ESC HEART FAILURE
ESC Heart Failure 2021; **8**: 399–407
Published online 25 November 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13088

ORIGINAL RESEARCH ARTICLE

Endothelial dysfunction and C-reactive protein predict the incidence of heart failure in hypertensive patients

Raffaele Maio[1], Maria Perticone[2], Edoardo Suraci[2], Angela Sciacqua[2], Giorgio Sesti[3] and Francesco Perticone[2]*

[1]*Division of Geriatrics, Azienda Ospedaliero—Universitaria Mater Domini, Catanzaro, Italy;* [2]*Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Viale Europa, Catanzaro, 88100, Italy;* [3]*Department of Clinical and Molecular Medicine, University of Rome-La Sapienza, Rome, Italy*

Abstract

Aims Endothelial dysfunction and heart failure are associated, but no prospective studies demonstrated that impaired endothelium-dependent vasodilation predicts incident heart failure. We designed this study to test whether endothelial dysfunction is associated with incident heart failure in a group of hypertensives.

Methods and results We enrolled 735 White never-treated hypertensive outpatients free from heart failure, diabetes, chronic kidney disease, and previous cardiovascular events. Endothelium-dependent vasodilation was investigated by intra-arterial infusion of acetylcholine, and laboratory determinations were obtained by standard procedures. During the follow-up [median 114 months (range 26–206)], there were 208 new cases of heart failure (3.1 events/100 patient-years). Dividing the study population in progressors and non-progressors, we observed that progressors were older, showed a higher prevalence of being female, and had a higher baseline heart rate, glucose, insulin, Homeostatic Model Assessment (HOMA), creatinine, and high-sensitivity C-reactive protein (hs-CRP) mean values, while estimated glomerular filtration rate and maximal acetylcholine-stimulated forearm blood flow were lower. In the multiple Cox regression analysis, female gender [hazard ratio (HR) = 1.454, 95% CI = 1.067–1.981], fasting glucose (HR = 1.186, 95% CI = 1.038–1.357), hs-CRP (HR = 1.162, 95% CI = 1.072–1.259), HOMA (HR = 1.124, 95% CI = 1.037–1.219), acetylcholine-stimulated forearm blood flow (HR = 0.779, 95% CI = 0.695–0.874), and estimated glomerular filtration rate (HR = 0.767, 95% CI = 0.693–0.849) maintained an independent association with the outcome. Successively, testing the interaction between forearm blood flow and hs-CRP, we observed that patients who have hs-CRP values above the median and forearm blood flow under the median show a higher risk of developing heart failure (HR = 7.699, 95% CI = 4.407–13.451).

Conclusions The present data demonstrate that an impaired endothelium-dependent vasodilation and hs-CRP predict development of incident heart failure in hypertensives.

Keywords Hypertension; Heart failure; Endothelium; Inflammation; Prognosis

Received: 7 September 2020; Revised: 14 October 2020; Accepted: 23 October 2020
**Correspondence to: Francesco Perticone, Department of Medical and Surgical Sciences, University Magna Graecia of Catanzaro, Viale Europa, 88100 Catanzaro, Italy. Tel: +39.09613647149; Fax: +39.09613647634. E-mail: perticone@unicz.it*
Raffaele Maio and Maria Perticone have contributed equally to this work.

Introduction

Endothelial dysfunction, characterized by a reduced nitric oxide (NO) bioavailability, is usually considered as a consequence of the exposure to cardio-metabolic risk factors that, promoting oxidative stress and pro-inflammatory pathways, induce the appearance and progression of atherosclerotic vascular damage.[1–6] On the other hand, we and others demonstrated that endothelial dysfunction is a potent and independent prognostic factor for adverse fatal and nonfatal cardiovascular outcomes.[7,8] In addition, some evidences demonstrated that endothelial dysfunction predicts the appearance of new diabetes[9] and the progression of subclinical target organ damage, such as atherosclerotic vascular injury,[10] hypertensive cardiac hypertrophy,[11] and renal impairment,[12] demonstrating a possible causative effect in the appearance and progression of cardiovascular continuum.

Heart failure (HF), whose incidence is increasing worldwide, is a complex clinical syndrome characterized by impairment of both cardiac structure and function and neurohormonal regulation. HF is one of the most common causes of hospitalization recurrence and death,[13] remaining thus a challenge to be overcome in order to increase patients' life expectancy and reduce health care costs. As a consequence of the worldwide increase of cardiovascular risk factors, both coronary artery disease and diabetes represent the major underlying pathogenetic mechanisms involved in the appearance and progression of HF. It is likely that endothelial dysfunction contributes to HF appearance with different mechanisms, such as the reduction of vasoreactivity of epicardial and small coronary vessels, the after-load increase, myocardial oxidative stress, and fibrotic process.[14,15] Conversely, several findings demonstrated that peripheral endothelial dysfunction is present in patients with chronic HF, in those with both reduced and preserved ejection fraction.[15–18] All these observations consent to affirm that HF-related endothelial dysfunction is, at the same time, a marker and a risk factor for HF. Furthermore, recent data demonstrated that inflammation plays a key role in the appearance and progression of HF, especially in patients with reduced ejection fraction,[19–21] confirming the strong interplaying between endothelial dysfunction and inflammation itself.

