High Prevalence of a Pathological Response to Acetylcholine Testing in Patients With Stable Angina Pectoris and Unobstructed Coronary Arteries

The ACOVA Study (Abnormal Coro{}nary VA{}somotion in patients with stable angina and unobstructed coronary arteries)

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Objectives
This study aimed at determining the prevalence of epicardial and microvascular coronary spasm in patients with anginal symptoms, despite angiographically normal coronary arteries.

Background
Despite a typical clinical presentation with exercise-related anginal symptoms (chest pain or dyspnea) with or without occasional attacks of resting chest pain suggestive of coronary artery disease, 40% of patients undergoing diagnostic angiography have normal or “near” normal coronary arteriograms. Many of these patients are given a diagnosis of noncardiac chest pain, and some are considered to have microvascular angina. However, we speculate that abnormal coronary vasomotion (reduced vasodilatation with exercise = reduced coronary flow reserve and/or vasospasm at rest) might also represent a plausible explanation for the symptoms of the patient.

Methods
This was a prospective study in 304 consecutive patients (50% men, mean age 66 ± 10 years) with exertional anginal symptoms undergoing diagnostic angiography. A total of 139 patients (46%) had 50% coronary artery disease in at least 1 coronary artery, 21 patients (7%) had luminal narrowings ranging from 20% to 49%, and 144 patients (47%) had normal coronary arteries or only minimal irregularities (<20% diameter reduction).

Results
One hundred twenty-four patients of the latter (86%) underwent intracoronary acetylcholine (ACH) testing, which elicited coronary spasm in 77 patients (62%), 35 patients (45%) with epicardial spasm (≥75% diameter reduction with reproduction of the symptoms of the patient) and 42 patients (55%) with microvascular spasm (reproduction of symptoms, ischemic electrocardiographic changes, and no epicardial spasm).

Conclusions
Nearly 50% of patients undergoing diagnostic angiography for assessment of stable angina had angiographically normal or near normal coronary arteriograms. The ACH test triggered epicardial or microvascular coronary spasm in nearly two-thirds of these patients. Our results suggest that abnormal coronary vasomotion plays a pathogenic role in this setting and that the ACH test might be useful to identify patients with cardiac symptoms, despite normal coronaries. (Abnormal Coronary Vasomotion in Patients With Suspected CAD But Normal Coronary Arteries: NCT00921856) (J Am Coll Cardiol 2012;59:655–62) © 2012 by the American College of Cardiology Foundation
shown that both abnormal epicardial and abnormal microvascular vasomotor responses can lead to myocardial ischemia in patients without atherosclerotic coronary artery obstructions (7–10). It is not known, however, how often this mechanism can explain the presence of anginal symptoms in individuals referred to diagnostic angiography for the assessment of stable angina who are found to have nonobstructive CAD or completely normal coronary arteriograms. Therefore, the present study sought to assess the prevalence of epicardial and microvascular coronary spasm among patients presenting with stable anginal symptoms but who had angiographically normal coronary arteries or only minimal irregularities (<20% narrowings).

**Methods**

**Patients.** The present study, ACOVA (Abnormal COronary VAsomotion in patients with stable angina and unobstructed coronary arteries), is a prospective trial involving 376 consecutive patients undergoing diagnostic coronary angiography between December 2007 and December 2008, for the assessment of stable angina pectoris. For inclusion in the study, patients had to have exercise-related anginal symptoms suggestive of CAD (chest pain or dyspnea, with or without occasional attacks of resting chest pain) and a left ventricular ejection fraction ≥50%, as assessed by left ventriculography. Patients with acute coronary syndrome, valvular heart disease, cardiomyopathy, myocarditis, chronic obstructive pulmonary disease, chronic renal failure, or a history of dysphagia or gastroesophageal reflux were not included in the study. Furthermore, we excluded patients with a history compatible with Prinzmetal’s variant angina (i.e., purely rest angina, despite a preserved exercise capacity, and documented transient ST-segment elevation during chest pain, relieved spontaneously or by nitrates and/or angiographic evidence of focal coronary spasm). Traditional cardiovascular risk factors, including hypertension, diabetes, hypercholesterolemia, current cigarette smoking, and a positive family history for cardiovascular disease (i.e., myocardial infarction or stroke in a first-degree relative <65 years of age), were recorded in every patient.

