Coronary Microvascular Dysfunction by Myocardial Contrast Echocardiography in Nonelderly Patients Referred for Computed Tomographic Coronary Angiography

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Background: Microvascular dysfunction (MVD) is a potential cause of chest pain in younger individuals. The authors hypothesized that nonelderly patients referred for computed tomographic angiography (CTA) but without significant stenosis would have a high prevalence of MVD by myocardial contrast echocardiography (MCE). Secondary aims were to test whether the presence of nonobstructive coronary artery disease (CAD) or reduced brachial flow-mediated dilation (FMD) predicted MVD.

Methods: Subjects ≤60 years of age undergoing CTA were recruited if they had either no evidence of coronary plaque or evidence of mild CAD (<50% stenosis) and at least one high-risk plaque feature. Subjects underwent quantitative perfusion imaging using MCE at rest and during regadenoson vasodilator stress. MVD was defined as global or segmental delay of microvascular refill (≥2 sec) during regadenoson. FMD of the brachial artery was also performed.

Results: Of the 29 patients in whom MCE could be performed, 12 (41%) had MVD. These subjects, compared with those with normal microvascular function, had lower hyperemic perfusion (mean, 236 ± 68 vs 354 ± 161 intensity units/sec; $P = .02$) and microvascular flux rate (mean, 1.6 ± 0.4 vs 2.5 ± 0.9 sec^{-1}; $P = .002$) on quantitative MCE. The degree of FMD was not significantly different in those with or without MVD (mean, 11 ± 4% vs 9 ± 4%; $P = .32$), and there was a poor correlation between results on stress MCE and FMD. Only eight of the 29 subjects were classified as having nonobstructive CAD. There were no groupwise differences in the prevalence of MVD function in those with versus without CAD (43% vs 38% for negative and positive findings on CTA, respectively, $P = .79$).

Conclusions: MVD is a common finding in the nonelderly population referred for CTA for evaluation of possible CAD but without obstructive stenosis. Neither the presence of noncritical atherosclerotic disease nor abnormal FMD increases the likelihood for detecting MVD in this population. (J Am Soc Echocardiogr 2019;32:817-25.)

Keywords: CT coronary angiography, Microvascular dysfunction, Myocardial contrast echocardiography

Noninvasive cardiovascular imaging is commonly used for the evaluation of suspected coronary artery disease (CAD) in symptomatic but stable patients. Approaches include those that evaluate coronary anatomy or detect ischemia during physiologic or pharmacologic stress. The sensitivity and specificity for the echocardiographic detection of CAD by either contractile reserve or myocardial perfusion are both generally between 80% and 90%.[1-3] Yet false-positive results occur, the rate of which is influenced by the prevalence of disease in the study population.[2,4] Positive results on stress echocardiography in those without significant coronary stenosis can result from abnormal microvascular function, defined as a vasoconstrictor-vasodilator imbalance,[5-7] which may be responsible

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Abbreviations

CAD = Coronary artery disease

CT = Computed tomographic

CTA = Computed tomographic angiography

FMD = Flow-mediated vasodilation

LV = Left ventricular

MCE = Myocardial contrast echocardiography

for the similar rate of clinical outcomes as in those with CAD.[4] Anatomic imaging with computed tomographic angiography (CTA) can directly image stenosis but is unlikely to detect microvascular dysfunction, even when performed with new fractional flow reserve algorithms that rely on computational fluid dynamics derived from anatomy.

In this study, we hypothesized that patients referred for CTA for suspected CAD, particularly the adult nonelderly, who are less likely to have severe obstructive CAD, would have a high prevalence of microvascular dysfunction assessed on perfusion imaging using vasodilator stress myocardial contrast echocardiography (MCE). A secondary hypothesis was that microvascular dysfunction would be more common in those with nonobstructive (<50%) epicardial coronary disease on CTA and at least one high-risk feature. This secondary hypothesis was based on the wide spectrum of pathways that predispose to abnormal vasomotor tone in patients with atherosclerosis or its risk factors[8,9] and the potential for ischemia to occur modest but diffuse arterial narrowing.[10] To test our hypotheses, vasodilator stress MCE was performed in subjects <60 years of age who were referred for CTA but had either no evidence of CAD or nonobstructive coronary atherosclerotic lesions and at least one established high-risk feature.

METHODS

Study Subjects

The study was approved by the investigational review board at Oregon Health & Sciences University and registered with ClinicalTrials.gov (NCT02465554). Subjects between the ages of 19 and 60 years without known CAD who had been referred for CTA by health care providers for evaluation of symptoms within 6 months were prospectively screened for eligibility over a 2-year period. The computed tomographic (CT) angiographic studies were reviewed by an expert blinded to all other data to ensure eligibility on the basis of the results of CTA. Patients were recruited to undergo microvascular testing using vasodilator MCE if they had either (1) no evidence of CAD, defined as lack of coronary plaque or stenosis on CTA and a coronary artery calcium score of 0, or (2) evidence of coronary plaque in at least one coronary artery but with stenosis severity <50% of luminal diameter, and at least one of the following high-risk plaque features: (a) positive remodeling, (b) low CT attenuation plaque, (c) spotty calcium, and (d) a "napkin ring" sign.[11,12] Subjects were excluded for vasculitis, pregnancy, presence of a myocardial bridge, contraindications to regadenoson, or allergy to ultrasound contrast agents. Clinical data including comorbidities, medications, and laboratory values were collected by taking a comprehensive history and through the electronic medical record. Blood for fasting plasma lipid levels was drawn on the day of the study.

