Relation of Plasma Coagulation Factor VII and Fibrinogen to Carotid Artery Intima-Media Thickness

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Summary

Plasma clotting factor VII and plasma fibrinogen have been claimed as independent risk factors for occlusive cardiovascular disease. The aim of this study was to assess the effect of these coagulation parameters on early atherosclerosis, additional to their possible effect on arterial thrombosis.

Introduction

A relation between high levels of coagulation factors as factor VII and fibrinogen and the risk of ischemic heart disease has been shown in several studies. Therefore, coagulation parameters have been suggested to be independent risk factors for occlusive cardiovascular disease (1–4). Oclusive cardiovascular disease generally originates from thrombus formation on an atherosclerotic plaque. It is the result of a decades-long chronic atherosclerotic process, combined with the acute phenomenon of arterial thrombosis (5). The clotting factor level may have an effect on the progression of atherosclerosis, on arterial thrombosis, or on both.

Materials and Methods

Study Design

We studied 121 healthy volunteers, aged 19–56 years. They were all free of clinical signs of cardiovascular disease, diabetes mellitus or any other chronic disorder, and did not use anticoagulants or lipid-lowering drugs. Use of oral...
contraceptives was not an exclusion criterion. We measured intima media thickness (IMT) of the carotid arteries, classical cardiovascular risk factors and plasma levels of factor VII and fibrinogen. In addition, we determined polymorphisms of the factor VII and fibrinogen genes.

**Assessment of Carotid Intima-media Thickness**

Ultrasoundographic scanning of the carotid arteries was performed with the Aloka Echo Camera SSD 650 equipped with a high density linear array probe with 7.5 MHz transducer frequency in B mode. The axial resolution was at least 0.3 mm. The subjects were lying in a supine position with the head slightly extended and rotated 45 degrees away from the side which was scanned. Scanning was performed in the anteposterior plane, imaging the carotid bifurcation and the common carotid artery. Three B-mode images of the left and right common carotid artery were frozen at peak diastole on sight, and recorded on a SVHS video cassette tape. Images were coded to ensure that later intima media thickness (IMT) measurements were performed blinded for subjects' identity, coagulation factor levels or risk factor status.

Intima media thickness was measured later on in one session for all subjects with the Cardiovascular Measurement System (CMS) (12). Frozen images were digitized at a resolution of 512 × 512 pixels with 8 bits of grey level. Calibration of the images was performed by manually identifying a 4 cm distance on a cm scale, typical pixel size in the non-magnified mode was 0.1 mm. A one-centimeter strip of the posterior carotid wall, one centimeter proximal to the carotid bifurcation was enlarged four times by cubic spline interpolation. Next, six intima-media thickness measurements were performed over this one centimeter range at 6 measurement sites in each recording. The thickness of the intima-media complex as defined by Pegoraro (6) was measured as the distance between the lumen intima interface and the media-adventitia interface on the B-mode image. The actual measurement at each of these six sites was carried out with the digital caliper on the CMS. The intima-media thickness of each of the six measurement sites was determined by manually defining the right and left intima-media thickness at each of these six distance measures. This multiple measurement approach results in a high degree of accuracy and precision. In this way, in each subject 36 intima-media thickness measurements (6 measurements × 6 images) were performed, the average value of these 36 measurements was defined as the intima media thickness (IMT) in each subject.

**Other Measurements**

Information on subject’s smoking habits (current smoking, package years of cigarette smoking), and presence of cardiovascular disease in first-grade relatives before the age of 60 years (history of myocardial infarction, intermittent claudication) were obtained by means of a questionnaire. Family history was coded as either negative or positive. Body mass index was calculated as Quetelet index (wt/ht² in kg/m²). Systolic and diastolic blood pressure were measured three times in each subject before venepuncture with a Hawksley random zero mercury sphygmomanometer after a minimum of ten minutes of rest. The mean was used in the analysis.

In fresh blood samples total serum cholesterol and HDL serum cholesterol concentration were determined by standard enzymatic assays, against the WHO standard. Plasma fibrinogen concentration and plasma clotting factor VII activity were determined by the methods according to Claus (13) (fibrinogen, g/l) and Owren (14) (factor VII, % of standard). Polymorphisms of the β-chain of fibrinogen and of factor VII were determined with the use of HaellI and Mspl restriction enzymes as reported by Thomas (10) and Green (11). The alleles with the restriction site were designated H1 (fibrinogen) and M1 (factor VII), and the non-cleavable alleles were designated H2 and M2.

