Delayed flow-mediated vasodilation and critical coronary stenosis

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ABSTRACT

Endothelial dysfunction, wall thickening and plaque are progressive manifestations of atherosclerosis. Delayed or absent brachial artery dilation after ischemic stimulus has been associated with severity of extracoronary and coronary atherosclerosis. In the current study, we aimed to verify if delayed or absent dilation associates with critical coronary stenosis. We also evaluated the association between coronary stenosis, carotid artery wall thickness and peripheral artery disease. Endothelial function was investigated by flow-mediated dilation of the brachial artery up to 3 min after ischemia, and patients classified as early, late or no dilators. Coronary angiography was performed through transradial or femoral artery approach. Computerized quantitative angiography was used to obtain percent stenosis of all lesions, while the Gensini score was used to evaluate the severity of coronary atherosclerosis. Seventy-four patients were enrolled. Carotid wall thickness and plaque, and peripheral artery disease were detected by ultrasound. Subjects with critical coronary stenosis showed a higher prevalence of delayed or absent dilation (coronary stenosis ≥70 per cent: late dilators 50 per cent, no dilators 35 per cent; coronary stenosis ≤70 per cent : late dilators 27 per cent, no dilators 6 per cent). The Gensini score was progressively higher in late dilators and no dilators compared with early dilators (early: 4.5±13.5; late 17.5±27.1; no 39.7±55.0; P<0.02). Carotid atherosclerosis and peripheral artery disease were more prevalent in subjects with critical coronary stenosis. Delayed or absent dilation associates with coronary stenosis and different degree of coronary atherosclerosis. The kinetic of arterial dilation seems to be relevant as the magnitude of dilation.

Significance of this study

What is already known about this subject?

- Carotid atherosclerosis is a marker of coronary atherosclerosis and incident cardiovascular events.
- Endothelial dysfunction, defined as reduced or absent arterial vasodilation after ischemia, associates with cardiovascular disease and incident cardiovascular events.
- Delayed but normal vasodilation after ischemia associates with asymptomatic atherosclerosis and cardiovascular risk.

What are the new findings?

- Delayed but normal vasodilation after ischemia detected by ultrasound associates with critical coronary stenosis detected by angiography in subjects without previous known cardiovascular disease.
- The kinetic of arterial dilation seems to be relevant as the magnitude of dilation in the identification of subjects with coronary disease.

How might these results change the focus of research or clinical practice?

- Ultrasound study of arterial function is a widely used technique to study endothelial function.
- A comprehensive approach involving measurement of arterial vasodilation and evaluation of the kinetic of flow-mediated dilation is reliable to identify subjects with critical coronary stenosis.

INTRODUCTION

Atherosclerosis is a progressive disease that affects the entire arterial system, with more frequent involvement of coronary, femoral and carotid arteries.[1] It is typically silent for decades until its advanced vascular manifestations become clinically evident, with consequent negative impact on patients' prognosis. Hence, there is an unmet need to detect the early signs of arterial damage in order to identify subjects that could benefit from more intensive prevention strategies. In this regard, non-invasive ultrasound-based techniques may offer the opportunity to detect atherosclerosis at an early clinical stage, thus increasing the chances to prevent clinical events. Flow-mediated dilation (FMD) and carotid intima-media thickness measurement (CIMT) are non-invasive ultrasound-based diagnostic techniques allowing the detection of early signs of atherosclerosis.[2–6] Association of CIMT with cardiovascular disease has been consistently reported by several independent groups. Furthermore, it has also been used in clinical research to