Coronary CT Imaging

Coronary CTA was performed using a prospective electrocardiographically triggered protocol on a 256-multidector-row scanner (Philips iCT; Philips, Cleveland, Ohio) with the injection of iodinated contrast agent (70 mL of Omnipaque 350 mg I/mL; GE Healthcare, Little Chalfont, United Kingdom). The imaging parameters included tube current of 150 mA, tube potential of 120 kVp, and gantry rotation time of 280 msec. Images were reconstructed with a slice thickness of 0.8 mm and an increment of 0.4 mm and transferred to the core laboratory for interpretation. Image analysis was performed by two expert readers in a consensus evaluation using a cardiac workstation (IntelliSpace Portal 9.0; Philips, Amsterdam, the Netherlands).

Coronary CT angiographic images were assessed for the presence of coronary stenosis and plaque in all four major epicardial coronary arteries, inclusive of the left main coronary artery, according to the guidelines of the Society of Cardiovascular Computed Tomography.[13] In patients with calcified coronary plaque, we quantified the total amount of coronary calcium using Agatston score on noncontrast computed tomography.[14] Patients were classified as having high-risk plaque features if at least one high-risk plaque feature was present, defined as positive remodeling (remodeling index > 1.1 assessed on multiplanar reformatted images reconstructed in long-axis and short-axis views of the vessel), low CT attenuation plaque (presence of areas of low CT attenuation in noncalcified plaque with mean CT number in three regions of interest < 30 HU), napkin-ring sign (a ringlike peripheral higher attenuation of the noncalcified portion of the coronary plaque), and spotty calcium (calcified plaque with diameter < 3 mm in any direction, length of the calcium < 1.5 times the vessel diameter, and width of the calcification less than two-thirds of the vessel diameter).[11,12]

Quantitative measurements of coronary plaque volume were performed using semiautomated analysis software QAngio CT Research Edition version 3.1 (Medis Medical Imaging, Leiden, the Netherlands). Analysis began with the automatic detection of the coronary arteries, followed by the segmentation of luminal and outer vessel boundaries. If needed, manual adjustments of the vessel centerline and boundaries were performed. The total coronary plaque volume was measured in all coronary segments that were determined to have coronary plaque on qualitative assessment.

Microvascular Testing with MCE

Patients abstained from all caffeinated products for 48 hours before the study. Perfusion imaging on MCE was performed with a contrast-specific multipulse amplitude modulation imaging algorithm (iE33; Philips Ultrasound, Andover, MA) at a centerline frequency of 2.0 MHz. Imaging was performed at a mechanical index of 0.12 to 0.14, and gain was set at a level that just eliminated background myocardial speckle. Images were acquired in the apical four-chamber, two-chamber, and long-axis imaging planes. Lipid-shelled octafluoropropane microbubbles (Definity; Lantheus Medical Imaging, North Billerica, MA) were diluted to 30 mL total volume in normal saline and infused at a constant rate of 1.0 to 1.5 mL/min. A five-frame high-power (mechanical index > 0.9) sequence was applied to destroy microbubbles in the imaging sector through inertial cavitation, after which electrocardiographically triggered end-systolic frames were acquired to assess contrast replenishment. MCE was performed at rest and during vasodilator stress. For the latter, images were acquired between 1 and 4 min after intravenous administration of regadenoson (0.4 mg). Microvascular dysfunction was defined by global or segmental lack of complete transmural or subendocardial microvascular refill within 2 sec during vasodilator stress.[15]

For quantitative analysis, digital video clips were transferred to a personal computer and analyzed using validated intensity analysis

HIGHLIGHTS

- Regadenoson MCE can be used to assess coronary microvascular dysfunction.
- Microvascular dysfunction is common in those referred for CTA.
- Coronary microvascular dysfunction is poorly predicted by FMD results.

software (iMCE; Narnar, Portland, OR). Data were averaged for the three major coronary vascular territories. A region of interest was drawn to derive information transmurally, even when defects seen were only subendocardial. The first postdestructive frame was digitally subtracted from all subsequent frames, and background-subtracted time-intensity data were fit to the function

$$y = A(1 - e^{-\beta t}),$$

where y is signal intensity at time t, A is the plateau intensity reflecting relative microvascular blood volume, and β is the rate constant reflecting microvascular blood flux rate. Microvascular blood flow was quantified by the product of microvascular blood volume and β.[16] Flow reserve and β reserve were calculated as the ratio of values obtained during regadenoson to those at rest.

Echocardiography

Echocardiography (iE33) was performed to assess chamber dimensions and left ventricular (LV) function according to guidelines published by the American Society of Echocardiography.[17] LV ejection fraction and volumes were calculated using the modified Simpson method, while LV mass was calculated using the linear formula. Stroke volume was calculated using the product of LV outflow tract area and the time-velocity integral measured using pulsed-wave spectral Doppler. Myocardial work was calculated by the product of stroke volume index, systolic blood pressure, and heart rate.