However, despite the large amount of data regarding the bidirectional association between endothelial dysfunction and HF, there are currently no prospective studies demonstrating that reduced endothelium-dependent vasodilation may predict incident HF. Thus, we designed this study to assess whether endothelium-dependent vasodilation, tested by pharmacologic stimulation of muscarinic receptor, may be considered an independent predictor of incident HF in a group of never-treated hypertensive patients.

Methods

From a large cohort of 812 newly diagnosed hypertensives participating in the CATanzaro Metabolic Risk factors (CATAMERI) study, we recruited a total of 735 Caucasian patients [372 men and 363 women aged 22–73 years (mean age 48.1 ± 10.6 years)] with systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic 90 mmHg. From the initial cohort, 17 patients died, 41 were lost to follow-up, and nine refused to continue the study. Exclusion criteria were as follows: previous cardiovascular events, diabetes mellitus defined as HbA1c $\geq$ 6.5%, or fasting plasma glucose $\geq$ 126 mg/dL, chronic kidney disease [estimated glomerular filtration rate (e-GFR) $<$ 60 mL/min/1.73 m^2], liver and peripheral vascular disease, and HF (diagnosed according to both clinical and echocardiographic criteria).

The CATAMERI study was submitted and approved on 17 October 2012 (approval number 2012.63) by the Ethics Committee of the Azienda Ospedaliero—Universitaria Mater Domini of Catanzaro. The investigation conforms with the principles outlined in the *Declaration of Helsinki*. All the participants gave their informed written consent for study participation.

Blood pressure measurements

After an initial BP measurement in both arms to detect a possible difference between them, readings of clinic BP were obtained in accordance with current guidelines at the moment of the evaluation, after 5 min of quiet rest, with a mercury sphygmomanometer. A minimum of three BP readings were taken on three separate occasions at least 2 weeks apart. SBP and diastolic BP (DBP) were recorded at the first appearance (Phase I) and the disappearance (Phase V) of Korotkoff sounds. Baseline BP values were the average of the last two of the three consecutive measurements obtained at intervals of 3 min. Patients with a clinic BP $\geq$140 mmHg systolic and/or $\geq$90 mmHg diastolic were defined as hypertensive. Secondary forms of hypertension were excluded by systematic testing according to a standard clinical protocol, which included measurement of plasma renin activity, aldosterone, Doppler studies of the renal arteries, and/or renal scintigraphy or renal angiography.

Laboratory determinations

At the first eligibility visit, all laboratory measurements were performed after a fasting period of at least 12 h. Plasma glucose was determined by the glucose oxidase method (glucose analyser, Beckman Coulter SpA, Milan, Italy). Triglyceride and cholesterol concentrations were measured by enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). Serum creatinine was measured by an automated technique based on the measurement of Jaffe chromogen and by the URICASE/POD method (Boehringer Mannheim, Mannheim, Germany) implemented in an auto-analyser. Values of e-GFR were calculated by using the equation proposed by investigators in the Chronic Kidney Disease Epidemiology. High-sensitivity C-reactive protein (hs-CRP) was measured, in 653 patients, by a turbidimetric immunoassay (Behring).

Follow-up and incident heart failure

New cases of HF were confirmed on the basis of the following criteria: signs and symptoms as dyspnoea on exertion, difficulty exercising and oedema; echocardiographic or other imaging tests to evaluate cardiac structure and function, particularly ejection fraction to define HF with reduced (HFrEF)

or preserved (HFpEF) left ventricular function; natriuretic peptides; and, for hospitalized patients, *International Classification of Diseases*, Tenth Revision codes as any diagnosis: HF (I50), cardiomyopathies (I42.0, I42.6, I42.8, and I42.9), and hypertension-induced heart disease (I11.0, I13.0, and I13.2).

Follow-up included periodic control visits at least every 6 months in the outpatient clinic. To improve long-term follow-up, a questionnaire was also mailed to family physicians, and patients were contacted by phone every 6 months. All clinical events had to be validated by a local committee on the basis of source data (hospital records, death certificates, or other original documents).

Vascular function evaluation

Vascular function assessments were performed at the first observation. All studies were performed by the same experienced investigators (R. M., M. P., and A. S.), at 9:00 a.m. after overnight fasting, with the subjects lying supine in a quiet air-conditioned room (22–24°C). The investigators were unaware of the patients' clinical and laboratory parameters. The subjects were instructed to continue their regular diet, while caffeine and alcohol were all prohibited 24 h before the study. To test vascular reactivity, we used the protocol previously described by Panza et al.[1] and subsequently employed by our group.[6,7,9,11,12] All patients underwent measurement of forearm blood flow (FBF) and BP during intra-arterial infusion of saline, acetylcholine, and sodium nitroprusside at increasing doses. Measurements of FBF and vascular resistance were repeated every 5 min until stable. Endothelium-dependent and endothelium-independent vasodilations were assessed by a dose–response curve to intra-arterial acetylcholine infusions (7.5, 15, and 30 μg/min, each for 5 min) and sodium nitroprusside infusions (0.8, 1.6, and 3.2 μg/min, each for 5 min), respectively. The infusion rate was 1 mL/min to avoid forearm volume modification. Forearm vascular resistance, expressed in arbitrary units (U), was calculated by dividing mean BP by FBF. For the present study, the maximal response to acetylcholine was considered for statistical analysis.

