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E-selectin and TFPI are associated with carotid intima-media thickness in stable IHD patients: The baseline findings of the MIAMI study

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Abstract *Objective:* MIAMI was a prospective multicenter clinical study designed to investigate the relationship between changes in carotid intima-media thickness (C-IMT) and those in the levels of circulating markers of inflammation, thrombosis and endothelial dysfunction. The study was performed in a group of stable coronary patients treated for two years with a moderate dosage of atorvastatin (20 mg/day). In this paper the cross-sectional relationship between C-IMT and the same circulating markers of inflammation, thrombosis and endothelial dysfunction measured at baseline was investigated.
Methods: Eighty-five subjects that had not used statins for at least two months were enrolled in the study. At time of enrollment, the levels of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), E-selectin, interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, high-sensitivity C-reactive protein (hs-CRP), tissue factor (TF), tissue factor pathway inhibitor (TFPI), von Willebrand factor (vWF), fibrinogen, total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and triglycerides were measured, in parallel with C-IMT assessment.

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Results: In cross-sectional analyses, markers of endothelial perturbation (i.e. E-selectin) and TFPI were more strongly correlated with atherosclerotic burden than markers of inflammation. The baseline picture in this study indicates that E-selectin and TFPI are linked with atherosclerotic burden.

Introduction

Inflammation plays an essential role in the initiation and progression of atherosclerosis [1]. A variety of markers of the systemic inflammatory response have been proposed to predict cardiovascular events in apparently healthy subjects and in patients with unstable angina or a history of previous myocardial infarction [2]. The inflammatory response primarily involves endothelial activation, with the release of a range of pro-inflammatory mediators and thrombotic factors into the circulation, reflecting endothelial dysfunction, which in turn may contribute to the propagation of vascular damage [3].

The association between plasma levels of inflammatory markers and coronary disease is further confirmed by the fact that many of them are important components of the key mechanisms underlying atherogenesis and its complications. Moreover, the net benefit of anti-atherosclerotic drugs such as statins, the most powerful lipid-lowering drugs available to date, has been at least partly attributed to their influence on the pro-inflammatory/prothrombotic pathways involved in atherothrombosis [4]. However, no single marker has been found to serve as the 'gold standard' against which to evaluate endothelial dysfunction, and epidemiological data to establish the association between a cluster of plasma levels of soluble markers and the risk of cardiovascular disease (CVD) are sparse or contradictory [5—8].

Intima-media thickness (IMT) is considered a marker of early morphological changes in extracranial carotid arteries. Carotid IMT (C-IMT) correlates significantly with traditional cardiovascular risk factors and is a powerful predictor of vascular events [9—11]. Thus, it can be used as a marker of atherosclerosis in other vascular diseases [12], and as a surrogate endpoint in pharmacological interventional trials performed in dislipidemic patients [13,14] as well as in patients with coronary artery disease (CAD) [15—17].

Information on the relationship between pro-inflammatory/prothrombotic markers and C-IMT in stable or unstable ischemic heart disease (IHD) remains sparse. However, it appears that some soluble markers could be mainly linked with atherosclerosis progression whereas others reflect plaque instability. The MIAMI (Markers of Inflammation and Atorvastatin effect in previous Myocardial Infarction) study was designed to investigate, the relationship between changes in C-IMT progression and those in circulating markers of inflammation, thrombosis and endothelial dysfunction in patients with stable IHD treated with a moderate dosage of atorvastatin (20 mg/day) for two years. In accordance with the literature [18], we established that at least two months had to have elapsed between the myocardial infarction and enrollment so as to avoid any influence of that critical event on levels of inflammatory markers.

We measured a panel of pro-inflammatory mediators (high-sensitivity C-reactive protein [hs-CRP], tumor necrosis factor [TNF]-α, interleukin [IL]-6, IL-8, prothrombotic molecules (tissue factor/tissue factor pathway inhibitor [TF/TFPI], von Willebrand factor [vWF], fibrinogen) and endothelial cell activation markers (soluble intercellular adhesion molecules-1 [sICAM-1], soluble vascular cell adhesion molecules-1 [sVCAM-1] and sE-selectin). In contrast to other pathological conditions, no information—with the exception of CRP [19] and E-selectin [20]—is available on the relationship between these variables and cross-sectional C-IMT or C-IMT progression in IHD patients.

In this study we have investigated the cross-sectional relationships between the plasma markers considered at baseline in the MIAMI study and the extent of atherosclerosis on the basis of C-IMT.