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monitor the efficacy of anti-atherosclerotic treatment.[2] On the other hand, although FMD is potentially able to identify functional arterial alterations, that is, the earliest hallmark of vascular disease, its clinical application has produced conflicting results.[7–9] Indeed, FMD does not associate with coronary stenosis and is not predictive of cardiovascular events in all studies. As a matter of the fact the American College of Cardiology and American Heart Association Task Force Guidelines recommend the measurement of CIMT as a reasonable approach to improve cardiovascular risk assessment in asymptomatic subjects at intermediate risk, while use of FMD is not currently endorsed due to its limited reproducibility.[10–12] We have recently demonstrated that FMD has a fairly different kinetic among individuals, which might explain the elevated inter-rater and intra-rater variability of FMD measurements. Specifically, we have found three different patterns of dilation after an ischemic stimulus: early dilation, late dilation and no dilation. Subjects with delayed dilation seem to have a higher prevalence of carotid atherosclerosis and cardiovascular events, and a higher cardiovascular risk score, than subjects without dilation.[13–15] Hence, we have designed the present study to evaluate if the latency of endothelium-dependent vasodilator response associates with critical coronary stenosis (CoS), detected by angiography, independently of the magnitude of dilation, the presence of carotid atherosclerosis and peripheral arterial disease.

MATERIALS AND METHODS

This is a cross-sectional study including consecutive inpatients admitted to the hospital in a 6-month period to perform coronary angiography as suggested by current guidelines.[16] Exclusion criteria were myocardial infarction, acute coronary syndrome or unstable angina, coronary revascularization, stroke, acute or chronic heart failure (New York Heart Association > II), dilated cardiomyopathy, hemodynamic relevant valvular heart disease or heart valve replacement, arrhythmias, rheumatic diseases, severe liver and kidney diseases, and cancer. Participants, eligible for the study, were informed about the aim of the study and enrolled on signing the informed consent. At the first visit, subjects underwent bodyweight and height measurement to calculate body mass index (BMI). After the patient has rested for 5 min, blood pressure (BP) was measured twice on the right arm and the average used for the analyses. Medical history and pharmacological treatment were also recorded.

All subjects underwent blood sample collection for measurement of blood lipids (total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides) and fasting glucose. Blood lipids and fasting glucose were measured using standardized methods. Subjects with fasting plasma glucose ≥126 mg/dL and no previous diagnosis of diabetes underwent a second blood collection to repeat blood glucose measurement. Hypertension, diabetes and hyperlipidemia were defined based on current guidelines.[17–19] Subjects who smoked regularly during the previous 12 months were classified as smokers. BMI was calculated according to the formula kg/m^2.

All patients underwent preliminary physical examination including palpation, auscultation of access site and assessment of radial artery pulse and ulnar artery blood supply by means of the Allen test for transradial procedures. A 6F sheath was used for vascular access. Coronary angiography and percutaneous coronary intervention (PCI) were performed as clinically indicated. Standard 5F Judkin, Amplatz or Tiger catheters were used for diagnostic procedures, while 6F E.B.U., Amplatz or Judkins catheters were used for PCI. In the presence of any reason not to perform the coronary procedure through a transradial approach, the right femoral artery represented the alternative approach. Unprolonged diagnostic trans-femoral procedures were performed without any heparin bolus. On the contrary, a weight-adjusted heparin bolus was administered for transradial diagnostic procedures. During interventional procedures, a proper anticoagulation level was maintained checking the activated clotting time. The arterial sheath was removed at the end of the procedure and a complete hemostasis was obtained in the catheterization laboratory.

Quantitative measurements were performed by computerized quantitative angiography (QCA) to obtain percent stenosis of all lesions using the validated CAAS-II software (Pie Medical Imaging, Maastricht, The Netherlands), as previously described.[20] Briefly, the contrast agent iomerol (Iomeron 400, Bracco Imaging) was injected by means of a power injector (ACIST CMS2000, Bracco Imaging) at 3 mL/s (right coronary artery) or 4 mL/s (left coronary artery) injection rate to obtain good quality standardized angiograms. QCA was performed offline by two independent operators and mean values were used for the analyses. CoS was defined as a stenosis ≥70 per cent at QCA in at least one major coronary artery.[21]

The ultrasound study was performed using echo-Doppler Philips HD 11XE (Royal Philips Electronics, The Netherlands) equipped with a 12–3 MHz high-resolution linear array, steerable pulsed wave Doppler and simultaneous ECG recording. The examination was conducted in a different day from blood sample collection and clinical examination. Patients assumed a light dinner the evening before the examination and were invited to fast until the morning of the study protocol. Morning ongoing therapy was administered after the study was performed. Patients in multiple daily insulin injections assumed the last dose of insulin at bedtime the day before vascular examination.

