Clinical Investigations

PREVENTION

Coronary Microvascular Dysfunction Assessed by Intracoronary Acetylcholine Provocation Testing Is a Frequent Cause of Ischemia and Angina in Patients With Exercise-Induced Electrocardiographic Changes and Unobstructed Coronary Arteries

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ABSTRACT

Background: The exercise electrocardiogram (ECG) is a standard examination in patients with suspected coronary artery disease. However, despite a pathologic result, many patients undergoing diagnostic coronary angiography do not have any significant epicardial stenosis. In this study, we assessed the relation between a pathologic exercise ECG and coronary microvascular dysfunction in response to intracoronary acetylcholine (ACh) provocation in patients without any relevant epicardial stenosis.

Hypothesis: Coronary microvascular dysfunction is significantly more often in patients with angina, unobstructed coronary arteries and a pathologic exercise stress test compared to those without pathologic stress test.

Methods: This study recruited 137 consecutive patients with exertional angina pectoris who underwent diagnostic coronary angiography between September 2008 and April 2011 (68% women; mean age, 63 ± 10 years). In none of the patients was there a stenosis of $>50\%$. All patients underwent an exercise ECG before angiography and intracoronary ACh provocation testing for assessment of coronary vasomotor responses directly after angiography.

Results: The exercise ECG showed an abnormal result in 69 patients (50%; ST-segment depression ≥ 0.1 mV and/or reproduction of the patient's usual symptoms). The ACh test revealed a coronary vasomotor abnormality (reproduction of the patient's symptoms, ischemic ECG shifts $\pm$ diffuse distal vasoconstriction) in 87 patients (64%). Such a result was significantly more often found in patients with a pathologic exercise ECG (50/69 [72%] vs 19/69 [28%], $P = 0.034$). There were no other statistically significant differences between patients with and those without pathologic exercise ECG.

Conclusions: Coronary microvascular dysfunction is frequently found in patients with exertional angina pectoris and unobstructed coronary arteries. Such a finding is found significantly more often in presence of a pathologic exercise ECG.

Introduction

Patients with angina pectoris (AP) and unobstructed coronary arteries remain a diagnostic challenge in everyday clinical cardiology. Recently, we were able to show that up to 62% of these patients suffer from a coronary vasomotor disorder that can be unmasked by intracoronary acetylcholine (ACh) provocation testing.[1] Thus, coronary vasomotor disorders represent a frequent condition in daily clinical routine. Nevertheless, they are often not considered or diagnosed. Studies have proposed that noninvasive measurements of microvascular function (eg, Endo-PAT) correlate with ACh-induced vasomotor disorders in Japanese patients,[2] but this has so far not been shown in Caucasian patients. Moreover, an association between inflammatory markers and coronary microvascular dysfunction has been reported in this setting.[3] However, there is currently no reliable noninvasive test available for the diagnosis of coronary microvascular dysfunction. In this study, we speculated that a pathologic exercise stress test in patients with unobstructed coronary arteries may be an indicator of coronary microvascular dysfunction rather than a false-positive test. Consequently, we assessed the

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relation between a pathologic exercise stress test (exercise tolerance test [ETT]) ECG and coronary microvascular dysfunction in response to intracoronary ACh provocation testing in patients with AP despite unobstructed coronary arteries.

Methods

Patients

From September 2008 to June 2011, a total of 137 consecutive patients (44 men; mean age, 63 ± 11 years) who underwent diagnostic coronary angiography and were found to have unobstructed coronary arteries (no epicardial stenosis ≥50%) were included in the study. They had to fulfill the following inclusion criteria: exertional AP and ETT before coronary angiography (bicycle stress test). Intracoronary ACh provocation testing was performed directly after diagnostic coronary angiography. Subjects were excluded and the provocation test was not performed if patients had severe chronic obstructive pulmonary disease or impaired renal function (creatinine >2.0 mg/dL), or if spontaneous spasm was observed. The following information was recorded in every patient: cardiovascular risk factors including hypertension, diabetes, hypercholesterolemia, a history of smoking, and a positive family history for cardiovascular events (myocardial infarction or stroke in a parent or sibling); results of the ETT (a positive response was defined as transient ischemic ECG changes ≥0.1 mV in ≥2 contiguous leads, 80 ms after the J point, and/or reproduction of angina during the stress test).