Methods

Study population and design

MIAMI is a spontaneous, prospective, open-label, multicenter study, which involve four recruiting centers in Milan (Italy). Eighty-five patients with stable IHD (i.e. previous myocardial infarction more than two months before entry) were enrolled. The exclusion criteria were: serum triglycerides $>$250 mg/dl, diabetes mellitus, renal impairment, liver dysfunction, malignancy, underlying inflammatory disease, previous stroke, congestive heart failure (NYHA class III or IV), unstable angina, coronary revascularization within the previous two months, and current therapy with

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steroids, calcium channel blockers or amiodarone. Previous adverse reactions to statins and an elevation in serum creatine kinase above the normal limit were two additional exclusion criteria. Patients were maintained on their existing medications, which included beta-blockers, diuretics and low-dosage angiotensin-converting enzyme (ACE) inhibitors throughout the study.

Enrollment in the MIAMI study started at the beginning of 2003. At that time, it was not mandatory to start statin treatment in the acute phase of the disease. In fact, despite the publication of the MIRACL trial [21], which indicated the benefit of this therapeutic approach, the European guidelines [22] reported that the MIRACL findings should be replicated before considering it mandatory to start statins in the acute phase of the disease or immediately thereafter, and suggested that the drug could be postponed for three months. In view of the lack of mandatory guidelines, the institutional review boards that approved the MIAMI protocol considered it acceptable to begin treatment with atorvastatin two months after the acute phase of the disease, and approved the two-month washout for patients already taking statins at enrollment.

Hypertension was defined as systolic blood pressure (BP) $\geq$ 140 mmHg or diastolic BP $\geq$ 90 mmHg at repeated measurements, or current use of antihypertensive drugs. Patients were considered smokers if they were current smokers or had stopped smoking less than one month before entry into the study. Patients were assigned to a 24-month treatment with 20 mg/day atorvastatin (kindly provided by Pfizer Italia). The study was approved by independent ethics committees for each clinical cardiologic center. All patients gave written informed consent.

On enrollment, patients had a baseline evaluation that included collection of demographic information, data on medication use and smoking and alcohol consumption, and a physical examination with BP and body mass index (BMI) measurements and fasting blood sampling. Electrocardiograms (ECG) were also recorded.

The design of the MIAMI study involved a 24-month follow-up with visits and plasma plus ultrasonic variables recorded at fixed intervals. This report illustrates the baseline picture.

Laboratory tests

At baseline, blood samples for central laboratory assays of plasma markers and lipid profile analysis were collected after a 12-h (overnight) fast by an atraumatic puncture of the antecubital vein.

For serum preparation, blood was collected without anticoagulants, allowed to coagulate then centrifuged to obtain serum, according to routine laboratory procedures. Citrated or EDTA-anticoagulated blood samples were centrifuged at 1700*g* at 4 °C for 10 min to obtain platelet-poor plasma. Serum and plasma were aliquoted and stored at −80 °C prior to analysis. Samples were frozen and thawed only once.

Total and high-density lipoprotein cholesterol (TC, HDL-C) and triglycerides were assayed by enzymatic techniques [23]. Low-density lipoprotein cholesterol (LDL-C) was calculated with Friedewald's formula [24]. Plasma levels of IL-6, IL-8, TNFα, sVCAM-1, sE-selectin, sICAM-1 (R&D), hs-CRP (Hyphen BioMed), vWF (Gradipore), TF and total TFPI (American Diagnostica), were determined using specific ELISA kits according to the manufacturer's instructions. Fibrinogen was determined according to Clauss [25].

All intra- and inter-assay coefficients of variation were <6%.

Carotid IMT

Carotid ultrasound was performed by a single operator (M.A.) using an ACUSON Aspen system equipped with a 10—13 MHz linear array probe and recorded on sVHS videotapes. The far wall of the left and right common carotid (CC), bifurcation (Bif) and internal carotid artery (ICA) were visualized in anterior, lateral and posterior projections.

The ultrasonic variables considered were the mean IMT of the CC (CC-IMT), Bif (Bif-IMT), and ICA (ICA-IMT), and the mean and maximum of the whole carotid tree (IMT_{mean} and IMT_{max}). All of these ultrasonic variables incorporate the plaque(s). This was done because, in agreement with other authors [26], we believe that IMT can only be considered a marker of atherosclerosis when plaques are incorporated into the measurements. Ultrasonography does not have sufficient axial resolution to distinguish between intimal and medial thickness, so if atherosclerotic plaques are excluded it is unclear whether the IMT measurement represents intimal thickening and thus atherosclerosis, or medial thickening and thus vascular hypertrophy, or both.

