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Sex-Related Differences in Vasomotor Function in Patients With Angina and Unobstructed Coronary Arteries

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ABSTRACT

BACKGROUND Coronary vasomotor dysfunction is an important mechanism for angina in patients with unobstructed coronary arteries.

OBJECTIVES The purpose of this study was to determine sex differences in the prevalence and clinical presentation of vasomotor dysfunction in a European population and to examine sex differences in the dose of acetylcholine leading to a positive acetylcholine provocation test (ACH test).

METHODS Between 2007 and 2014, we included 1,379 consecutive patients with stable angina, unobstructed coronaries and ACH test performed for epicardial vasospasm or coronary microvascular dysfunction (CMD) due to microvascular spasm. The predictive value of sex, risk factors, symptoms, and noninvasive test results was analyzed by means of logistic regression.

RESULTS The mean patient age was 62 years, and 42% were male. There were 813 patients (59%) with a pathological ACH test, 33% for CMD and 26% for epicardial vasospasm. A pathological test was more common in females (70% vs. 43%; $p < 0.001$). In a multivariable logistic regression model the sex difference was statistically significant with a female-male odds ratio for CMD and epicardial vasospasm of 4.2 (95% confidence interval: 3.1 to 5.5; $p < 0.001$) and 2.3 (95% confidence interval: 1.7 to 3.1; $p < 0.001$), respectively. Effort-related symptoms, but neither risk factors nor noninvasive stress tests, contributed to predicting a pathological test. Female patients were more sensitive to acetylcholine with vasomotor dysfunction occurring at lower ACH doses compared with male patients.

CONCLUSIONS Vasomotor dysfunction is frequent in patients with angina and unobstructed coronaries in a European population. Female patients have a higher prevalence of vasomotor dysfunction (especially CMD) compared with male patients. A pathological ACH test was observed at lower ACH doses in women compared with men.
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The majority of patients with angina pectoris without obstructive coronary artery disease (CAD) might still have myocardial ischemia as the cause of their symptoms (1–4). Coronary vasomotor disorders comprising both epicardial and microvascular dysfunction have been proposed as important pathophysiological mechanisms causing symptoms of myocardial ischemia (2). Moreover, an association between stable angina pectoris with nonobstructive CAD and major adverse cardiovascular events is well-demonstrated (2,5), and it is likely that patients with vasomotor disorders carry the major part of this adverse prognosis (2,6–8).

Angina pectoris without obstructive CAD is more common in female compared with male patients (5,9–11). The importance of recognizing sex differences is enhanced by the fact that angina pectoris in female patients can present differently from the symptoms among male patients (12). This distinction may lead to an underestimation of cardiac causes of chest-related symptoms in female patients, in particular if coronary angiography (CAG) is normal,

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ABBREVIATIONS AND ACRONYMS

ACH = acetylcholine
AUC = area under the curve
CAD = coronary artery disease
CAG = coronary angiography
CMD = coronary microvascular dysfunction
ECG = electrocardiograph
EV = epicardial vasospasm

the symptoms are atypical and further investigations beyond the CAG are not performed (13). Female patients are also reported to differ in the result of noninvasive tests compared with male patients with a much greater proportion of female patients diagnosed with the now abandoned term cardiac syndrome X, a syndrome of exertional angina pectoris and no obstructive CAD (14). Conversely, some recent studies indicate that the sex difference in coronary microvascular dysfunction (CMD) is not as great as indicated in prior studies (15).

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Several studies have indicated that epicardial vasospasm (EV) and CMD according to acetylcholine (ACH) test are much more frequent in Asian than in European patients (16–18). However, we have previously shown that the prevalence in European patients might be underestimated (3). The Japanese population is well-described, and ACH tests are done routinely at many Japanese centers (19–21). In contrast, ACH tests are rarely performed in Europe and the United States. In Japanese studies, coronary vasomotor disorders are a frequent finding among patients with angina pectoris without obstructive CAD (21,22). In Japan, CMD seems to be more frequent in female patients, and EV more frequent in male patients (6,17,22). Furthermore, vasomotor dysfunction is correlated with a prior history of obstructive CAD and, in most studies, patients with CAD have been part of the study population (3,16,19,22,23).

We sought to determine prevalence of epicardial and microvascular vasomotor dysfunction in a large European population with neither prior history nor present evidence of obstructive epicardial CAD (no epicardial stenosis >50%) or other cardiac reasons for their symptoms. Furthermore, we sought to compare the prevalence of vasomotor dysfunction in male and female patients and whether sex differences were related to the clinical presentation, presence of cardiovascular risk factors, or results of noninvasive stress tests. In addition, we investigated whether there was a sex difference in the dose needed to achieve a pathological ACH test.

