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High plasma levels of endothelin-1 enhance the predictive value of preclinical atherosclerosis for future cerebrovascular and cardiovascular events: a 20-year prospective study

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Background and purpose **Clinical and experimental evidence suggests that endothelin-1 (Et-1) plays a role in cardiac and vascular disease. In the present study, we investigated the prognostic significance of Et-1 for cerebrovascular and cardiovascular outcome, in a 20-year follow-up.**

Methods **We studied 82 originally healthy individuals, referred to our Unit of Cardiovascular Prevention, to evaluate the presence of asymptomatic carotid lesions. We subdivided these individuals into two groups, according to the plasma values of Et-1 (respectively $\leq$ or $>$2.7 pg/ml). Traditional cardiovascular risk factors were investigated, and by carotid ultrasound examination, we distinguished between normal individuals and those with intima–media thickening or asymptomatic carotid plaque.**

Results **Major cardiac and cerebral events (all-cause death, myocardial infarction, revascularization procedures, fatal and nonfatal stroke) were registered in 41 individuals and significantly more in those with high vs. low Et-1 levels (95 vs. 5%; $P<0.0001$). Furthermore, by logistic multivariate regression analysis, we found that among all evaluated baseline clinical and laboratory variables, hypertension [odds ratio (OR): 20.4 (3.3–127), $P=0.001$], high Et-1 concentrations [OR: 1.4 (1.0–1.8), $P=0.02$] and the presence of intima–media thickness or asymptomatic carotid plaque [OR: 3.7 (1.14–12.1), $P=0.02$] were independent predictors of future events. Finally, integrating technical and laboratory data, high levels of Et-1 have defined a high risk of major cardiac and cerebral event and stroke at follow-up, which increased in relation to the progression of carotid atherosclerosis ($P<0.05$).**

Conclusion **Et-1 plasmatic levels significantly influence the cardiovascular and cerebrovascular risk profile, beyond traditional cardiovascular risk factors and preclinical carotid atherosclerosis.**

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Keywords: cardiovascular events, cerebrovascular events, endothelin-1, follow-up

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Introduction

The endothelial function is strictly dependent on anatomical continuity of the monolayer cell as well as its functional integrity. In the presence of cardiovascular risk factors, the protective role of endothelium seems to deteriorate, configuring the so-called 'endothelial dysfunction',[1] characterized by an impaired vasomotor response to main endothelium-dependent vasodilating stimuli, as well as a proinflammatory and anticoagulant action. Endothelial dysfunction represents the earliest event in the development of atherosclerotic plaque; it actually occurs when a structural lesion is still not evident. Therefore, its evaluation could be useful for an early stratification of patients at risk of cardiovascular events.[2]

Endothelin-1 (Et-1) is a peptide secreted primarily by vascular endothelial cells, with potent vasoconstrictor[3] and mitogenic properties, and it is involved in the hydrosaline homeostasis through its interactions with the renin–angiotensin–aldosterone system (RAAS), stimulating the sympathetic nervous system too. A large number of studies have reported the pleiotropic action of this peptide on cardiac and renal function, as well as on vascular cell growth. Et-1 has been related to the progression of atherosclerotic disease, and a pathogenic role has been suggested for heart failure[4]; in addition, other studies have suggested the short-term prognostic significance of Et-1 in patients with acute myocardial infarction[5] (AMI) after primary angioplasty as well as in those with ischemic stroke.[6] These findings highlight the role of Et-1 as an important mediator of cardiovascular and cerebrovascular diseases and further provide important therapeutic insights into the prevention of atherosclerosis, vascular remodeling, left ventricular hypertrophy and hypertensive nephropathy.

In the present study, we aimed to assess whether baseline Et-1 levels may be predictors of future cardiovascular and

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cerebrovascular events in a cohort of baseline healthy individuals, screened for the presence of asymptomatic carotid lesions in our Centre for Early Diagnosis of Preclinical and Multifocal Atherosclerosis and for Cardiovascular Prevention. The follow-up period was 20 years. We, therefore, aimed to test the hypothesis that measuring levels of this peptide can provide information not only on the progression of atherosclerosis disease, but can also be considered to be predictive of the risk of developing ischemic stroke and/or cardiovascular events, helping to define the prognosis, independently of common risk factors.

