation to record mortality, subsequent MI, and stroke. The examination at onset included each patient's history, physical examination, electrocardiogram (ECG) at rest, and a laboratory blood analysis. The study began in 1979. Two years later the determination of plasma fibrinogen and factor VIIc was included, and 10 581 individuals (7540 men and 3041 women) were recruited (Table 1). As expected, relevant numbers of MI or coronary heart disease (CHD) death occurred only in men aged 40 years and over. The analysis described below was therefore confined to the 2116 male participants between 40 and 65 years of age without a prior history of MI or stroke who had completed their 6-year follow-up.

Diagnostic criteria and the definition of the end points have been published in detail. Two end points were considered: definite nonfatal MI and definite coronary death including sudden cardiac death and fatal MI. Sudden cardiac death was diagnosed if a subject was observed to have died within 1 hour from onset of symptoms. Fatal MI was diagnosed when a death certificate or hospital record described the cause of death accordingly or when an autopsy finding of acute MI was available. Definite nonfatal MI was diagnosed if one or more of the following conditions were fulfilled: (1) diagnostic ECG at the time of event; (2) ischemic cardiac pain plus diagnostic enzymes; (3) ischemic cardiac pain plus equivocal enzymes and equivocal ECG; or (4) an ECG that was diagnostic for MI while a previous one was not.

The blinded members of the Critical Event Committee (see “Appendix”) verified diagnoses and causes of death of all event cases.

Blood Sample Collection
Blood samples for the coagulation tests were collected in 1/10 of 3.13% trisodium citrate from 7 to 9 AM in a fasting
state. Within 60 minutes plasma was separated by centrifugation of blood samples at room temperature for 15 minutes at 2500g. Serum for clinical chemistry was prepared from blood samples without additives by centrifugation for 10 minutes at 3000g. Serum and plasma, transferred in aliquots into plastic tubes, were kept in liquid nitrogen until they were stored in the laboratory at -70°C.

Fibrinogen

Clottable plasma fibrinogen was determined according to Claus4 by using thrombin and control plasma from Behringwerke, Marburg, FRG, and a plasma pool.

Factor VIIc

Plasma factor VIIc was determined by a one-step assay15 using thromboplastin (Thromborel), factor VIIc-deficient plasma, and control plasma from Behringwerke and a plasma pool.

Total, HDL, and LDL Cholesterol and Triglycerides

Triglycerides and total and high-density lipoprotein cholesterol (HDL-C) were measured in serum by using enzymatic assays and (for the latter) a precipitation method from Boehringer Mannheim, FRG, on a Hitachi 737 autoanalyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula.

Statistics

An explorative analysis of data was performed. Continuous variables were compared by t test (triglycerides after logarithmic transformation), and discrete parameters were compared by the χ² test. In all analyses, except where age was included as an independent variable in the multiple logistic regression, results are age adjusted using regressions derived from the entry data. For additional description of the relation between a specific variable and the probability of suffering a cardiovascular event, the numbers in tertiles of the variable are given. In addition, a multiple logistic analysis was performed; variables included were age, body mass index, systolic blood pressure, cholesterol, HDL-C, triglycerides, uric acid, fasting blood glucose, diabetes mellitus, angina pectoris, smoking, and a family history of MI. The level of significance was fixed at P<.05. The statistical analysis was done with the software packages SPSS and SAS.

Results

The 2116 subjects were observed for 72 months. Their mean age at entry was 48.9 (SD, 6.2; range, 40 through 65) years. In this group 82 coronary events were observed (9 sudden cardiac deaths and 14 fatal and 59 nonfatal MIs), which corresponds to an annual incidence rate of 0.65% (Table 2). Causes other than CHD accounted for 61 deaths (8 from other diseases of the circulatory system, 27 from malignant neoplasms, 15 from accidents or violence, and 11 from other diseases). Additionally, 11 nonfatal strokes were observed. A total of 1962 subjects did not experience a stroke or MI during the 6 years after examination.