Ultrasonic measurements were made using a specific software (M'ath, Metris SRL France), which allows automatic edge detection of the echogenic lines of the intima-media complex. Readings were taken at the far wall of the whole CC, Bif, and the first proximal centimeter of the ICA. Each ultrasonic scan was repeated twice (within two weeks); the mean of these two

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independent IMT determinations was adopted for statistical analysis and to assess reproducibility. The absolute differences (mean ± SD) between replicate scans were 0.012 ± 0.010, 0.044 ± 0.045, 0.043 ± 0.046, 0.020 ± 0.025 and 0.085 ± 0.109 mm for CC-IMT, Bif-IMT, ICA-IMT, IMT_{mean} and IMT_{max}, respectively.

Statistical analysis

The sample size of 85 patients was calculated in order to assess with a power of 80% and an alpha error of 0.004 (accounting for multiple comparisons with Bonferroni correction), a correlation coefficient (r) of at least 0.4 between each soluble marker and carotid IMT.

Continuous data are expressed as mean ± SD and median (interquartile range), whereas categorical data are expressed as number or percentage. Variables were tested for normal distribution using the Kolmogorov–Smirnov test. Correlations between clinical characteristics, plasma markers and IMT parameters were analysed by the Spearman correlation and by partial correlation adjusted for age, sex, pack-year and lipids. Multiple regression analysis was used to confirm the independence of the relationships observed. Variables with a skewed distribution were included in parametric models after logarithmic transformation. Statistical analysis was performed using Statistical Analysis System (SAS) version 8.0 software.

Results

Table 1 summarizes the patients' clinical characteristics. On average patients were middle-aged with a prevalence of males (61.2%) and about half of them had a history of hypertension or a parental history of CVD. All were being treated with cardiovascular drugs (diuretics, beta-blockers, aspirin and low-dosage ACE inhibitors).

TC and LDL-C levels (Table 2) were at the upper limits, and HDL-C and triglycerides within the normal range. The biological markers listed in Table 2 did not correlate significantly with sex, age, smoking or BMI.

Partial correlation analysis, adjusted for age and sex, showed a negative correlation between HDL-C and sICAM-1 ($r = -0.45$; $p < 0.0001$), sVCAM-1 ($r = -0.31$; $p < 0.05$), sE-selectin ($r = -0.26$; $p < 0.05$), hs-CRP ($r = -0.29$; $p < 0.05$), IL-6 ($r = -0.31$; $p < 0.05$), and a positive correlation between triglycerides and sICAM-1 ($r = 0.36$; $p < 0.001$), sE-selectin ($r = 0.33$; $p < 0.05$), TF ($r = 0.22$; $p < 0.05$) and TFPI ($r = 0.25$; $p < 0.05$).

Table 1 Patients' clinical characteristics

Characteristic	Value
Gender (male/female)	72/13
Age (yrs, mean ± SD)	57.5 ± 8.1
BMI (kg/m^2, mean ± SD)	26.2 ± 3.2
Systolic BP (mmHg, mean ± SD)	127.3 ± 11.3
Diastolic BP (mmHg, mean ± SD)	78.3 ± 7.0
Current smoker (%)	12.9
Pack-year (mean ± SD)	51.2 ± 33.9
Hypertension (%)	44.7
Diabetes (%)	0[a]
Parental history of CVD (%)	53.7
History of angina (%)	7.10
Previous coronary revascularization	
PTCA (%)	75.3
CABG (%)	8.2
Current medication	
ACE inhibitors (low-dosage) (%)	43.0
Beta-blockers (%)	83.7
Aspirin (%)	97.6
Diuretic drugs (%)	11.8
Anti-arrhythmic drugs (%)	22.4

CVD, cardiovascular disease; BMI, body mass index; PTCA, percutaneous transcatheter coronary angioplasty; and CABG, coronary artery bypass graft.
[a] Exclusion criterion.

Table 3 explores the relationships between C-IMT, age and all soluble markers reported in Table 2 by both Spearman and partial correlations, adjusted for age, sex, pack-year and lipids. All C-IMT variables were closely correlated with age ($p < 0.05$ always), but only CC-IMT_{mean} and IMT_{mean} reached the threshold p-value ($p < 0.004$) selected to account for multiple comparisons. A positive, unadjusted correlation between IL-6, TFPI and some ultrasonic variables were observed, but they were no longer significant after data adjustment for age, sex, pack-year and lipids. In contrast, sE-selectin was not correlated with IMT in the unadjusted analyses, but became highly correlated with IMT at different carotid sites when partial correlation was considered.

