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, leukocytes, 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%, $P=.01$) and factor VII (FVII) (133.9±36.4% versus 107.0±27.3%, $P=.001$) in the patients with increased CC-IMT. CC-IMT increase correlated positively with plasma levels of FVII ($r=.31$, $P<.01$) and vWF ($r=.31$, $P<.01$). Multiple stepwise regression analysis identified FVII as the only independent variable associated with an increase in CC-IMT ($\beta=.83$, $P=.01$).

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 • hemostatics • 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,[1-4] 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.[5-10] CC-IMT has been found to correlate positively with the presence of atherosclerotic plaques in the carotid and femoral regions[11,12] and with coronary artery disease as well as or better than conventional risk factors.[13-15] Moreover, it has been associated with major cardiovascular risk factors, such as age, hypertension, hypercholesterolemia, and smoking,[11,16-18] but only one hemostatic factor, fibrinogen.[18-20]

Even more interesting is the issue of the relation between risk factors and the progression of carotid atherosclerosis. Age; high values of low-density lipoprotein cholesterol, leukocytes, hemoglobin, and serum copper; low serum selenium; (log)triglycerides; and coronary artery disease have been found to be independent risk factors for increasing CC-IMT in population-based studies.[21-23] The involvement of low-density lipoprotein cholesterol[8,24,25] and hypertension[26] in carotid atherosclerosis progression has been confirmed in clinical trials investigating the effects of lipid-lowering or antihypertensive treatments. In contrast, no association has been found between such progression and fibrinogen levels in middle-aged women.[23]

The PLAT study is a prospective, multidisciplinary study designed to assess the association between hemostatic variables, conventional risk factors, extension of atherosclerosis, and the risk of atherothrombotic events in a population with a history of ischemic disease in at least one of three main arterial regions.[2,27] 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.[2,27] 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

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Selected Abbreviations and Acronyms

CC-IMT = common carotid intima-media thickness
FVII = factor VII
PAD = peripheral arterial disease
PLAT = Progetto Lombardo Atero-Trombosi
vWF = von Willebrand factor

(16 months). 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 and/or >95 mm Hg diastolic blood pressure and/or current use of antihypertensives); 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 hemostatic 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 1250*g* 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 PLAT study,[2] 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.

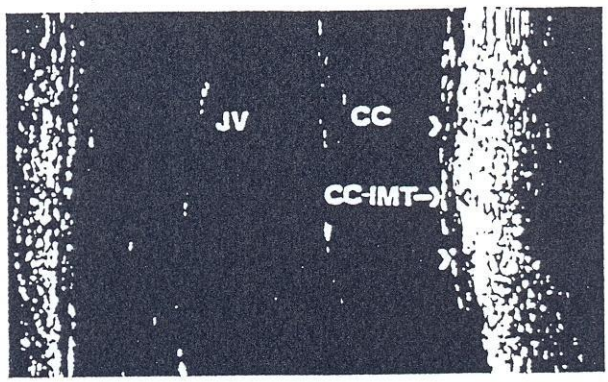
Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were determined by enzymatic methods. High-density lipoprotein cholesterol was separated by selective precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate magnesium chloride.[28,29] Low-density lipoprotein cholesterol levels were calculated according to Friedewald's formula.[30]

Fibrinogen level was determined as described by Clauss[31] 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 internal-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 internal-standard plasma pool. vWF and protein C levels were determined with commercial enzyme-linked immunosorbent assays (Boehringer Mannheim), and these results were also expressed as percentages of the internal-standard plasma pool. All tests were performed in duplicate by one member of the laboratory staff. Reagents from a single batch were used to avoid batch-to-batch variability.

TABLE 1. Baseline Characteristics of Patients

Men/women	61/3
Age, y	58.7±6.6
Body mass index, kg/m²	24.6±2.9
Family history of ischemic vascular disease	29 (45.3)
History of or current hypertension	21 (32.8)
History of or current diabetes mellitus	8 (12.5)
Current smokers	37 (57.8)
Drug treatment	
Lipid-lowering drugs	3 (4.68)
β-Blockers	3 (4.68)
Calcium antagonists	16 (25.0)
Diuretics	8 (12.5)
Anticoagulants	2 (3.12)
Antiaggregants	40 (62.5)

Continuous variables are mean±SD; values in parentheses are percentages.