METHODS

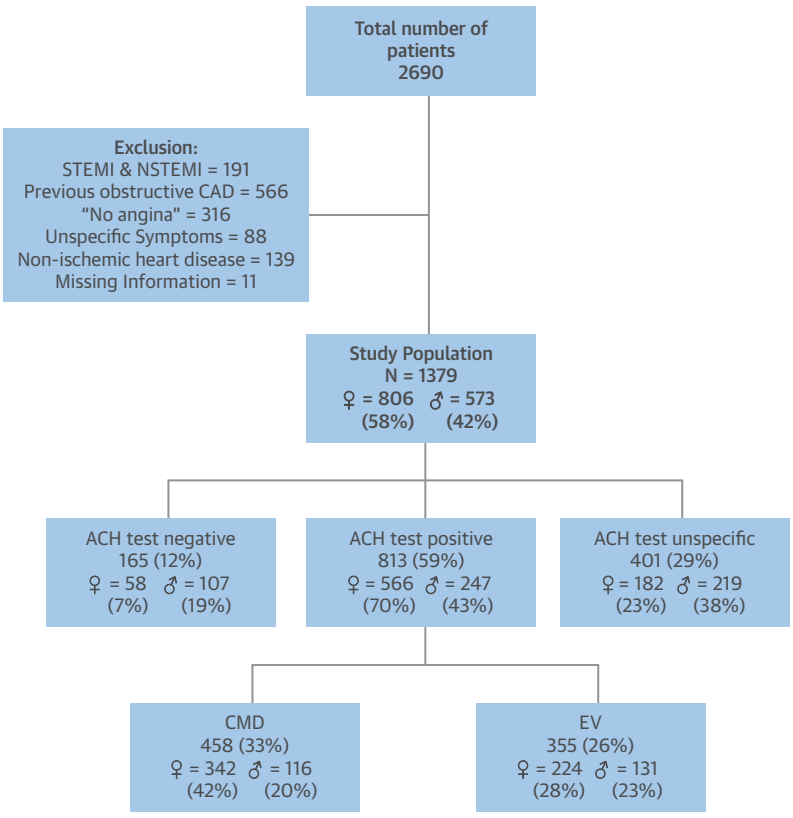
STUDY POPULATION. This retrospective study included consecutive patients with angina pectoris undergoing an ACH test at Robert-Bosch-Krankenhaus in Stuttgart, Germany, from September 2007 to December 2014 (**Figure 1**). Patients undergoing CAG for suspected or progression of known CAD who were found to have unobstructed coronary arteries (no epicardial stenosis >50% by visual assessment) underwent an ACH test. The test was not performed in patients with severe chronic obstructive pulmonary disease or impaired renal function. Patients undergoing an ACH test between 2007 and June 2010 were also included in our prior study (3).

A total of 2,690 ACH tests were performed in the study period. To ensure a homogeneous population, 191 patients with non-ST-segment elevation myocardial infarction and ST- segment elevation myocardial infarction were excluded. There were 566 patients with a prior history of obstructive CAD and 404 patients with other symptoms (n = 316) such as palpitations, syncope, or another reason (n = 88) leading to the invasive assessment. All these patients were excluded from the final population, as were 139 patients with nonischemic cardiac disease such as cardiomyopathies or severe valvular disorders. Furthermore, another 11 patients were excluded due to a missing electrocardiograph (ECG) or a lack of description of the symptoms during the ACH test. Our final population thus consisted of 1,379 patients with either angina pectoris or unexplained dyspnea considered to be an angina equivalent (**Figure 1**).

CLINICAL DATA. The previous medical history was derived from patient charts and included the classical risk factors hypertension, family history of cardiovascular disease, diabetes mellitus, hypercholesterolemia, and a history of smoking. A history of smoking was considered positive if the patient had a history of habitual smoking of at least 5 years in the past or was an active smoker. Patients on statin treatment or with a clinical indication for statin treatment based on serum lipids at admission were defined as having hypercholesterolemia. Family history was considered positive if at least 1 first- or second-degree relative had a cardiovascular event at a margin of <10 years from the age of the patient. If a noninvasive stress test was performed before the ACH test, the result was recorded. Furthermore, it was recorded whether the patient had symptoms at rest, during exercise, or a combination of resting and effort-related symptoms. Dyspnea, which could not be explained by known lung disease, was considered as an angina equivalent.

ACH TESTING. The ACH test was performed immediately after the CAG if no coronary artery stenosis of

FIGURE 1 Study Flow Chart



The study cohort. The percent in the study population represents the distribution between male and female patients. ACH = acetylcholine; CAD = coronary artery disease; CMD = coronary microvascular dysfunction; EV = epicardial vasospasm; NSTEMI = non-ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction.

50% or greater was found as described previously (3). Incremental doses of 2, 20, 100, and 200 μg of ACH were infused over a period of 3 min each into the left coronary artery via the angiography catheter. In patients who remained asymptomatic and showed no diagnostic ST-segment changes during the assessment of the left coronary artery, 80 μg of ACH was injected into the right coronary artery. The waiting time before injecting the next higher dosage was approximately 1 min. A coronary angiogram was performed after each dose. The effect of ACH was reverted by administration of glyceroltrinitrate 0.2 mg in the respective coronary artery.

The ACH test was evaluated according to the diagnostic criteria for EV defined by the COVADIS (Coronary Vasomotion Disorders International Study Group) (24) with one exception. We considered the test positive for EV, if a diffuse or focal vasoconstriction of an epicardial vessel of 75% or greater was obtained compared with 90% proposed by COVADIS. Thus, the ACH test was regarded as positive for EV when meeting all 3 criteria namely: 1) reproduction of the usual symptoms; 2) ischemic ECG changes; and 3) more than 75% vasoconstriction on angiography by visual assessment. The dose of ACH leading to EV was registered. Ischemic ECG changes were defined as significant (≥1 mm horizontal or downsloping) ST-segment depression or ST=segment elevation or pathological T-wave inversions. If no significant epicardial spasm (>75%) was produced, but a reproduction of the usual symptoms was achieved together with ischemic ECG changes, the test was interpreted as CMD due to microvascular spasm. The dosage of ACH leading to microvascular spasm was registered. Any other reaction was considered as an unspecific reaction. The test was classified as negative if all of the 3 criteria were negative (i.e., no vasospasm,