Statistical analysis

Data are expressed as means ± SD or as percentage frequency, and comparisons between groups were made by one-way ANOVA, Student's *t*-test, or the χ^2 test, as appropriate. A value of $P \leq 0.05$ was considered statistically significant. The events rate is reported as the number of events per 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 the patients without events, the date of censor was that of the last contact with the patient. Survival curves were estimated by use of the Kaplan–Meier product-limit method and compared by using the mantel (log-rank test).

The association between endothelial function and incidence risk of HF was analysed by univariate and multiple Cox regression analyses. Tested covariates included maximal vasodilatory response to acetylcholine as well as traditional [age, gender, smoking, fasting glucose, serum cholesterol, SBP, and body mass index (BMI)] and emerging [fasting insulin, Homeostatic Model Assessment (HOMA) index, and hs-CRP] cardiovascular risk factors. The multiple Cox regression model was constructed by including all variables that turned out to be associated with incident risk of HF ($P < 0.10$) at univariate Cox regression analysis. By this strategy, we constructed a Cox model of adequate statistical power (at least 10 events for each variable into the final model). Data are expressed as hazard ratio (HR), 95% CI, and *P* value.

Point estimates of the probability of HF occurrence associated with maximal vasodilatory response to acetylcholine were calculated by using the equation derived from the multiple Cox regression analysis. Analysis of biological interaction between acetylcholine-stimulated FBF and hs-CRP in a subgroup of 653 patients was performed, as previously described by Greenland and Rothman,[22] by dividing patients into four groups in relation to the median of acetylcholine-stimulated FBF and hs-CRP.

Results

Baseline characteristics of patients who progressed toward HF (progressors) and those remaining free of HF (non-progressors) are reported in *Table 1*. There were no statistically significant differences between the two groups in BMI, smoking habit, SBP and DBP, total cholesterol, triglyceride, uric acid, and basal FBF. On the contrary, progressors were older, showed a higher prevalence of being female, and had a higher baseline heart rate, glucose, insulin, HOMA, and creatinine mean values, while e-GFR values were lower. Similarly, mean hs-CRP values, measured in 653 patients, were significantly higher in progressors than in the control group (4.44 ± 1.47 vs. 3.40 ± 1.70 mg/L; $P < 0.0001$). In addition, the highest response in acetylcholine-stimulated FBF was significantly lower in progressors compared with the non-progressor group (222 ± 130 vs. 332 ± 187%; $P < 0.0001$); in contrast, no significant differences were observed in maximal vasodilation induced by sodium nitroprusside (312 ± 113 vs. 320 ± 111%; $P = 0.401$).

At the first eligibility visit, none of the patients had been treated with anti-hypertensive drugs. In the whole study population, baseline BP values were 148.5/90.3 ± 17.2/12.0 mmHg, with a little but not significant difference in

Table 1 Baseline characteristics of the study population stratified as progressors and non-progressors to heart failure

	All	Progressors	Non-progressors	*P*
n	735	208	527	
Gender, female (%)	362 (49.3)	130 (62.5)	232 (44.0)	0.0001
Age, years	48.1 ± 10.6	50.0 ± 11.0	47.4 ± 10.4	0.004
BMI, kg/m^2	27.4 ± 3.6	27.9 ± 4.1	27.2 ± 3.4	0.250
Current smokers, *n* (%)	111 (15.1)	36 (17.3)	74 (14.2)	0.294
Systolic BP, mmHg	148.5 ± 17.2	150.3 ± 16.3	147.8 ± 17.5	0.078
Diastolic BP, mmHg	90.3 ± 12.0	90.9 ± 10.9	90.1 ± 12.4	0.436
Heart rate, b.p.m.	72.5 ± 9.7	70.1 ± 9.1	73.2 ± 9.8	0.002
Fasting glucose, mg/dL	95.2 ± 10.6	97.0 ± 11.1	94.5 ± 10.3	0.004
Fasting insulin, U/L	13.9 ± 7.2	17.0 ± 8.1	12.8 ± 6.5	0.0001
HOMA	3.3 ± 1.8	4.1 ± 2.1	3.0 ± 1.6	0.0001
Total cholesterol, mg/dL	204.8 ± 31.4	203.7 ± 32.3	205.3 ± 31.1	0.534
LDL cholesterol	129.3 ± 31.5	128.9 ± 32.4	129.5 ± 30.7	0.822
HDL cholesterol	51.9 ± 12.3	51.2 ± 13.1	52.2 ± 11.9	0.534
Triglyceride, mg/dL	115.9 ± 39.1	117.3 ± 40.6	115.4 ± 38.5	0.560
Creatinine, mg/dL	0.95 ± 0.19	1.1 ± 0.2	0.9 ± 0.2	0.0001
e-GFR, mL/min/1.7 m^2	84.9 ± 20.0	69.9 ± 17.7	90.8 ± 17.6	0.0001
Uric acid, mg/dL	5.0 ± 1.7	5.2 ± 1.6	5.0 ± 1.7	0.932
hs-CRP, mg/dL	3.70 ± 1.71	4.44 ± 1.47	3.40 ± 1.70	0.0001
New diabetes, *n* (%)	99 (13.5)	43 (20.7)	56 (10.6)	0.0001
New coronary events, *n* (%)	217 (29.5)	128 (61.5)	89 (16.9)	0.0001
Forearm blood flow				
Basal, mL·100 ml tissue^{-1}·min^{-1}	3.36 ± 0.66	3.27 ± 0.61	3.40 ± 0.67	0.998
Acetylcholine, % increase	301 ± 180	222 ± 130	332 ± 187	0.0001
Sodium nitroprusside, % increase	318 ± 112	312 ± 113	320 ± 111	0.401
Anti-hypertensive drugs				
ACE-i/ARBs, *n* (%)	570 (77.5)	160 (76.9)	410 (77.8)	0.797
Calcium antagonists, *n* (%)	255 (34.7)	71 (34.1)	184 (34.9)	0.841
Beta-blockers, *n* (%)	63 (8.6)	18 (8.6)	45 (8.5)	0.960
Alpha-blockers, *n* (%)	18 (2.4)	6 (2.4)	12 (2.3)	0.631
Diuretics, *n* (%)	122 (16.6)	35 (16.8)	87 (16.5)	0.916
Associations, *n* (%)	411 (55.9)	116 (55.7)	295 (55.9)	0.959