Three hundred four patients (80%) were found to be eligible for inclusion in the study. They were divided into 3 groups according to the degree of epicardial narrowing on angiography: Group 1: 0% to 20% narrowings (n = 144), Group 2: ≥50% stenosis (n = 139), Group 3: >20% to 49% stenosis (n = 21) (Fig. 1). Our study focused on groups 1

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**Figure 1** Study Flow Chart

The flow chart visualizes the number of patients in the respective groups according to the degree of epicardial stenosis and the result of the acetylcholine (ACH) test in patients with normal or “near” normal coronary arteries. CAD = coronary artery disease.
and 2 only. Thus, we report results on 283 patients (137 men; mean age 66 ± 10 years), of whom 240 (85%) underwent exercise stress testing before angiography, which showed ischemic electrocardiographic (ECG) changes in 151 (53%) patients. The remainder underwent diagnostic coronary angiography on the basis of a high pre-test likelihood for obstructive CAD. The study complied with the Declaration of Helsinki, and the study protocol was approved by the local ethics committee. All patients gave written informed consent before study entry. Trial registry information is given after abstract.

**Study protocol.** The study flowchart is shown in Figure 1. Patients with normal coronary arteries or only minimal irregularities on baseline diagnostic angiography (Group 1) underwent standardized intracoronary acetylcholine (ACH) provocation for the assessment of coronary vasomotor function (ACH test). We have previously reported our standardized procedure for the assessment of coronary vasomotor abnormalities in our institution (4,11). As per our institutional protocol, cardiovascular medications able to affect coronary vasomotor responses (calcium channel blockers, beta blockers, long acting nitrates) were discontinued at least 24 h before coronary angiography. Sublingual glyceryl trinitrate administration, however, was permitted for the relief of chest pain at all times. None of the patients required this treatment <4 h before angiography.

Incremental doses of ACH 2, 20, 100, and 200 μg (11,12) were administered over a period of 3 min each into the left coronary artery (LCA) via the angiographic catheter. In the patients who did not develop symptoms or ischemic ECG changes, we subsequently injected ACH into the right coronary artery (RCA) (80 μg ACH over 3 min). After the administration of ACH or when chest pain and/or coronary spasm developed, 0.2 mg glyceryl trinitrate (Perrignanit, Schwarz Pharma, Monheim, Germany) were injected into both the LCA and RCA. Heart rate, blood pressure, and the 12-lead-ECG were continuously monitored during ACH testing.

**Measurements.** The angiographic images were quantitatively analyzed with QCA-CMS (version 7.0, Medis-Software, Leiden, the Netherlands). The following binary variables were obtained for analysis: reproduction of the usual chest pain of the patient, presence of coronary vasoconstriction ≥75%, and ischemic ECG changes (i.e., ST-segment depression or ST-segment elevation of ≥0.1 mV or T-wave peaking in at least 2 contiguous leads), in the absence of epicardial spasm (≥75% diameter reduction) (Figs. 2C and 2D) (5).

**Statistical analysis.** Data analysis was carried out with SPSS (version 16.0, SPSS, Inc., Chicago, Illinois). Results are expressed as mean ± SD. The 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’s exact test was used for categorical variables. After univariate analysis, multivariate logistic regression analysis was performed in a forward likelihood fashion to identify independent predictors for obstructive CAD as well as abnormal coronary vasomotion. A 2-tailed p value of <0.05 was considered statistically significant.

**Results**

**Clinical characteristics.** The clinical characteristics of all patients included in the study (i.e., type of anginal symptoms, cardiovascular risk factors, and their response to noninvasive exercise stress testing) are shown in Table 1. Univariate and multivariate analysis comparing patients with and without CAD are shown in Tables 1 and 2, respectively. Compared with patients in Group 1, those with obstructive CAD (Group 2) were more likely to be male and older. They also had a higher prevalence of hypercholesterolemia. In addition, they more often complained of purely effort-induced chest pain than effort chest pain or dyspnea with occasional attacks of chest pain at rest. Moreover, they showed ischemic ECG changes during noninvasive stress testing more often than patients in Group 1 (p < 0.01, respectively).