TABLE 1 Patient Characteristics and Findings in ACH Test

	All Patients (N = 1,379)	Female (n = 806, 58.4%)	Male (n = 573, 41.6%)	p Value
Age, yrs	61.87 ± 11.9	63.9 ± 11.1	59.0 ± 11.6	<0.001
Hypertension	970 (70.3)	599 (74.3)	371 (64.8)	<0.001
Smoking	502 (36.4)	211 (26.2)	291 (50.8)	<0.001
Family history of CVD	392 (28.4)	254 (31.5)	138 (24.1)	0.003
Diabetes	237 (17.2)	135 (16.8)	102 (17.8)	0.61
Dyslipidemia	841 (61.0)	485 (60.2)	356 (62.1)	0.46
Symptoms				
Rest	486 (35.2)	272 (33.8)	214 (37.4)	0.168
Effort	395 (28.6)	228 (28.3)	167 (29.1)	0.729
Both rest and effort	396 (28.7)	244 (30.3)	152 (26.5)	0.130
Dyspnea	102 (7.4)	62 (7.7)	40 (7.0)	0.619
Duration of symptoms, days	101 [337]	101 [448]	101 [344]	0.07
Response to ACH test				<0.001
EV	355 (25.7)	224 (27.8)	131 (22.9)	
CMD	458 (33.2)	342 (42.4)	116 (20.2)	
Unspecific reaction	401 (29.1)	182 (22.6)	219 (38.2)	
Negative	165 (12.0)	58 (7.2)	107 (18.7)	
Noninvasive stress test	763 (55.0)	434 (53.9)	329 (57.4)	0.189
Positive tests	210 (27.5)	132 (30.4)	78 (23.7)	

Values are mean ± SD, n (%), or median [interquartile range]. The p values are given for the difference between female and male patients.

ACH = acetylcholine; AP = angina pectoris; CVD = cardiovascular disease; EV = epicardial vasospasm; CMD = coronary microvascular dysfunction.

no symptoms, and no ECG changes). Any complication occurring during the ACH was registered.

STATISTICAL ANALYSIS. Data analysis was performed using Stata/IC version 14.0 (Stata Corp., College Station, Texas). Continuous variables were compared by using 2-sample *t*-test when normally distributed and 2-sample Wilcoxon rank-sum for not normally distributed data. The chi-square test was used for categorical variables. Simple logistic regression with CMD and EV as outcomes was performed for sex, age, symptom characteristics, results from noninvasive testing, dosage of ACH, and cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, smoking, and family history), investigating the association of each of these factors with a pathological ACH test. When performing logistic regression with CMD as the outcome, patients with EV were excluded and vice versa. Factorial interaction between sex and some of the predictors of both CMD and EV were examined in a multivariable logistic regression model adjusted for age. A p value of <0.05 was considered significant. From a logistic regression model with age, sex, type of symptoms, and result of noninvasive tests as predictors, receiver operating characteristic curves were constructed for both EV and CMD to see whether these factors could predict the result of ACH test. The area under the curve (AUC) was calculated for CMD and EV. First, we created receiver operating characteristic curves for the entire population and then repeated the process for male and female sex individually.

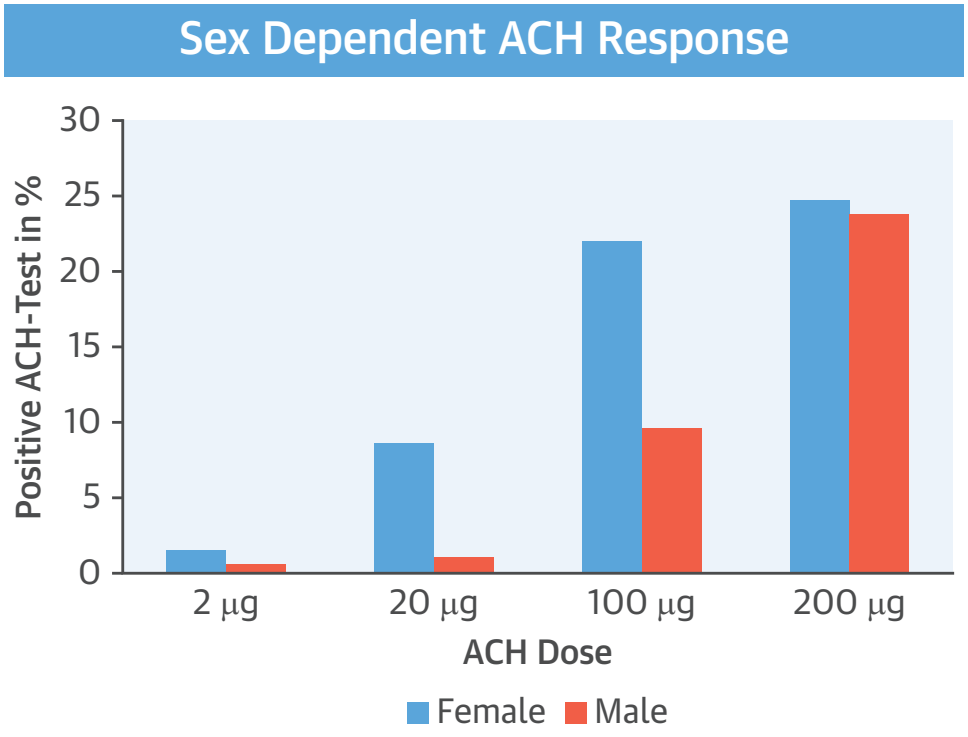
RESULTS

PATIENT CHARACTERISTICS. A total of 806 female (58%) and 573 male (42%) patients were included. Female patients were significantly older, had higher prevalence of hypertension, and a greater proportion had a positive family history, whereas a larger proportion of the men were smokers. The 2 groups did not differ significantly in other risk factors, indication for the ACH test, duration of symptoms, or symptom characteristics (Table 1). After adjusting for age difference, the occurrence of hypertension was no longer significant.