Table 4 shows five stepwise, multiple regression models performed by using all ultrasonic variables as dependent variables (one at time) and gender, age, BMI, systolic and diastolic BP, history of hypertension, smoking habits (pack-year), therapy (beta-blockers, ACE inhibitors, diuretics and anti-arrhythmic drugs) and all of the plasma markers shown in Table 2 as independent variables. These analyses confirmed the strong association of sE-selectin and TFPI with some but not all IMT parameters.

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Table 2 Lipid profiles, plasma markers and C-IMT

Variables	Mean ± SD	Median (interquartile range)	Normal range
Lipids			
TC (mg/dl)	229.8 ± 43.8	223.0 (206.5–250.0)	<200
HDL-C (mg/dl)	46.7 ± 11.2	43.5 (40.0–53.0)	>40
LDL-C (mg/dl)	153.2 ± 37.8	150.0 (129.3–170.8)	<130
Triglycerides (mg/dl)	152.5 ± 69.2	149.5 (95.3–182.0)	<150
Inflammatory markers			
Hs-CRP (μg/ml)	1.82 ± 2.19	1.04 (0.52–2.11)	0.2–10
Fibrinogen (mg/dl)	370.3 ± 60.9	363.0 (333.0–409.8)	250–350
IL-6 (pg/ml)	2.23 ± 1.71	1.60 (1.10–2.71)	0.4–9.5
IL-8 (pg/ml)	8.79 ± 6.93	7.52 (3.48–12.22)	<10
TNFα (pg/ml)	1.75 ± 2.29	1.03 (0.69–1.62)	N.D.–4.2
Cell adhesion molecules			
sICAM-1 (ng/ml)	248.2 ± 52.7	241.3 (210.5–273.4)	115–306
sVCAM-1 (ng/ml)	584.5 ± 159.3	551.9 (476.0–661.3)	340–900
sE-selectin (ng/ml)	33.7 ± 15.6	32.4 (21.7–41.8)	29–63
Procoagulant markers			
TF (pg/ml)	83.0 ± 49.8	72.2 (58.0–95.5)	29–112
TFPI (ng/ml)	57.3 ± 17.7	53.4 (45.3–67.5)	75–120
vWF (IU/ml)	1.24 ± 0.36	1.21 (0.99–1.48)	05–2.3
C-IMT (mm)			
CC-IMT_{mean}	0.72 ± 0.11	0.69 (0.65–0.75)	[a]
Bif-IMT_{mean}	1.13 ± 0.38	1.04 (0.87–1.33)	[a]
ICA-IMT_{mean}	0.97 ± 0.38	0.83 (0.68–1.15)	[a]
IMT_{mean}	0.90 ± 0.18	0.88 (0.75–1.01)	[a]
IMT_{max}	2.08 ± 0.68	2.03 (1.49–2.54)	[a]

[a] Unavailable because age- and sex-dependent [40].

Discussion

We report here for the first time that, in stable IHD patients, E-selectin and TFPI are closely and independently correlated with IMT at different carotid sites. Unlike ICAM-1 and VCAM-1, E-selectin is expressed only on the endothelial surface after cytokine stimulation [27] and it can therefore be considered a specific marker of endothelial dysfunction in cardiovascular and inflammatory diseases. Experimental results suggested that E-selectin may influence intimal hyperplasia through the regulation of inflammatory cell infiltration [28].

A relationship between E-selectin and C-IMT has already been reported in healthy subjects [29], in

Table 3 Spearman and partial (adjusted for age, gender, pack-year and lipids) correlation coefficients between some plasma markers and C-IMT

	CC-IMT_{mean}		Bif-IMT_{mean}		ICA-IMT_{mean}		IMT_{mean}		IMT_{max}	
	R	Adjusted R	R	Adjusted R	R	Adjusted R	R	Adjusted R	R	Adjusted R
Age	0.42*	[a]	0.34	[a]	0.23	[a]	0.43*	[a]	0.27	[a]
IL-6	0.34*	0.14	0.18	0.14	0.15	0.12	0.29*	0.18	0.22	0.21
sE-selectin	0.10	0.12	0.24	0.25	0.18	0.37*	0.28	0.36*	0.27	0.37*
TFPI	0.17	0.10	0.26	0.24	0.30*	0.20	0.39*	0.27	0.35*	0.30

*$p < 0.004$.

