

Noninvasive assessment of endothelial function and ST segment changes during exercise testing in coronary artery disease

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Endothelial function (EF) plays an important role in the onset and clinical course of atherosclerosis, although its relationship with the presence and extent of coronary artery disease (CAD) has not been well defined. We evaluated EF and the ST segment response to an exercise test in patients with a broad spectrum of CAD defined by coronary angiography. Sixty-two patients submitted to diagnostic catheterization for the evaluation of chest pain or ischemia in a provocative test were divided into three groups according to the presence and severity of atherosclerotic lesions (AL): group 1: normal coronaries (N = 19); group 2: CAD with AL <70% (N = 17); group 3: CAD with AL ≥70% (N = 26). EF was evaluated by the percentage of flow-mediated dilatation (%FMD) in the brachial artery during reactive hyperemia induced by occlusion of the forearm with a pneumatic cuff for 5 min. Fifty-four patients were subjected to an exercise test. Gender and age were not significantly correlated with %FMD. EF was markedly reduced in both groups with CAD (76.5 and 73.1% vs 31.6% in group 1) and a higher frequency of ischemic alterations in the ST segment (70.8%) was observed in the group with obstructive CAD with AL ≥70% during the exercise test. Endothelial dysfunction was observed in patients with CAD, irrespective of the severity of injury. A significantly higher frequency of ischemic alterations in the ST segment was observed in the group with obstructive CAD. EF and exercise ECG differed among the three groups and may provide complementary information for the assessment of CAD.

Key words: Coronary artery disease; Endothelial function; Exercise testing

A.L. Ribeiro was supported by research grants from CNPq (#305395/2005-0) and FAPEMIG (#PPM-00328-08).

Received May 9, 2008. Accepted January 16, 2009

Introduction

Atherosclerosis is the anatomical substrate of most cardiovascular diseases and coronary artery disease (CAD) continues to be the main cause of death throughout America, Europe and most of Asia. The endothelium plays a central role in the process of plaque formation and instabilization, with a direct effect on the clinical course of atherosclerotic disease and other cardiovascular disorders, such as arterial hypertension and cardiac insufficiency.

Since the end of the 1980's, a series of studies have

evaluated endothelial function in healthy subjects in the presence of risk factors for vascular damage or in patients with CAD. Basically, what is tested is the capacity of the endothelium to secrete nitric oxide (NO) in response to physiological or pharmacological stimuli. Although this is only one of the functions of the endothelium, a decrease in intravascular NO concentration might be the biological link between endothelial dysfunction and atherosclerosis (1), since NO, in addition to being a potent vasodilator, inhibits leukocyte and platelet activation and maintains smooth muscle cells in a non-proliferative state (2).

Endothelial function in the coronary circulation was first evaluated in 1986 by Ludmer and colleagues (3) by the intracoronary injection of acetylcholine. In normal arteries, acetylcholine stimulates the release of NO by the endothelium followed by vasodilatation. Paradoxical vasoconstriction is observed in the absence of normal endothelial function.

In 1992, Celermajer and colleagues (4) described a technique for the noninvasive diagnosis of endothelial dysfunction by ultrasound of the brachial artery (brachial artery reactivity test, BART), which permitted the elaboration of new concepts such as: 1) risk factors determine endothelial dysfunction in apparently healthy asymptomatic individuals, and 2) endothelial dysfunction can be reversed by the administration of drugs, such as statins and angiotensin-converting enzyme inhibitors (2). BART is a reproducible method, which shows a good correlation with the intracoronary acetylcholine test since the process of functional damage to the endothelium seems to be systemic and not restricted to the coronary arteries (5-8).

The ST segment response during dobutamine stress echocardiography has been proposed as a marker for endothelial dysfunction (9), whereas stress ECG continues to be the most frequently used screening method in obstructive CAD when the atherosclerotic plaque occupies more than 70% of the arterial lumen. A correlation has been reported between BART and the severity of CAD as defined by coronary angiography (10). The diagnostic performance of the method has been compared to exercise testing and myocardial scintigraphy (11) and it is known that vascular reactivity is altered during a very early stage of atherosclerosis before the formation of plaque.

The relationship between endothelial dysfunction and the severity of atherosclerotic injury has not been well characterized in patients with CAD. The objective of the present study was to evaluate endothelial function by the BART and the ST segment response methods during an exercise test in patients presenting a wide range of CAD defined by coronary angiography.