ACH TEST. Overall, the ACH test was positive in 813 patients (59%) and conclusively negative in 165 (12%). There were 401 (29%) patients with an unspecific reaction. A larger proportion of female than male patients had coronary vasomotor dysfunction, 70.2% versus 43.1% ($p < 0.001$). This sex difference was greater for CMD with female patients having CMD more than twice as often as male patients (42.4% vs. 20.2%; $p < 0.001$). Furthermore, there was a significant sex difference in the ACH dose leading to a positive ACH test (Central Illustration). Female patients had a significantly higher grade of coronary vasomotor dysfunction at lower doses of ACH compared with male patients. The difference was statistically significant for all doses <200 μg except 20 μg, indicating that women are more sensitive to ACH and have a lower threshold for EV as well as CMD.

EPICARDIAL VASOSPASM. In a logistic regression with EV as the outcome, none of the classical risk factors were associated with EV. Female sex was significantly associated with EV with an age-adjusted female-male odds ratio (OR) of 2.3 (95% confidence interval [CI]: 1.7 to 3.1; $p < 0.001$). Thus, EV was not significantly associated with age, smoking, family history, diabetes, or hypertension. Patients with EV had a higher prevalence of angina during effort or a mix of effort and resting symptoms compared with resting symptoms only or predominantly resting symptoms. The finding was just only statistically significant for effort symptoms with an OR of 1.4 (95% CI: 1.0 to 2.0; $p = 0.046$). The OR for a mix of symptoms was 1.6 (95% CI: 1.1 to 2.2; $p = 0.008$). Both findings remained statistically significant in a multivariable logistic regression model

CENTRAL ILLUSTRATION **Sex-Dependent ACH Response**



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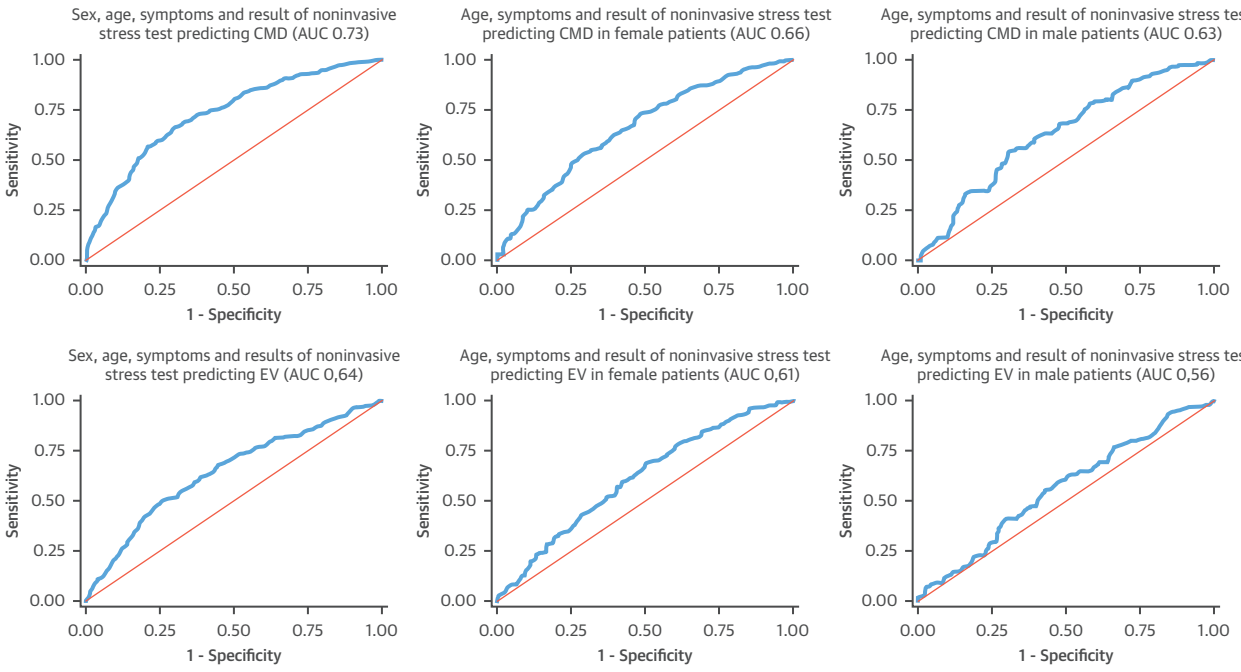
Dose of acetylcholine (ACH) needed to elicit a positive ACH test result in relation to sex. Female patients are more sensitive to ACH, achieving a positive ACH test at lower doses of ACH compared with male patients. The difference was statistically significant for all doses between 200 and 20 µg.

TABLE 2 **Predictors of Epicardial Vasospasm and Coronary Microvascular Dysfunction in 1,379 Consecutive Patients With Angina With Nonobstructive CAD**

