Clinical research

Interaction between vascular dysfunction and cardiac mass increases the risk of cardiovascular outcomes in essential hypertension

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Received 7 August 2004; revised 22 November 2004; accepted 9 December 2004; online publish-ahead-of-print 2 February 2005

Aims To investigate the additive prognostic impact of both forearm endothelial dysfunction and left ventricular mass (LVM) for future cardiovascular events.

Methods and results We enrolled 324 Caucasian, never treated, hypertensive outpatients. Endothelial function, by intra-arterial infusion of acetylcholine (ACh), and echocardiographic LVM were investigated. Patients were divided into tertiles on the basis of their increase in ACh-stimulated forearm blood flow (FBF) and LVM indexed by body surface area (LVMi). Cardiovascular events assessed were: fatal and non-fatal myocardial infarction, fatal and non-fatal stroke, transient cerebral ischaemic attack, unstable angina, coronary revascularization procedures, and symptomatic aorto-iliac occlusive disease. During a mean follow-up of 45.2 ± 23.6 months, there were 47 new cardiovascular events (3.8 events/100 patient-years). The event rate was 6.8, 2.8, and 1.6% in the tertiles of ACh-stimulated FBF (log-rank test, \(P = 0.0009\)), and 1.4, 3.4, and 6.6% in the tertiles of LVMi (log-rank test, \(P = 0.0002\)), respectively. Besides, a significant interaction was documented between FBF and LVMi. In fact, the cardiovascular risk increases up to 11.4% in patients with low FBF and high LVMi.

Conclusion For the first time, we demonstrate that the co-existence of LVH and endothelial dysfunction in hypertensive patients increases significantly the risk of subsequent cardiovascular events.

KEYWORDS Atherosclerosis; Endothelium; Hypertension; Cardiac hypertrophy; Prognosis

Introduction Normal endothelium possesses different vasoprotective effects such as inhibition of platelet aggregation, suppression of adhesion of leukocytes and monocytes on the endothelial surface, and inhibition of migration and proliferation of vascular smooth muscle cells.1–3 Thus, a dysfunctioning endothelium plays a key pathophysiological role in the development and progression of atherosclerosis because it loses the ability to protect the vascular system by reducing its anti-atherosclerotic and antithrombotic actions. Besides, impaired endothelial function has been demonstrated in patients with both traditional4–8 and emerging9,10 risk factors for coronary artery disease (CAD), suggesting that endothelial cells may be both a target and a mediator of atherosclerosis.
Recently, some studies have demonstrated that endothelial dysfunction in both coronary\textsuperscript{11–14} and peripheral\textsuperscript{15} vasculature provides prognostic information for future clinical events.

Similarly, left ventricular hypertrophy (LVH) is recognized as a powerful and independent risk factor for cardiovascular mortality and morbidity\textsuperscript{16,17} because it predisposes to arrhythmias\textsuperscript{18} and maximizes the consequences of acute myocardial ischaemia.\textsuperscript{19} Data from the Framingham Heart Study,\textsuperscript{16} as well as from a recent investigation,\textsuperscript{20} showed that the relationship between left ventricular mass (LVM) and cardiovascular risk is direct and continuous. In addition, some interventional studies have demonstrated that regression of LVM is associated with reduction of cardiovascular events, thus confirming the prognostic value of LVH.\textsuperscript{21,22} Finally, in a previous paper we reported that echocardiographic hypertensive LVM is inversely related to endothelial function, confirming that both LVM and vascular endothelium are damaged by hypertension.\textsuperscript{23}

However, at this moment no data are available to demonstrate a possible additive effect of both endothelial dysfunction and LVH on the cardiovascular prognosis in essential hypertension. The aim of this study was to investigate, in a group of previously never treated hypertensive patients and without previous complications, the prognostic impact of both forearm endothelial dysfunction and LVM for future cardiovascular events.

Methods

Study population

A total of 324 Caucasian hypertensive outpatients at Catanarzo University Hospital, 149 men and 175 women, aged 35–58 years (mean ± SD = 47.7 ± 9.1) were enrolled for this study. None of the patients had history or clinical evidence of CAD [angina or myocardial infarction (MI)], valvular heart disease, diabetes, hyperlipidaemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon. No participants had ever been treated with antihypertensive drugs.