Patients and Methods

In a cross-sectional study, 62 patients recruited from two centers were evaluated at the homodynamic laboratory after coronary angiography for the investigation of chest pain or ischemic alterations in a provocative test. Patients subjected to coronary angiography during the course of acute coronary syndrome and with any other chronic disease, such as cardiac insufficiency, valvulopathy, chronic renal failure with serum creatinine higher than 2.0 mg%, liver diseases, thyroid diseases, connective tissue

diseases, or neoplasms were excluded.

The study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais. After signing an informed consent form, patients were submitted to standard clinical evaluation, brachial artery ultrasound, and a treadmill exercise test.

Stable angina was defined as typical chest pain supposedly related to CAD. Systemic arterial hypertension was defined on the basis of information provided by the patient or the use of antihypertensive medication. The presence of diabetes mellitus was diagnosed on the basis of one of the following findings: information provided by the patient, use of hypoglycemic medication, two fasting glycemia measurements higher than 126 mg% or one glycemia measurement higher than 200 mg%. Patients with a smoking habit for the last three months were considered to be smokers. Hereditary CAD was defined as the presence of disease in first-degree relatives younger than 55 years for males and younger than 65 years for females. Dyslipidemia was classified as serum low-density lipoprotein cholesterol levels greater than 160 mg%.

Coronary angiography

Angiography was performed with a Phillips Integris H3000 system (The Netherlands) and routine projections were obtained for the definition of coronary anatomy. The films were analyzed by two independent interventional cardiologists who were unaware of the clinical data and BART results. When the opinions were divergent, the films were analyzed by a third observer. The patients were divided into three groups according to the presence and severity of atherosclerotic lesions: group 1: normal coronaries; group 2: coronaries with non-obstructive lesions (degree of stenosis <70%); group 3: obstructive CAD (degree of stenosis \geq 70%).

Brachial artery reactivity test

Endothelial function was evaluated by the BART according to the method of Celermajer et al. (4). Calcium channel blockers, nitrates and angiotensin-converting enzyme inhibitors were withdrawn 24 h before the test and beta-blockers were discontinued 48 h before the test. The tests were performed in the morning (between 8:00 and 11:00 am) after a minimum fast of 8 h. All tests were conducted by the same examiner with experience in vascular ultrasound who was unaware of clinical and angiographic data. The Aspen ultrasound system (Acuson, USA) equipped with a linear 7.0-MHz transducer was used.

The patient was kept in dorsal decubitus for 10 min before the test. With the transducer positioned 2 to 15 cm above the cubital fossa, the cross-sectional diameter of

the brachial artery was measured (in mm) under basal conditions. Next, a pneumatic cuff positioned 5 cm below the cubital fossa was inflated at 250 mmHg for 5 min in order to produce distal ischemia for the stimulation of NO secretion by the endothelium. Sixty seconds after deflation of the cuff, new images of brachial artery diameter in the reactive hyperemia state were obtained. After a 15-min waiting period to allow the vessel to return to basal condition, sublingual nitroglycerine spray (400 µg) was administered and a new recording of arterial diameter was obtained 4 min later. All measurements were made in diastole and the mean value of four measurements was calculated for each situation. The variation in arterial diameter during rest and after reactive hyperemia is reported as the percentage of flow-mediated dilatation (%FMD), a variable that defines endothelial dysfunction. The administration of nitroglycerine was used to evaluate endothelium-independent vasodilatation by the direct action of the drug on smooth muscle cells, thus excluding a possible confounding factor in the evaluation of endothelial function.

Exercise test

The exercise test was performed on a treadmill within a maximum interval of 6 months before or after coronary angiography using the protocol considered to be the most appropriate in each case (Bruce or modified Bruce) (12). The ST segment depression during exercise was defined as ischemic when ≥ 1 mm from the J point with flat or down sloping morphology and ≥ 1.5 mm from the Y point with up sloping (slope of more than 1 mV/s) morphology, measured at least 60 to 80 ms after the end of the QRS complex. Patients with baseline ECG abnormalities (pre-excitation syndromes, electronically paced rhythm, resting ST depression greater than 1 mm, and complete left bundle branch block) were excluded from this analysis. Common confounders for stress ECG interpretation were also analyzed for each case. Other ischemic criteria, such as chest pain, hemodynamic abnormalities, arrhythmias, and ST elevations were also monitored.