ACE-i, angiotensin converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; LDL, low-density lipoprotein.

SBP between the two groups (150.3 ± 16.3 vs. 147.8 ± 17.5 mmHg). All patients were treated to reduce clinical BP < 140/90 mmHg using standard lifestyle and pharmacological treatment. Diuretics, beta-blockers, angiotensin converting enzyme inhibitors, calcium channel blockers, angiotensin II receptor antagonists, and α1-blockers were used alone or in various associations without significant differences between the groups. Anti-hypertensive drugs used in the study population are reported in *Table 1*. No significant differences between groups were observed in the percentage of patients reaching recommended BP target (64 vs. 66% in the progressor and non-progressor patients, respectively).

Follow-up and incident events

During follow-up [median 114 months (range 26–206)], there were 208 new cases of HF (3.1 events/100 patient-years); of these, 107 had an ischaemic aetiology, 18 were attributable to diabetic cardiomyopathy, and 83 had a hypertensive aetiology. In particular, we documented 137 cases of HFpEF and 71 cases of HFrEF; patients with HFrEF were older (53.4 ± 8.9 vs. 47.8 ± 9.3 years; $P < 0.0001$) with lower percentage of women (39.4 vs. 60.6%) and, obviously, ejection fraction value (37.4 ± 4.2 vs. 63.2 + 6.5%; $P < 0.0001$).

In addition, we recorded 99 new cases of diabetes (1.9 events/100 patient-years) and 217 (3.0 events/100 patient-years) new cases of vascular morbid events at the cardiac (*n* = 152), cerebrovascular (*n* = 57), or peripheral vascular (*n* = 8) level. In particular, there were 54 patients with myocardial infarct (three fatal), 58 with unstable angina pectoris, 40 with coronary revascularization procedures, 47 with stroke (5 fatal), 10 with transient cerebral ischaemia, and eight with new onset peripheral occlusive disease.

Vascular function

In the whole population, intra-arterial acetylcholine infusion caused a significant dose-dependent increase in FBF and decrease in vascular resistance. The FBF increments from basal (3.36 ± 0.66 mL·100 mL tissue^{-1}·min^{-1}) at the three incremental doses were 1.9 ± 1.2 (55.8%), 5.4 ± 3.5 (158.8%), and 10.2 ± 6.3 mL·100 mL tissue^{-1}·min^{-1} (301%). At the highest dose of acetylcholine (30 μg/min), FBF increased to 13.6 ± 3.9 mL·100 mL tissue^{-1}·min^{-1}, and vascular resistance decreased to 10.4 ± 4.9 units. Interestingly, dividing the study

population in progressors and non-progressors, we observed (*Table 1*) a significant difference in maximal acetylcholine-stimulated FBF: 222.4 ± 130.1 vs. 332.0 ± 187.3 mL·100 mL tissue^{-1}·min^{-1}, as well as at first (42.7 ± 31.5 vs. 60.1 ± 36.9 mL·100 mL tissue^{-1}·min^{-1}) and second dose of acetylcholine infusion (115.3 ± 77.1 vs. 175.3 ± 108.1 mL·100 mL tissue^{-1}·min^{-1}). The curves under the area were significantly different (135 ± 87 vs. 201 ± 119; $P < 0.0001$). Event-free survival curves, crude and adjusted, above and under maximal FBF mean value (260%) are graphically reported in *Figure 1*.

Similarly, sodium nitroprusside infusion induced (*Table 1*) a significant increase in FBF (maximal increment from the basal, +318%) and a decrease in vascular resistance (−72%), without significant differences between groups. As expected, intra-arterial infusion of vasoactive substances caused no changes in BP or heart rate values; in fact, doses of infused vasoactive substances are very low and aimed to only modify FBF, without a systemic effect.