**ACH testing.** The ACH test was performed in 124 of the 144 patients in Group 1 (86%). In 20 patients, the test could not be performed due to logistic reasons. There were no serious complications during ACH testing (e.g., myocardial infarction, refractory spasm, sustained ventricular arrhythmias, or need for resuscitation). Transient atrioventricular block was frequently observed and occurred mostly during provocation of the RCA; it always resolved within seconds.
after reducing the speed of the ACH injection, due to the short half-life of ACH. Coronary spasm was seen in 77 patients (62%). Thirty-five (45%) patients had epicardial coronary spasm, and 42 had microvascular spasm (55%) (Fig. 1).

Among patients with epicardial spasm, 30 (86%) had diffuse and 5 (14%) had focally pronounced spasm. Epicardial spasm was usually confined to the distal segments of the coronary arteries (diffuse spasm in 25, and focal in 1 patient; 74%). Proximal segment spasm was only seen in 1 patient (focal), mid-coronary segment spasm was seen in 3 (all focal), and spasm in all segments was seen in 5 cases (all diffuse).

Fifty-two percent of the patients with microvascular spasm had <25% epicardial constriction during ACH-provocation. Intermediate constriction (25% to 50% epicardial narrowing) was seen in 36%, and the remaining 12% showed epicardial constriction between 50% and 74%.

The ACH test was negative according to our definitions in 47 patients (38%). Twenty-nine of these patients, however, reported some chest pain (n = 14), had ischemic ECG changes (n = 7), or had diffuse asymptomatic epicardial narrowing ≥75% (n = 8) during the test.

Univariate analysis revealed that, compared with patients with a “negative” ACH test (n = 47), those with evidence of abnormal coronary vasomotion (n = 77) were more likely to be female. Furthermore, they more often had effort-induced chest pain, and they also had a higher prevalence of a positive family history for cardiovascular disease, compared with patients with a “negative” ACH test (Table 3). On multivariate analysis, the same variables (female gender, effort-induced chest pain, and a positive family history) were identified as significant independent predictors for abnormal coronary vasomotion (Table 2). Because diabetes and hypertension have long been recognized to be associated with structural changes in the microvasculature, both variables were included into the multivariate analysis, although they did not reach statistical significance on univariate analysis. Moreover, the presence of both hypertension and diabetes was tested. Either alone or in combination, these variables did not reach statistical significance in the multi-
Multivariate Logistic Regression Analysis

Table 1  Clinical Characteristics in Study Patients

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>All Patients (N = 283)</th>
<th>CAD (&gt;50% Coronary Stenosis) (n = 139 [49%])</th>
<th>Unobstructed Coronary Arteries (n = 144 [51%])</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>137 (48%)</td>
<td>93 (67%)</td>
<td>44 (31%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>66 ± 10</td>
<td>69 ± 10</td>
<td>64 ± 10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Exertional chest pain pectoris</td>
<td>141 (50%)</td>
<td>97 (70%)</td>
<td>44 (31%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Exertional dyspnea with occasional chest pain pectoris at rest</td>
<td>32 (11%)</td>
<td>5 (4%)</td>
<td>27 (19%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Exertional chest pain with occasional symptoms at rest</td>
<td>110 (39%)</td>
<td>37 (27%)</td>
<td>73 (51%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Noninvasive test for ischemia performed</td>
<td>240 (85%)</td>
<td>122 (88%)</td>
<td>118 (82%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Ischemic ECG changes during noninvasive test for ischemia</td>
<td>151 (53%)</td>
<td>95 (68%)</td>
<td>56 (39%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Risk factors

- Hypertension: 214 (76%) vs. 112 (81%) vs. 102 (71%) p = 0.07
- Diabetes mellitus: 66 (23%) vs. 35 (26%) vs. 31 (22%) p = 0.49
- Hypertension and diabetes mellitus: 55 (19%) vs. 31 (22%) vs. 24 (17%) p = 0.29
- Hypercholesterolemia: 191 (67%) vs. 108 (78%) vs. 83 (58%) p < 0.01
- Smokers: 38 (13%) vs. 16 (12%) vs. 22 (15%) p = 0.39
- Positive family history for CVD: 107 (38%) vs. 47 (34%) vs. 60 (42%) p = 0.18

Values are n (%) or mean ± SD.