	Epicardial Vasospasm						Coronary Microvascular Dysfunction					
	Univariable Analysis			Multivariable Analysis			Univariable Analysis			Multivariable Analysis		
	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value	OR	95% CI	p Value
Female	2.3	1.7–3.1	<0.001	2.3	1.7–3.1	<0.001	4.3	3.3–5.7	<0.001	4.2	3.1–5.5	<0.001
Age, yrs	1.0	0.99–1.00	0.087	1.0	0.99–1.00	0.625	0.9	0.99–1.00	0.053	1.0	0.9–1.0	0.028
Smoking	0.9	0.7–1.2	0.368	1.0	0.5–1.3	0.788	0.6	0.4–0.8	<0.001	0.7	0.6–1.1	0.055
Hypercholesterolemia	1.3	1.0–1.7	0.095	1.3	0.8–1.9	0.070	1.0	0.8–1.3	0.911	1.1	0.8–1.4	0.576
Hypertension	0.9	0.7–1.2	0.584	0.9	0.5–1.3	0.464	0.9	0.7–1.2	0.526	0.8	0.6–1.1	0.111
Family history of CVD	1.2	0.9–1.6	0.215	1.1	0.6–1.4	0.588	1.1	0.9–1.5	0.401			
Diabetes	0.7	0.5–1.0	0.089	0.9	0.6–1.7	0.115	0.8	0.6–1.1	0.261	0.9	0.6–1.3	0.618
Symptoms												
Rest		Ref.			Ref.			Ref.			Ref.	
Effort	1.4	1.0–2.0	0.046	1.4	1.0–2.0	0.047	2.2	1.6–3.1	<0.001	2.2	1.6–3.1	<0.001
Both rest and effort	1.6	1.1–2.2	0.008	1.6	1.1–2.3	0.006	2.0	1.4–2.8	<0.001	2.0	1.4–2.7	<0.001
Dyspnea	0.9	0.5–1.6	0.760	0.9	0.5–1.6	0.802	1.1	0.6–1.8	0.819	1.1	0.6–1.9	<0.001
Duration of symptoms, days	1.0	0.99–1.00	0.766				1.0	0.99–1.00	0.094			
Result of noninvasive stress test	1.1	0.7–1.7	0.666	1.1	0.7–1.7	0.676	1.5	1.0–2.3	0.043	1.5	1.0–2.1	0.044

CAD = coronary artery disease; CI = confidence interval; OR = odds ratio; other abbreviations as in Table 1.

FIGURE 2 ROC Curves for Prediction of EV and CMD



Receiver operating characteristics (ROC) curves with area under the ROC curve (AUC) values indicating modest ability of age, sex, symptoms, and result of noninvasive stress test in predicting EV and coronary microvascular dysfunction in patients with angina pectoris with nonobstructive CAD. Abbreviations as in Figure 1.

with all the prior mentioned risk factors as predictors.

CORONARY MICROVASCULAR DYSFUNCTION. A logistic regression with CMD as the outcome found that the occurrence of CMD was not associated with family history, age, diabetes, hypertension, or smoking (Table 2). The age-adjusted female-male OR was 4.3 (95% CI: 3.1 to 5.5; $p < 0.001$). Like in EV, patients with effort-related symptoms and a mix of effort and resting symptoms were more likely to have CMD compared with patients with symptoms only during rest or predominantly during rest. The OR for effort related symptoms was 2.2 (95% CI: 1.6 to 3.1; $p < 0.001$) and for mix of effort-related and resting symptoms, the OR was 2.0 (95% CI: 1.4 to 2.8; $p < 0.001$). Repeated analyses for both sex individually revealed similar results.

NONINVASIVE TESTS. A total of 763 patients (55%) had undergone a noninvasive stress test before CAG. Noninvasive stress tests consisted of single-photon emission computed tomography with pharmacologic (adenosine or dobutamine) stress, cardiac stress-magnetic resonance imaging, exercise stress echocardiography, and exercise ergometry. Of the 763 tests, 210 (27.5%) were abnormal. There were no sex differences in the indication for or result of the stress tests. There was no association between an abnormal noninvasive stress test and an overall positive ACH test, for either CMD or EV. Given that both EV and CMD were correlated to effort-related symptoms, we analyzed the AUC with sex, age, symptoms, and result of noninvasive stress test as predictors of a positive ACH test. The predictive abilities of the model were poor to moderate: the AUC for CMD was 0.73 and for EV the AUC was 0.64 (Figure 2). The predictive ability was primarily driven by the sex difference. Analyzing AUC for both EV and CMD separately for male and female patients reduced the ability of the model to predict a positive test (Figure 2). For female patients, the AUC for CMD was 0.66 and for male patients it was 0.63. For EV, the AUC for female patients was 0.61 and for male patients it was 0.56.

COMPLICATIONS DURING ACH TESTING. Forty-nine patients (0.04%) experienced complications during the ACH test. None of them were fatal. One patient had ventricular tachycardia during intubation of the

left coronary artery before application of ACH and required cardioversion. One patient had a catheter-induced spasm in the proximal right coronary artery, which was resolved after removing the catheter. One patient had fast paroxysmal atrial fibrillation that resolved spontaneously after discontinuing the ACH injection. The observation of short phases of atrial fibrillation during ACH testing is not uncommon, but was not registered as a complication in this study. Three patients had symptomatic hypotension, which was also resolved by discontinuing the injection. The remaining 44 patients experienced symptomatic bradycardia. In 2 of them, intravenous injection of atropine was necessary to stabilize the heart rhythm. Otherwise, no other action than discontinuing the ACH injection was needed.

DISCUSSION

This is the largest European study looking at sex differences in coronary vasomotor dysfunction comprising EV and CMD in symptomatic patients with no present or prior history of obstructive CAD. The study confirms that vasomotor dysfunction is a common cause of angina in European men and women (3). The main new findings are that EV and in particular CMD are more prevalent in women and that women are more sensitive to ACH. Importantly, as shown in some earlier studies, the presence of EV and CMD cannot be predicted from noninvasive testing (25,26). The reasons for this finding are multiple and are most likely related to: 1) different clinical presentations of our patients (effort vs. rest symptoms); 2) different underlying mechanisms such as enhanced vasoconstriction versus impaired vasodilation; and 3) different sensitivity and specificity for the reported noninvasive stress tests to detect myocardial ischemia.

In the present study, we could not confirm our previous finding of resting angina to be a predictor of EV (3); in contrast, patients with EV as well as CMD seem to have symptoms during effort more frequently than during rest. One explication for this finding may be that our patients with EV did not have focal spasm with concomitant ST-segment elevation (i.e., Prinzmetal's variant angina). In contrast, most of our patients with EV had distal and diffuse spasm. It is conceivable that a significant proportion of these patients suffered from a microvascular disorder involving the distal epicardial segments (25). Such patients more often complain of exertional symptoms rather than pure resting angina. The fact that EV in our study was more frequent in women compared with men is in line with previous studies showing an increased rate of diffuse spasm in women compared with men (20,22,25).