Cox regression analyses

On univariate analysis (*Table 2*), incident risk of HF was inversely related with maximal vasodilatory response to acetylcholine [100% increase, HR = 0.720 (95% CI = 0.648–0.799), $P < 0.0001$], e-GFR [10 mL/min/1.73 m^2 increase, HR = 0.694 (95% CI = 0.639–0.755), $P < 0.0001$] and directly with serum creatinine [HR = 7.899 (95% CI = 4.016–15.534), $P < 0.0001$], gender [HR = 1.504 (95% CI = 1.134–1.995), $P < 0.005$], HOMA [HR = 1.249 (95% CI = 1.166–1.338), $P < 0.0001$], hs-CRP [HR = 1.236 (95% CI = 1.149–1.328), $P < 0.002$], fasting glucose [10 mg/dL increase, HR = 1.219 (95% CI = 1.074–1.284), $P < 0.002$], age [10 years' increase, HR = 1.152 (95% CI = 1.008–1.316), $P = 0.038$], and fasting insulin [HR = 1.050 (95% CI = 1.031–1.068), $P < 0.0001$]. No association was found between occurrence of HF and SBP, BMI, cholesterol, and smoking.

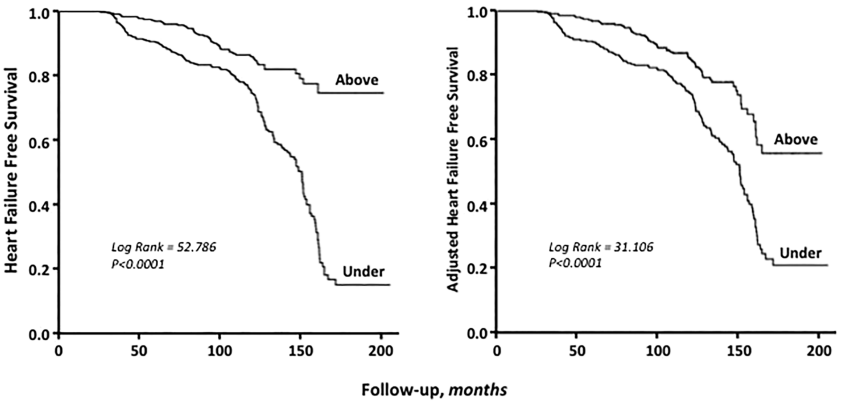
Table 2 Cox regression analysis for incident heart failure

	Hazard ratio	95% CI	*P*
Univariate			
Creatinine, 1 mg/dL	7.899	4.016–15.534	0.0001
Gender, female	1.504	1.134–1.995	0.005
HOMA	1.249	1.166–1.338	0.0001
hs-CRP, mg/dL	1.236	1.149–1.328	0.0001
Fasting glucose, 10 mg/dL	1.219	1.074–1.284	0.002
Age, 10 years	1.152	1.008–1.316	0.038
Fasting insulin, U/L	1.050	1.031–1.068	0.0001
FBF, 100% increase	0.720	0.648–0.799	0.0001
e-GFR, 10 mL/min/1.7 m^2	0.694	0.639–0.755	0.0001
Systolic BP, 10 mmHg	1.042	0.961–1.131	0.321
BMI, kg/m^2	1.038	0.999–1.078	0.053
Smoking	0.997	0.695–1.429	0.985
Total cholesterol, 10 mg/dL	0.975	0.933–1.019	0.267
Multivariate, model 1			
Gender, female	1.536	1.145–2.059	0.004
Fasting glucose, 10 mg/dL	1.147	1.011–1.301	0.033
HOMA	1.124	1.037–1.219	0.005
FBF, 100% increase	0.753	0.676–0.838	0.0001
e-GFR, 10 mL/min/1.7 m^2	0.748	0.679–0.824	0.0001
Multivariate, Model 2			
Gender, female	1.454	1.067–1.981	0.018
Fasting glucose, 10 mg/dL	1.186	1.038–1.357	0.012
hs-CRP, mg/dL	1.162	1.072–1.259	0.0001
HOMA	1.120	1.032–1.218	0.005
FBF, 100% increase	0.779	0.695–0.874	0.0001
e-GFR, 10 mL/min/1.7 m^2	0.767	0.693–0.849	0.0001
Multivariate, Model 3			
Coronary events	3.312	2.274–4.825	0.0001
Gender, female	1.695	1.168–2.462	0.006
hs-CRP, mg/dL	1.208	1.092–1.336	0.0001
Fasting glucose, 10 mg/dL	1.167	1.006–1.354	0.041
HOMA	1.118	1.028–1.216	0.009
FBF, 100% increase	0.868	0.756–0.996	0.044

HOMA, Homeostatic Model Assessment; Model 2, Model 1 + hs-CRP; Model 3, Model 2 + diabetes and coronary events.

In the multiple Cox regression analysis, including the variables reaching the statistical significance at univariate analysis, female gender [HR = 1.454 (95% CI = 1.067–1.981),

Figure 1 Kaplan–Meier analysis for heart failure. We graphically reported the crude (left) and adjusted event-free survival curves in hypertensive patients subdivided into above and under median of maximal acetylcholine-stimulated forearm blood flow.



