Relation Between Hemostatic Variables and Increase of Common Carotid Intima-Media Thickness in Patients With Peripheral Arterial Disease

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Background and Purpose. Increases in common carotid intima-media thickness (CC-IMT), as measured by B-mode ultrasonography, have been widely used in both population studies and clinical trials in the search for risk factors for early atherosclerosis progression and have been found to correlate with age and with high concentrations of low-density lipoprotein cholesterol, leucocytes, and hemoglobin. We have now investigated the relation between several baseline hemostatic and conventional risk factors and CC-IMT changes over 16 months in 64 patients with peripheral arterial disease randomly selected from the prospective PLAT study series.

Methods. Samples from 24 patients (37.5%) who showed increases in CC-IMT during the follow-up period were compared with those from 40 (62.5%) in whom CC-IMT remained unchanged.

Results. Baseline conventional risk factors and coagulation variables were similar in the two groups except for higher plasma concentrations of von Willebrand factor (vWF) (178.3±53.6% versus 141.2±53.7%, P=0.01) and factor VII (FVII) (133.9±36.4% versus 107.0±27.3%, P=0.01) in the patients with increased CC-IMT. CC-IMT increase correlated positively with plasma levels of FVII (r=0.31, P<0.01) and vWF (r=0.31, P<0.01). Multiple stepwise regression analysis identified FVII as the only independent variable associated with an increase in CC-IMT (β=0.33, P=0.01).

Conclusions. High plasma concentration of FVII and vWF may be associated with the progression of early carotid atherosclerosis in patients with peripheral arterial disease. (Stroke. 1996;27:450-454.)

Key Words. Carotid arteries, hemostasis, lipids, risk factors, arteriosclerosis

An increasing body of evidence indicates that hemostatic factors play a role in ischemic arterial disease. Plasma fibrinogen, FVII, factor VIII, and vWF have all been shown to be associated with atherothrombotic events in prospective studies, but whether hemostatic factors are associated only with the thrombotic component of cardiovascular disease or also with the atherosclerotic process is still debated.

Ultrasonographically determined CC-IMT is a useful method to study early atherosclerosis and has been extensively validated by both direct and indirect methods. CC-IMT has been found to correlate positively with the presence of atherosclerotic plaques in the carotid and femoral regions and with coronary artery disease as well as or better than conventional risk factors. Moreover, it has been associated with major cardiovascular risk factors, such as age, hypertension, hypercholesterolemia, and smoking, but only one hemostatic factor, fibrinogen, has been shown to be associated with atherothrombotic events in a population with a history of ischemic disease in at least one of three main arterial regions. It also included a pilot study of CC-IMT changes in a subgroup of patients with PAD, which we report here.

Subjects and Methods

Patients. From March 1986 to December 1987, 953 patients with documented coronary, cerebral, or peripheral atherosclerotic disease were enrolled and studied at six specialized university and hospital centers in the Milan area. The design, organization, and preliminary and principal results of the PLAT study have been described elsewhere. As established in the study protocol, 75 of the 335 PAD patients, recruited in two centers, were randomly selected for carotid B-mode ultrasonography to be performed at baseline and at the end of the follow-up period.
Complete ultrasonographic reassessment was available for 64 patients (85% of those eligible).

PAD was classified as Fontaine stage IIA (intermittent claudication, >1 block) in 42 patients, IIB (intermittent claudication, <1 block) in 21, and III (rest pain) in 1. Table 1 shows the baseline demographic and clinical characteristics. No restrictions were placed on drugs or diet. Oral informed consent was obtained from all patients.