Methods

Study designs and participants

We performed a 20-year prospective study in all the patients through periodic outpatient visits for detecting the occurrence of cerebrovascular and cardiovascular events eventually. The considered endpoints were two: the first composite endpoint included the following major adverse cardiovascular and cerebrovascular events (MACCEs): cardiovascular death, AMI, new revascularization procedures (coronary angioplasty, coronary artery bypass graft) and cerebrovascular events (transient ischemic attack and major or minor stroke); the second endpoint included the occurrence of major stroke only.

AMI was defined by a prolonged episode of chest pain with electrocardiogram and/or specific myocardial enzyme changes, involving hospitalization and presumed new ischemic electrocardiographic changes or new left bundle branch block, occurred before cardiac biomarkers were obtained or before cardiac biomarker values were increased[7]; as to the second outcome, major stroke was defined by the modified Rankin Scale[8]; finally sudden cardiac death was defined by cardiac death with or without symptoms suggestive of myocardial ischemia. The causes of death were retrieved directly from the families and were confirmed in all the cases by the general practitioners on the basis of their own records (which may include hospital records).

Our cohort included 82 adults, men and women, from 30 to 75 years (mean age of the whole population was 58.6 years; 56 ± 15.97 in the first group with lower levels of Et-1 and 61 ± 14.18 in the second group with higher levels of Et-1 – difference not significant). They were baseline healthy individuals, all referred between 1990 and 1992 to our Centre for Early Diagnosis of Preclinical and Multifocal Atherosclerosis and for Cardiovascular Prevention for evaluating the presence of asymptomatic carotid lesions through an ultrasound scan. Patients were excluded if they had a history of peripheral artery disease, coronary revascularization, angina pectoris, myocardial infarction, carotid surgery or cerebrovascular event.

Patients underwent physical examination, biochemical analysis and ultrasonography of the carotid arteries in order to verify the clinical wellness and the presence of atherosclerotic carotid lesions and to evaluate the impact of main cardiovascular risk factors. The adopted procedures were in agreement with the Helsinki Declaration of 1975 as revised in 1983 and were approved by the Department Ethic Council. All patients gave their informed consent for participating in the study, and at admission answered a questionnaire on personal and medical items, including age, medical history and use of medications. Among the main cardiovascular risk factors, the presence of family history of cardiovascular diseases (in a first-degree relative before 55 years), hypertension (SBP or DBP respectively $\geq$140 or $\geq$90 mmHg or pharmacological therapy with antihypertensive drugs), diabetes (fasting glucose plasma concentrations higher than 126 mg/dl or pharmacological therapy with antidiabetic drugs or insulin), dyslipidemia (total cholesterol >200 mg/dl or pharmacological therapy with statins) and smoking habits were considered. Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. The average of two measurements obtained on one occasion, separated by a count of the pulse rate, was used in the present analysis. Height and weight were recorded and BMI was expressed as kg/m^2. Participants were categorized as having obesity if BMI was 30 kg/m^2 or higher.

Biochemistry

A blood sample was drawn in the morning, before the medical examination, after 12–14 h overnight fast. Total cholesterol was quantified by standard enzymatic-colorimetric methods.[9] Et-1 was measured by a sensitive enzyme immunoassay for human endothelin.[10] The assay is based on a sandwich method that uses two differing capture and detection antiendothelin antibodies. A monoclonal antiendothelin antibody, which did not react with an endothelin C-terminal heptapeptide, was used as an immobilized antibody. The Fab fragment of rabbit antibodies against the endothelin C-terminal heptapeptide was used as an enzyme-labeled detector antibody after being coupled with horseradish peroxidase 10.[8] As there is still no general agreement on which values can be considered elevated for Et-1, we referred to the work of Morganti *et al.*[11] that considered increased Et-1 plasma levels higher than 2.7 pg/ml for a population living at sea level.

Ultrasound examination of the carotid arteries

B-mode real-time ultrasound was used to evaluate the arterial wall thickness in the carotid arteries using a Toshiba 270 SS machine with a built-in software and with a 7.5–10.0 MHz probe. The power output, focus, depth of measurement and gain were standardized by using the preset program incorporated within the software package of the ultrasound equipment. The intima–media thickness (IMT) was defined as the distance between the echogenic line representing the intima–blood interface

and the outer echogenic line representing the adventitia junction. After freezing the image, the measurement was made with electronic callipers.