Study Protocol

The study protocol complied with the Declaration of Helsinki and all patients gave written informed consent before angiography. All patients in the study underwent intracoronary provocation with ACh in accordance to a standardized protocol immediately after diagnostic angiography.[1] Cardiovascular medications (β-blockers, calcium channel blockers, and nitrates) were discontinued 48 hours before coronary angiography. Sublingual glyceryltrinitrate administration was permitted for the relief of chest pain at all times; however, none of the patients required this treatment <4 hours prior to angiography. Heart rate, blood pressure, and the 12-lead ECG were continuously monitored during ACh testing. Ischemic ECG changes were defined as transient ST-segment depression or elevation ≥0.1 mV in ≥2 contiguous leads.

Acetylcholine Testing

Incremental doses of 2 μg, 20 μg, 100 μg, and 200 μg of ACh were manually infused over a period of 3 minutes into the left coronary artery (LCA) via the angiographic catheter. In patients who remained asymptomatic and showed no diagnostic ST-segment changes during LCA ACh infusion, 80 μg of ACh was injected into the right coronary artery (RCA).[1]

Transient atrioventricular block was frequently observed, mostly during provocation of the RCA. It almost always resolved within seconds after reducing the speed of the manual injection. Therefore, we did not test the RCA with a pacing catheter in the right ventricle avoiding potential complications. A bolus of glyceryltrinitrate 0.2 mg (Perlinganit; Schwarz Pharma, Monheim, Germany) was injected into the LCA or RCA to relieve angina and/or severe epicardial constriction. Nitrates were also infused routinely at the end of the ACh test into the RCA and LCA.

Acetylcholine Test Assessment

Angiographic responses during the ACh test were analyzed using computerized quantitative coronary angiography (QCA-CMS, version 6.0; Medis, Leiden, the Netherlands). The ACh test was considered positive for coronary microvascular dysfunction when typical ischemic ST-segment changes and AP developed in the absence of epicardial coronary constriction ≥75%-diameter reduction.[1] Additionally, epicardial vasoconstriction was measured, and ≥75%-diameter reduction compared with the relaxed state following intracoronary nitroglycerine was recorded as significant. Patients who experienced no AP, constriction, or ST-segment shifts were considered to have a negative ACh test response (normal coronary vasoreactivity).

Statistical Analysis

Data analysis was carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD. The Student *t* test was used to compare continuous variables. For values without normal distribution, median and interquartile ranges are stated and the Mann-Whitney *U* test was used for analysis. The Fisher exact test was used for categorical variables. Multiple logistic regression analysis was performed using forward variable selection based on likelihood ratios to identify predictors for a pathologic exercise stress test result. A 2-tailed *P* value <0.05 was considered significant.

Results

Overall Results

The summary of the results of all patients is shown in Table 1. The ACh test was completed in all patients without any complications. The ACh test revealed coronary microvascular dysfunction in 87 patients (64%), of whom 33 showed distal and diffuse epicardial vasoconstriction at higher doses of ACh (Figure 1). In the remaining 50 patients, the ACh test was uneventful. The Fisher exact test revealed that patients with a pathologic ETT significantly more often had a pathologic ACh test result (69/50 compared with 68/37, $P = 0.034$; Figure 2A). Statistical comparison of patients with and those without a pathologic ETT revealed no significant differences regarding age, sex, left ventricular ejection fraction, and cardiovascular risk factors. However, when comparing patients with a pathologic ACh test with those without a pathologic ACh test, there were significantly more women with a positive family history for cardiovascular disease among the patients with a pathologic ACh test ($P < 0.0001$ and $P = 0.035$, respectively; Table 1 and Table 2).