Flow-Mediated Vasodilation

Brachial artery flow-mediated vasodilation (FMD) was performed using a linear-array probe (5–9 MHz). At baseline, the brachial artery diameter proximal to the antecubital fossa was measured using two-dimensional ultrasound and real-time spatial compounding of coplanar angled beams. Centerline blood velocities were measured at the same location using pulsed-wave Doppler and angle correction software. A blood pressure cuff was placed around the proximal forearm and insufflated to 50 mm Hg over systolic blood pressure for 5 min. After release of the cuff, Doppler velocity measurements were measured immediately, and brachial diameter measurements were repeated at 30-sec intervals for 4 min. FMD was calculated as the ratio of maximum hyperemic to baseline diameter. This value was also expressed normalized to hyperemic flow, expressed as the absolute increase in blood velocity.

Statistical Analysis

Data were analyzed using Prism version 5.0 (GraphPad Software, San Diego, CA). Groupwise differences were assessed using the Mann-Whitney U test for data that were determined to be nonnormally distributed using the D'Agostino and Pearson omnibus test. For data with normal distribution, groupwise differences were assessed using the nonpaired Student's t test for groupwise comparisons and the paired Student's t test for pre- and postregadenoson data. Differences in proportions were assessed using a χ^2 or Fisher exact test. Correlations were made by least squares fit and linear regression analysis. Differences were considered significant at $P < .05$ (two sided).

Table 1 Clinical characteristics

Variable	Total cohort (N = 29)	No CAD (n = 21)	CAD (n = 8)	P*
Age (y)	48 ± 6	47 ± 6	51 ± 6	NS
Male/female	10/19	6/15	4/4	NS
BMI (kg/m^2)	28.7 ± 6.6	28.6 ± 7.0	29.1 ± 5.8	NS
Family history	16 (55)	10 (48)	6 (75)	NS
Dyslipidemia	10 (34)	5 (24)	5 (63)	NS
Diabetes mellitus	2 (7)	1 (5)	1 (13)	NS
Hypertension	7 (24)	4 (19)	3 (38)	NS
Smoking history	4 (14)	4 (19)	0 (0)	NS
Medications				
ACE inhibitor/ARB	8 (28)	6 (29)	2 (25)	NS
β-blocker	9 (31)	5 (24)	4 (50)	NS
CCB	3 (10)	1 (5)	2 (25)	NS
Antiplatelet	8 (28)	3 (14)	5 (63)	NS
Antithrombotic	2 (7)	1 (5)	1 (13)	NS
Statin	8 (28)	3 (14)	5 (63)	.01
Other lipid agent	1 (3)	0 (0)	1 (13)	NS
Long-acting nitrate	0 (0)	0 (0)	0 (0)	NS
Insulin	0 (0)	0 (0)	0 (0)	NS

ACE, Angiotensin-converting enzyme; *ARB*, angiotensin receptor blocker; *BMI*, body mass index; *CCB*, calcium channel blocker.
Data are expressed as mean ± SD or as number (percentage).
*P value for comparison of cohorts without and with CAD.

RESULTS

Of the 30 patients recruited, vasodilator stress MCE could be performed in 29. Clinical data are shown in Table 1. At least one traditional CAD risk factor was present in 18 of the subjects (62%), and 15 (51%) were taking at least one vasoactive antihypertensive medication. On average, plasma lipid values (Table 2) were largely normal, although four subjects had elevated low-density lipoprotein cholesterol (14%) and six subjects (21%) had elevated lipoprotein(a) at the time of the study. A total of eight subjects were taking statins. On average, echocardiographic measurements, including measurements of stroke work and myocardial work, which influence oxygen demand, were normal (Table 3). For the entire cohort, a total of 21 subjects were classified as having no coronary plaque and eight as having nonobstructive CAD (<50%), with at least one high-risk plaque feature. Coronary plaque was present in one vessel in four subjects, two vessels in two subjects, three vessels in one subject, and four vessels in one subject.

Table 2 Plasma lipid values on the basis of absence or presence of CAD

Variable	Total cohort (*N* = 29)	No CAD (*n* = 21)	CAD (*n* = 8)	*P**
Triglycerides (mg/dL)	138 ± 53	140 ± 55	132 ± 50	NS
Total cholesterol (mg/dL)	190 ± 36	199 ± 30	168 ± 42	.03
HDL cholesterol (mg/dL)	55 ± 15	57 ± 15	50 ± 13	NS
LDL cholesterol (mg/dL)	108 ± 27	114 ± 22	92 ± 34	.05
Lipoprotein(a) (mg/dL)	23 ± 31	13 ± 10	51 ± 50	.003

HDL, High-density lipoprotein; *LDL*, low-density lipoprotein.
Data are expressed as mean ± SD.
**P* value for comparison of cohorts without and with CAD.

Table 3 Echocardiographic measurements

Variable	Total cohort (*N* = 29)	No CAD (*n* = 21)	CAD (*n* = 8)	*P**
LVIDd (cm)	4.7 ± 0.6	4.7 ± 0.6	4.6 ± 0.6	NS
LVIDs (cm)	3.2 ± 0.8	3.3 ± 0.8	3.0 ± 0.7	NS
IVSd (cm)	0.9 ± 0.3	0.9 ± 0.3	1.0 ± 0.2	NS
PWd (cm)	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	NS
LVEDV (mL)	100 ± 22	100 ± 24	99 ± 20	NS
LVESV (mL)	38 ± 12	39 ± 11	38 ± 14	NS
LVEF (%)	63 ± 7	63 ± 6	63 ± 9	NS
LV mass index (g/m^2)	77 ± 25	74 ± 24	85 ± 28	NS
Stroke volume (mL)	72 ± 19	73 ± 22	72 ± 12	NS
Cardiac output (L/min)	5.1 ± 1.5	5.1 ± 1.7	5.0 ± 1.1	NS
Stroke work (mL × mm Hg)	8,729 ± 1,906	8,581 ± 2,001	9,101 ± 1,717	NS
Myocardial work (×1,000 mL × mm Hg/min)	1,032 ± 328	978 ± 333	1,170 ± 294	NS