Common, internal and external carotid arteries were studied. The presence of plaque and/or stenosis was evaluated and blood flow velocity of internal carotid artery measured. Plaque was defined as a focal thickening ≥1.5 mm measured from the media–adventitia interface to the intima–lumen interface.[22] Internal carotid artery stenosis was defined as follows: stenosis 16–50 per cent spectral broadening during all the cardiac cycle and systolic blood velocity <140 cm/s; stenosis >50 per cent as systolic blood velocity ≥140 cm/s and spectral broadening during all the cardiac cycle; occlusion as absence of Doppler signal.[23] Carotid atherosclerosis was defined as the presence of plaque and/or stenosis and/or occlusion at the carotid vascular tree. Images of the common carotid artery were recorded for offline IMT measurement as previously described.[24] Briefly, IMT, defined as the distance between the leading edge of the lumen–intima interface and the inner edge of the media–adventitia interface of the far wall, was measured 1 cm proximal to the bulb, in the three projections (anterior, lateral and posterior), at the end of

the systole of the cardiac cycle. The average of the three measurements of IMT (mean IMT) for each side was calculated and, along with the maximal IMT, used for statistical analyses.

Endothelial function was evaluated at the brachial artery of the non-dominant arm. The artery was imaged ≈10 cm above the elbow. In order to obtain a clear image showing near and far intima–lumen interface, gain setting and transducer position were adjusted and kept fixed throughout the study. The skin was marked and the transducer clamped in a probe holder. To evaluate brachial artery endothelial function, FMD test was performed. The pneumatic cuff was placed around the forearm and inflated to 250 mm Hg for 5 min. After deflating the cuff (reactive hyperemia), brachial artery imaging was recorded for 3 min. Images of the artery at baseline, and 50 s, 2 and 3 min after cuff deflation were captured and analyzed for offline internal brachial artery diameter (ID) measurement. ID was defined as the distance between intima–lumen interface of the near wall and lumen–intima interface of the far wall. Brachial artery ID was measured at the end of the diastole, using dedicated software (Autodesk Design Review). The software allows to carefully measure distances between selected points. An expert single-blind investigator performed the analysis. Images captured from the recording of brachial artery at baseline, and 50 s, 2 and 3 min after cuff deflation were displayed on the computer screen. For each image, ID was measured at three different locations of the vessel wall using a caliper placed manually by the operator. The average of three measurements was calculated. The caliper revealed automatically the distance from the intima–lumen interface of the near wall and the lumen–intima interface of the far wall. The caliper distance was calibrated based on the known distance scale previously displayed on the echo-Doppler screen. FMD was expressed as percentage change of arterial diameter from baseline to postreactive hyperemia and calculated using the following formula: {[post-deflation (50 s, 2, 3 min) ID-baseline ID/baseline ID] x100}. Peak dilation was defined as the maximal dilation calculated among three observations. Based on the time to peak, dilation subjects were defined as 'early dilators' if peak FMD was at 50 s; 'late dilators' if peak FMD was >50 s. 'No dilators' were defined as those subjects who did not dilate at all during the observation points.[13–15] The reproducibility of the method has been published elsewhere.[25] Briefly, the coefficient of variation of FMD was 7.4 per cent, the FMD technical error of measurement (TEM) was 0.55, and the % TEM was 6.7 per cent. To test the reproducibility of FMD patterns, we studied 15 subjects twice in two different occasions. Five subjects were defined as early, six as late and four as no dilators on both studies. The observation time (50 s, 2 and 3 min) set to define subjects as early, late and no dilators was determined from a preliminary observation (data not published) when we measured in a sample of 15 healthy subjects brachial artery diameter every 5 s, and up to 180 s, after the release of the forearm cuff. Out of 15 subjects, 7 showed peak FMD between 50 and 65 s; 5 showed peak FMD between 115 and 125 s; 3 subjects did not have any dilation nor had constriction by cuff release up to 180 s. The mean difference detected among 50–65 s and 115–125 s was respectively 0.09 and 0.13 mm. Based on this finding we decided to set observation time at 50 s, plus 2 min and 3 min.