Patients were examined in the supine position, and each carotid wall and segment were examined to identify the thickest intimal–medial site. Three segments were identified and measured in anterior and posterior planes on each side: the distal 1 cm of the common carotid proximal to the bifurcation, the bifurcation itself and the proximal 1 cm of the internal carotid artery. At each of these sites, we have determined the IMT, automatically measured, and detected any possible plaque. We primarily used the maximum carotid IMT value, which was determined as the mean of the maximum IMT of near-wall and far-wall measurements of both the left and right side arteries for each of the three arterial segments. If data on one of the walls or one of the sides were missing, the maximum thickness of the available wall and side was used. Ultrasound examination was performed by one investigator (C.P.) blinded to the study and with no possibility of reproducing the IMT measurement, to limit the risk of a large interobserver variability.[12]

According to the guidelines of the joint European Society of Hypertension/European Society of Cardiology, we distinguished between 'normal individuals', if IMT was less than 0.9 mm, individuals with IMT if IMT was more than 0.9, but less than 1.5 mm and individuals with asymptomatic carotid plaque (ACP), if IMT was more than 1.5 mm.[13]

Statistical analysis

The statistical analysis was performed using the software Staview Program (SAS Institute Inc., Cary, North Carolina, USA). Differences in the investigated parameters among study groups were assessed by the unpaired Student's *t*-test (for numeric variables) and by the χ^2 test (for nominal variables). Data were expressed as mean ± standard deviation or as percentages. Multivariate analysis (logistic regression) was used to assess the potential independent effects of baseline variables on the incidence of future events. A P value less than 0.05 was considered statistically significant.

Results

Baseline clinical characteristics, laboratory data and ultrasonography findings in relation to plasma values of Et-1 are summarized in Table 1. Individuals with elevated Et-1 levels[11] ($n = 60$) had higher prevalence of most of the traditional cardiovascular risk factors, including hypertension ($P = 0.03$), carotid IMT or ACP ($P = 0.001$) in relation to individuals with low Et-1 concentrations. There were no significant differences in age and in the presence of smoking, obesity and the other risk factors.

Table 2 shows the baseline clinical characteristics, laboratory data and ultrasonography findings in relation to the occurrence of clinical events ($n = 41$), as registered in the 20-year follow-up in the group with elevated Et-1 levels. At univariate analysis, individuals with high Et-1 levels were mainly women ($P < 0.004$), were of older age ($P < 0.0001$), had a higher prevalence of hypertension ($P < 0.0001$) and carotid atherosclerosis ($P < 0.001$), and had higher levels of Et-1 ($P < 0.0001$). Furthermore, by logistic multivariate regression analysis, we found that among all evaluated baseline clinical and laboratory variables, only hypertension [odds ratio (OR): 20.4, (3.3–127) $P = 0.001$], high Et-1 concentrations [OR: 1.4, (1.0–1.8) $P = 0.02$] and the presence of IMT or ACP [OR: 3.7, (1.14–12.1) $P = 0.02$] were independent predictors of future events.

Table 1 Baseline clinical characteristics, laboratory data and ultrasonographic findings in relation to plasma values of endothelin-1

	Et-1 ≤2.7 pg/ml ($n = 22$)	Et-1 >2.7 pg/ml ($n = 60$)	*P* value
Female [*n* (%)]	11 (50%)	38 (63%)	NS
Age (years)	56 ± 15.97	61 ± 14.18	0.15
Hypertension [*n* (%)]	13 (59%)	49 (81.6%)	0.03
Diabetes [*n* (%)]	10 (45.4%)	34 (56.6%)	NS
Dyslipidemia [*n* (%)]	6 (27.3%)	22 (36.6%)	NS
Smoking [*n* (%)]	3 (13.6%)	6 (10%)	NS
Obesity [*n* (%)]	6 (27.3%)	12 (20%)	NS
IMT/ACP [*n* (%)]	9 (41%)	48 (80%)	0.001
Et-1 (pg/ml)	1.49 ± 0.68	6.09 ± 2.79	<0.0001

Et-1, endothelin-1; IMT/ACP, intima–media thickening or asymptomatic carotid plaque.

According to the results on multivariate analysis, relating high Et-1 plasma values and the presence of IMT/ACP (intima–media thickening or ACP), we therefore wanted to test whether the occurrence of MACCE ($n = 41$) at follow-up was better defined integrating instrumental data and plasma values of Et-1.

We found not only a higher prevalence of events among individuals with higher values of Et-1 (95 vs. 5%, $P < 0.001$) or in the presence of carotid plaque (90 vs. 12%, $P < 0.001$), but also that in the group with higher Et-1 levels, the number of events increased in those with carotid plaque at baseline, whereas in patients with lower Et-1 levels, this relationship was not confirmed (Fig. 1).