In our study, 70% of female patients had a pathological ACH test response, indicating the presence of cardiac causes for their symptoms even though no obstructive CAD could be revealed. In comparison, only 43% of male patients had a positive ACH test. CMD was found more than twice as often in female compared with male patients. A similar sex distribution was seen in prior studies, especially regarding CMD even though CMD was defined differently in most of these studies (12,15,19,22,27).

Coma-Canella et al. (28) published a prior European study regarding angina and provocation testing in 2006. In this Spanish study of 162 stable angina patients, EV was found in 52.5% of the patients compared with 25% in the present study (28). They used ergonovine as the provocation agent, which is known to induce coronary spasms less frequently than ACH (29). The high prevalence of EV in their study is due to a more lenient definition of a positive provocation test with induction of EV as sole criteria regardless of ECG changes or patient symptoms. They defined EV as complete or subtotal occlusion of one or more segments of the coronary artery, if a plaque was presented. If the coronary artery was angiographically normal a 50% segmental reduction of the lumen was considered significant. Like in our study, Coma-Conella et al did not find any correlation between results of noninvasive stress testing and ergonovine provocation test. Their population was comparable to ours except a higher prevalence of patients with diabetes and lower prevalence of hypertension. As in the present study, male patients had a higher prevalence of smoking compared with female patients. In their study EV was much more common in male patients compared with female (61% vs. 34%) and smoking was found to be an individual risk factor for EV. Whether the study population included patients with prior history of CAD is not described.

In 2013 Sato et al. published a retrospective study with a Japanese population of 1,877 patients with angina pectoris without obstructive CAD (22). Their population was comparable to ours according to most risk factors but they did not exclude patients with prior history of CAD. In their study, the maximum dose of ACH was 100 μg in the left and 50 μg in right coronary artery. They found a pathological ACH response in 53% of the patients. Male patients had a much higher prevalence of EV and female patients a much higher prevalence of diffuse vasoconstriction defined as a lumen reduction between 76% to 90% together with ischemic ECG changes. Symptoms

during the test were not described as a part of the diagnostic criteria. Even though the prevalence of a pathological ACH test is comparable to our study, the definition of a positive test and dose of ACH used differ in the 2 studies. In 2015 Di Fiore et al. published data from 183 Australian patients undergoing ACH provocation test (30). They reported the prevalence of EV of 44% and CMD of 32%. The Australian population differed from our study population in several ways. A total of 77% of the patients were female, they were much younger with a mean age of 54 years, and 51% of the patients had prior history of IHD. A total of 82% of the patients were Caucasian. The ACH doses for the provocation test were lower than in the present study and they did not describe any significant difference between male and female patients in the outcome of the ACH test. Recently, Sueda et al. published the results of 461 Japanese patients undergoing provocation testing with both ACH and ergonovine (31). They also described a much higher rate of EV among male (54.4%) compared with female patients (35.5%), but women had a higher rate of diffuse vasoconstriction as described earlier. In this study, resting angina was significantly associated with EV, while unspecific symptoms were associated with diffuse vasoconstriction.

Common for all studies presented is that they have a much higher prevalence of EV among men compared with women. This difference compared with the present study might be explained by exclusion of patients with prior history of CAD in our study. Male patients have a higher incidence of CAD and it has been shown that patients with prior history of CAD have a higher tendency to EV (3,22,23,30,32). Another explanation may be that different definitions for the diagnosis of EV were applied. Indeed, as shown previously by our group, women suffering from CMD often develop distal and diffuse epicardial spasm at higher doses of ACH (25). Thus, it may be possible that a substantial number of women in the present study were classified as EV even though CMD was the leading pathologic finding. In addition, our finding that a positive family history was more often found in women compared with men points towards a different genetic background among the sexes. This, however, needs to be examined in future studies.

The sex differences observed in the present paper might have several reasons. Prior studies have shown a greater burden of microvascular dysfunction among women (8,11–13,33,34). Several mechanisms have been proposed for this observation including smaller body size and myocardial mass (11). The coronary arteries of women have a smaller diameter, thinner walls and more winding course (35,36). Some studies suggest a higher grade of diastolic dysfunction in women as a possible reason (12), which may however be an observation of reversed causality (37). We believe that various factors contribute to a difference in coronary remodeling. Body size and size of the coronary arteries may play an important role, but hormonal factors should also be considered (34).

In our study, we did not find an association between symptoms at rest and EV. The finding might give rise to a discussion of the indications for performing provocative coronary artery spasm testing defined by COVADIS, where resting angina is classified as class I criteria (24). Our study shows that provocative testing might also be indicated in patients with CMD and thus in patients with exercise related symptoms. The lack of association between pathological findings in ACH testing and positive stress testing also questions the relevance of requiring a positive stress test for diagnosing ischemic heart disease with no obstructive CAD, as recently proposed in a paper regarding a Western European population (38). The findings of this paper indicate that functional provocative testing is indicated in all patients in whom a cardiac source of chest symptoms is suspected yet no coronary artery stenosis is found.

This study illustrates the difficulties in diagnosing and predicting vasomotor dysfunction in patients with angina. We found no correlation between cardiovascular risk factors or results of noninvasive stress testing and vasomotor dysfunction. The poor to moderate AUC confirmed that neither the risk factors nor clinical manifestations could help detecting the patients with a positive ACH test. The association between classical risk factors and ACH test has been discussed in several other studies with somewhat inconsistent findings (4,39–41). The low rate of complications is comparable to prior studies (3,19,27,42,43) and even comparable to reported complication rates for diagnostic CAG in other studies (44,45).