The local ethics committee approved the study, and all participants gave written informed consent for all procedures.

Blood pressure measurements

Clinic blood pressure (BP) was measured in the left arm of supine patients, after 5 min of quiet rest, with a mercury sphygmomanometer. Three BP readings were taken on three separate occasions at least 2 weeks apart. Patients with a clinic BP > 140 mmHg systolic and/or 90 mmHg diastolic were defined as hypertensive.\textsuperscript{24} All patients underwent 24-h ambulatory BP monitoring (A&D TM-2420/2421 recorders) during normal daily activities. Recordings were taken every 10 min during the day and every 20 min during the night.

Echocardiograms

Echocardiographic readings were made, with the patient in partial left decubitus position, by an investigator who had no knowledge of patient BP and other clinical data. Reproducibility of measurements was optimized by having the same experienced sonographer performing all studies in a dimly lit and quiet room.\textsuperscript{25} Tracings were recorded under two-dimensional guidance and measurements were taken at the tip of the mitral valve or just below. Measurements of interventricular septum thickness, posterior wall thickness and left ventricular internal dimension were made at end-diastole and end-systole as recommended by the American Society of Echocardiography.\textsuperscript{26} LVM was calculated with the Devereux formula\textsuperscript{27} and indexed by body surface area (g/m\textsuperscript{2}) (LVMI).

Vascular function

All studies were performed by the same experienced investigators (R.M., A.S.), at 09:00 h after subjects had fasted overnight, in a quiet air-conditioned room (22–24°C), using the protocol previously described by Panza et al.\textsuperscript{28} and, subsequently, employed by our group.\textsuperscript{15,23} All patients underwent measurement of forearm blood flow (FBF) and BP during intra-arterial infusion of saline, acetylcholine (ACh), and sodium nitroprusside (SNP) at increasing doses. Measurements of FBF and vascular resistance (VR), expressed in units (U), were repeated every 5 min until stable. Endothelium-dependent and endothelium-independent vasodilatation were assessed by a dose–response curve to intra-arterial ACh infusions (7.5, 15, and 30 μg/mL/min, each for 5 min) and SNP infusions (0.8, 1.6, and 3.2 μg/mL/min, each for 5 min), respectively. The drug infusion rate, adjusted for forearm volume of each subject, was 1 mL/min. Inter- and intra-observer variability was 3.3 and 2.7%, respectively.

Follow-up and cardiovascular events

All patients were treated to reduce clinic BP < 140/90 mmHg using standard lifestyle and pharmacological treatment. Diuretics, β-blockers, ACE-inhibitors, calcium channel blockers, angiotensin II receptor antagonists, and α\textsubscript{1}-blockers were used alone or in various combinations. Follow-up included periodic control visits and a questionnaire mailed to family physicians. All information regarding potential clinical events was validated by source data including hospital record forms, death certificates, and other available original documents.

Cardiovascular events assessed during long-term follow-up were: fatal and non-fatal MI, fatal and non-fatal stroke, transient cerebral ischaemic attack (TIA), unstable angina, coronary revascularization procedures, and symptomatic aorto-iliac occlusive disease. Diagnosis of acute MI was based on chest pain history, cardiac enzyme measurement, and new ST elevation in at least two leads. Unstable angina was defined by typical chest pain associated with ischaemic electrocardiographic changes and, successively, documented by provocative tests. TIA was defined by clinical diagnosis of any sudden focal neurological deficit that cleared completely in <24 h. Cardiovascular events were validated by physicians not involved in this study.

Statistical analysis

To test differences between clinical and biological data, we used the analysis of variance for continuous variables and the χ\textsuperscript{2} test for categorical variables. Differences between means were compared by the unpaired Student’s t-test. The responses to ACh and SNP were compared by ANOVA for repeated measurements and, when analysis was significant, Tukey’s test was applied. Event rate is reported as the number of events/100 patient-years based on the ratio of the number of events...
observed to the total number of patient-years of exposure up to the terminating event or censor. For patients without events, the date of censor was that of the last contact. For the patients who experienced multiple events, survival analysis was restricted to the first event. Survival curves were estimated by using the Kaplan–Meier product-limit method and compared by using the Mantel (logistic-rank) test. The effect of prognostic factors on survival was evaluated by using a multivariable Cox regression model. We tested the following variables: age, gender, serum cholesterol, smoking habits (previous or never smokers, current smokers), and clinic and ambulatory BP. For categorical variables, proportional hazards were assessed both by visual inspection and by the log-log method. For continuous variables, the proportional risk assumption was tested by relating the Schoenfeld residuals of the Cox analysis with the statistical package SPSS 10.0 for Windows.