IVSd, Diastolic interventricular septal thickness; *LVEDD*, LV end-diastolic volume; *LVEF*, LV ejection fraction; *LVESD*, LV end-systolic volume; *LVIDd*, diastolic LV diameter, *LVIDs*, systolic LV diameter; *PWd*, diastolic posterior wall thickness.
Data are expressed as mean ± SD.
**P* value for comparison of cohorts without and with CAD.

Table 4 Vital signs at rest and during vasodilator stress*

	HR (beats/min)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Double product (mm Hg/min)
Entire cohort				
Rest	71 ± 12	117 ± 15	68 ± 11	8,325 ± 2,199
Stress	84 ± 17†	118 ± 22	71 ± 14	9,577 ± 3,467†
No CAD on CTA				
Rest	71 ± 14	113 ± 15	68 ± 11	8,175 ± 2,324
Stress	86 ± 18†	117 ± 24	71 ± 15	9,569 ± 3,788
CAD on CTA				
Rest	69 ± 8	126 ± 14	69 ± 10	8,718 ± 1,911
Stress	78 ± 12	121 ± 18	74 ± 14	9,603 ± 2,497
Normal microvascular function				
Rest	69 ± 11	118 ± 15	69 ± 10	8,172 ± 2,060
Stress	86 ± 19†	117 ± 25	73 ± 14	10,139 ± 3,545
Abnormal microvascular function				
Rest	73 ± 15	114 ± 16	66 ± 12	8,404 ± 2,570
Stress	82 ± 12	117 ± 19	69 ± 15	9,593 ± 3,353

Data are expressed as mean ± SD.
BP, Blood pressure; *HR*, heart rate.
**P* = NS for all comparisons between presence and absence of CAD and normal and abnormal microvascular function.
†*P* < .05 versus resting condition.