STUDY LIMITATIONS. Our study is a single-center study. Not all ACH tests were done early in the morning, as proposed by other protocols. This might have lowered the incidence of vasospasm. Angiographies read by clinicians were not blinded to the patient condition, symptoms, ECG-changes, etc. It was difficult to compare findings with previous studies as the definition of EV and CMD and the ACH dose applied varied among the studies. Furthermore, our definition of CMD was not based on coronary flow reserve measurements as done by other investigators but rather on our definition of microvascular spasm during ACH testing. Although the latter has not yet

been broadly accepted as a form of CMD it has been recently suggested (46). In addition, the strength of our study is a homogeneous patient population with consecutive inclusion and meticulous exclusion of patients with a prior history of CAD and other cardiac disease.

CONCLUSIONS

This is the largest European study in symptomatic patients with no present or prior history of obstructive CAD. It confirms that an abnormal response to ACH testing is common and shows that EV and especially CMD occur more often in female compared with male patients. Moreover, women are more sensitive to ACH leading to abnormal test results at lower doses. Neither risk factors nor results from noninvasive testing can detect patients with a positive ACH test. The ACH test should be considered in all patients with angina pectoris and unobstructed coronary arteries.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Epicardial vasospasm and coronary microvascular dysfunction are common in patients who have angina pectoris without coronary artery obstruction.

TRANSLATIONAL OUTLOOK: Better methods are needed to identify and measure coronary microvascular dysfunction.

REFERENCES

1. Schoenenberger AW, Felber S, Gujer S, et al. Invasive findings in patients with angina equivalent symptoms but no coronary artery disease; results from the heart quest cohort study. Int J Cardiol 2013;167:168–73.

2. Pepine CJ, Anderson RD, Sharaf BL, et al. Coronary microvascular reactivity to adenosine predicts adverse outcome in women evaluated for suspected ischemia results from the National Heart, Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. J Am Coll Cardiol 2010;55:2825–32.

3. Ong P, Athanasiadis A, Borgulya G, et al. Clinical usefulness, angiographic characteristics, and safety evaluation of intracoronary acetylcholine provocation testing among 921 consecutive white patients with unobstructed coronary arteries. Circulation 2014;129:1723–30.

4. Patel MB, Bui LP, Kirkeeide RL, Gould KL. Imaging microvascular dysfunction and mechanisms for female-male differences in CAD. J Am Coll Cardiol Img 2016;9:465–82.

5. Jespersen L, Hvelplund A, Abildstrom SZ, et al. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. Eur Heart J 2012;33:734–44.

6. Hoshino M, Yonetsu T, Mizukami A, et al. Moderate vasomotor response to acetylcholine provocation test as an indicator of long-term prognosis. Heart Vessels 2016;31:1943–9.

7. Cortigiani L, Rigo F, Gherardi S, et al. Coronary flow reserve during dipyridamole stress echocardiography predicts mortality. J Am Coll Cardiol Img 2012;5:1079–85.

8. Murthy VL, Naya M, Taqueti VR, et al. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. Circulation 2014;129:2518–27.

9. Kobayashi Y, Fearon WF, Honda Y, et al. Effect of sex differences on invasive measures of coronary microvascular dysfunction in patients with angina in the absence of obstructive coronary artery disease. J Am Coll Cardiol Intv 2015;8:1433–41.

10. Bugiardini R, Bairey Merz CN. Angina with "normal" coronary arteries: a changing philosophy. JAMA 2005;293:477–84.

11. Paul TK, Sivanesan K, Schulman-Marcus J. Sex differences in nonobstructive coronary artery disease: recent insights and substantial knowledge gaps. Trends Cardiovasc Med 2017;27:173–9.

12. Crea F, Battipaglia I, Andreotti F. Sex differences in mechanisms, presentation and management of ischaemic heart disease. Atherosclerosis 2015;241:157–68.

13. Shaw LJ, Bairey Merz CN, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. J Am Coll Cardiol 2006;47 Suppl:S4–20.

14. Kaski JC. Overview of gender aspects of cardiac syndrome X. Cardiovasc Res 2002;53:620–6.

15. Sara JD, Widmer RJ, Matsuzawa Y, Lennon RJ, Lerman LO, Lerman A. Prevalence of coronary microvascular dysfunction among patients with chest pain and nonobstructive coronary artery disease. J Am Coll Cardiol Intv 2015;8:1445–53.

16. Bertrand ME, LaBlanche JM, Tilmant PY, et al. Frequency of provoked coronary arterial spasm in 1089 consecutive patients undergoing coronary arteriography. Circulation 1982;65:1299–306.

17. Sueda S, Ochi N, Kawada H, et al. Frequency of provoked coronary vasospasm in patients undergoing coronary arteriography with spasm provocation test of acetylcholine. Am J Cardiol 1999;83:1186–90.

18. Wakabayashi K, Suzuki H, Honda Y, et al. Provoked coronary spasm predicts adverse outcome in patients with acute myocardial infarction: a novel predictor of prognosis after acute myocardial infarction. J Am Coll Cardiol 2008;52:518–22.

19. Takagi Y, Yasuda S, Takahashi J, et al. Clinical implications of provocation tests for coronary artery spasm: safety, arrhythmic complications, and prognostic impact: multicentre registry study of the Japanese Coronary Spasm Association. Eur Heart J 2013;34:258–67.

20. Sueda S, Kohno H, Fukuda H, et al. Clinical impact of selective spasm provocation tests: comparisons between acetylcholine and ergonovine in 1508 examinations. Coron Artery Dis 2004;15:491–7.