**Results**

**Study population**

Demographic, clinical, and haemodynamic characteristics of the study population, stratified by tertiles of both ACh-stimulated vasodilation and LVMI, are reported in Table 1. The baseline clinic and ambulatory BP values were 158.2/92.3 ± 14.1/9.4 and 149.8/88.4 ± 9.1/9.0 mmHg. After 6 months and 1 year of treatment, clinic BP decreased to 138.9/86.4 ± 11.7/8.1 and 134.7/83.2 ± 9.9/8.7 mmHg, respectively, and the reduction was similar in the three tertiles. In particular, BP values were 134.2/83.0 ± 9.7/8.8, 135.1/83.4 ± 9.9/8.8, and 134.9/83.3 ± 10.0/8.6 mmHg, respectively. Drugs used in the study population, divided by tertiles of ACh-stimulated FBF and cardiac mass, are reported in Table 2.

**Vascular function**

Intra-arterial infusions of ACh caused a dose-dependent and significant (P < 0.0001) increase in FBF and decrease in forearm VR. The FBF increments from basal at the three incremental doses of ACh were 2.2 ± 1.9 (+69%), 5.0 ± 3.5 (+156%), and 10.4 ± 5.7 mL/100 mL tissue/min (+320%). At the highest dose of ACh (30 µg/min), FBF increased to 14.1 ± 6.0 mL/100 mL tissue/min, and VR decreased to 9.9 ± 4.8 U.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics by tertiles of ACh-stimulated FBF and cardiac mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st 2nd 3rd</td>
</tr>
<tr>
<td>Age, years</td>
<td>48.5 ± 9.1</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>66/42</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.4 ± 3.4</td>
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<tr>
<td>Current smokers, %</td>
<td>37.1</td>
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<tr>
<td>Cholesterol, mmol/L</td>
<td>5.1 ± 0.7</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.31 ± 0.30</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.1 ± 0.5</td>
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<tr>
<td>BP, mm Hg</td>
<td></td>
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<tr>
<td>Clinic systolic</td>
<td>160 ± 14</td>
</tr>
<tr>
<td>Clinic diastolic</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>24-h systolic</td>
<td>151 ± 13</td>
</tr>
<tr>
<td>24-h diastolic</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>Mean</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>Heart rate, b.p.m.</td>
<td>75 ± 7</td>
</tr>
<tr>
<td>Basal FBF, 100 mL tissue/min</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>ACh-stimulated FBF, % increase</td>
<td>14.4 ± 49</td>
</tr>
<tr>
<td>ACh-stimulated VR, U</td>
<td>14.0 ± 4.1</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>143 ± 36</td>
</tr>
<tr>
<td>SNP-stimulated FBF, % increase</td>
<td>356 ± 95</td>
</tr>
<tr>
<td>SNP-stimulated VR, U</td>
<td>7.6 ± 2.0</td>
</tr>
</tbody>
</table>

BMI = body mass index; HRT = hormone replacement therapy.
Intra-arterial infusion of ACh caused no changes in BP or heart rate values. The ACh-stimulated FBF was considered both as a continuous variable and according to specific tertiles: ≤216% increase from basal (first), >216% to <369% increase from basal (second), and ≥369% increase from basal (third).

During SNP infusion, a significant increase in FBF and a decrease in forearm VR were observed, but no significant differences were found between tertiles. Peak percentage increases in FBF in the three tertiles were 356 ± 95, 355 ± 99, and 356 ± 86%, respectively (P = 0.614 by ANOVA); the VR values were 7.6 ± 2.0, 7.5 ± 2.8, and 7.6 ± 2.1 (P = 0.287 by ANOVA).