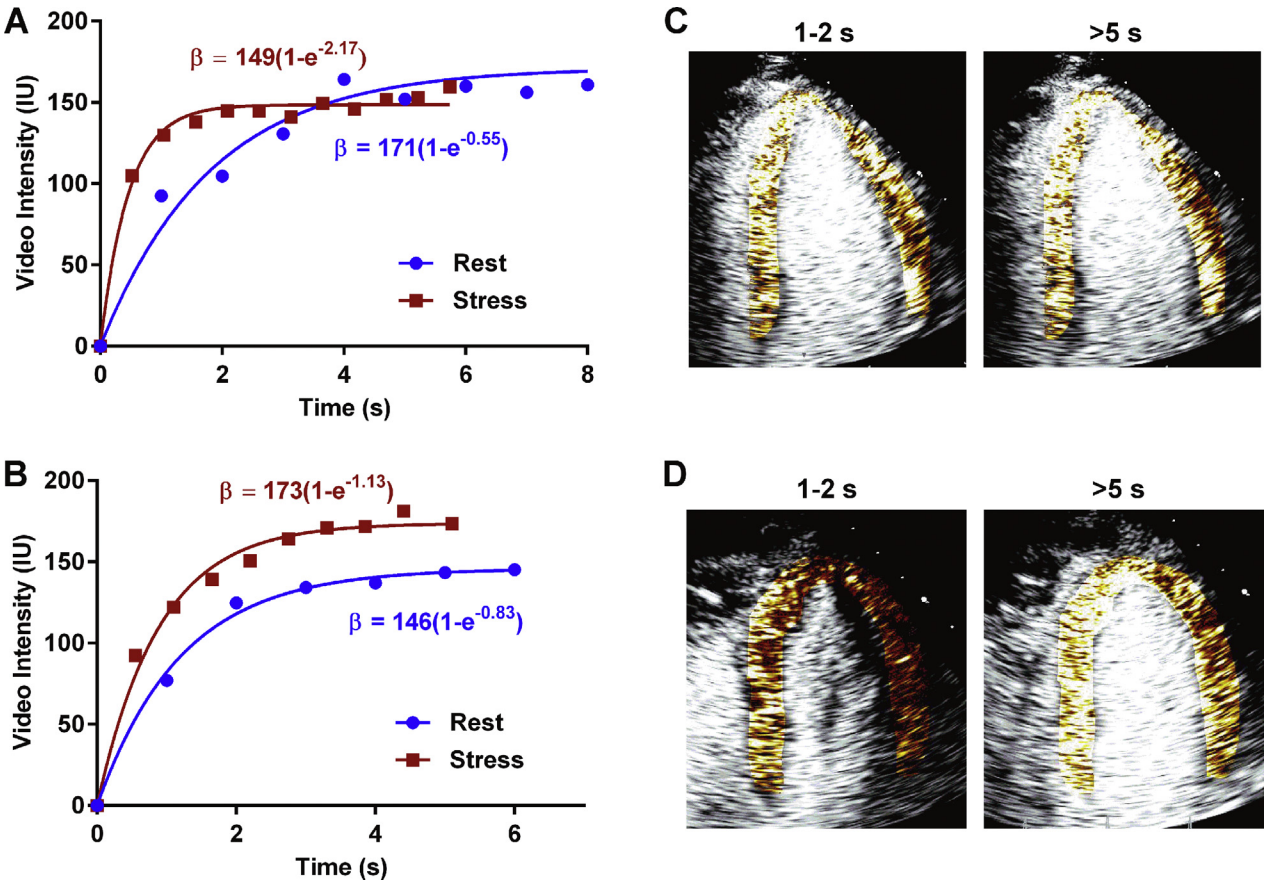

Figure 1 Examples of time-intensity data from the myocardium at rest and during regadenoson stress from a patient categorized as having **(A)** normal microvascular function or **(B)** abnormal microvascular function. During stress, the subject with normal microvascular function exhibits a plateau of video intensity between 1 and 2 sec and a nearly fourfold increase in β value, whereas the subject with abnormal microvascular function has a plateau of video intensity between 3 and 4 sec and a small (<40%) increase in β value. **(C,D)** The corresponding background-subtracted color-coded myocardial contrast echocardiographic images from the apical four-chamber view during stress at 1 to 2 or >5 sec after the destructive pulse sequence illustrate the incomplete replenishment by 1 to 2 sec in the patient with microvascular dysfunction.

Table 5 Plasma lipid values on the basis of microvascular functional status

Variable	Normal (n = 17)	MVD (n = 12)	P
Triglycerides (mg/dL)	139 ± 52	136 ± 57	NS
Total cholesterol (mg/dL)	183 ± 29	201 ± 43	NS
HDL cholesterol (mg/dL)	52 ± 14	59 ± 15	NS
LDL cholesterol (mg/dL)	103 ± 20	114 ± 35	NS
Lipoprotein(a) (mg/dL)	28 ± 40	17 ± 12	NS

HDL, High-density lipoprotein; *LDL*, low-density lipoprotein; *MVD*, microvascular dysfunction.
Data are expressed as mean ± SD.

During regadenoson vasodilator stress, for the entire cohort there were significant drug-related increases in both heart rate and double product, with no change in blood pressure (Table 4). No patients experienced hypotension, defined as systolic blood pressure < 90 mm Hg, thereby excluding the possibility of reduced aortic perfusion pressure as a cause for impaired hyperemic perfusion. No patients had diagnostic ST-segment changes on electrocardiography.

During regadenoson MCE, 12 subjects (41%) had evidence of impaired microvascular function, defined as delayed microvascular replenishment of contrast on MCE during vasodilator stress in at least one vascular territory (Figure 1). Seven patients (24%) had severe microvascular dysfunction, defined as contrast replenishment that required ≥5 sec during regadenoson in at least one segment. In these patients, the spatial pattern was divided nearly evenly between those with global versus segmental abnormalities in stress microvascular perfusion. No significant differences in either heart rate or blood pressure at rest or during stress were found for subjects classified as having normal versus abnormal microvascular function (Table 4). There were no major demographic or risk factor differences between those with normal versus abnormal microvascular function on MCE, except that there was a nonsignificant trend for greater proportion of women in abnormal microvascular function group (83% vs 53%; P = .09). There were no significant differences in blood lipid values for those with normal versus abnormal microvascular function (Table 5).

Quantitative analysis of myocardial perfusion on MCE, which for each patient was averaged for at least two vascular territories, showed no differences in resting MBF, microvascular blood volume, or microvascular flux rate (β) between subjects classified as having normal versus abnormal microvascular function (Figure 2). During

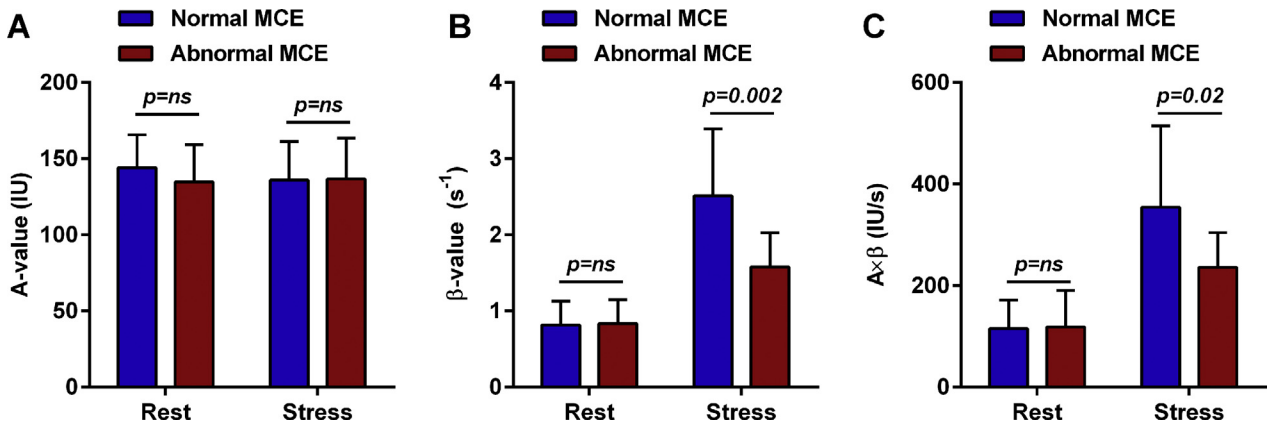

Figure 2 Quantitative myocardial contrast echocardiographic data (mean ± SD) averaged for at least two vascular territories, including **(A)** microvascular blood volume (*A*), **(B)** microvascular flux rate (β), and **(C)** microvascular blood flow ($A \times \beta$) at rest and during regadenoson in patients subjectively determined to have normal microvascular response versus microvascular dysfunction. *IU*, Intensity units.