Table 3, which compares the event and nonevent groups, shows significantly higher levels of fibrinogen, total cholesterol, LDL-C, triglycerides, and systolic blood pressure in men who suffered a coronary event. Their HDL-C was significantly lower. The mean fibrinogen concentration was 0.25 g/L higher in the event group than in the healthy, asymptomatic population (P=.001). Factor VIIc activity did not differ significantly (112.3% [SD, 19.9] versus 108.4% [SD, 21.6], respectively; P=.09). However, when only fatal events were considered, this difference was more pronounced (117.0% [SD, 11.7] versus 108.4% [SD, 21.6], respectively; P=.06). The number of coronary events in tertiles of variables is shown in Table 4. It confirms the information of Table 3 except for the body mass index. Body mass index, diastolic blood pressure, and factor VIIc were associated with an approximately 1.5-fold higher number of events when comparing the lower with the upper tertile.

In the event group, 45.1% of the individuals were active cigarette smokers compared with 27.8% in the nonevent group. A total of 1089 nonsmokers had low or medium fibrinogen levels (<2.77 g/L); only 29 (2.7%) of
TABLE 3. Age-Adjusted Mean Values of Coagulation, Lipid, and Other Variables in the Groups With and Without Coronary Events

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Event (n=1962)</th>
<th>Event (n=82)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.63 (0.63)</td>
<td>2.88 (0.68)</td>
<td>.001</td>
</tr>
<tr>
<td>Factor Vlcl, %</td>
<td>108.4 (21.6)</td>
<td>112.3 (19.9)</td>
<td>.086</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>225.0 (40.8)</td>
<td>251.6 (48.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45.6 (11.6)</td>
<td>40.2 (10.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>148.5 (35.6)</td>
<td>175.0 (38.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>136.4</td>
<td>163.4</td>
<td>.003</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>132.5 (18.3)</td>
<td>137.9 (21.5)</td>
<td>.03</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>87.2 (11.0)</td>
<td>89.8 (12.3)</td>
<td>.06</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 (3.0)</td>
<td>26.4 (3.0)</td>
<td>.44</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BP, blood pressure; and BMI, body mass index. Numbers in parentheses are standard deviations.

*Geometric mean.

them suffered a coronary event. In contrast, 24 (7.9%) of 304 smokers with fibrinogen levels >2.77 g/L belonged to the event group (Table 5).

To elucidate the interdependence of different variables to an increased coronary risk, the multiple logistic function (MLF) was applied. The standardized coefficient of logistic regression for fibrinogen was .00465 ± .00134 (P<.001). Applying the MLF analysis, this coefficient remained greater than zero (.00352 ± .00140, P=.02) after taking into account age, systolic blood pressure, smoking, diabetes mellitus, angina pectoris, and family history of MI. Allowing in addition for total cholesterol and HDL-C, significance was still reached (.00299 ± .00147, P=.05). The distribution of coronary events among quintiles of the estimated risk by MLF resulted in only 26.9 CHD cases/1000 in 6 years in the three lowest quintiles, taking into account the following factors: age, systolic blood pressure, total cholesterol, HDL-C, diabetes mellitus, angina pectoris, smoking, a family history of MI, and fibrinogen (Fig 1). Thus, in 60% of all participants only 13% of all events occurred. However, not taking into account fibrinogen, 59% of all events were found in the highest quintile of the MLF. The addition of fibrinogen resulted in 129.6 events/1000 in 6 years, ie, 65% of all cases.

Three-dimensional analysis of event rate, fibrinogen, and LDL-C, with the latter two depicted in their tertiles, revealed a 6.1-fold higher coronary risk for individuals with high LDL-C and high fibrinogen compared with those with high LDL-C but low fibrinogen (103.1 versus 16.9 events/1000 in 6 years, respectively; Fig 2). Individuals with high fibrinogen values had a more than twofold risk even if LDL-C was low (48.4 versus 19.0 events, respectively). However, low fibrinogen was still associated with a lower event rate even if the LDL values were high.

Discussion

Several prospective studies report an association of plasma fibrinogen with the risk of cardiovascular disease. Differences between the annual incidence rates in these studies are mainly dependent on the age at entry. This could account for minor differences in the results. The sample studied here had a comparatively low mean...
age at entry of 48.9 years and a low annual incidence rate of 0.65%, whereas the corresponding figures for the Northwick Park Heart (NPH) study were 52.4 years and 0.8%, respectively.7

The distribution of events to tertiles of plasma fibrinogen was similar in the NPH and PROCAM studies; the odds ratio of incidences in the lower and upper tertiles was 2.4 in our study. In the NPH study the odds ratio was 3.2 for ischemic heart disease events within 5 years of entry and 2.1 for events in the whole observation period, respectively.7