CAD = coronary artery disease; CVD = cardiovascular disease; ECG = electrocardiographic.

Discussion

We found a high prevalence of patients with normal or “near” normal coronary arteriograms among patients with stable angina referred for diagnostic coronary angiography, as previously reported by other investigators (1–3,14). The novel finding in our study, however, is the high prevalence of epicardial and coronary microvascular spasm in this group of patients.

Patients with typical anginal symptoms and unobstructed coronary arteries are usually considered by the treating physician to have noncardiac chest pain or are sometimes diagnosed as having microvascular angina, a condition thought to be caused by a reduced vasodilatory ability of the coronary microcirculation (6). Microvascular spasm has mainly been seen in Japanese patients where functional testing is an integrated part of invasive coronary angiography. It has been reported that microvascular spasm was less common in Caucasian cohorts (5) but functional coronary testing is not broadly applied in Europe or the United States. However, in the present prospective study in a contemporary European patient cohort referred for diagnostic coronary angiography and found to have normal or near normal coronary arteries, we observed microvascular spasm in a large proportion of our patients. The results of our study are of clinical importance, because we have shown for the first time that both epicardial (mainly distal) and microvascular spasm can be responsible for or associated with anginal symptoms and ST-segment changes in a large proportion of patients with stable angina and unobstructed coronary arteries.

The observation in our study that patients with unobstructed coronary arteries were more often women than men is in line with previous work from Bell et al. (14), who showed that, among 22,795 patients undergoing coronary angiography, 24% of men and 48% of women had unobstructed coronary arteries. Similar observations were made in the large American College of Cardiology National Cardiovascular Data Registry database of 565,504 patients (3), where the median rate of obstructive CAD (any epicardial stenosis ≥50%) was only 45%. Although one cannot necessarily apply our results to such a large population in the United States, one can still speculate about the implications for the health care system if approximately 30% to 50% of patients with anginal symptoms and unobstructed coronary arteries would have a coronary vasomotor abnormality as an explanation for their symptoms.

In both of the large studies quoted in the previous paragraph, women were more likely than men to have unobstructed coronary arteries, despite the presence of
angina symptoms. This corresponds to our finding that, among patients with unobstructed coronary arteries in our study, women were also more often found to have abnormal coronary vasomotion than men. To assess the potential reasons for this was beyond the remit of our study, but this is an issue that requires further investigation.

Our finding that a positive family history for cardiovascular disease was more often present in patients with abnormal coronary vasomotion compared with those without is in line with previous studies (15), underpinning the potential impact of the genetic background in the setting of coronary vasomotor disorders. Various genetic mutations have been described as potential explanations (16,17).

Previous studies have suggested a higher prevalence of epicardial spasm among Asian compared with Caucasian patients (18). However, recent studies on Caucasian patients have cast doubt on this discrepancy (19). The finding of our study that 28% of patients with unobstructed coronary arteries were found to have epicardial spasm is in line with data from Asian patients (20) supporting the notion of abnormal coronary vasomotion in response to ACH ranging from subtle changes via findings compatible with microvascular spasm to severe distal and diffuse epicardial spasm (8).

Microvascular spasm has been described as a pathogenic mechanism for resting chest pain in patients with normal coronary angiograms, albeit mainly Japanese (5,25). The high prevalence of microvascular spasm in the present study suggests an important role of coronary microvascular dysfunction also as a possible cause of exercise-induced angina in patients with chest pain and unobstructed coronary arteries (26). Although microvascular dysfunction cannot be visualized directly, it has been shown that abnormal coronary vasomotion in response to ACH is associated with perfusion defects on cardiac stress magnetic resonance imaging with adenosine (27). Moreover, impaired microvascular reactivity to adenosine has been shown to be predictive of major adverse outcomes among women evaluated for suspected ischemia (28), underpinning the prognostic implications of microvascular dysfunction.