Cardiac mass and FBF

The mean value of L VMI was 128.7 ± 35.5 g/m² in the whole population. For the analysis, LVM was considered both as a continuous variable and according to specific tertiles: ≤112 g/m² (first), >112 to <138 g/m² (second), and ≥138 g/m² (third). The correlational analysis showed an inverse and significant relationship (r = −0.415; P < 0.00001) between L VMI and peak percentage increase in ACh-stimulated FBF.

Outcome events

During the follow-up period (42 months median; range, 8–108 months), there were 47 new cardiovascular morbidity events (3.8 events/100 patient-years) at the cardiac (n = 28), cerebrovascular (n = 15), or peripheral vascular (n = 4) level. In particular, there were nine patients with MI (one fatal), 15 with unstable angina pectoris, four with coronary revascularization procedures, 11 with stroke (one fatal), four with TIA, and four with new onset aorto-iliac occlusive disease.

The event rate in our population could, probably, be due to the high prevalence of second/third grade of hypertension (55%) and LVH (49%). In addition, patients in the lower, in comparison with those in the upper tertile, of ACh-stimulated FBF, showed a greater prevalence of males (61 vs. 32%; P < 0.0001) and, even if the differences were not statistically significant, were older (+2 years) and with higher systolic BP (+3 mmHg). Similar findings were obtained dividing the population by tertiles of L VMI. Patients in the third tertile were older (+2.1 years), with a greater prevalence of males (59 vs. 36%; P < 0.001) and higher systolic (+2 mmHg) and diastolic (+2 mmHg) BP. Finally, it is necessary to remark that in our analysis we included both soft and hard vascular events; excluding 12 soft clinical outcomes (coronary revascularization procedures, TIA, and aorto-iliac occlusive disease), the event rate decreases to 2.8/100 patient-years, a value compatible with the cardiovascular risk profile of our patients.

Total event rate and event-free survival curves by tertiles of ACh-stimulated FBF and LVM are graphically reported in Figure 1. In particular, there were 6.8 (first), 2.8 (second), and 1.6 (third) cardiovascular events (per 100 patient-years) in the tertiles of ACh-stimulated FBF (log-rank test = 14.28; P = 0.0009), and 1.4 (first), 3.4 (second), and 6.6 (third) in the tertiles of L VMI (log-rank test = 16.70; P = 0.0002). Differences across tertiles of ACh-stimulated FBF (P = 0.0001) and L VMI (P = 0.03) also remained significant after exclusion of soft cardiovascular events. Dividing the study population for tertiles of SNP, the event rate (per 100 patient-years) was 3.6, 4.6, and 3.2 (log-rank = 1.30; P = 0.520).

Multivariable analysis

To identify the effect of ACh-stimulated FBF, cardiac mass, and the classic risk factors for atherosclerosis (e.g. age, gender, BMI, serum cholesterol, smoking, BP) as independent predictors of cardiovascular events, we performed a multivariable Cox regression analysis. This model retained only L VMI and ACh-stimulated FBF as independent predictors of clinical events (Figure 2). In particular, for each 10 g/m² of L VMI, the risk increases by 15%; in contrast, for each 1% increase in ACh-stimulated FBF, the risk reduces by 0.4%. A marked risk gradient for adverse events was also noted across the tertiles of L VMI. In fact, in the second and third tertiles, event risk increases more than two- and three-fold, respectively, even if the significance is reached only in the third tertile (HR = 3.52, 95% CI = 1.39–8.90; P = 0.007). In contrast, a low-risk gradient for clinical outcome was observed across the tertiles of ACh-stimulated FBF. In particular, the event risk was
reduced by about 50% in the second tertile and by about two-thirds in the third tertile, even if the variation is significant only in the latter (HR = 0.34, CI = 0.13–0.86; P = 0.023). Age, smoking, and cholesterol were correlated with endothelial dysfunction, but did not reach statistical significance, probably due to the homogeneity of the enrolled population.

Figure 3 shows that for every tertile of ACh-stimulated FBF, the rate of total events significantly increases from the first to the third tertile of LVMI (log-rank test; all P < 0.005). Furthermore, we demonstrated by a Cox regression analysis, including age, gender, and monitored systolic BP, a clear interaction between FBF and LVMI because the event rate increases to 11.4% in patients with low FBF (first tertile) and high LVMI (third tertile) (Table 3).