Gender, age, BMI, systolic and diastolic blood pressure and all plasma markers shown in Table 2 have been analysed, but only those showing at least one significant correlation with at least one of IMT variables ($p < 0.004$) are reported in the table. Only correlation with a p-value <0.004 were considered as significant in order to take into account Bonferroni correction for multiple comparisons.

[a] Adjustment variable.

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Table 4 Multiple linear regression analyses with carotid IMTs as dependent variables (5 models)

		B	Standard error	Beta	*t*	Sig
CC-IMT_{mean}	Age	0.006	0.001	0.421	4.520	0.000
	Pack-year	0.025	0.007	0.310	3.321	0.001
	DBP	0.003	0.001	0.211	2.271	0.026
Bif-IMT_{mean}	Age	0.017	0.005	0.366	3.628	0.000
	sE-selectin	0.006	0.003	0.236	2.336	0.022
ICA-IMT_{mean}	Age	0.011	0.004	0.245	2.682	0.009
	Gender	0.223	0.104	0.213	2.136	0.036
	HDL	0.017	0.004	0.491	4.678	0.000
	sE-selectin	0.008	0.002	0.334	3.535	0.001
	Beta-blockers	−0.319	0.096	−0.305	−3.311	0.001
IMT_{mean}	Age	0.012	0.002	0.545	6.429	0.000
	Log-TFPI	0.463	0.119	0.332	3.897	0.000
	sE-selectin	0.004	0.001	0.299	3.426	0.001
	Log-Triglycerides	−0.213	0.085	−0.241	−2.733	0.008
	Beta-blockers	−0.161	0.041	−0.317	−3.906	0.000
	ACE inhibitors	0.087	0.031	0.239	2.808	0.006
	Diuretics	−0.119	0.050	−0.210	−2.368	0.02
IMT_{max}	Log-TFPI	1.749	0.476	0.340	3.674	0.000
	Age	0.032	0.008	0.387	4.175	0.000
	sE-selectin	0.011	0.004	0.249	2.689	0.009
	ACE inhibitors	0.399	0.127	0.296	3.146	0.002
	Beta-blockers	−0.404	0.168	−0.217	−2.408	0.018
	Diuretics	−0.428	0.205	−0.205	−2.088	0.04

DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol. The 5 models (one for each IMT variables as dependent variables) were performed by using gender, age, BMI, systolic and diastolic blood pressure, history of hypertension, pharmacological treatments and all variables reported in Table 2 as independent variables.

the general population [20] and in diabetic patients [7]. The finding reported here of a correlation in stable IHD patients also suggests that the pro-atherogenic role of E-selectin is not limited to the early steps of atherosclerotic disease, but rather affects all stages, from the earliest to the most advanced. Although supported by a study showing that circulating E-selectin concentrations are also related to coronary atherosclerosis detected by angiography in IHD patients in stable condition [30], this possibility does however conflict with at least two population-based studies: the first showing no relationship between E-selectin and progression of peripheral atherosclerosis [31], and the second showing that E-selectin cannot predict clinical CHD in a multivariate analysis [20]. Further studies are needed to clarify this. Atherosclerosis risk factors may affect the CC-, Bif- and ICA-IMT differently [32]. The present study did in fact find that, at variance with other carotid districts, CC-IMT did not correlate with circulating concentrations of E-selectin. This suggests that E-selectin may serve as a marker of localized atherosclerosis, which rarely occurs in the CC artery.

TFPI is a serine protease inhibitor that regulates the TF-dependent pathway of blood coagulation [33]. It is synthesized primarily by the vascular endothelium under normal physiologic conditions [34], but various inflammatory stimuli can influence its expression in different cell types (monocytes/macrophages, megakaryocytes, etc.) [35]. Previous studies have shown that TFPI co-localized with TF in focal areas such as atherosclerotic plaques, thus suggesting a potentially active role of TFPI in the regulation of atherosclerotic lesion thrombogenicity either before or after plaque rupture [33,36,37]. Besides its anticoagulant activity, TFPI inhibits the proliferation of the cells within the vascular wall by inducing apoptosis [38]. Thus, together with plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), prothrombin fragment F1 + 2 (F1 + F2), factor VII, factor VIII and antithrombin, TFPI may play a role in the development of CVD by modulating the balance between coagulation and fibrinolysis [39].

Only a few studies have investigated the correlation between TFPI and the degree of atherosclerosis evaluated by C-IMT. In population-based

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studies, IMT did not correlate with TFPI [40—42] whereas in combined hyperlipidemia, TFPI was a determinant of IMT variance [43,44]. In agreement with studies in patients with IHD and unstable angina [45,46] our findings of an independent correlation of this serine protease inhibitor with carotid atherosclerosis in stable IHD patients are in-line with an *in vivo* pro-atherogenic role, especially in pathological states involving endothelial activation.