Statistical analysis

Discrete and continuous variables obtained for the three groups were submitted to descriptive analysis. The presence or absence of normal distribution of each variable was evaluated by the Shapiro-Wilk test and the Bartlett test was used for the evaluation of variance homogeneity. Continuous variables were compared among

groups by ANOVA and discrete variables were compared by the chi-square test. %FMD and ST changes in the exercise test were considered to be outcome (dependent) variables, with the severity or absence of coronary atherosclerotic injury being defined as the independent (predictor) variable. Significant covariables were the severity of atherosclerotic injury, left ventricular function, age, gender, and the presence of risk factors. When necessary, adequate mathematical transformations were performed for analysis of variance, with the comparison of means by the Fisher test and adjustment for covariables. A P value < 0.05 was considered to be statistically significant (13).

Results

Sixty-two patients were studied, 19 in group 1 (normal coronary arteries), 17 in group 2 (atherosclerotic lesions $< 70\%$) and 26 in group 3 (atherosclerotic lesions $\geq 70\%$). The demographic characteristics of the patients are reported in Table 1. There was a predominance of males in groups 2 and 3 compared with group 1 (58.8 vs 73.1 vs 26.3%, respectively, $P = 0.007$) and the mean age tended to be higher in these groups (59.2 ± 8.8 vs 61.3 ± 9.7 vs 54.1 ± 6.6 years, respectively, $P = 0.025$), but these variables were not correlated with %FMD. The three groups were similar in terms of the distribution of cardiovascular risk factors (arterial hypertension, diabetes, dyslipidemia, smoking, and family history of CAD). A higher percentage of patients in group 3 presented a history of at least one coronary event (acute myocardial infarction or myocardial revascularization surgery; 0.0 vs 11.8 vs 42.3%, $P = 0.001$). Atypical chest pain was significantly more prevalent in groups 1 and 2 (63.2 vs 76.5 vs 23.1%, $P = 0.001$). However, stable angina was more prevalent in group 3

Table 1. Clinical and demographic features and risk factors for coronary artery disease.

	Group 1 (N = 19)	Group 2 (N = 17)	Group 3 (N = 26)	Group comparisons
Age (years)	54.1 \pm 6.6	59.2 \pm 8.8	61.3 \pm 9.7	1 < 2 = 3*
Gender (males)	26.3%	58.8%	73.1%	1 < 2 = 3*
Hypertension	84.2%	82.4%	84.6%	
Diabetes mellitus	0%	17.6%	26.9%	
Dyslipidemia	21.1%	29.4%	34.6%	
Smoking	5.3%	17.6%	3.8%	
Atypical chest pain	63.2%	76.5%	23.1%	1 = 2 > 3*
Stable angina	10.5%	5.9%	46.2%	1 = 2 < 3*
Previous coronary event	0%	11.8%	42.3%	1 = 2 < 3*

Data are reported as mean \pm SD or percent. Group 1: normal coronaries; group 2: atherosclerotic lesions $< 70\%$; group 3: atherosclerotic lesions $\geq 70\%$. * $P < 0.05$ (ANOVA for continuous variables and chi-square test for discrete variables).

(10.5 vs 5.9 vs 46.2%, $P = 0.003$).

The results of the BART and the exercise test are shown in Table 2. With respect to the most important BART variable, mean %FMD was $9.0 \pm 5.8\%$ in group 1, $4.5 \pm 3.6\%$ in group 2, and $4.9 \pm 3.6\%$ in group 3 ($P = 0.003$). Using a receiver operating characteristic (ROC) curve, endothelial dysfunction was defined as an FMD $<7.0\%$ (area under the curve = 0.72 ± 0.06 ; Figure 1).

When considering endothelial dysfunction as a binary variable that is present when %FMD is $<7.0\%$, the fre-

quency of endothelial dysfunction was 31.6% in group 1, 76.5% in group 2, and 73.1% in group 3 ($P = 0.006$). These results demonstrate that the prevalence of endothelial dysfunction was high in this selected sample, with significantly more altered values in the groups presenting some degree of CAD. After the administration of nitroglycerine, %FMD was 12.1 ± 4.0 , 11.2 ± 4.6 and $8.5 \pm 3.5\%$ in groups 1, 2, and 3, respectively ($P = 0.011$).

Fifty-four patients were subjected to the exercise test. The test was not possible in 7 patients because of some

motor limitation, and was inappropriate in the case of one patient because of the finding of a left branch bundle block on baseline ECG. The frequencies of the ST segment depression types observed were: down sloping: 5 (17.2%), flat: 20 (69.0%), and up sloping: 4 (13.8%). The ST depression, when analyzed quantitatively, was not statistically different between groups 1, 2 and 3: 1.58 ± 0.80 , 1.60 ± 0.42 and 1.96 ± 0.98 , respectively ($P = 0.574$). A higher frequency of an ischemic ST segment response was observed in the group with obstructive CAD (40.0 vs 40.0 vs 70.8% , $P = 0.048$). No significant differences were observed

Table 2. Results of the brachial artery reactivity test and the exercise test.