P = 0.018], fasting glucose [HR = 1.186 (95% CI = 1.038–1.357), P = 0.012], serum hs-CRP [HR = 1.162 (95% CI = 1.072–1.259), $P < 0.0001$], HOMA [HR = 1.120 (95% CI = 1.032–1.218), $P < 0.005$], FBF [HR = 0.779 (95% CI = 0.695–0.874), $P < 0.0001$], and e-GFR [HR = 0.767 (95% CI = 0.693–0.849), $P < 0.0001$] maintained an independent association with the outcome (*Table 2*). In this model, we included only HOMA index to avoid a possible colinearity with fasting insulin and because the insulin resistance condition is very frequent in essential hypertension. Successively, according to recent evidences demonstrating the association between HF and subclinical inflammation, we tested the possible interaction between FBF and hs-CRP. In particular, we observed that patients who have hs-CRP values above the median and FBF under the median show a higher risk of developing HF [HR = 7.699 (95% CI = 4.407–13.451), $P < 0.0001$] (*Table 3*). Interestingly, diabetes and coronary events were added into the analysis, only coronary events were retained as independent predictors of incident HF modifying the impact of other covariates without excluding acetylcholine FBF and hs-CRP.

Table 3 Interaction between acetylcholine-stimulated forearm blood flow and high-sensitivity C-reactive protein in predicting incident heart failure

	HR	CI 95%	*P*
0	1		
1	3.459	1.893–6.655	0.0001
2	3.423	1.845–6.352	0.0001
3	7.699	4.407–13.451	0.0001

hs-CRP median, 3.9 mg/L; acetylcholine-stimulated median 260%. 0, hs-CRP under median; acetylcholine above median (reference group); 1, hs-CRP above median; acetylcholine above median; 2, hs-CRP under median; acetylcholine under median; 3, hs-CRP above median; acetylcholine under median.

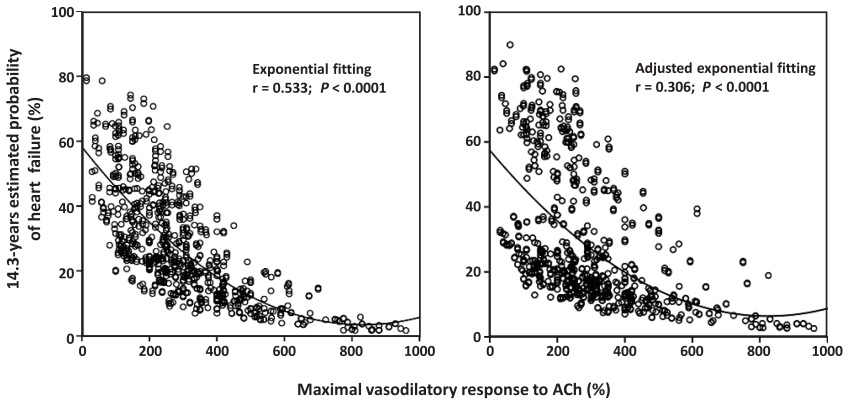
In *Figure 2*, we graphically reported the relationship, expressed as exponential fitting curve, crude and adjusted, between maximal vasodilatory response to acetylcholine and probability of HF occurrence. In addition, we calculated the best cut-off of FBF for predicting the appearance of HF, that is, 261% (area under the receiver operating characteristic curve 0.694, sensitivity 75.5%, and specificity 60.3%).

Discussion

The results of this study demonstrated, for the first time, that endothelial dysfunction is an independent and strong predictor of incident HF in hypertensive patients, allowing to hypothesize its causative role in the cardiovascular continuum, from hypertension to clinical events. Interestingly and clinically relevant, after adjustment for some well-established HF risk factors—such as age, gender, and e-GFR—vasodilatory response to acetylcholine remained a significant predictor of incident HF. Present data provide evidence for a strong association between baseline endothelial function and subsequent development of HF in essential hypertension. Thus, according to this, the true novelty of this study relies in the fact that, for the first time, endothelial dysfunction has been demonstrated to predict HF development, preceding—rather than following—its onset, contrary to what has been reported so far. In fact, in contrast to our results, previous literature data have merely shown that endothelium-dependent vasodilation is blunted in patients with HF, which, in turn, contributes to the worsening of its progression.[14–18]

The underlying pathogenetic mechanisms involved in this association may be recognized in an excess of oxidative stress that reduces the bioavailability of NO, induces a neurohormonal activation with associated release of inflammatory

Figure 2 Relationship between endothelial function and risk of incident heart failure. Crude (left) and adjusted relationship between peak percentage increase in endothelium-dependent vasodilation and the possibility of developing incident heart failure. ACh, acetylcholine.



mediators, and produces alterations of local shear stress due to low cardiac output.[14] In addition, pre-existing traditional cardiovascular risk factors also contribute, through the increase in oxidative stress again, to the vascular motricity impairment, whose association with endothelial dysfunction has well established from long time.[1–4,6] In fact, it is well recognized that reduced endothelium-dependent vasodilation is present in other clinical conditions, such as hypertension, diabetes, and chronic kidney disease, thus providing a plausible explanation of the key role of endothelial dysfunction contributing to HF development.[15,16]

Another important finding obtained in this study is that also hs-CRP was retained, in the multivariate Cox regression analysis, as independent predictor of incident HF, confirming recent evidences supporting the association between them. In fact, several studies demonstrated that failing patients, independent from prevalent systolic or diastolic dysfunction, have increased levels of pro-inflammatory cytokines probably induced by the co-existence of traditional cardiovascular risk factors and maintained by other mechanisms such as immune system activation.[19–21] Of interest, and prognostically relevant, endothelial dysfunction and hs-CRP, interacting between them, strongly increased the risk of incident HF, with HR reaching 7.699 (95% CI = 4.407–13.451) (*Table 3*). Notably, because both inflammation and endothelial dysfunction are present in hypertensive patients,[4,5,9] it is likely that these two conditions represent, among others, the possible pathogenetic mechanisms contributing to the progression from hypertension to HF.