The results of this study may have substantial clinical implications. First, they demonstrate that carotid IMT remains able to recognize the effect of vascular risk factors, even in the late phases of atherosclerosis. Second, they indicate that both E-selectin and TFPI have the potential for use as molecular markers to further stratify the risk of atherosclerosis in patients already considered at high risk because they are in secondary prevention. In fact, when patients with stable IHD are stratified according to tertiles of TFPI or E-selectin (Fig. 1), those in the highest tertile have significantly higher IMT values than those in the lowest tertile.

Finally, our findings should add to the current strategies aimed at preventing the recurrence of cardiovascular events in patients in secondary prevention, through the management of non-conventional vascular risk factors. The prospective part of the MIAMI study will allow investigations into whether the anti-atherosclerotic effect of atorvastatin is mainly due to its lipid-lowering effect, or whether there is a pleiotropic effect on circulating soluble markers, including E-selectin or TFPI.

The free medical treatment offered to these patients, which could have had a confounding effect on the outcome measurements, may be a limitation to the study. However, patients treated with beta-blockers, ACE inhibitors, diuretics and anti-arrhythmic drugs did not differ from those without treatment in any of the variables measured. In addition, the correlations between the thickening of carotid artery IMT and E-selectin or TFPI remained significant even after data adjustment for pharmacological treatments, thus indicating that they are both true independent predictors of carotid atherosclerosis.

In conclusion, the baseline data from the MIAMI study show a good correlation between E-selectin, TFPI and IMT of the extracranial carotid arteries in off-statin patients with stable IHD. The final results of the prospective part of the MIAMI study should add to our knowledge of the effects of statins on clustering of pro-inflammatory and prothrombotic markers, and the morphological changes of the arterial wall evaluated by B-mode measurement of C-IMT.

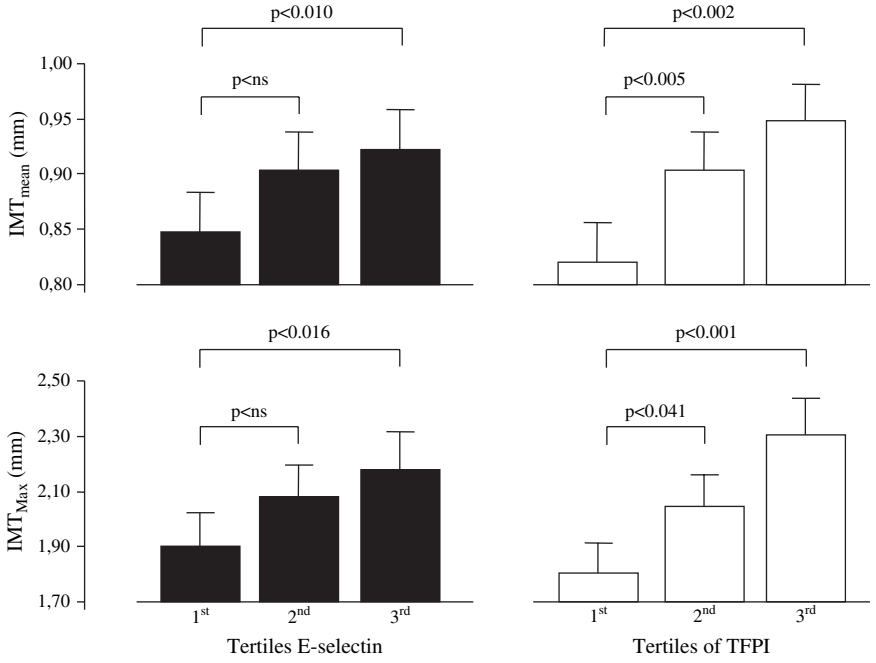

Figure 1 Average (IMT_{mean}, top panel) and maximal (IMT_{max}, bottom panel) carotid IMT of patients stratified according to tertiles of E-selectin or TFPI. The significances were calculated after data adjustment for: sex, age, BMI, systolic and diastolic BP, history of hypertension, smoking habits (pack-year) and therapy (beta-blockers, ACE inhibitors, diuretics and anti-arrhythmic drugs). Results are means ± SM.