Therefore, higher values of Et-1 define a higher risk of events at follow-up in patients with carotid atherosclerosis, whereas lower values of Et-1 do not significantly influence the distribution of events at follow-up in patients with carotid atherosclerosis ($P < 0.05$).

Similar data were found as it concerns the occurrence of stroke ($n = 24$) at follow-up time. In fact, we found a higher prevalence of events among individuals with higher values of Et-1 (87.5 vs. 12.5%, $P < 0.000$). In the group of patients with higher values of Et-1, the distribution of events was significantly rising according to the progression of carotid disease severity ($P < 0.05$),

Table 2 Logistic regression analysis of the baseline clinical characteristics, laboratory data and ultrasonographic findings in relation to the occurrence of major cardiac and cerebral events in the group with higher levels of endothelin-1, as registered in the 20-year follow-up

	Patients with MACCE ($n=41$)	*P* value	Patients without events ($n=41$)	Logistic regression analysis or (95% CI); *P* value
Female [*n* (%)]	14 (34)	0.004	35 (85)	1.248 (1.08–1.74); 0.012
Age (years)	69 ± 11	<0.0001	51 ± 12	1.00 (0.97–1.04); 0.69
Hypertension [*n* (%)]	39 (95)	<0.0001	23 (56)	20.431 (3.28–127); 0.001
Diabetes [*n* (%)]	17 (42)	0.04	27 (66)	0.734 (0.23–2.28); 0.59
Dyslipidemia [*n* (%)]	8 (20)	0.01	20 (49)	0.418 (0.11–1.50); 0.18
Smoking [*n* (%)]	3 (7)	NS	6 (15)	0.858 (0.14–5.70); 0.86
Obesity [*n* (%)]	6 (15)	NS	12 (29)	0.894 (0.23–3.34); 0.86
IMT/ACP [*n* (%)]	37 (90)	<0.0001	20 (49)	3.730 (1.14–12.12); 0.028
Et-1 >2.7 pg/ml [*n* (%)]	39 (95)	<0.0001	21 (51)	1.372 (1.03–1.82); 0.029

CI, confidence interval; Et-1, endothelin-1; IMT/ACP, intima–media thickening or asymptomatic carotid plaque; MACCE, major cardiac and cerebral event.

whereas in the group with lower Et-1 levels, it was not (Fig. 2).

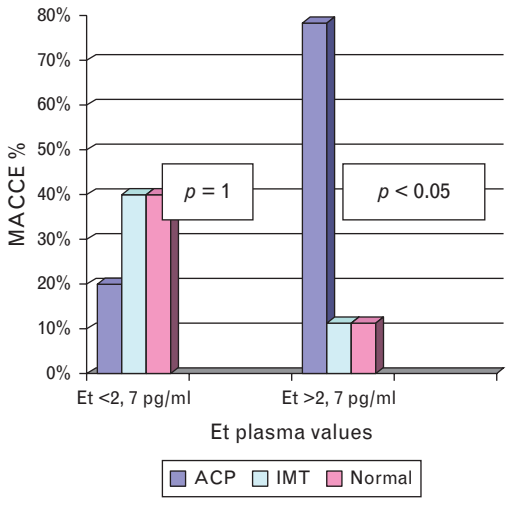
Discussion

According to our results, Et-1 plasmatic levels significantly influence the cardiovascular and cerebrovascular risk profile, beyond traditional cardiovascular risk factors and preclinical carotid atherosclerosis.

Soon after the discovery of Et-1, this vasoactive substance was reported to have several biological actions on cardiac tissue. In human myocardium, in-vitro Et-1 has been shown to exert a positive inotropic[12–15] effect via sensitization of cardiac myofilaments to calcium and activation of the sodium proton exchanger. The positive inotropic effect is accompanied by prolongation of the duration of the action potential.[16,17] This pharmacological action of Et-1 may contribute to the proarrhythmogenic property of the peptide. Et-1 also affects heart function indirectly via profound coronary vasoconstriction and it is involved in the mechanism by which angiotensin induces cardiac hypertrophy.