Multivariate logistic regression analysis incorporating all parameters from Table 1 revealed that the only significant

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Table 1. Patient Characteristics

	All Patients, N = 137	Uneventful ETT, n = 68 (50)	Pathologic ETT, n = 69 (50)	*P* Value
Male sex	44 (32)	27 (40)	17 (25)	0.069
Age, y	63 ± 11	63 ± 11	63 ± 11	0.870
LVEF, %	73 ± 9	73 ± 8	73 ± 10	0.584
ACh provocation testing abnormal	87 (64)	37 (54)	50 (72)	0.034
Hypertension	105 (77)	53 (78)	52 (75)	0.840
T2DM	27 (20)	17 (25)	10 (14)	0.138
Hypercholesterolemia	73 (53)	36 (53)	37 (54)	0.999
Current smoking	38 (28)	18 (26)	20 (29)	0.849
Positive family history of CVD	74 (54)	33 (49)	41 (59)	0.232

Abbreviations: ACh, acetylcholine; CVD, cardiovascular disease; ETT, exercise tolerance (stress) test; LVEF, left ventricular ejection fraction; SD, standard deviation; T2DM, type 2 diabetes mellitus.
Data are presented as mean ± SD or n (%).

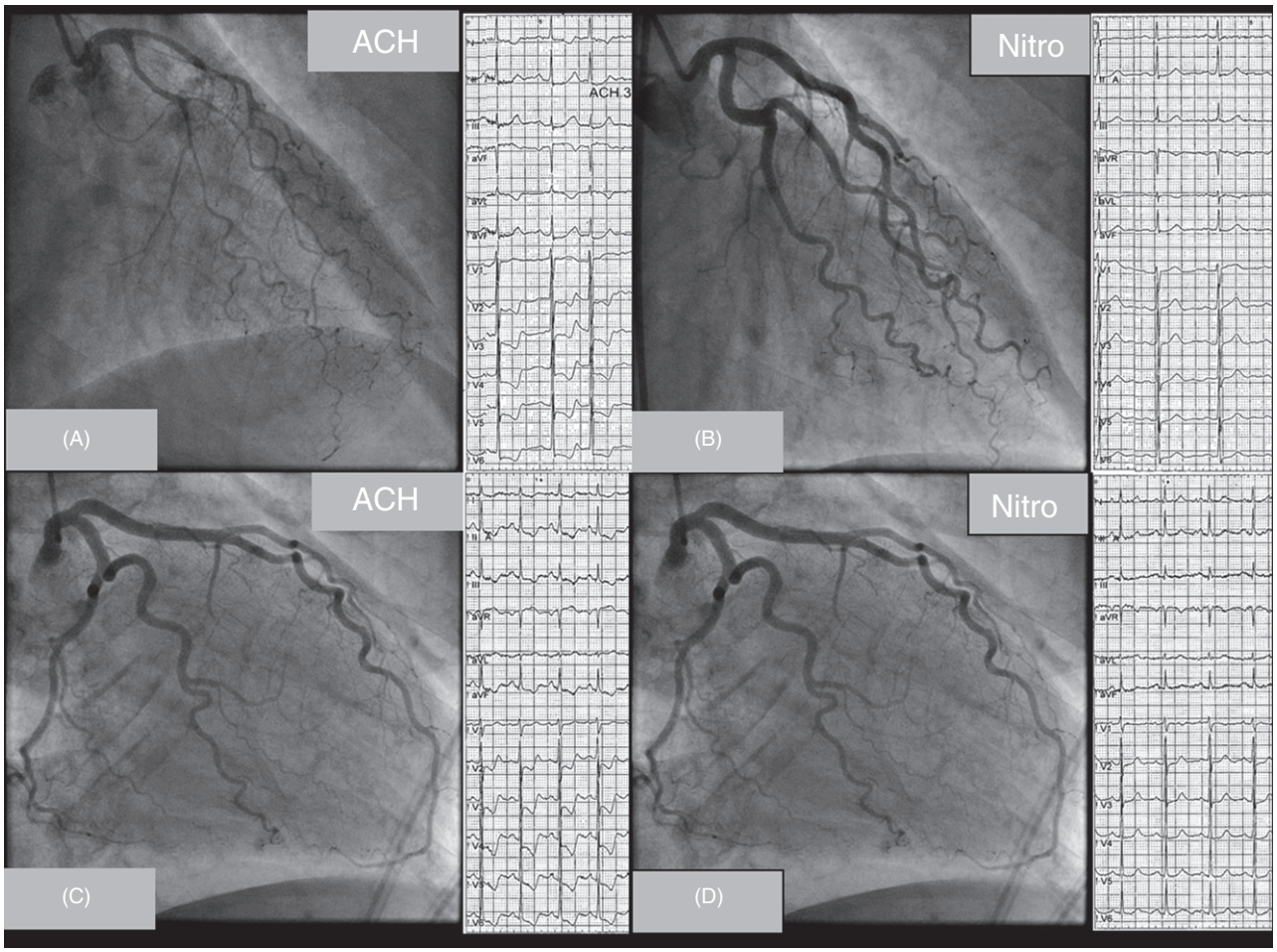

Figure 1. The upper panel shows LCA angiograms and ECGs of a patient with epicardial vasoconstriction following signs of coronary microvascular dysfunction. Note the diffuse but distally accentuated narrowing of the LAD during i.c. ACh infusion together with ischemic ECG shifts (A) and (B) resolution of both findings after nitroglycerine i.c. The lower panel shows an example of a patient with coronary microvascular dysfunction. (C) During ACh, the patient had reproduction of angina and ischemic ECG changes but no epicardial vasoconstriction. (D) After nitroglycerine i.c., chest pain and ECG changes resolved. Abbreviations: ACh, acetylcholine; ECG, electrocardiogram; i.c., intracoronary; LAD, left anterior descending artery; LCA, left coronary artery. With permission from Ong et al. (Ong P, Athanasiadis A, Mahrholdt H, Borgulya G, Sechtem U, Kaski JC (2012) Increased coronary vasoconstrictor response to acetylcholine in women with chest pain and normal coronary arteriograms (cardiac syndrome X). Clin Res Cardiol 101:673–681.)