	Group 1 (N = 19)	Group 2 (N = 17)	Group 3 (N = 26)	Group comparisons
%FMD	9.0 ± 5.8	4.5 ± 3.6	4.9 ± 3.6	$1 > 2 = 3^*$
%NMD	12.1 ± 4.0	11.2 ± 4.6	8.5 ± 3.5	$1 = 2 > 3^*$
Endothelial dysfunction	31.6%	76.5%	73.1%	$1 < 2 = 3^*$
ST depression (mm)	1.58 ± 0.80	1.60 ± 0.42	1.96 ± 0.98	
Abnormal exercise test (% of patients)	40.0%	40.0%	70.8%	$1 = 2 < 3^*$

Data are reported as mean \pm SD or percent. Group 1: normal coronaries; group 2: atherosclerotic lesions $<70\%$; group 3: atherosclerotic lesions $\geq 70\%$; FMD = flow-mediated dilatation. NMD = nitroglycerin-mediated dilatation. $*P < 0.05$ (ANOVA for continuous variables and chi-square test for discrete variables).

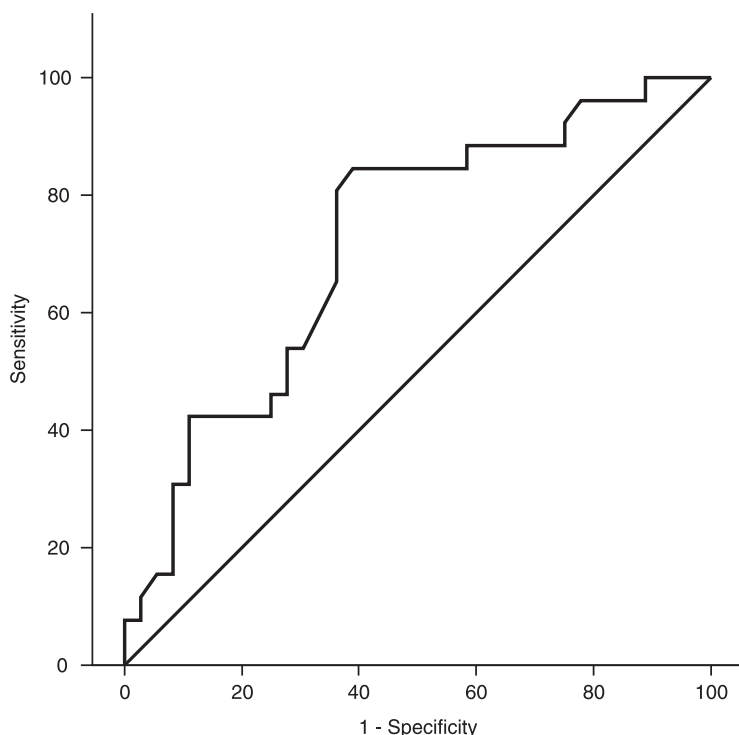


Figure 1. Accuracy of the brachial artery reactivity test in recognizing obstructive coronary artery disease evaluated by a receiver operating characteristic curve (area under the curve = 0.72 ± 0.06). On the basis of these data, endothelial dysfunction was defined as flow-mediated dilatation $<7.0\%$.

among the three groups with respect to the other exercise test variables such as presence of symptoms, heart rate and blood pressure response during exercise, functional capacity, or arrhythmias.

Discussion

There is a growing interest in the study of the early subclinical stages of atherosclerotic disease, and the diagnosis of endothelial dysfunction can be considered to be part of these new methods.

The differences in some demographic and clinical characteristics observed among the three groups are inherent to the method used, i.e., since the patients were divided according to the severity of injury defined by coronary angiography, a higher mean age and higher prevalence of males were observed in groups 2 and 3 compared to the control group. It is interesting to note that the prevalence of atypical chest pain was higher in the control group and the group without obstructive CAD and the prevalence of stable angina was higher in patients with obstructive CAD, a finding supporting the value of a clinical history as an important diagnostic resource in CAD.

Endothelial function was found to be significantly altered in groups 2 and 3 (%FMD of 4.4 and 4.7%, respectively), with the mean FMD being well below the cut-off point of 7.0%. This finding confirms the hypothesis that endothelial dysfunction is present since the early stages of CAD, irrespective of the severity of atherosclerotic injury. In the present study, the BART was able to discriminate patients with any degree of CAD, with the observation of lower %FMD values in groups 2 and 3 compared to the control group. However, similar values were obtained for groups 2 and 3, demonstrating that the test is able to discriminate patients with CAD but not the severity of atherosclerotic injury defined by angiography.