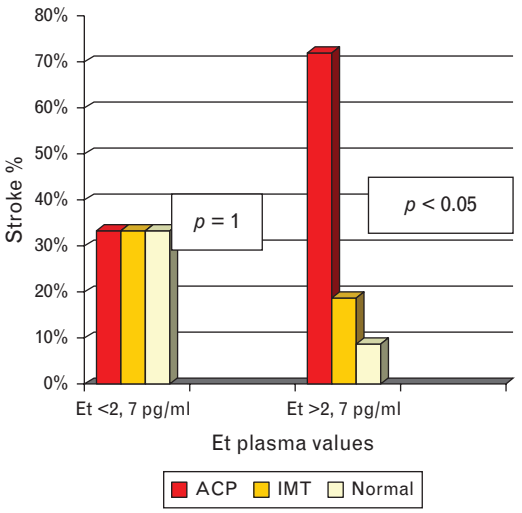
As regards the relation between plasma levels of Et-1 and myocardial infarction, the role of Et-1 is both beneficial and detrimental. Et-1 seems to play an important role in causation of myocardial infarction,[18] postinfarct scar formation,[19] left ventricular remodeling[20] and in the prognosis of myocardial infarction. In a study[21] performed in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention, plasma levels of Et-1 measured after myocardial infarction were found to be a strong predictor of 1-year survival, independently of clinical and biochemical variables previously associated with a poor prognosis. The above-mentioned studies, therefore, provided interesting insights into the potential benefits of a therapy with Et-1 blockers, and showed not only a pathophysiological role of this peptide, but also a prognostic significance, particularly related to cardiovascular mortality.[22]

Fig. 1



Distribution of major cardiac and cerebral events (MACCEs) (%) according to carotid ultrasound findings and plasma values of endothelin-1.

Fig. 2



Distribution of stroke (%) according to carotid ultrasound findings and plasma values of endothelin-1.

Moreover, it should be highlighted that the pathophysiological role of this peptide in hemorrhagic as well as ischemic stroke has already been reported.[23] Stroke causes an interruption in the normal blood flow of cerebral vessels in a specific area. Et-1 receptors localized in neurons, glial cells, and microvessel endothelial cells accounted for a large volume of endothelin in the cerebral circulation; under inflammatory conditions caused by cytokines, the production of Et-1 from smooth muscle cells is increased. Endothelin has a sustained vasoconstrictive effect on cerebral vessels, which opposes the homeostatic mechanism of the body that attempts to dilate the vessels and move to the devoid area oxygen and nutrients.[9–23]

Levels of Et-1 have been discovered actually to drastically increase in the cerebral spinal fluid of stroke patients 18 h after a stroke[24] and have been shown to influence the neurological outcome. Although strokes stem from a variety of factors, hypertension and inflammation are the most prominent contributors to stroke pathophysiology. So the intense vasoconstrictive effect of Et-1 that feeds the mechanisms of hypertension increases even more the already detrimental vascular consequences associated with hypertension and atherosclerosis.[25] Therefore, the development of pharmacological strategies for modifying the production and activity of this potent vasoconstrictor peptide may be beneficial in improving the outcome of brain and heart ischemia.[26,27] The role of carotid IMT or ACP is consistent to previous studies.[28–30]

In the present study, we performed a 20-year follow-up in a group of 82 baseline healthy individuals, to evaluate the occurrence of future cerebrovascular and cardiovascular events in relation to the levels of Et-1.

So the limitations of our study are related to the small sample of patients examined.

In conclusion, our study confirmed the presence of a strong association between known cardiovascular risk factors such as hypertension, carotid preclinical atherosclerosis[27–30] and subsequent cardiovascular or cerebrovascular events. Nevertheless, the present study suggested a possible role of Et-1, as a marker of endothelial dysfunction, influencing primarily cerebrovascular outcome. Moreover, it seems that elevated levels of Et-1 strongly enhance the role of preclinical carotid atherosclerosis as a predictor of MACCE.[28–30]

Therefore, recent studies[31–36] have shown the importance of different laboratory markers in risk stratification of patients with coronary artery disease.

Similarly, several recent studies have demonstrated the predictive validity of new markers. In particular, soluble suppression of tumorigenicity 2 has a role as an early marker of cardiovascular diseases, above all in heart failure and ischemic heart diseases.[37] Moreover, the serum osteoprotegerin is important in predicting coronary artery disease.[38] Recent study evaluated also the dosage of salivary N-terminal probrain natriuretic peptide as a predictive method for heart failure.[39]

The results of our study suggest the possibility of using a new laboratory parameter, which is endothelin, to better define the cerebrovascular and cardiovascular risk.

Given the demonstrated relationship to events at the follow-up, a possible therapeutic intervention on Et-1 (e.g. with receptor antagonists for the Et-1 as bosentan and ambrisentan, usually used for pulmonary hypertension) may not only have a positive effect on cardiac function, but also an important role in preventing new cardiovascular events, through a more accurate stratification of patients, as well as avoiding permanent effects that can result from a cerebral vascular accident.

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