Risk Factors and Blood Analysis

The risk factors evaluated in this study included body mass index (weight [kilograms] divided by height [meters] squared); family history of ischemic vascular disease (defined as positive if a first-degree relative had a coronary, cerebral, or peripheral atherosclerotic event); hypertension (defined as a history of >160 mm Hg systolic or >95 mm Hg diastolic blood pressure and/or current use of antihypertensive agents); diabetes mellitus (defined as a history of diabetes and/or current use of oral hypoglycemic agents or insulin); current smokers (patients who had been regular smokers for at least the previous 3 months); and plasma concentrations of lipids and lipoproteins and hemorrhagic factors. Blood samples for all tests were drawn by clean venipuncture without stasis with a 20-gauge needle: from the antecubital vein between 8 and 9:30 AM after the subject had rested supine for 10 minutes. For hemostatic tests, blood was collected into 3.8% trisodium citrate (9:1 vol/vol) and immediately centrifuged at 1250g for 15 minutes at room temperature. The supernatant plasma was snap-frozen and stored in small aliquots at −80°C until assayed. For lipid assays, samples were collected into disposable plastic tubes; serum was separated after coagulation and stored at −80°C. Within 2 weeks, frozen plasma samples were transported in polystyrene containers with adequate amounts of dry ice to two central laboratories, one of which performed the coagulation tests and the other the lipid assays. Transport took no longer than 90 minutes. Tubes containing plasma were inspected on arrival to ensure that the sample had not thawed and were then stored at −80°C. In the original FLAT study, eight samples of FVII levels greater than 200%, suggesting cold activation during transport, were discarded. At the time of assay, the frozen samples were transferred to a 37°C water bath and then handled at room temperature.

Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined by enzymatic methods. High-density lipoprotein cholesterol was separated by selectivity precipitation of apolipoprotein B-containing lipoproteins with deheparin-sulfate magnesium chloride. Low-density lipoprotein cholesterol levels were calculated according to Friedewald's formula.

Fibrinogen level was determined as described by Clauss with the use of fibrinogen reagent (Boehringer Mannheim). FVII and factor VIII coagulant were determined by a one-stage biological assay in which reagents and factor-deficient plasmas from Instrumentation Laboratory were used; reference curves were prepared daily with an international standard pool, obtained from 150 healthy blood donors and snap-frozen and stored in liquid nitrogen, which was used throughout the study. Antithrombin III level was measured by a kinetic chromogenic method (Coatest Antithrombin, Ortho), and the results were expressed as percentages of the international standard plasma pool.

Ultrasonic Imaging and Image Analysis

The B-mode measurements here reported were obtained from far-wall echoes of the two common carotid arteries. B-mode imaging was centralized and all initial and follow-up scans and measurements were done with a single sonograph and by a single observer who was blinded about the results of blood analysis.

Real-time B-mode imaging of extracranial carotid arteries was performed with the use of a Biosound echotomographic system (model Phase-one, Bio Dynamics) connected to a probe that generates a wide-band ultrasound pulse with midfrequency of 8 MHz. This instrument gives axial and lateral resolutions of approximately 0.385 and 0.500 mm, respectively. The images obtained were recorded on VHS videotapes and processed by a computer-assisted technique.
Table 2. Far-Wall CC-IMT at Baseline and After 16-Month Follow-up of PAD Patients

<table>
<thead>
<tr>
<th>Far-Wall CC-IMT</th>
<th>Baseline (n=64)</th>
<th>Follow-up (n=64)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>0.80±0.16</td>
<td>0.82±0.18</td>
<td>.002</td>
</tr>
<tr>
<td>Left</td>
<td>0.88±0.26</td>
<td>0.93±0.31</td>
<td>.012</td>
</tr>
<tr>
<td>Mean</td>
<td>0.85±0.19</td>
<td>0.89±0.22</td>
<td>.001</td>
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</tbody>
</table>

Values are mean±SD, expressed in millimeters. Comparison was performed by paired Student's t test.

The individual subject's CC-IMT values of each carotid were calculated as the quotient of the sum of the areas (A) of different sectors and sum of the lengths (L). For the anterior projection in which "a" sectors are visualized, the CC-IMT was calculated as follows:

$$(A1 + A2 + A3 + ... + An)/(L1 + L2 + L3 + ... + Ln)$$

Values of the different projections (anterior, lateral, and posterior) were averaged, and measurements of left and right carotid arteries were also combined to obtain mean wall thickness.