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References

[1] Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med 1999;340:115—26.
[2] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135—43.
[3] Libby P. Inflammation and atherosclerosis. Nature 2002; 420:868—74.
[4] Schonbeck U, Libby P. Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as anti-inflammatory agents? Circulation 2004;109(21 Suppl. 1):II18—26.
[5] Hunt KJ, Pankow JS, Offenbacher S, Kritchevsky SB, Duncan BB, Shahar E, et al. B-mode ultrasound-detected carotid artery lesions with and without acoustic shadowing and their association with markers of inflammation and endothelial activation: the Atherosclerosis Risk in Communities Study. Atherosclerosis 2002;162:145—55.
[6] Del Rincon I, Williams K, Stern MP, Freeman GL, O'Leary DH, Escalante A. Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects. Arthritis Rheum 2003;48:1833—40.
[7] Leinonen ES, Hiukka A, Hurt-Camejo E, Wiklund O, Sarna SS, Mattson Hulten L, et al. Low-grade inflammation, endothelial activation and carotid intima-media thickness in type 2 diabetes. J Intern Med 2004;256:119—27.
[8] Lowe GD. Circulating inflammatory markers and risk of cardiovascular and non-cardiovascular disease. J Thromb Haemost 2005;3:1618—27.
[9] Burke GL, Evans GW, Riley WA, Sharret AR, Howard G, Barnes RW, et al. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Stroke 1995;26:386—91.
[10] Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, et al. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 1998;128:262—9.
[11] van der Meer I, Bots ML, Hofman A, del Sol AI, van der Kuip D, Witteman J. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation 2004;109:1089—94.
[12] Hulthe J, Wikstrand J, Emanuelsson H, Wiklund O, de Feyter PJ, Wendelhag I. Atherosclerotic changes in the carotid artery bulb as measured by B-mode ultrasound are associated with the extent of coronary atherosclerosis. Stroke 1997;28:1189—94.
[13] Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, Stalenhoef AF. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. Lancet 2001;357:577—81.
[14] Taylor AJ, Kent SM, Flaherty PJ, Coyle LC, Markwood TT, Vernalis MN. ARBITER: arterial biology for the investigation of the treatment effects of reducing cholesterol. A randomized trial comparing the effects of atorvastatin and pravastatin on carotid intima media thickness. Circulation 2002;106:2055—60.
[15] De Groot E, Jukema JW, Montauban van Swijndregt AD, Zwinderman AH, Ackerstaff RG, van der Steen AF, et al. B-mode ultrasound assessment of pravastatin treatment effect on carotid and femoral artery walls and its correlations with coronary arteriographic findings: a report of the Regression Growth Evaluation Statin Study (REGRESS). J Am Coll Cardiol 1998;31:1561—7.
[16] Riegger G, Abletshauser C, Ludwig M, Schwandt P, Widimsky J, Weidinger G, et al. The effect of fluvastatin on cardiac events in patients with symptomatic coronary artery disease during one year of treatment. Atherosclerosis 1999;144(1):263—70.
[17] Sidhu JS, Kaposzta Z, Markus HS, Kaski JC. Effect of rosiglitazone on common carotid intima-media thickness progression in coronary artery disease patients without diabetes mellitus. Arterioscler Thromb Vasc Biol 2004;24(5):930—4.
[18] James SK, Oldgren J, Lindback J, Johnston N, Siegbahn A, Wallentin L. An acute inflammatory reaction induce by myocardial damage is superimposed on a chronic inflammation in unstable coronary artery disease. Am Heart J 2005;149:619—26.
[19] Orem C, Durmus I, Kilinc K, Baykan M, Gokce M, Orem A, et al. Plasma fibronectin level and its association with coronary artery disease and carotid intima-media thickness. Coron Artery Dis 2003;14:219—24.
[20] Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96:4219—25.
[21] Schwartz GG, Olsson AG, Ezekowitz MD, Gaur P, Oliver MF, Waters D, et al. Effects of atorvastatin on early recurrent ischaemic events in acute coronary syndromes: the MIRACL