That microvascular spasm was demonstrated in patients with effort-induced anginal symptoms indicates that abnormal microvascular vasomotion might be triggered by physical exercise. It is conceivable that the full spectrum of abnormal coronary vasomotion operates in these patients.
(i.e., a background of endothelial and/or medial smooth muscle dysfunction involving the whole coronary tree, which can lead to severe coronary constriction in response to a diversity of stimuli, including exercise, anxiety, and yet unidentified triggers).

Intracoronary ACH provocation testing has been reported to be a sensitive and safe test for the assessment of coronary vasomotor function in the catheterization laboratory (29). A stepwise infusion approach with incremental doses, as in the present study, ensures a very low rate of serious complications (<1%) (30), which is in the order reported for diagnostic coronary angiography (31). With such a protocol, the infusion of ACH did not result in any serious complications in more than 2,500 such tests performed at our institution since 2006.

**Clinical implications of our findings.** The high prevalence of epicardial and microvascular coronary spasm in our study suggests that functional abnormalities of the coronary arteries should regularly be considered as a differential diagnosis in patients with stable angina but angiographically unobstructed coronary arteries. Moreover, they should be suspected especially in female patients with effort-induced chest pain, a positive family history for cardiovascular disease, and coronary arteriograms with only minimal irregularities. Intracoronary provocation tests (e.g., with ACH) might represent a useful clinical tool to establish the diagnosis of a functional abnormality underlying the symptoms of the patient. Such a diagnostic algorithm might also represent a cost-effective approach, minimizing the need for repeated invasive and noninvasive tests for myocardial ischemia and CAD commonly performed in this type of patient in the attempt to establish a diagnosis, which often proves elusive (32,33).

**Study limitations.** We focused, for inclusion in the study, on patients with exercise-related anginal symptoms, but an abnormal exercise stress test was not required. This might have led to different results with regard to the prevalence of unobstructed coronary arteries and abnormal coronary vasomotion on ACH testing. However, the aim of our study was to assess a representative real-world clinical cohort of patients, and it is common practice that patients with exertional chest pain and cardiovascular risk factors undergo coronary angiography without noninvasive testing for transient myocardial ischemia (34).

For the administration of ACH, we used the same protocol as in the ENCORE II (Evaluation of Nifedipine and Cerivastatin on the Recovery of Endothelial Function) study (11), with the exception that, whereas investigators in the ENCORE II study selectively injected incremental ACH doses of 2, 20, and 100 μg into the left anterior descending and left circumflex arteries, respectively, we administered graded doses of ACH through the diagnostic catheter into the left main stem, with a maximum dose of 200 μg. This slight modification of the protocol was carried out for logistic reasons (to avoid instrumentation of the coronary vessels) and is unlikely to have resulted in major differences in the coronary responses.

Because direct visualization of the coronary microcirculation in the clinical setting is not possible at present, microvascular spasm remains a diagnosis of exclusion. However, we used an accepted definition of microvascular spasm as proposed by Mohri et al. (5). Due to the paucity of data with regard to coronary microvascular spasm, the definition by Mohri et al. (5) has not been validated in larger trials. However, according to our experience, this definition is practically useful when performing ACH provocation testing, because the response described by Mohri et al. (5) is frequently encountered. Furthermore, the fact that a pathological ACH test result is associated with perfusion defects on cardiac stress magnetic resonance imaging (27) underlines the ischemic origin of such disorders.

**Conclusions**

Nearly 50% of patients undergoing diagnostic angiography for the assessment of stable angina had angiographically unobstructed coronary arteries. Intracoronary ACH provocation triggered epicardial as well as microvascular coronary spasm leading to reproduction of the symptoms of the patient combined with ischemic ST-segment changes in nearly two-thirds of these patients. Our results suggest that intracoronary ACH provocation might be a useful clinical tool for the assessment of patients with suspected CAD who are found to have unobstructed coronary arteries.

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**REFERENCES**


Pathological ACH Response in Stable Angina

Ong et al.

Key Words: acetylcholine • coronary spasm • normal coronary arteries • stable angina.

APPENDIX

For an accompanying video, please see the online version of this article.