Mean CC-IMT = (Left CC-IMT + Right CC-IMT)/2

Measurements of area and length were made with a graphic table equipped with appropriate software (graphic table software). In previous studies the reproducibility of the IMT measurement determined on two scans performed by the same sonographer (D.B.) on 14 subjects 2 weeks apart was evaluated, giving a correlation of 0.94. Videotape images and IMT measurements were processed by another observer under blinded conditions. The difference between the first and second measurements ranged from 0.01 to 0.18 mm (0.023±0.088 mm [SD]). The accuracy and reproducibility of CC-IMT determination were 4.5% and 5.0%, respectively (10 Patients who at the end of the follow-up presented an increase of mean CC-IMT compared with the baseline value of more than 3% were referred to as progressors. Patients in whom no change or even a decrease in mean CC-IMT was detected were referred to as nonprogressors.

Statistical Analysis

Whether the distribution of hemostatic and biochemical variables was normal was ascertained by establishing the index of kurtosis and skewness and comparing the frequencies of distribution of the expected versus observed data with the use of the $\chi^2$ test. Means and SD values were used when variables were normally distributed, but the distribution of most variables was not normal and the two patient groups, which differed in size, were compared by nonparametric statistics (Mann-Whitney U test). Sonographic findings were compared between subgroups by one-way ANOVA and Student's t test; baseline values and those at the end of follow-up were compared by paired Student's t test. Differences in categorical variables were analyzed by the $\chi^2$ test. Correlation was determined by the nonparametric Spearman correlation. Multiple stepwise regression analysis was applied to determine the relative importance of the independent variables.

Results

In the total group of patients, far-wall thickness of left and right common carotid arteries and mean CC-IMT increased significantly during the 16-month follow-up (Table 2). CC-IMT changes of the left and right arteries were similar in magnitude, as were the relations between IMT changes and lipid or hemostatic variables in the two arteries, so that analyses were henceforth based on the mean IMT.

The patients were divided into progressors (increase in CC-IMT >5%, ie, 0.11±0.08 mm [SD]; n=24) and nonprogressors (increase in CC-IMT <5%, ie, -0.01±0.04 mm [SD]; n=40). No difference was found in any demographic or clinical characteristics or conventional risk factors between the two groups of patients (Table 3). They were also similar in terms of drug treatment, with the exception of two patients in the progressor group who were receiving oral anticoagulants and were therefore excluded from the analysis of FVII and protein C.

When baseline lipid levels and hemostatic variables were compared, there were no significant differences between the two groups except in vWF and FVII, which were higher in progressors (Table 4). The association between lipid or hemostatic variables and CC-IMT increase was investigated by multivariate and multiple stepwise regression analysis. Baseline CC-IMT values correlated positively with triglycerides ($r=.24$; $P<.05$), whereas CC-IMT increase correlated with plasma FVII ($r=.32$; $P<.01$) and vWF levels ($r=.31$; $P<.01$) and to a lesser extent triglycerides ($r=.23$; $P=.06$). Finally, plasma FVII correlated with vWF ($r=.26$; $P<.05$).

Multiple stepwise regression analysis, in which CC-IMT change was used as the dependent variable and independent variables were those listed in Table 4, identified FVII as the only independent variable significantly associated with increased CC-IMT ($\beta=.33$; $P=.01$), accounting for 10% of the variability in the extent of CC-IMT progression ($R^2=.10$).

Discussion

The prospective design of the PLAT study allowed us to study CC-IMT in selected patients with PAD over time. At entry CC-IMT was related only to plasma triglyceride levels, a lipid variable now emerging as a potential predictive risk factor in cardiovascular dis-