The Ankle Brachial Index (ABI) was measured using a standard BP cuff and a hand-held Doppler device with a vascular digital probe. Systolic BP was measured in both arms and both ankles after 10 min rest in the supine position. To evaluate brachial pressure, the cuff was placed on the patient's upper arm with the lower edge approximately 1 cm above the antecubital fossa. To evaluate ankle pressure, the cuff was placed at the patient's leg approximately 2 cm above the ankle's medial malleolus. For pressure measurement, the tip of the probe was placed into conductivity gel at a 45–60° angle until clear arterial pulse sound was heard. The cuff was then inflated to the point that pulse sound disappeared, and over for further 20 mm Hg. The cuff was then slowly deflated at a rate of 2 mm Hg/s and the point when arterial pulse sound resumed was recorded defining the brachial and ankle pressure. ABI was calculated by dividing ankle and brachial pressure for each side. Subjects were defined as having peripheral arterial obstructive disease (PAOD) if ABI was <0.94 at least in one artery examined and as having arterial calcification if ABI was >1.3.[26]

Individual coronary angiography results were used to calculate the Gensini score. It was computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its geographic importance (reductions of 25, 50, 75, 90, 99 per cent, and complete occlusion, were given Gensini scores of 1, 2, 4, 8, 16 and 32, respectively). Each coronary segment was assigned a multiplier, depending on the position and the functional relevance of the downward myocardial area supplied by that segment, as follows: ×5 for the left main coronary artery; ×2.5 for the proximal segment of left anterior descending coronary artery (LAD); ×2.5 for the proximal segment of the circumflex artery; ×1.5 for the mid-segment of the LAD; ×1 for the right coronary artery, the distal segment of the LAD, the posterolateral artery and the obtuse marginal artery; and ×0.5 for all other segments.[27]

The analyses were performed using SPSS V.17.0. For the aim of the study, subjects were divided into two groups according to the presence or absence of coronary atherosclerosis ≥70 per cent. Carotid atherosclerosis and PAOD were considered as dichotomous variables (0=absence and 1=presence). Carotid atherosclerosis was defined as the presence of plaque and/or stenosis and/or occlusion at the carotid vascular tree; PAOD was defined as ABI <0.94 at least in one of the studied peripheral arteries (anterior and posterior tibial artery, right and left); and arterial calcification as ABI >1.3 at least in one peripheral artery.

Based on our previous published data, the mean percentage of late plus no dilators among subjects comparable for age and clinical characteristics to subjects enrolled in the current study was approximately 40 per cent. Therefore, the number of subjects to be enrolled to detect a difference of late plus no dilators=50 per cent between those with and without CoS was estimated to be at least 26 subjects in each group for $\alpha=0.05$ and $\beta=0.10$.

Triglycerides and Gensini score were not normally distributed, and therefore log transformed before applying parametric tests. Statistical significance was set at $P<0.05$. The t-test for unpaired data was applied to compare difference between continuous variables, and the χ^2 test to compare prevalence. The differences among FMD groups (early,

Table 1 Clinical and biochemical characteristics of study patients

Variables	All n=74
Age (years)	64±9
Male sex (%)	57
Total cholesterol (mg/dL)	181±38
Triglycerides (mg/dL)	133±64
High-density lipoprotein-cholesterol (mg/dL)	52±15
Fasting glucose (mg/dL)	125±47
Body mass index (kg/m^2)	29±5
Systolic blood pressure (mm Hg)	138±15
Diastolic blood pressure (mm Hg)	79±8
Hypertension (%) (yes)	84
Diabetes (%) (yes)	42
Hyperlipidemia (%) (yes)	82
Smokers (%)	15