**Statistical Analysis**

We analyzed the relation of the factors of interest with carotid wall thickness by linear regression techniques. The regression coefficient obtained by this method, indicates the increase (or decrease, dependent on the sign of the coefficient) in intima media thickness per unit increase in the factor studied. All continuous variables (e.g., blood pressure, cholesterol, age) were entered into the regression equation as such, and therefore the coefficients indicate the change in wall thickness (in μm) per one mmHg increase in blood pressure, one mmol/l increase in cholesterol and one year increase in age. To facilitate interpretation and comparison between variables we calculated the change in intima media thickness for meaningful increments of the independent variables, e.g. 10 mmHg for blood pressure, 1 mmol/l for cholesterol, 10 years for age and 10 package-years for smoking. The original regression coefficients can easily be derived by dividing the change in IMT by 10 for blood pressure, age and pack-years. Discrete variables were coded as 0 or 1. In this instance, the regression coefficient indicates the difference in intima media thickness between the two categories.

Since several of the variables may be associated, we subsequently set up a multivariate model which yields mutually adjusted regression coefficients.

**Results**

**Baseline Values and Relations between Determinants**

Baseline characteristics in our study showed the expected mean values for healthy subjects, with a broad range that facilitated the evaluation of possible effects of risk factors (Table 1).

The average carotid intima media thickness was 519 μm (SD 72, range 397–781 μm). Left and right intima media thickness showed a Pearson correlation coefficient of r = 0.83, with average values of 521 μm and 517 μm, respectively.

Several of the determinants were correlated. Factor VII plasma level was associated with sex (mean 99.4% in men, and 111.4% in women), and was positively correlated with the total cholesterol level (regression coefficient 0.64% FVII per mmol/l cholesterol) and fibrinogen level (regression coefficient 15.8% FVII per g/l fibrinogen). Fibrinogen showed, in addition to its association with the Factor VII level, increasing values with age (regression coefficient 0.13 g/l per 10 yrs). Systolic and diastolic blood pressure were higher in men than in women (mean SBP 124 mmHg in men, and 117 mmHg in women; mean DBP 81 mmHg in men, and 76 mmHg in women), and in the obese (regression coefficient 1.6 mmHg systolic and 1.6 mmHg diastolic per kg/m²).

**Clotting Factor Gene Polymorphism and Clotting Factor Plasma Levels**

The Mspl polymorphism of factor VII had an allele-frequency for the M2 allele of ten percent, 18 percent of the individuals were carriers.
The HaeIII fibrinogen polymorphism did not fulfill our expectations in this respect.

**Relation of Determinants and Vessel Wall Thickness**

The intima-media thickness of the carotid artery (mean of 18 measurements at each side), was higher for men than for women, and increased with age, blood pressure (both systolic and diastolic), total serum cholesterol, cholesterol/HDL ratio and Quetelet-index, whereas it decreased with HDL-cholesterol (Table 3). No relation with package-years of smoking, nor with a positive family history of cardiovascular disease was found.

The intima-media thickness increased with higher levels of fibrinogen, whereas the fibrinogen polymorphism showed a strong trend of association with intima-media thickness, albeit in the unexpected direction (lower IMT for carriers of the H2-allele). Intima-media thickness was not related to factor VII activity, nor to the factor VII polymorphism genotype (Table 3). Scatterplots of factor VII and fibrinogen versus intima-media thickness with the regression lines are depicted in figure 1. It may be noted that the association of IMT with fibrinogen levels (20.7 μm per 1 g/l increase in fibrinogen) was to a high extent determined by one outlying observation. Leaving out this point (fibrinogen 4.26 g/l, IMT 752 μm) reduced the slope of the regression line to 13.7 μm per 1 g/l (CI95 -8.5 to 36.0 μm per g/l increase in fibrinogen)

**Multivariate Analysis**

Since several of the determinants appeared to be interrelated, we performed a multivariate regression analysis. The regression coefficients of this analysis are to be interpreted similarly to those of a simple univariate regression, but now the effects of other variables are adjusted for.