21. Sueda S, Kohno H, Ochi T, Uraoka T. Overview of the acetylcholine spasm provocation test. Clin Cardiol 2015;38:430–8.

22. Sato K, Kaikita K, Nakayama N, et al. Coronary vasomotor response to intracoronary acetylcholine injection, clinical features, and long-term prognosis in 873 consecutive patients with coronary spasm: analysis of a single-center study over 20 years. J Am Heart Assoc 2013;2:e000227.

23. Nobuyoshi M, Abe M, Nosaka H, et al. Statistical analysis of clinical risk factors for coronary artery spasm: identification of the most important determinant. Am Heart J 1992;124:32–8.

24. Beltrame JF, Crea F, Kaski JC, et al. International standardization of diagnostic criteria for vasospastic angina. Eur Heart J 2017;38:2565–8.

25. Ong P, Athanasiadis A, Mahrholdt H, Borgulya G, Sechtem U, Kaski JC. Increased coronary vasoconstrictor response to acetylcholine in women with chest pain and normal coronary arteriograms (cardiac syndrome X). Clin Res Cardiol 2012;101:673–81.

26. Cassar A, Chareonthaitawee P, Rihal CS, et al. Lack of correlation between noninvasive stress tests and invasive coronary vasomotor dysfunction in patients with nonobstructive coronary artery disease. Circ Cardiovasc Interv 2009;2:237–44.

27. Wei J, Mehta PK, Johnson BD, et al. Safety of coronary reactivity testing in women with no obstructive coronary artery disease: results from the NHLBI-sponsored WISE (Women's Ischemia Syndrome Evaluation) study. J Am Coll Cardiol Intv 2012;5:646–53.

28. Coma-Canella I, Castano S, Macias A, Calabuig J, Artaiz M. Ergonovine test in angina with normal coronary arteries. Is it worth doing it? Int J Cardiol 2006;107:200–6.

29. Goto A, Ito S, Kondo H, et al. Evaluation of adjunctive intracoronary administration of acetylcholine following intravenous infusion of ergonovine to provoke coronary artery spasm. J Cardiol 1999;34:309–16.

30. Di Fiore DP, Zeitz CJ, Arstall MA, Rajendran S, Sheikh AR, Beltrame JF. Clinical determinants of acetylcholine-induced coronary artery spasm in Australian patients. Int J Cardiol 2015;193:59–61.

31. Sueda S, Miyoshi T, Sasaki Y, Sakaue T, Habara H, Kohno H. Gender differences in sensitivity of acetylcholine and ergonovine to coronary spasm provocation test. Heart Vessels 2016;31:322–9.

32. Pepine CJ. Provoked coronary spasm and acute coronary syndromes. J Am Coll Cardiol 2008;52:528–30.

33. Humphries KH, Izadnegahdar M, Sedlak T, et al. Sex differences in cardiovascular disease - impact on care and outcomes. Front Neuroendocrinol 2017;46:46–70.

34. Ong P, Athanasiadis A, Sechtem U. Gender aspects in patients with angina and unobstructed coronary arteries. Clin Res Cardiol Suppl 2013;8:25–31.

35. Kitzman DW, Scholz DG, Hagen PT, Ilstrup DM, Edwards WD. Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity): a quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. Mayo Clin Proc 1988;63:137–46.

36. Paulsen S, Vetner M, Hagerup LM. Relationship between heart weight and the cross sectional area of the coronary ostia. Acta Pathol Microbiol Scand A Pathol 1975;83:429–32.

37. Crea F, Bairey Merz CN, Beltrame JF, et al. The parallel tales of microvascular angina and heart failure with preserved ejection fraction: a paradigm shift. Eur Heart J 2017;38:473–7.

38. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and no obstructive coronary artery disease (INOCA): developing evidence-based therapies and research agenda for the next decade. Circulation 2017;135:1075–92.

39. Mygind ND, Michelsen MM, Pena A, et al. Coronary Microvascular function and cardiovascular risk factors in women with angina pectoris and no obstructive coronary artery disease: the iPOWER study. J Am Heart Assoc 2016;5:e003064.

40. Teragawa H, Mitsuba N, Ishibashi K, Nishioka K, Kurisu S, Kihara Y. Evaluation of coronary microvascular function in patients with vasospastic angina. World J Cardiol 2013;5:1–7.

41. Wessel TR, Arant CB, McGorray SP, et al. Coronary microvascular reactivity is only partially predicted by atherosclerosis risk factors or coronary artery disease in women evaluated for suspected ischemia: results from the NHLBI Women's Ischemia Syndrome Evaluation (WISE). Clin Cardiol 2007;30:69–74.

42. Sueda S, Kohno H, Miyoshi T, Sakaue T, Sasaki Y, Habara H. Maximal acetylcholine dose of 200 mug into the left coronary artery as a spasm provocation test: comparison with 100 mug of acetylcholine. Heart Vessels 2015;30:771–8.

43. Sueda S, Miyoshi T, Sasaki Y, Sakaue T, Habara H, Kohno H. Safety and optimal protocol of provocation test for diagnosis of multivessel coronary spasm. Heart Vessels 2016;31:137–42.

44. Chandrasekar B, Doucet S, Bilodeau L, et al. Complications of cardiac catheterization in the current era: a single-center experience. Catheter Cardiovasc Interv 2001;52:289–95.

45. Laskey WK, Kimmel S, Krone RJ. Contemporary trends in coronary intervention: a report from the Registry of the Society for Cardiac Angiography and Interventions. Catheter Cardiovasc Interv 2000;49:19–22.

46. Ong P, Camici P, Beltrame JF, et al. International standardization of diagnostic criteria for microvascular angina. Int J Cardiol 2017. in press.

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