Values are expressed as mean±SD or percentage.

late and no dilators) were evaluated by analysis of variance. Logistic regression analysis was used to evaluate variables predictive of CoS (stenosis ≥70 per cent). Independent variables included in the analysis were sex, age, BMI, diabetes, hyperlipidemia, hypertension, smoking habit, FMD pattern (early dilators=reference group), peak FMD and FMD calculated at 50 s, carotid atherosclerosis, IMT max (we have included the maximum value measured) and PAOD.

RESULTS

Seventy-four subjects were enrolled after signing the informed consent, all positive to stress testing (ECG and imaging) performed before the enrollment, and meeting current indication for coronary angiography. Among these, 42 were men (57 per cent) and 32 women (43 per cent); overall age range was 42–80 years. Clinical and biochemical baseline characteristics are displayed in table 1.

Subjects were divided into two groups according to presence of CoS ≥70 per cent in at least one major coronary artery since this represents the indication for coronary angioplasty (table 2).

The prevalence of male sex and the concentration of triglycerides were significantly higher, and HDL-cholesterol significantly lower, in subjects with critical CoS. Other variables and medications' use were comparable between groups. As far as antihypertensive drugs are concerned, the prevalence of different class was the following in subjects with and without CoS≥70 per cent: with CoS, RAS-inhibitors in 100 per cent of cases, calcium channel antagonists in 14 per cent, diuretics in 24 per cent and beta blockers in 38 per cent; without CoS, RAS-inhibitors in 97 per cent of cases, calcium channel antagonists in 27 per cent, diuretics in 48 per cent and beta blockers in 46 per cent. The difference was not statistically significant.

Morphological and functional parameters of carotid, brachial and lower limb arteries detected by ultrasound are shown in table 3.

Carotid atherosclerosis and PAOD were more prevalent in subjects with critical CoS, who also had significantly higher mean and maximal IMT of the left common carotid artery.

Table 2 Clinical and biochemical characteristics of study patients, classified according to the presence or absence of critical coronary stenosis (CoS)

Variables	With CoS≥70%	Without CoS≥70%
Number (%)	26 (35)	48 (65)
Age (years)	64±8	63±9
Sex (% M)	81*	44
Total cholesterol (mmol/L)	186±35	179±39
Triglycerides (mmol/L)	161±75*	120±54
High-density lipoprotein-cholesterol (mmol/L)	44±11*	56±15
Fasting blood glucose (mmol/L)	130±49	123±46
Body mass index (kg/m^2)	29±4	30±5
Systolic blood pressure (mm Hg)	141±16	136±15
Diastolic blood pressure (mm Hg)	81±8	78±8
Diabetes (%)	50	37
Antidiabetic drugs (%)	31	38
Hypertension (%)	84	85
Antihypertensive drugs (%)	83	85
Hyperlipidemia (%)	85	83
Hypolipidemic drugs (%)	64	73
Antiplatelet drug (%)	96	88
Smokers (%)	26	11

Values are expressed as mean±SD or percentage.
*P<0.01.

Furthermore, subjects with critical CoS had significantly lower peak FMD of brachial artery than subjects without CoS.

Based on time to peak FMD, 36 (49 per cent) subjects were classified as early dilators, 26 (35 per cent) as late dilators and 12 (16 per cent) as no dilators. Subjects with CoS ≥70 per cent had significantly higher prevalence of delayed or absent dilation compared with subjects without critical stenosis (figure 1). Furthermore, the Gensini score (mean±SD) in early, late and no dilators was respectively 5±13, 18±27, 43±56 (Kruskal-Wallis test P<0.001).