When all classical determinants were entered into the model, age, blood pressure, total cholesterol and HDL-cholesterol emerged as independent determinants of intima-media thickness. When diastolic blood pressure was entered in the analysis instead of systolic blood pressure, the diastolic regression coefficient became 1.10 μm/10 mmHg (95% CI -0.90 to 22.9). The effect of sex was attenuated as was that of fibrinogen, whereas the Quetelet index had no independent effect, nor had factor VII plasma levels. In this analysis, as in univariate analysis, no effect of smoking or a positive family history could be observed (Table 4).

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**Table 2**  Factor VII and fibrinogen polymorphisms and plasma levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Perc of subjects</th>
<th>Mean clotting factor level (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VII</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1M1</td>
<td>99</td>
<td>82%</td>
<td>109 6% (26 0)</td>
</tr>
<tr>
<td>M1M2</td>
<td>19</td>
<td>16%</td>
<td>85 1% (17 5)</td>
</tr>
<tr>
<td>M2M2</td>
<td>3</td>
<td>2%</td>
<td>82 3% (6 4)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1H1</td>
<td>86</td>
<td>71%</td>
<td>2 88 g/l (5 8)</td>
</tr>
<tr>
<td>H1H2</td>
<td>33</td>
<td>27%</td>
<td>2 86 g/l (5 5)</td>
</tr>
<tr>
<td>H2H2</td>
<td>2</td>
<td>2%</td>
<td>3 60 g/l (7 4)</td>
</tr>
</tbody>
</table>

1Increment compared to H1H1 genotype

2Increment compared to M1M1 genotype

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**Table 3**  Multivariate analysis of risk factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change in IMT (μm)</th>
<th>95%-confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (10 yrs)</td>
<td>37.7</td>
<td>25.4 to 50.2</td>
</tr>
<tr>
<td>Sex (m = 0, f = 1)</td>
<td>-28.6</td>
<td>-54.0 to -3.2</td>
</tr>
<tr>
<td>Systolic BP (10 mmHg)</td>
<td>17.9</td>
<td>9.5 to 26.3</td>
</tr>
<tr>
<td>Diastolic BP (10 mmHg)</td>
<td>21.6</td>
<td>9.8 to 33.8</td>
</tr>
<tr>
<td>Serum cholesterol (1 mM)</td>
<td>23.2</td>
<td>12.2 to 34.2</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (1 mM)</td>
<td>-21.0</td>
<td>-51.7 to 9.7</td>
</tr>
<tr>
<td>Cholesterol/HDL-cholesterol ratio</td>
<td>17.5</td>
<td>9.0 to 6.0</td>
</tr>
<tr>
<td>Smoking (10 package years)</td>
<td>6.6</td>
<td>-8.8 to 22.0</td>
</tr>
<tr>
<td>Quetelet index (1 kg/m²)</td>
<td>6.6</td>
<td>2.2 to 11.0</td>
</tr>
<tr>
<td>Fam history (neg = 0, pos = 1)</td>
<td>-13.8</td>
<td>-47.9 to 20.3</td>
</tr>
<tr>
<td>Plasma fibrinogen (1 g/l)</td>
<td>20.7</td>
<td>-1.6 to 43.0</td>
</tr>
<tr>
<td>Factor VII activity (10%)</td>
<td>2.0</td>
<td>-2.9 to 6.9</td>
</tr>
<tr>
<td>Fibrinogen polymorphism H1H2/H2H2</td>
<td>-25.6</td>
<td>-53.7 to 2.6</td>
</tr>
<tr>
<td>Factor VII polymorphism M1M2/M2M2</td>
<td>-7.8</td>
<td>-41.4 to 25.7</td>
</tr>
</tbody>
</table>

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**Fig 1** Scatterplot of fibrinogen (left) and factor VII (right) with intima-media thickness. The regression line is indicated in the figures (fibrinogen 459 + 20.7 × fibrinogen level, factor VII 498 + 20 × factor VII level)
Discussion

We performed a cross sectional study to assess whether ultrasound measurement of carotid artery wall thickness could be of value in investigating the role of clotting factor levels in the development and progression of atherosclerosis.