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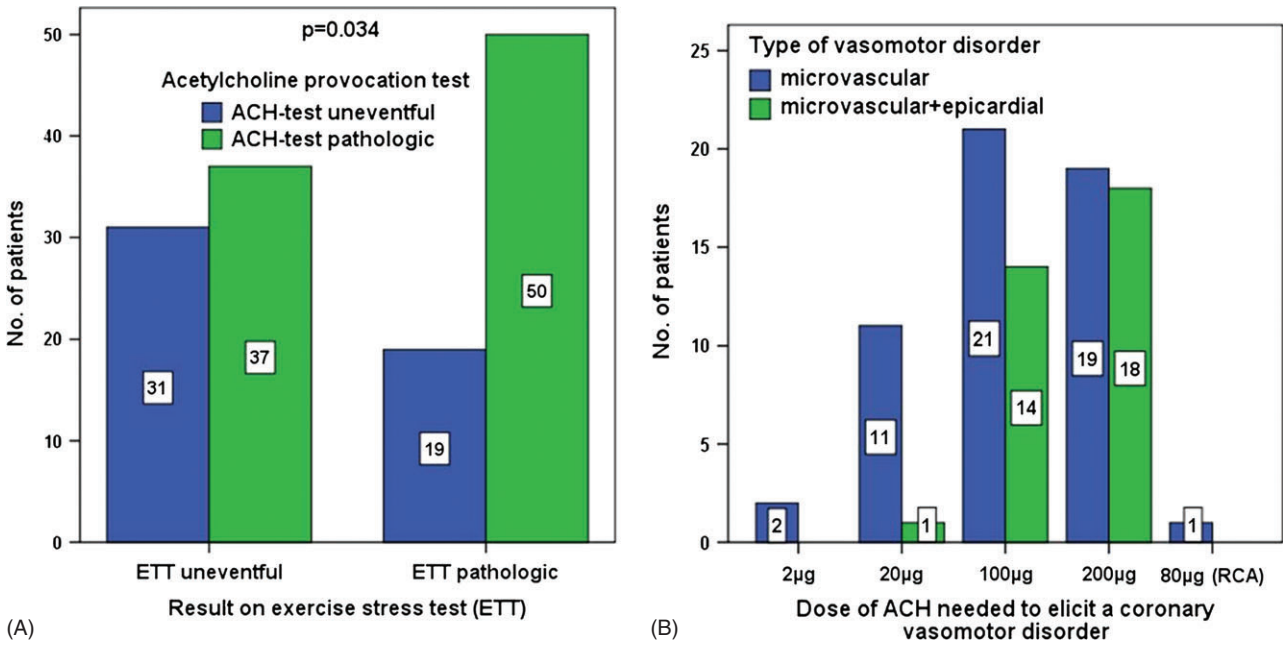

Figure 2. (A) Distribution of patients with pathologic vs uneventful ETT compared with pathologic vs uneventful ACh test, showing that patients with pathologic ETT significantly more often had a pathologic ACh test ($P = 0.034$). (B) Distribution of doses of ACh needed to elicit a coronary vasomotor disorder. Abbreviations: ACh, acetylcholine; ETT, exercise tolerance (stress) test.

Table 2. Patient Characteristics According to ACh Test Result

	Uneventful ACh Test, n = 50 (36)	Pathologic ACh Test, n = 87 (64)	*P* Value
Male sex	28 (56)	16 (18)	<0.0001
Age, y	63 ± 11	63 ± 11	0.870
LVEF, %	73 ± 8	73 ± 10	0.584
ETT abnormal	19 (38)	50 (57)	0.034
Hypertension	38 (76)	67 (77)	0.999
T2DM	12 (24)	15 (17)	0.376
Hypercholesterolemia	25 (50)	48 (55)	0.597
Current smoking	16 (32)	22 (25)	0.432
Positive family history of CVD	21 (42)	53 (61)	0.035

Abbreviations: ACh, acetylcholine; CVD, cardiovascular disease; ETT, exercise tolerance (stress) test; LVEF, left ventricular ejection fraction; SD, standard deviation; T2DM, type 2 diabetes mellitus.
Data are presented as mean ± SD or n (%).

Table 3. Multivariable Analysis[a]

Pathologic ETT vs Uneventful ETT	OR, 95% CI, *P* Value
Coronary microvascular dysfunction on intracoronary ACh testing	1.546, 1.076-1.777, 0.029

Abbreviations: ACh, acetylcholine; CI, confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; ETT, exercise tolerance (stress) test; LVEF, left ventricular ejection fraction; OR, odds ratio.
[a]The following univariate parameters were considered for the multivariable model: hypertension, DM, cigarette smoking, hypercholesterolemia, positive family history of CVD, sex, LVEF, age, ACh test result.

predictor for a pathologic ETT was a pathologic ACh test (Table 3).

The doses of ACh needed to elicit coronary microvascular dysfunction are shown in Figure 2B. Patients without distal and diffuse epicardial vasoconstriction mainly reacted at doses between 2 µg and 100 µg, whereas those with concomitant epicardial constriction reacted mostly at 100 µg and 200 µg of ACh.