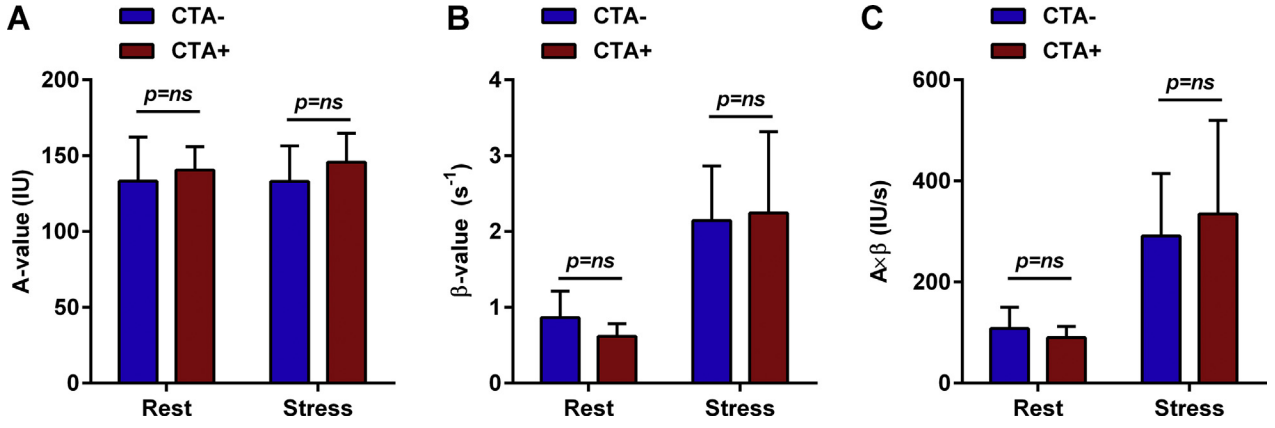

Figure 3 Quantitative myocardial contrast echocardiographic data (mean ± SD) including **(A)** microvascular blood volume (*A*), **(B)** microvascular flux rate (β), and **(C)** microvascular blood flow ($A \times \beta$) at rest and during regadenoson in patients subjectively determined to have normal versus abnormal results on CTA. *IU*, Intensity units.

regadenoson stress, however, subjects with microvascular dysfunction had significantly lower MBF and microvascular flux rate (β) and lower β reserve (1.95 ± 0.58 vs 3.67 ± 2.96, $P = .01$), thereby corroborating the subjective classification of these patients.

Separate analysis was performed for subjects with and without CAD, with the caveat that the power from these studies was limited by the small number of subjects with CAD. In subjects with CAD, the median Agatston score was 41.8 (interquartile range, 10.5–84.8). High-risk features included spotty calcium in all eight subjects, positive remodeling in three subjects, low CT attenuation plaque in two subjects, and napkin-ring sign in two subjects. The mean total plaque volume in subjects with coronary plaque was 32.5 mm^3. The clinical characteristics of the patients stratified according to results of CTA (Table 1) did not show any major groupwise differences, except those with CAD had a trend toward higher prevalence of a history of dyslipidemia ($P = .08$). Plasma lipids (Table 2) revealed that those with nonobstructive CAD had lower total and low-density lipoprotein cholesterol than those without CAD, likely reflecting a greater proportion of subjects treated with statins (63% vs 14%, $P < .01$). However those with CAD had significantly higher levels of lipoprotein(a), a known CAD risk factor. Results on resting echocardiography (Table 3) were similar between groups, as were hemodynamic variables at rest or during vasodilator stress (Table 4). When microvascular function was analyzed according to the computed tomographic angiographic stratification, there were no groupwise differences in the prevalence of microvascular dysfunction function (43% vs 38% for patients with negative and positive findings on CTA; $P = .79$, Fisher exact test), or by quantitative myocardial contrast echocardiographic perfusion during stress (Figure 3). The proportion of patients who had CAD on CTA was similar for those with and without microvascular dysfunction (29% vs 38%; $P = .90$, χ^2 test). Mean total plaque volume was also similar in subjects with versus without microvascular dysfunction (9.3 ± 20.4 vs 6.6 ± 15.7 mm^3, $P = .84$).

Brachial FMD was performed in all subjects and was expressed either as absolute value or as FMD normalized to hyperemic flow velocity, the major contributor of shear augmentation after reperfusion. There were no groupwise differences in these FMD values when analyzed on the basis of the presence versus absence of microvascular function on MCE or on the presence versus absence of CAD (Figure 4). On continuous analysis, the relationships between MCE-derived perfusion and FMD were weak, with a significant but poor relationship between microvascular flux rate (β) during vasodilator stress and FMD ($r^2 = 0.16$, SEE = 0.04). Neither hyperemic blood flow nor β reserve correlated with FMD.