Discussion

In this study we demonstrate that both ACh-stimulated vasodilation and echocardiographic LVMI are independent predictors of cardiovascular events in a group of never treated hypertensives. In addition, for the first time, we documented an evident biological interaction between FBF and LVMI, because the event rate significantly increases in patients with low FBF and increased LVMI. In particular, the co-existence of both LVH and endothelial dysfunction almost doubles the risk for future vascular events in hypertensives. This condition might have clinical importance because the detection of LVH and endothelial dysfunction contributes to defining the global cardiovascular risk in essential
hypothesis, our data extend previous findings because increased risk in hypertensives. In accordance with this, clinically relevant, may also be supported by the fact that regression of LVH appears to be a favourable prognostic marker independent of the treatment-induced reduction in BP.

Similarly, we and others have recently reported that forearm and coronary endothelial dysfunction can provide prognostic value independent of that provided by assessment of the traditional cardiovascular risk factors. In accordance with this, a noteworthy clinical importance might be attributed to recent results obtained by Modena et al., demonstrating that therapy-related endothelial improvement reduces cardiovascular outcomes in hypertensive post-menopausal women.

Clinical implications and conclusions

We demonstrate, for the first time, that the co-existence of LVH and endothelial dysfunction in hypertensives significantly increases the risk of future cardiovascular events. In addition, we and others have reported that hypertensives with LVH have attenuated forearm and coronary endothelium-dependent vasodilation, showing that both the endothelium and left ventricle are damaged by hypertension. In keeping with this, because measurements of LVM may easily be obtained by echocardiography, we suggest that patients with LVH be considered at higher cardiovascular risk for the probable co-existence of endothelial damage. Taken together, because both LVH and endothelial dysfunction are reversible disorders, it seems attractive and clinically useful to use, in these patients, an aggressive therapeutic strategy in order to reduce LVM and to improve endothelial function. However, we remark that tight control of the established and modifiable cardiovascular risk factors still remains the primary strategy to improve the prognosis in patients at high risk for vascular diseases.

Endothelial dysfunction and cardiovascular risk

Cardiovascular risk factors are associated with cardiovascular morbidity and mortality. The mechanism by which risk factors promote atherosclerosis and lead to clinical events is not entirely defined, even if endothelial activation can now be considered the pathogenetic link between these conditions. In fact, endothelial dysfunction, an early event in atherogenesis, may often be associated with erosion and/or rupture of the atherosclerotic plaque, which promotes plaque instability and acute vascular syndromes.

The endothelium contributes to vascular homeostasis by producing some vasoactive substances that act in the blood vessel wall and lumen. Normally, all these endothelial substances regulate vascular tone, thrombogenesis, lipid breakdown, vascular inflammation, and smooth muscle cell proliferation. However, in the presence of vascular risk factors, the endothelium changes its phenotype, promoting vasoconstriction, thrombosis, vascular inflammation, and cell proliferation and, consequently, plays a key pathophysiological role in the development and progression of the atherosclerotic process.

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted hazard ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.713</td>
<td>0.590–4.968</td>
<td>0.321</td>
</tr>
<tr>
<td>2</td>
<td>2.065</td>
<td>0.802–5.315</td>
<td>0.132</td>
</tr>
<tr>
<td>3</td>
<td>4.978</td>
<td>2.394–10.349</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

0 = Reference group (1st tertile of LVM + 3rd tertile of FBF). 1 = 3rd tertile of LVM + 3rd tertile of FBF. 2 = 1st tertile of LVM + 1st tertile of FBF. 3 = 3rd tertile of LVM + 1st tertile of FBF.
Study limitations

Our findings have been obtained in initially untreated white subjects, so results may not be extended to different racial groups or to subjects receiving antihypertensive treatment at the time of the qualifying evaluation. Another limitation of the study was the lack of assessment of the serial changes in FBF, BP, and cardiac mass over time. Finally, the invasiveness of the method used to evaluate endothelial function, even if minimal, may represent another limitation of this study. In fact, the method cannot be easily applicable in a large prospective human study.

Acknowledgements

This study was supported in part by grants from PRIN-COFIN 2000 (MM06A92341-002) – Ministero dell’Università e Ricerca Scientifica e Tecnologica.

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