To evaluate variables independently associated with critical CoS, the logistic regression analysis was performed. Sex, age, diabetes, hyperlipidemia, hypertension, smoking habit, FMD pattern (early dilators=reference group), peak FMD, FMD calculated at 50 s, carotid atherosclerosis and PAOD were included as independent variables. As shown in table 4, FMD pattern was significantly and independently associated with the presence of CoS ≥70 per cent.

Variables significantly and independently associated with coronary stenosis ≥70 per cent (dependent variable). Independent variables sex, age, BMI, diabetes, hyperlipidemia, hypertension, smoking habit, FMD pattern, FMD at 50 s and peak FMD, carotid atherosclerosis, IMT max and PAOD.

DISCUSSION

The main finding of the present study is the independent association between the kinetic of postischemic brachial artery dilation and CoS. Indeed, 85 per cent of subjects with stenosis ≥70 per cent had a delayed or absent dilation while only 15 per cent showed a dilation after 50 s. Baseline brachial artery as well as FMD at 50 s were not

Table 3 Carotid artery measurements, peripheral artery disease and flow-mediated dilation (FMD) of study patients, classified according to the presence or absence of critical coronary stenosis (CoS)

Variables	With CoS≥70%	Without CoS≥70%
Number (%)	26 (35)	48 (65)
Baseline brachial artery diameter (mm)	3.94±0.6	3.71±0.6
FMD 50 s (% dilation)	2.4±8.0	6.2±5.7
Peak FMD (% dilation)	4.4±7.9*	8.0±4.4
Mean right CCA IMT (µm)	913±192	867±184
Mean left CCA IMT (µm)	955±252*	816±174
Maximal right CCA IMT (µm)	1037±243	1011±246
Maximal left CCA IMT (µm)	1057±280*	963±194
Carotid atherosclerosis (%)	88†	50
Peripheral artery disease (%)	42*	21

Values are expressed as mean ±SD.
*P<0.05.
†P=0.001.
CCA, common carotid artery; IMT, intima-media thickness measurement.

significantly different between the two groups. The extent of the ischemic area, as measured by the Gensini score, progressively and significantly increased from early to no dilators. The kinetic of brachial artery dilation after ischemia seems to be predictive of CoS over the magnitude of postischemic dilation traditionally measured at 50 s. The results of the present study support the usefulness to evaluate FMD response to ischemia for a longer time than the recommended 50–60 s.[13 15]

The heterogeneity of the time to peak dilation is known. Age, training status and obstructive sleep apnea influence both the magnitude and the kinetic of FMD.[28–31] The methodological approach used in the current study does not reveal the exact timing of the maximum vasodilation. However, it allows to discriminate between subjects who dilate at the traditional observation time of 50 s and those who dilate later. In addition to that, the preliminary data we have obtained examining the kinetic of dilation support the selected observation times. Despite the different approaches used to evaluate FMD, the technique is definitely considered a useful tool to detect early vascular damage and identify subjects requiring a better

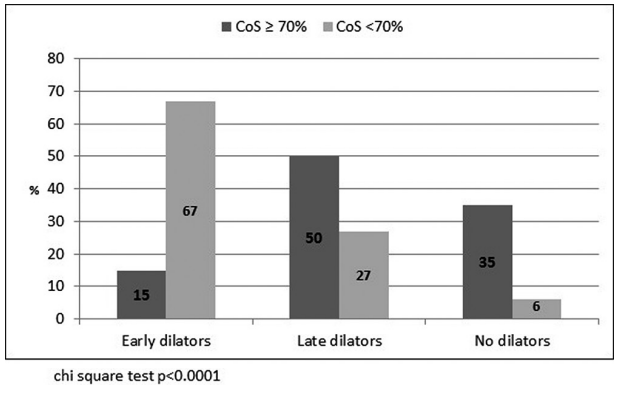

Figure 1 Prevalence of critical coronary stenosis (CoS) in early, late and no dilators. Comparison χ^2 test P<0.001.