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study — a randomized controlled trial. JAMA 2001;285: 1711—8.
[22] De Backer G, Ambrosioni E, Borch-Johnsen K, Borotons C, Cifkova R, Dallongeville J, et al. European guidelines on cardiovascular disease prevention in clinical practice. European Journal of Cardiovascular Prevention and Rehabilitation 2003;10(Suppl. 1):S1—S78.
[23] Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19: 476—82.
[24] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499—502.
[25] Clauss A. Gerinnungsphysiologishe Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol 1957;17:237—46.
[26] Simon A, Gariepy J, Chironi G, Megnien JL, Levenson J. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. J Hypertens 2002;20:159—69.
[27] Roldàn V, Marìn F, Lip GYH, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors. Thromb Haemost 2003;90:1007—20.
[28] Gotoh R, Suzuki J-I, Kosuge H, Kakuta T, Sakamoto S, Yoshida M, et al. E-selectin blockade decreases adventitial inflammation and attenuates intimal hyperplasia in rat carotid arteries after balloon injury. Arterioscler Thromb Vasc Biol 2004;24:2063—8.
[29] Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R, et al. Plasma tumour necrosis factor-α and early carotid atherosclerosis in healthy middle-aged men. Eur Heart J 2002;23:376—83.
[30] Nasuno A, Matsubara T, Hori T, Higuchi K, Imai S, Nakagawa I, et al. Levels of soluble E-selectin and ICAM-1 in the coronary circulation of patients with stable coronary artery disease. Association with the severity of coronary atherosclerosis. Jpn Heart J 2002;43:93—101.
[31] Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, Fowkes FG. C-reactive protein, interleukin-6, and soluble adhesion molecules as predictors of progressive peripheral atherosclerosis in the general population: Edinburgh Artery Study. Circulation 2005;112(7):976—83.
[32] Ludwig MM, Jörger U, Lund S, Stumpe KO. Divergent responses of common carotid artery intimal-medial thickness to high BP and hypercholesterolaemia in relation to age. J Hypertens 1993;11:130—1.
[33] Crawley J, Lupu F, Westmuckett AD, Severs NJ. Expression, localization, and activity of tissue factor pathway inhibitor in normal and atherosclerotic human vessels. Arterioscler Thromb Vasc Biol 2000;20:1362—73.
[34] Bajaj MS, Kuppuswamy MN, Saito H, Spitzer SG, Bajaj SP. Cultured normal human hepatocytes do not synthesize lipoprotein-associated coagulation inhibitor: evidence that endothelium is the principal site of its synthesis. Proc Natl Acad Sci U S A 1990;87:8869—73.
[35] Ameri A, Kuppuswamy MN, Basu S, Bajaj SP. Expression of tissue factor pathway inhibitor by cultured endothelial cells in response to inflammatory mediators. Blood 1992; 79:3219—26.
[36] Kaikita K, Takeya M, Ogawa H, Suefuji H, Yasue H, Takahashi K. Colocalization of tissue factor and tissue factor pathway inhibitor in coronary atherosclerosis. J Pathol 1999;188:180—8.
[37] Cortellaro M, Cofrancesco E, Arbustini E, Rossi F, Negri A, Tremoli E, et al. Atorvastatin and thrombogenicity of the carotid atherosclerotic plaque: the ATROCAP study. Thromb Haemost 2002;88:41—7.
[38] Hamuro T, Kamikubo Y, Nakahara Y, Miyamoto S, Funatsu A. Human recombinant tissue factor pathway inhibitor induces apoptosis in cultured human endothelial cells. FEBS Lett 1998;421:197—202.
[39] Haverkate F. Levels of haemostatic factors, arteriosclerosis and cardiovascular disease. Vascul Pharmacol 2002;39: 109—12.
[40] de Maat MP, Bladbjerg EM, Drivsholm T, Borch-Johnsen K, Moller L, Jespersen J. Inflammation, thrombosis and atherosclerosis: results of the Glostrup study. J Thromb Haemost 2003;1:950—7.
[41] Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. Circulation 1998;97:1467—73.
[42] Sakata T, Mannami T, Baba S, Kokubo Y, Kario K, Okamoto A, et al. Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries. Atherosclerosis 2004;176:355—60.
[43] Lavrencic A, Kosmina B, Keber I, Videcnik V, Keber D. Carotid intima-media thickness in young patients with familial hypercholesterolaemia. Heart 1996;76:321—5.
[44] Sebestjen M, Zegura B, Videcnik V, Keber I. Determinants of endothelial dysfunction and carotid intima-media thickness in combined hyperlipidemia. Coron Artery Dis 2005; 16:175—80.
[45] Falciani M, Gori AM, Fedi S, Chiarugi L, Simonetti I, Dabizzi RP, et al. Elevated tissue factor and tissue factor pathway inhibitor circulating levels in ischaemic heart disease patients. Thromb Haemost 1998;79:495—9.
[46] Freeburn JC, Wallace JM, Strain JJ, Sinnamon DG, Craig BM, Johnson D, et al. Monocyte tissue factor-like activity in post myocardial infarction patients. Br J Haematol 1998;102:605—8.

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