Table 3. Baseline Clinical Characteristics and Conventional Risk Factors in Patients With (Progressors) and Without (Nonprogressors) Increase in CC-IMT at End of Follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonprogressors (n=40)</th>
<th>Progressors (n=24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.7±5.6</td>
<td>58.8±6.4</td>
<td>.95</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.5±2.2</td>
<td>25.0±2.9</td>
<td>.50</td>
</tr>
<tr>
<td>Ankle/arm pressure index</td>
<td>77.1±29.7</td>
<td>84.0±17.9</td>
<td>.30</td>
</tr>
<tr>
<td>Family history of ischemic vascular disease</td>
<td>17 (42.9)</td>
<td>12 (50)</td>
<td>.75</td>
</tr>
<tr>
<td>History of or current hypertension</td>
<td>18 (45.5)</td>
<td>8 (33.3)</td>
<td>.83</td>
</tr>
<tr>
<td>History of or current diabetes mellitus</td>
<td>5 (12.5)</td>
<td>3 (12.5)</td>
<td>.70</td>
</tr>
<tr>
<td>Current smokers</td>
<td>25 (62.5)</td>
<td>12 (50)</td>
<td>.47</td>
</tr>
</tbody>
</table>

Continuous variables are mean±SD; values in parentheses are percentages.
Table 4. Baseline Lipid and Hemostatic Variables in Patients With (Progressors) and Without (Nonprogressors) Increase in CC-IMT at End of Follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonprogressors (n=40)</th>
<th>Progressors (n=24)</th>
<th>Total (n=64)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.21 ± 1.30</td>
<td>5.94 ± 0.97</td>
<td>6.11 ± 1.30</td>
<td>.02</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.31 ± 1.20</td>
<td>3.85 ± 0.87</td>
<td>4.13 ± 1.11</td>
<td>.10</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.05 ± 0.30</td>
<td>1.06 ± 0.25</td>
<td>1.05 ± 0.30</td>
<td>.95</td>
</tr>
<tr>
<td>Total/HDL-C</td>
<td>6.35 ± 2.46</td>
<td>5.91 ± 1.57</td>
<td>6.19 ± 2.19</td>
<td>.44</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.86 ± 1.36</td>
<td>2.28 ± 1.73</td>
<td>2.01 ± 1.84</td>
<td>.30</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43.5 ± 3.70</td>
<td>42.9 ± 3.04</td>
<td>43.5 ± 3.49</td>
<td>.31</td>
</tr>
<tr>
<td>Leukocytes, x10^3/μL</td>
<td>7.48 ± 1.9</td>
<td>7.29 ± 2.14</td>
<td>7.41 ± 1.99</td>
<td>.70</td>
</tr>
<tr>
<td>FVIII, %</td>
<td>107.0 ± 27.3</td>
<td>133.8 ± 36.4</td>
<td>117.3 ± 33.7</td>
<td>.001</td>
</tr>
<tr>
<td>FVIII:C, %</td>
<td>128.2 ± 51.2</td>
<td>152.5 ± 35.9</td>
<td>145.5 ± 46.7</td>
<td>.24</td>
</tr>
<tr>
<td>vWF, %</td>
<td>141.2 ± 53.7</td>
<td>178.3 ± 53.6</td>
<td>154.7 ± 50.5</td>
<td>.01</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>264.2 ± 59.4</td>
<td>297.0 ± 58.8</td>
<td>288.9 ± 59.5</td>
<td>.41</td>
</tr>
<tr>
<td>Antithrombin III, %</td>
<td>103.9 ± 14.2</td>
<td>103.3 ± 15.0</td>
<td>103.7 ± 14.5</td>
<td>.07</td>
</tr>
<tr>
<td>Protein C, %</td>
<td>110.7 ± 24.7</td>
<td>121.8 ± 28.7</td>
<td>114.8 ± 28.7</td>
<td>.10</td>
</tr>
</tbody>
</table>

LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and FVIII:C, factor VIII coagulant. Values are mean ± SD.

In conclusion, the finding that the association between CC-IMT progression and the risk factors for atherosclerosis was significant, underscores the importance of identifying these factors as potential targets for intervention. The presence of these risk factors may indicate a higher risk for future cardiovascular events in patients with PAD. Further research is needed to better understand the mechanisms underlying the association between these risk factors and CC-IMT progression.
sequent fibrin formation (by lower[er]-intensity oral anticoagulation) could be effective in hindering the progression of atherosclerotic disease.

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References