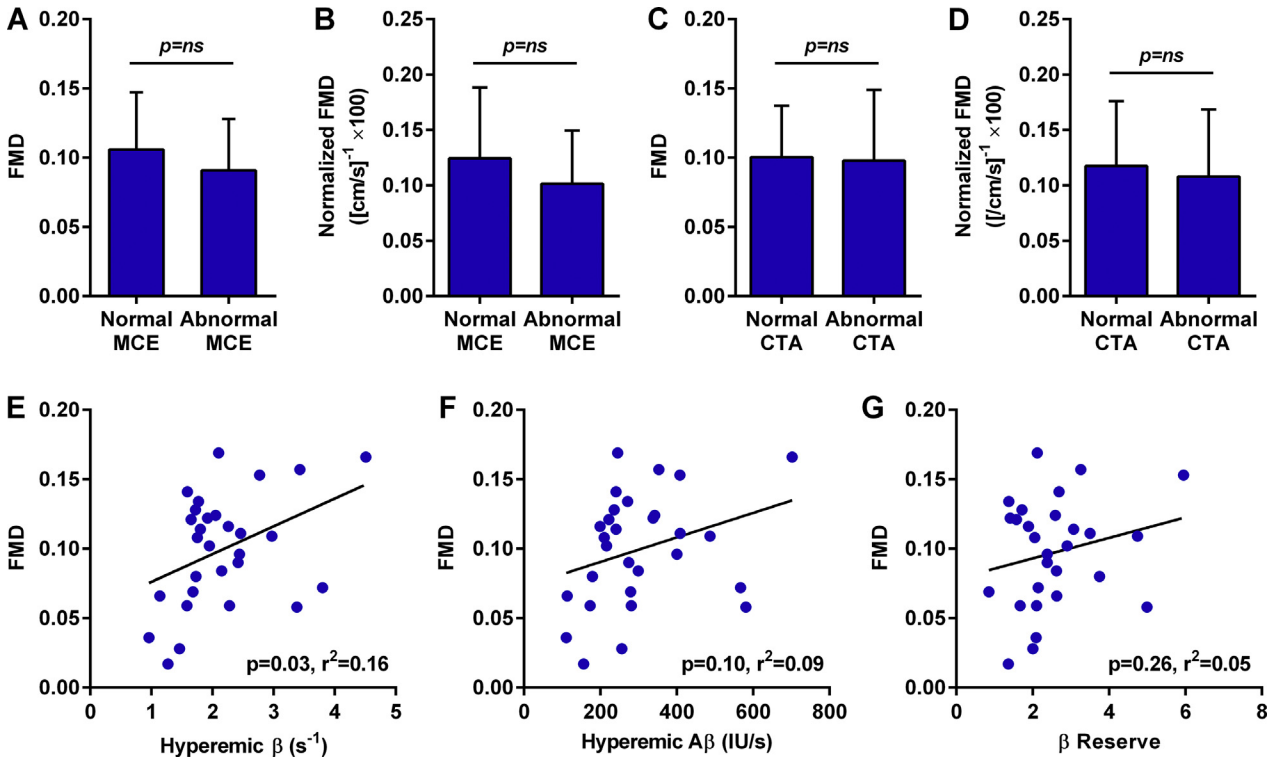

Figure 4 **(A–D)** Brachial artery FMD in the patient cohorts segregated on the basis of myocardial contrast echocardiographic microvascular response or on results of CTA. FMD data are expressed as a ratio to baseline diameter or as FMD ratio normalized to the increase in brachial artery velocity during hyperemia to control for the increase in arterial shear. **(E–G)** Correlation between brachial artery FMD and hyperemic microvascular flux rate (β), hyperemic microvascular blood flow (Aβ), or β reserve. *IU*, Intensity units.

DISCUSSION

It is increasingly recognized that abnormal microvascular function without significant obstructive CAD can be responsible for chest pain and for ischemia detected on cardiovascular stress testing. The importance of this phenomenon is underscored by studies that have suggested that patients with microvascular dysfunction or those with "false-positive" results on stress echocardiography have cardiovascular outcome rates that are similar to those of patients with CAD.[4,9,18] Although there is no consensus for the best technique for diagnosing microvascular dysfunction, approaches that have been used include evaluation of coronary diameter and flow velocity during intracoronary administration of various endothelial-dependent or independent vasodilators in the catheterization laboratory, or noninvasive assessment of global or regional microvascular perfusion during vasodilator stress using MCE or positron emission tomography.

For noninvasive perfusion imaging, methods that are able to quantify myocardial blood flow are desirable for several reasons. A reduction in flow reserve, which is often used to define coronary microvascular dysfunction, can occur solely from an increase in resting blood flow that occurs in the setting of anemia or any condition that increases resting myocardial oxygen demand, such as increased heart rate, contractility, or wall stress.[5] MCE in women with "syndrome X" has suggested that abnormal flow reserve solely from increased resting flow can occur without any increase in oxygen demand.[19] This finding suggests an insufficiency of oxygen or nutrient delivery could exist. Abnormal flow reserve can also occur when vascular resistance does not fall appropriately in response to vasodilatory stimuli.[5,8] From a physiologic perspective, this would result in a shallower slope to the relationship between coronary perfusion pressure and microvascular blood flow during vasodilator stimulus. Although this phenomenon is often attributed to abnormal vasodilator-vasoconstrictor balance, it can also occur from extrinsic microvascular forces when intramyocardial pressures are elevated, such as in high-preload states.[5] Although the diagnosis of coronary microvascular dysfunction is conventionally made in the absence of significant (>50%) coronary stenosis, mild but diffuse atherosclerotic narrowing has the potential to reduce flow reserve purely from an anatomic basis.[10]