Intima-media thickness was clearly associated with several classical cardiovascular risk factors like age, sex, systolic and diastolic blood pressure, total and HDL serum cholesterol. All these associations were positive or negative according to the expected (15) effect of the risk-factor on atherosclerosis. Although the effects themselves are small, the associations are more than striking, since they were observed in a small study of a heterogeneous group of mostly young individuals. We therefore conclude that ultrasound assessment of carotid intima-media thickness offers itself as a valuable research tool for non-invasive measurement of early atherosclerosis in healthy young and middle-aged subjects, as it is applied in studies with elderly or symptomatic subjects.

It is notable that at the relative young age of our subjects, alterations were already visible in the arterial wall wall, in a clear association with the presence or absence of cardiovascular risk factors.

Information from the literature about IMT in young healthy individuals is very scarce. When we compare our results to those of the healthy control groups in two other studies (7, 16), the mean intima-media thicknesses are very similar (about 0.5 mm), as is the age relation compared to two studies that also included young subjects (6, 17). Interestingly, a much higher mean IMT of 1 mm was found in the Kuopio Ischaemic Heart Disease Risk Factor Study (8), which is conducted in an area with a very high incidence of coronary artery disease (eastern Finland). No comparison can be made on the effects of risk factors on intima-media thickness in young subjects, as no such studies could be found in the literature.

We did not find associations of artery wall thickness with cardiovascular family history, nor with smoking, which is not in accordance with several other studies in which an effect of smoking on IMT had been found (7, 16). Although it has to be borne in mind that most studies on IMT, including ours, have been quite small which may easily have led to differences due to chance variation, it cannot be ruled out that these well-established risk factors for atherosclerosis may have a delayed effect, which will therefore not yet be apparent in young individuals.

Our study showed no association of the level of factor VII, or its polymorphism with intima-media thickness. Even in view of the limited number of subjects, an important effect of factor VII seems unlikely from these data. This is less clear for fibrinogen. Univariate analysis indicated a relation of fibrinogen with intima-media thickness that was (per 1 g/l) of about the same magnitude as for serum cholesterol (per 1 mM). This association largely disappeared in the multivariate analysis, which may be the result of correlations with other variables, especially age, on the other hand, it cannot be ruled out that part of the effect of age is mediated by fibrinogen. Finally, the confidence intervals for the association of fibrinogen levels and artery wall thickness remained wide, and do not allow firm conclusions.

The fibrinogen polymorphism had no relation with plasma fibrinogen levels in these 121 Dutch volunteers, whereas it was a clear predictor of fibrinogen levels in two studies among British and French individuals (9, 18). Recently, we also failed to find this association in an independent sample consisting of 199 patients with deep venous thrombosis and 199 healthy controls (19). Apparently, the association between this polymorphism and fibrinogen levels is not universal. We did find a relation between the Haem polymorphism and IMT, which was in the unexpected direction (i.e., lower IMT in carriers of the H2-allele). Obviously, since the polymorphism and the fibrinogen level were not associated, this effect cannot be interpreted as mediated by the plasma level. Still, this finding provokes thought, especially given our previous study on venous thrombosis (40 percent reduction of risk in H2-carriers) (19), and the results of the ECTIM study (10–20% reduction of myocardial infarction risk for those who carried the H2-allele) (18).

These findings suggest that factor VII and fibrinogen have no detectable influence on early atherosclerosis. Obviously, our study offers no information of the effect of clotting factor levels in advanced atherosclerosis with plaque formation. We have studied healthy volunteers with clotting factor levels that generally were in the usual range. We cannot exclude therefore, that extreme values of factor VII or fibrinogen (either at the lower or upper extreme of the spectrum) may have a noticeable and relevant effect on atherosclerosis.

Our method of measuring atherosclerosis can be extended to other superficial arteries such as the femoral or popliteal, although it is likely that for research purposes measurements at the carotid arteries are sufficient for an adequate overall picture (20). We believe that automatic (off-line) detection of intima-media boundaries and contours could further improve the accuracy and precision of our measurements. In addition, arterial vessel wall compliance may offer additional information in describing atherosclerosis.

Intima-media measurements are valuable in young and middle-aged subjects, to study the determinants of atherosclerosis in its earliest stages. Our study offers no support for the hypothesis that the clotting factor system is involved in the development and progress of early atherosclerosis.

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