Moreover, increased arterial stiffness may be considered another important pathogenetic mechanism linking endothelial dysfunction to the HF development. In fact, it is well established that the aortic stiffening results in the augmentation of left ventricular systolic workload as consequence of central SBP increase. These haemodynamic modifications are negatively reflected both in the left ventricle, by promoting cardiac hypertrophy[23] with associated diastolic dysfunction, and in the coronary circulation with reduction of coronary perfusion pressure.[24] According to this, we previously demonstrated that impaired endothelium-dependent vasodilation in hypertensive patients is inversely related to pulse pressure, which represents an important marker of vascular aging and arterial stiffness.[25] In addition, other pathogenetic mechanisms related to reduced NO bioavailability are involved in the cardiac hypertrophy process such as the alteration of both matrix metalloproteinases affecting cell migration and the redox-sensitive pathway in response to either chronic pressure overload or neurohumoral stimuli as demonstrated by several experimental data.[26–28] Particularly, mediators of oxidative stress participate in cardiac growth by activation of various mitogen protein kinases and the transcription factor nuclear factor-κB. The proliferative role of these pro-oxidant mediators is confirmed by *in vivo* evidences demonstrating the antioxidant effect in reducing the development of experimental pressure overload cardiac hypertrophy in mice or guinea pigs.[26,29] On the other hand, oxidative stress is well known to promote cardiac interstitial fibrosis excess, which is considered an important detrimental aspect of both left ventricular hypertrophy and subsequent HF.[26,30] Clinically relevant, we previously demonstrated that the increase of endothelial dysfunction parallels the increase of left ventricular mass in hypertensive patients[31] as well as that the preserved endothelium-dependent vasodilation predicts regression of cardiac mass, independently of traditional cardiovascular risk factors and anti-hypertensive therapy.[11] Interestingly and clinically relevant, the co-existence of left ventricular hypertrophy and endothelial dysfunction significantly increases the risk of subsequent fatal and nonfatal cardiovascular events,[32] confirming the importance of better stratifying the cardiovascular risk of the hypertensive patients.

Taken together, the present data clearly demonstrate that endothelial dysfunction is associated with incident HF, thus allowing to hypothesize its causative role in the cardiovascular continuum. Furthermore, given the observed new onset of both diabetes and coronary artery disease during the follow-up, it is plausible that these two clinical conditions, which are well-recognized determinants of both structural and functional cardiac alterations, also contribute to the progression from endothelial dysfunction to HF. These data support what is already known about the progression from hypertension to HF, retaining diabetic cardiomyopathy and ischaemic cardiac dysfunction as intermediate steps in this continuum. Obviously, because endothelial dysfunction is also associated, in a bidirectional manner, with diabetes and ischaemic heart disease, it is possible to affirm that a dysfunctional endothelium concurs to HF development with multiple pathogenetic mechanisms.

The strength of this study relies in the fact that, even if the evaluation of endothelial function is not a routine procedure, the relevance of our data is that we directly tested endothelial function by stimulating muscarinic cholinergic receptors by intra-arterial infusion of vasoactive agonist. The other strengths of this study are the wideness of the study population and the follow-up duration.

On the contrary, study limitations are as follows: it is an observational, nonrandomized, prospective study; our findings were obtained in untreated White hypertensives, so results may not be extended to different racial groups or to subjects receiving anti-hypertensive treatment at the time of the qualifying evaluation.

In conclusion, on the basis of our findings, it is important to reinforce the need to optimize the risk stratification strategies of hypertensive patients through careful phenotyping. Moreover, it may be prognostically useful to develop drugs that, in addition to their anti-hypertensive effect, could attenuate the subclinical inflammation present in these patients.

Conflict of Interest

None declared.

Funding

None.