Within this framework, in the present study quantitative MCE perfusion imaging during vasodilator challenge was performed to determine the prevalence of microvascular dysfunction in nonelderly subjects referred for CTA who did not have significant obstructive disease or myocardial bridging. The vast majority of these subjects were classified on the basis of Diamond-Forrester classification as having medium pretest likelihood for disease. We found that 41% subjects had evidence of microvascular impairment. The primary definition of microvascular dysfunction was made on the basis of abnormal hyperemic flow rather than abnormal flow reserve in order to eliminate those with abnormal flow reserve due solely to increased resting flow. Quantitative MCE and conventional echocardiography were useful for proving that there was no difference between those with normal versus abnormal microvascular function with regard to either resting blood flow or indices of myocardial work. Quantitative MCE was also useful for confirming a reduction in microvascular flux rate (β) during vasodilator challenge in those defined as having microvascular dysfunction. This finding aligns with the notion that impaired

vasodilatory capacity will increase arteriolar resistance and, hence, reduce precapillary driving pressure and microvascular flux rate.[20]

A recent study of patients with angina but no obstructive CAD found endothelial dysfunction or increased coronary resistance during vasodilator testing in the catheterization laboratory in about half, a prevalence similar to our own results.[21] Some of these patients had myocardial bridges identified, and all had some degree of atherosclerosis on intravascular ultrasound. Other studies have demonstrated that traditional atherosclerotic risk factors are associated with abnormal flow reserve on positron emission tomography. There are many potential pathways by which atherosclerotic disease itself and associated hyperlipidemia or diabetes mellitus can impair microvascular responses. Some of the better characterized processes include increased vascular production of reactive oxygen species, reduced endothelial nitric oxide synthase activity, endothelial nitric oxide synthase uncoupling, impaired purinergic receptor response, and increased production of and sensitivity to vasoconstrictors.[22] Accordingly we investigated whether microvascular dysfunction in our population was more prevalent in those with evidence of atherosclerosis on CTA. Unexpectedly, microvascular dysfunction was seen to the same extent in those with completely normal coronary arteries versus those with mild nonobstructive disease and at least one high-risk plaque feature on CTA, although these results must be tempered by the small number of subjects with CAD. These findings are in agreement with a study of subjects with atherosclerosis on CTA in whom positron emission tomographic perfusion imaging with vasodilator stress did not show any differences in hyperemic myocardial blood flow in segments subtended by arteries with 0% to 50% stenosis versus arteries with no plaque.[23] In another study, coronary flow reserve by transthoracic Doppler was associated with certain atherosclerotic risk factors, yet statistical modeling revealed that they affected flow reserve to only a minor degree.[24] In our study, there were no differences in lipid values on the basis of microvascular functional status.

Our results show a rather poor association between vasodilator myocardial contrast echocardiographic measurements of hyperemic myocardial perfusion and FMD. This finding, however, may not be entirely unexpected, as previous studies have clearly demonstrated that coronary and brachial reactivity are not tightly correlated.[25] The implications of our findings are that FMD may not be an appropriate method for identifying subjects who have symptoms from impaired coronary microvascular function.

There are important limitations to the present study that deserve comment. With prospective screening, the number of patients studied was small on the basis of regional trends of low utilization of CTA as a consequence of burdensome preauthorization policies and willingness to participate in the study by only half of those who met eligibility criteria. To evaluate whether significant atherosclerotic disease predisposes to abnormal microvascular function, we elected to study those who had evidence of atherosclerosis only if they also had at least one high-risk plaque feature on CTA. These criteria eliminated some subjects with nonsevere CAD but without high-risk features. Microvascular testing using MCE was performed with regadenoson rather than with other vasodilator agents that are purely endothelial dependent, such as acetylcholine. We selected regadenoson on the basis of the notion that vascular response to an adenosine A2a receptor agonist still requires the additional shear-mediated endothelial responses for full effect.[26] Similar to positron emission tomographic perfusion imaging, which has been used to assess microvascular function, MCE was not validated against a gold standard for microvascular dysfunction in patients. However, a close correlation is known to exist between these two techniques with respect to quantifying hyperemic response.[27] Finally, although the increase in heart rate and double product during regadenoson in the "abnormal microvascular function" group did not meet statistical significance, this is unlikely to be the reason for reduced hyperemic flow, as flow augmentation (normally several-fold) occurs primarily from direct coronary arteriolar adenosine A2a receptor effects rather than from the very modest increases in myocardial work.

CONCLUSION

Our data indicate that microvascular dysfunction is not an uncommon finding in the nonelderly population referred for CT angiographic evaluation of possible CAD in whom "significant" coronary stenosis is not found. Although the population of patients with evidence of atherosclerotic disease on CTA was small, the presence of atherosclerosis even with additional high-risk features did not increase the likelihood of abnormal microvascular response. Our results indicate that vasodilator MCE testing and measurement of peak hyperemic microvascular flux rate or replenishment time can provide a rapid, noninvasive method not only for explaining ischemic symptoms in patients who do not have significant disease on angiography but also for identifying those patients in whom prognosis is affected by abnormal microvascular function.[18] This information may have a clinical impact by identifying those who may benefit from therapies that have been shown to reduce symptoms in microvascular dysfunction or who should avoid medications or dietary supplements that adversely increase vasomotor tone.

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