Although the mean %FMD in the group with normal coronaries was 8.9%, well above the values found for groups 2 and 3, about one-third of the patients of this group presented endothelial dysfunction when considering a cut-off of 7.0%. This finding agrees with Celermajer et al. (4), who demonstrated altered endothelial function in young patients presenting risk factors when compared to the control group of young patients with no risk factors (%FMD: 4.0 vs 11.0%, respectively, $P < 0.001$). Witte et al. (14) also showed a close correlation between %FMD and the main cardiovascular risk factors in a population at an overall low risk for CAD.

BART has been used in many cross-sectional studies employing distinct inclusion criteria, ranging from the selection of young asymptomatic patients with or without risk

factors to patients with peripheral arterial disease or with a clinical suspicion of CAD referred for coronary angiography (4,11,15,16). Such a heterogeneous sample and the lack of a large-scale community study impair the definition of the performance of this method as a diagnostic test. No consensus exists regarding the reference value for %FMD that defines endothelial dysfunction. In a meta-analysis regarding the use of BART, %FMD ranged from 0.2 to 19.2% in healthy subjects and from -1.3 to 14.0% in patients with coronary disease (17). In 2001, the International BART Task Force published guidelines outlining an adequate method for the execution of the test but did not provide a %FMD value that would define endothelial dysfunction (18). Schroeder et al. (11) studied the diagnostic performance of BART compared to exercise testing and myocardial scintigraphy in 122 patients with a broad spectrum of CAD using coronary angiography as the gold standard and defined the presence of endothelial dysfunction to be when %FMD was $<4.5\%$ based on the ROC curve. The FMD $<7.0\%$ obtained in the present study is closer to the value reported by Jambrik et al. (19), who studied 198 patients submitted to coronary angiography and found a %FMD $\leq 8.8\%$ as a good cut-off using the ROC curve, with a sensitivity of 90% and specificity of 37%.

A different situation is observed when analyzing the prognostic value of BART: endothelial dysfunction is a strong and independent predictor of cardiovascular events in patients with chronic CAD referred for coronary angiography and in acute coronary syndromes (15,20-23).

No correlation was observed between %FMD and the degree of stenosis, in agreement with the findings reported by Enderle et al. (24) but in contrast to those of Neunteufl et al. (10). Other investigators compared BART with functional methods evaluating myocardial perfusion and demonstrated a good correlation between %FMD and alterations detected by PET scintigraphy and a reduction in coronary flow reserve using echo-Doppler (25,26). One limitation of our study and of most of those cited here is that the severity of CAD was established by subjective analysis of the coronary angiogram. In this respect, computerized quantitative coronary angiography would permit a more objective assessment and the comparison among studies. However, BART permits the detection of changes that anticipate plaque formation and that is why our data may not have demonstrated a clear correlation between endothelial dysfunction and the degree of coronary obstruction.

With respect to endothelium-independent vasodilatation mediated by nitroglycerine, as expected, the %FMD was higher than that obtained during endothelium-mediated vasodilatation, but a significant difference was observed among the three groups, with a less marked re-

sponse to nitroglycerine in the group with obstructive CAD. We have no definite explanation for this difference and the data reported in the literature are controversial, with wide variations in the percentage of dilatation mediated by nitroglycerine.

As expected, the response of the exercise test was different between groups 1 and 2 (without obstructive CAD) and group 3 (with obstructive CAD), since the ischemic response of the ST segment is related to the severity of coronary atherosclerotic injury. The significant difference in the ischemic response observed, which was more frequent in group 3, agrees with the established concept that usually only atherosclerotic lesions $\geq 70\%$ cause ischemia during provocative tests.

In the present study the two tests evaluated, BART and exercise test, provided different results for the groups with and without obstructive CAD, in contrast to the results

obtained by Pálincás et al. (9) when comparing the ST segment response using a stress echocardiogram and BART. Our data suggest that BART and the exercise test evaluated different stages of CAD and provided distinct but complementary information.

Despite the knowledge gathered over the last few years, it is difficult to define the clinical importance of data provided by the evaluation of endothelial function as discussed in a recently published review (27). Studies involving larger patient samples and different selection criteria may provide answers to essential questions over the next years. What is the role of the endothelial function noninvasive assessment in the evaluation of the overall cardiovascular risk of the patient? What %FMD value defines endothelial dysfunction? And, most important, are aggressive strategies for the prevention of cardiovascular events indicated for patients with endothelial dysfunction?

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