B-mode imaging of common carotid artery (CC). JV indicates jugular vein.

Ultrasound Imaging and Image Analysis

The B-mode measurements here reported were obtained from far-wall echoes of the two common carotid arteries. B-mode scanning 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 a 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.

For CC-IMT measurement, the full length of the common carotid was analyzed from distal to proximal end, starting from the crest of the bifurcation but excluding the first centimeter distal to the flare of the carotid bulb. Each common carotid was examined in anterior, lateral, and posterior planes. As previously described,[5] ultrasound investigation of the far wall of the common carotid arteries reveals two parallel echogenic lines separated by an anechoic space (Figure). The mean distance between these two lines is a reliable index of the thickness of the intima-media complex.[5]

Since the instrument visualizes anatomic structures in a field approximately 2 cm long, to obtain complete visualization of each common carotid artery, for each projection two to four carotid sectors, depending on the length of the artery, were produced during the complete scan, and the mean wall thickness for the sectors was calculated.

TABLE 2. Far-Wall CC-IMT at Baseline and After 16-Month Follow-up of PAD Patients

Far-Wall CC-IMT	Baseline (n=64)	16-mo Follow-up (n=64)	P
Right	0.80±0.16	0.83±0.18	.002
Left	0.88±0.26	0.93±0.31	.012
Mean	0.85±0.19	0.89±0.22	.001

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 "n" sectors are visualized, the CC-IMT was calculated as follows:

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

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:

$$\text{Mean CC-IMT}=(\text{Left CC-IMT}+\text{Right CC-IMT})/2$$

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 .94. Videotape images and IMT measurements were processed by another observer under blinded conditions. The difference between the first and second measurements ranged from −0.14 to 0.18 mm (0.023±0.088 mm [SD]). The accuracy and reproducibility of CC-IMT determination were 4.6% and 5.0%, respectively.[6,10] Patients who at the end of the follow-up presented an increase of mean CC-IMT compared with the baseline value of more than 5% 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 χ^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 χ^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 ($\beta=.83$, $P=.01$), accounting for 10% of the variability in the extent of CC-IMT progression ($R^2=.1006$).

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

Variable	Nonprogressors (n=40)	Progressors (n=24)	P
Men/women	38/2	23/1	.35
Age, y	58.7±6.6	58.8±6.4	.95
Body mass index, kg/m²	24.5±2.8	25.0±2.9	.50
Ankle/arm pressure index	77.1±29.7	84.0±17.9	.30
Family history of ischemic vascular disease	17 (42.5)	12 (50)	.75
History of or current hypertension	13 (32.5)	8 (33.3)	.83
History of or current diabetes mellitus	5 (12.5)	3 (12.5)	.70
Current smokers	25 (62.5)	12 (50)	.47

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

Variable	Nonprogressors (n=40)	Progressors (n=24)	Total (n=64)	*P*
Cholesterol, mmol/L	6.21±1.30	5.94±0.97	6.11±1.20	.38
LDL-C, mmol/L	4.31±1.20	3.85±0.87	4.13±1.11	.13
HDL-C, mmol/L	1.05±0.30	1.06±0.26	1.05±0.28	.95
Total/HDL-C	6.35±2.46	5.91±1.57	6.19±2.19	.44
Triglycerides, mmol/L	1.86±1.56	2.28±1.73	2.01±1.64	.30
Hematocrit, %	43.9±3.70	42.9±3.04	43.5±3.49	.31
Leukocytes, $\times 10^3$/L	7.48±1.9	7.29±2.14	7.41±1.99	.70
FVII, %	107.0±27.3	133.9±36.4	117.3±33.7	.001
FVIII-C, %	138.3±51.2	152.5±35.9	143.5±46.7	.24
vWF, %	141.2±53.7	178.3±53.6	154.7±56.5	.01
Fibrinogen, mg/dL	284.2±59.4	297.0±58.8	288.9±59.5	.41
Antithrombin III, %	103.9±14.2	103.3±15.0	103.7±14.5	.87
Protein C, %	110.7±24.7	121.8±26.7	114.8±26	.10