Table 4 Multivariable logistic regression

Independent variable	Beta coefficient	OR	Lower 95% CI	Upper 95% CI	P value
Late dilators*	2.9	17	1.4	229	0.03
No dilators*	5.2	173	2.2	13,331	0.02
Carotid atherosclerosis	2.7	15	1.3	184	0.03

*Risk versus early dilators.

control of cardiovascular risk factors. However, the predictive value of a blunted FMD response for incident cardiovascular disease seems confirmed in the long term and in old subjects with established atherosclerosis.[5] In subjects without known vascular disease, the association between FMD and incident cardiovascular events is still weak, as well as in subjects with established coronary artery disease and in treatment with vasoactive medications.[32 33] A recent paper has reported that an FMD <10 per cent is predictive of incident cardiovascular events in subjects without any previous cardiovascular disease during a follow-up of 4 years. The predictive value of FMD would seem to overcome the impact of major cardiovascular risk factors at least in healthy subjects of middle age.[34] Another paper published by Kuvin *et al*[35] has reported an interesting finding about the association between FMD and coronary atherosclerosis detected by a non-invasive diagnostic test. The authors have found that subjects with positive exercise myocardial perfusion imaging had a lower FMD, and FMD was predictive of coronary artery disease with an OR of 1.32 for each percent decrease in dilation.

Carotid atherosclerosis was significantly associated with coronary atherosclerosis and predictive of CoS in our study. On the other hand, the presence of PAOD was significantly associated with the presence of coronary atherosclerosis, but was not predictive of critical coronary stenoses. However, this latter finding might be explained by the low prevalence of PAOD observed. Indeed, while about 90 per cent of subjects with critical coronary stenoses had carotid plaques or stenoses, only about 40 per cent had PAOD. PAOD is definitely a late manifestation of atherosclerosis, and the ABI test has a diagnostic value only in the presence of significant lower limb arterial stenoses.

Finally, IMT of the left common carotid artery was significantly associated with the presence of coronary atherosclerosis. So far, many studies have shown that IMT measured by ultrasonography is a useful index of subclinical cardiovascular disease, marker of systemic atherosclerosis and predictor of cardiovascular events.[2 3 6] Among non-invasive imaging tests, only IMT measurement is currently recommended by the American Heart Association as a tool improving the assessment of individual risk. In our study, common carotid IMT was no longer associated with coronary atherosclerosis ≥70 per cent in the multiple logistic regression analysis model. Mean age in our sample was 64 years, comparable to the population recruited in the Cardiovascular Health Study, which has described a strong and significant association between increased common carotid IMT and cardiovascular risk factors but not with incident cardiovascular diseases. We

might argue that the predictive role of IMT is weaker in older subjects.[36]

The present study has, in our opinion, an interesting point. It was designed to verify if the kinetic of arterial dilation might be associated with severe coronary atherosclerosis diagnosed by angiography, independently of and over other non-invasive ultrasound-based diagnostic techniques, including traditional FMD. In fact, the results of the present study demonstrate that FMD measured at 50 s was not discriminant between subjects with and without critical coronary atherosclerosis, as the evaluation of the kinetic of FMD did. In addition, the significant association with the Gensini score also demonstrates that the delayed or absent dilation associates with the extent of coronary artery disease.

The mechanisms underlying arterial dilation are complex and need further studies to be fully understood. Techniques and procedures evaluating arterial dilation should be better standardized and should include a longer observation time in order to evaluate subjects with a delayed dilation. Based on our observation, the kinetic of FMD might offer additional information to define subjects likely at risk for coronary heart disease.

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Contributors CI and SDR planned the research, designed the study and written the paper. CI and CT performed the ultrasound studies. CT and CC collected and analyzed data. CC and EA selected patients and were responsible for IC. DT, GA, AM and CS performed coronary angiography. AG and CI were study supervisors and edited the manuscript.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study protocol was approved by local ethical committee and conducted in accordance with the Declaration of Helsinki.

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