References

1. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**: 22–27.
2. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J, Dzau VJ. Impaired vasodilation of forearm resistance vessel in hypercholesterolemic humans. *J Clin Invest* 1990; **86**: 228–234.
3. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent coronary arterial vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; **88**: 2510–2516.
4. Quyyumj AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. *Am J Med* 1998; **105**: 32S–39S.
5. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
6. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. *Diabetes* 2001; **50**: 159–165.
7. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 200; **104**: 191–196.
8. Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation* 2005; **111**: 363–368.
9. Perticone F, Maio R, Sciacqua A, Andreozzi F, Iemma G, Perticone M, Zoccali C, Sesti G. Endothelial dysfunction and C-reactive protein are risk factors for diabetes in essential hypertension. *Diabetes* 2008; **57**: 167–171.
10. Halcox JP, Donald AE, Ellins E, Witte DR, Shipley MJ, Brunner EJ, Marmot MG, Deanfield JE. Endothelial function predicts progression of carotid intima-media thickness. *Circulation* 2009; **119**: 1005–1012.
11. Perticone F, Maio R, Perticone M, Miceli S, Sciacqua A, Tassone EJ, Shehaj E, Tripepi G, Sesti G. Endothelial dysfunction predicts regression of hypertensive cardiac mass. *Int J Cardiol* 2013; **167**: 1188–1192.
12. Perticone F, Maio R, Perticone M, Sciacqua A, Shehaj E, Naccarato P, Sesti G. Endothelial dysfunction and subsequent decline in glomerular filtration rate in hypertensive patients. *Circulation* 2010; **122**: 379–384.
13. Seferovic PM, Ponikowski P, Anker SD, Bauersachs J, Chioncel O, Cleland JGF, de Boer RA, Drexel H, Ben Gal T, Hill L, Jaarsma T, Jankowska EA, Anker MS, Lainscak M, Lewis BS, McDonagh T, Metra M, Milicic D, Mullens W, Piepoli MF, Rosano G, Ruschitzka F, Volterrani M, Voors AA, Filippatos G, Coats AJS. Clinical practice update on heart failure 2019: pharmacotherapy, procedures, devices and patient management. An expert consensus meeting report of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail* 2019; **21**: 1169–1186.
14. Giannitsi S, Bougiakli M, Bechlioulis A, Naka K. Endothelial dysfunction and heart failure: a review of the existing bibliography with emphasis on flow mediated dilation. *JRSM Cardiovasc Dis* 2019; **8**: 1–7.
15. Zuchi C, Tritto I, Carluccio E, Mattei C, Cattadori G, Ambrosio G. Role of endothelial dysfunction in heart failure. *Heart Fail Rev* 2020; **25**: 21–30.
16. Colombo PC, Banchs JE, Celaj S, Talreja A, Lachmann J, Malla S, DuBois NB, Ashton AW, Latif F, Jorde UP, Ware JA, Le Jemtel TH. Endothelial cell activation in patients with decompensated heart failure. *Circulation* 2005; **111**: 58–62.
17. Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation* 1991; **84**: 1589–1596.
18. Taqueti VR, Solomon SD, Shan AM, Desai AS, Groarke JD, Osborne MT, Hainer J, Bibbo CF, Dorbala S, Blankstein R, Di Carli MF. Coronary microvascular dysfunction and future risk of heart failure with preserved ejection fraction. *Eur Heart J* 2018; **39**: 840–849.
19. Dick SA, Epelman S. Chronic heart failure and inflammation. What do we really know? *Circ Res* 2016; **119**: 159–176.
20. Shirazi LF, Bissett J, Romw F, Methta JL. Role of inflammation in heart failure. *Curr Atheroscler Rep* 2017; **19**: 27.
21. Perticone M, Zito R, Miceli S, Pinto A, Suraci E, Greco M, Gigliotti S, Hribal ML, Corrao S, Sesti G, Perticone F. Immunity, inflammation and heart failure: their role on cardiac function and iron status. *Front Immunol* 2019; **10**: 2315.
22. Greenland S. In Rothman K. J., ed. *In Modern Epidemiology*. Philadelphia: Lippincott Raven; 1998. p 329–342.
23. Khattar RS, Acharya DU, Kinsey C, Senior R, Lahiri A. Longitudinal association of ambulatory pulse pressure with left ventricular mass and vascular hypertrophy in essential hypertension. *J Hypertens* 1997; **15**: 737–743.
24. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, O'Rourke M, CAFE Investigators, Anglo-Scandinavian Cardiac Outcomes Trial Investigators, CAFE Steering Committee and Writing Committee. Differential impact of blood pressure—lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006; **113**: 1213–1225.
25. Ceravolo R, Maio R, Pujia A, Sciacqua A, Ventura G, Costa MC, Sesti G, Perticone F. Pulse pressure and endothelial dysfunction in never-treated hypertensive patients. *J Am Coll Cardiol* 2003; **41**: 753–758.
26. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signaling in cardiac hypertrophy and heart failure. *Heart* 2007; **93**: 903–907.
27. Marti CN, Gheorghiade M, Kalogeropoulos AP, Georgiopoulou VV, Quyyumi AA, Butler J. Endothelial dysfunction, arterial stiffness, and heart failure. *J Am Coll Cardiol* 2012; **60**: 1455–1469.
28. Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: the cytokine hypothesis. *J Card Fail* 1996; **2**: 243–249.
29. Li JM, Gall NP, Grieve DJ, Grieve DJ, Chen M, Shan AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* 2002; **40**: 477–484.
30. Johar S, Cave AC, Narayanapanicker A, Grieve DJ, Shah AM. Aldosterone mediates angiotensin II-induced interstitial cardiac fibrosis via a Nox2-containing NADPH oxidase. *FASEB J* 2006; **20**: 1546–1548.
31. Perticone F, Maio R, Ceravolo R, Cosco C, Cloro C, Mattioli PL. Relationship between left ventricular mass and endothelium-dependent vasodilation in never-treated hypertensive

patients. *Circulation* 1999; **99**: 1991–1996.
32. Sciacqua A, Scozzafava A, Pujia A, Maio R, Borrello F, Andreozzi F, Vatrano M, Cassano S, Perticone M, Sesti G, Perticone F. Interaction between vascular dysfunction and cardiac mass increases the risk of cardiovascular outcomes in essential hypertension. *Eur Heart J* 2005; **26**: 921–927.