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

ease.[32,33] During the 16-month follow-up a significant progression of carotid atherosclerosis occurred, as judged by increases in CC-IMT. Indeed, the mean increase of CC-IMT in the left and right carotid arteries was found to be approximately 0.0025 mm/mo, in line with the rate of carotid atherosclerosis progression recorded in the CLAS study over a 4-year follow-up.[8]

Of our 64 patients, carotid atherosclerosis progressed in 24, who also had higher baseline levels of vWF and FVII. Univariate regression analysis confirmed the relation between progression of carotid atherosclerosis and vWF, FVII, and to a lesser extent plasma triglycerides. At multiple regression analysis, only FVII was independently and significantly associated with increased CC-IMT; this might be explained by the significant correlation between FVII and vWF. The same associations were obtained if progressors were defined as patients showing a CC-IMT increase of >2 SD of the measurement error, although only 10 subjects fell into this category.

Earlier studies,[8,21,24-26] including one of ours,[6] documented a relation between plasma cholesterol levels or blood pressure and CC-IMT. In our group of PAD patients, however, no relation was found between these two risk factors and baseline CC-IMT or CC-IMT progression. The discrepancy may derive not only from the small sample size but also from the type of patients enrolled who usually do not present with hyperlipidemia or hypertension.

Data on the relation between CC-IMT and hemostatic variables are still limited. In a few studies fibrinogen has been shown to be the only hemostatic variable to correlate positively with echographically assessed carotid atherosclerosis,[18-20] but this association is still controversial[22,23,34-37]; we found no relation to either baseline CC-IMT or CC-IMT increase. This could be due to the method used to assess carotid atherosclerosis or the small numbers and type of population studied. In fact, PAD patients in the PLAT study showed an inverse relation between plasma fibrinogen levels and clinical events,[2] in contrast to patients after myocardial infarction or with cerebrovascular disease.

In agreement with larger cross-sectional studies,[18,20,35] we detected no relation between baseline FVII or vWF and baseline CC-IMT. Instead, these two variables correlated significantly with CC-IMT progression. This finding underlines the conceptual difference between cross-sectional and longitudinal studies, the latter allowing identification of variables reflecting the metabolic condition of patients during the period studied.

vWF is of interest not only as a marker of endothelial cell injury[38] but also because of its potential role in atherothrombosis; increased vWF has recently been related to major risk factors for this disease,[27,39] the extension of atherosclerosis,[4,27] and recurrence of ischemia in vascular disease patients.[2-4]

FVII is a vitamin K–dependent clotting factor synthesized principally in the liver. The active enzyme factor VIIa, generated by limited proteolysis of FVII, binds to tissue factor expressed on subendothelium and activated monocytes/macrophages. The factor VIIa–tissue factor complex initiates coagulation by limited proteolysis of factors IX and X,[40] thereby boosting the conversion of prothrombin to thrombin and of fibrinogen to fibrin. In current epidemiological and clinical studies, FVII plasma levels are considered a reliable index of hypercoagulability.[1,41] The association of FVII with an increase in CC-IMT reported here provides evidence of the potential role of hypercoagulability in atherosclerosis progression. However, multiple regression analysis of the full PLAT data set failed to demonstrate an association between FVII and ischemic events in PAD patients.[2] Such an association with progression of atherosclerosis but not atherothrombotic events may indicate that FVII is involved more with atherogenesis than the acute thrombotic component of vascular ischemic disease.

In the present study of a selected group of PAD patients, FVII and vWF proved predictive of carotid atherosclerosis progression. The limited sample size and lack of restrictions regarding medical treatment, which could have had a confounding effect on outcome measurements, might have represented a limitation to the study. However, the percentage of patients taking lipid-lowering drugs was negligible (4.7%), the two patients on oral anticoagulant treatment were excluded from the FVII and protein C analysis, and patients taking calcium antagonists were equally distributed in the two groups. We therefore tentatively suggest that blocking the action of circulating vWF (perhaps by interfering with its activity in aggregating platelets and/or mediating their adhesion to subendothelium) and/or FVII and conse-

quent fibrin formation (by low[er]-intensity oral anticoagulation) could be effective in hindering the progression of atherosclerotic disease.

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