In a recent meta-analysis of six prospective studies by Ernst and Resch10 surveying more than 90,000 person-years, plasma fibrinogen was confirmed as an independent risk factor for CHD. However, the studies surveyed differed in design. Most did not take HDL-C and family history of CHD into account. The latter variables have an important effect on the risk of MI and may also be related to plasma fibrinogen. In particular, factors affecting fibrinogen (eg, smoking and body weight) also alter HDL-C values. Moreover, fibrinogen concentrations may have a strong genetic component, identified by Humphries et al16 by using restriction fragment length polymorphism markers of the fibrinogen locus. In the PROCAM study the independent association of fibrinogen with the risk of CHD was demonstrated even after the addition of HDL-C and family history of CHD to the risk factors in the MLF analysis.

In the 21-year follow-up of the Gothenburg study,5 plasma fibrinogen was found to be an independent risk factor for stroke but not for CHD if smoking was included in the multivariate analysis. This is not confirmed by our findings; even after addition of smoking to the MLF, plasma fibrinogen remained significant.

The simultaneous consideration of event rate, serum LDL-C, and plasma fibrinogen confirmed the independent risk association of the two plasma factors (see the MLF analysis). High fibrinogen added markedly to the predictive power of high LDL. Unexpectedly, however, low fibrinogen appeared to counterbalance the clear-cut risk association of high LDL-C. This latter observation is so far unexplained and needs further analysis.

It is of further interest to consider the CHD frequency in nonsmokers and smokers in relation to fibrinogen (Table 5). A smoker with a high fibrinogen level had a fourfold elevated risk of CHD compared with a nonsmoker with low fibrinogen. This accords with other reports.17,18

The precise pathophysiological relation of fibrinogen and risk of CHD is currently unknown. Increased fibrinogen concentrations could adversely affect plasma viscosity and platelet aggregability. On the other hand, atherosclerosis could secondarily cause increases of fibrinogen as an acute-phase protein. In support of the latter hypothesis are observations of higher leukocyte counts in the event groups of other studies9,19-20 and significantly higher levels of C-reactive protein in angina pectoris patients who develop a coronary event within 2 years.21

In 1987, preliminary follow-up data from the coagulation part of the PROCAM study22 showed that the

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**TABLE 5. Absolute and Relative Numbers of Smokers and Nonsmokers Distributed by Coronary Events and Tertiles of Fibrinogen Level**

<table>
<thead>
<tr>
<th>Fibrinogen</th>
<th>Nonsmoker</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Middle</td>
</tr>
<tr>
<td>Without event</td>
<td>576</td>
<td>484</td>
</tr>
<tr>
<td>With event</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Individuals with event, %</td>
<td>1.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Cutting points for tertiles of fibrinogen level were 2.36 and 2.77 g/L.
mean factor VIIc activity in the coronary event group (n=15) was 10% higher compared with the group without an event (n=1659; P=.07). This trend toward higher factor VIIc activity for all CHD events was confirmed in the present study as well as having been shown after 5 years of observation in the NPH study (117.4% in event versus 107.0% in non-event cases; P<.001). When only fatal events were taken into account, the difference was more pronounced in both studies: 123.9% (SD, 27.7) versus 107.0% (SD, 25.1), P<.001, in the NPH study and 117.0% (SD, 11.7) versus 108.4% (SD, 21.6), P=.06, in the PROCAM study.

To evaluate possible differences of methodology, a laboratory comparison has been performed that includes the factor VII assays of the NPH, Atherosclerosis Risk in Communities, and PROCAM studies. Plasma samples enriched with activated factor VII (factor VIIa) expressed a proportionately greater factor VIIc in the NPH assay using mixed human and bovine thromboplastin than in the Münster pure human system. It could also be shown that the converse held in samples that were relatively deficient in factor VIIa. These differences in test results may be important if part of the clear association of the risk of CHD with factor VII activity, as found in the NPH study, is due to the presence of increased circulating levels of factor VIIa.

Based on our results, fibrinogen should be considered as a powerful independent risk factor of CHD and should be taken into account in CHD risk algorithms. In other words, the individual prediction of coronary risk may be markedly improved by applying risk scores that include plasma fibrinogen.

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**Appendix**

Members of the Critical Event Committee included K. Kochsieck, MD, Würzburg; B.E. Strauer, MD, Düsseldorf; U. Gleichmann, MD, Bad Oeynhausen; and R. Uebis, MD, Aachen, FRG.

**References**


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