Discussion

The results of our study show that a pathologic ETT in patients with unobstructed coronary arteries is significantly more often associated with ACh-induced coronary microvascular dysfunction compared to patients with an uneventful ETT. Thus, there is a substantial proportion of patients in whom the ETT may not be false-positive, but rather is an indicator of a dysfunctional microcirculation. In this setting, the ACh test may allow diagnosis of microvascular dysfunction, similarly to invasive measurements of coronary flow reserve as previously shown by other investigators.[4] Finally, coronary microvascular dysfunction may also be suspected in patients with a pathologic ETT and unobstructed coronary arteries in whom the ACh test may not be feasible, justifying a trial of treatment.

Traditionally, the clinical presentation with exertional AP, a pathologic ETT, and unobstructed coronary arteries has been called cardiac syndrome X, or microvascular angina.[5,6] Numerous studies have identified the coronary

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microcirculation as the main target in this condition, and it has been proposed that microvascular dysfunction can be due to chronic low-grade inflammation caused by various stimuli.[7,8] Mechanistically, there are structural and functional alterations present in the coronary tree and the myocardium in this condition.[9] From a functional point of view, impaired vasodilatation and/or enhanced vasoconstriction have been shown to play a pathogenetic role.[4,10] Furthermore, it has been proposed that coronary microvascular dysfunction can be patchily distributed along the microcirculation.[11] Therefore, the amount and the distribution of microvascular dysfunction may be decisive for an ETT to become pathologic. However, the exact mechanisms underlying this heterogenic condition are still incompletely understood.

Various techniques have been applied to assess the coronary microcirculation.[12,13] Panting et al showed, for example, that patients with microvascular angina had reversible perfusion defects on cardiac stress magnetic resonance imaging (MRI) with adenosine.[14] In addition, Yilmaz et al showed that perfusion defects on cardiac stress MRI are often present in patients with coronary vasomotor disorders assessed by ACh provocation testing.[15] Another noninvasive approach is assessment of coronary flow reserve via transthoracic Doppler echocardiography (TTDE). Galiuto et al[16] have shown that patients with microvascular angina frequently have impaired coronary flow reserved on TTDE. However, these noninvasive tests have limited diagnostic accuracy for identifying coronary microvascular dysfunction,[17] underpinning the need for novel more sensitive noninvasive imaging techniques aimed at visualization of the coronary microcirculation in vivo.

Clinical Implications

Patients with a pathologic exercise stress test and unobstructed coronary arteries should further be investigated for coronary microvascular dysfunction using, for example, the ACh test. Identification of patients with a coronary microvascular disorder is important, as targeted treatment with angiotensin-converting enzyme inhibitors, statins, and calcium antagonists may be able to improve symptoms[18–20] and alter prognosis.[21] Recently, it has been shown that even patients with unobstructed coronary arteries can have an elevated risk of coronary events[22] and disability pension.[23] Moreover, it has been shown that patients with microvascular dysfunction have more vulnerable plaque characteristic than those without.[24] In addition, the patients can be reassured that a cause for their symptoms has been found and further unnecessary investigations can be avoided. Finally, in patients with a pathologic ETT and unobstructed coronary arteries in whom an ACh test is not possible, a trial of treatment for coronary microvascular dysfunction may be justified.

Study Limitations

Because the coronary microcirculation cannot be visualized in vivo at present, coronary microvascular dysfunction remains a diagnosis of exclusion. In this study, we did not use coronary flow reserve measurements to document microvascular dysfunction, as previously done by other investigators.[4] This represents a limitation of this study, but this aspect is part of our ongoing work in this area. Instead, we used intracoronary ACh provocation testing with a definition for microvascular dysfunction previously proposed by our group.[1]

Conclusion

Acetylcholine-induced coronary microvascular dysfunction is frequently found in patients with exertional AP and unobstructed coronary arteries. Such a finding is significantly more often found in presence of a pathologic exercise ECG.

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