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ORIGINAL RESEARCH

Sex-Related Differences in the Prognostic Role of Acetylcholine Provocation Testing

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BACKGROUND: Intracoronary provocation testing with acetylcholine (ACh) is helpful to diagnose and risk-stratify patients with ischemia with nonobstructed coronary arteries (NOCA) and myocardial infarction with NOCA. This study explored potential sex-related disparities on the prognostic significance of ACh provocative testing.

METHODS: Consecutive patients with ischemia with NOCA and those with myocardial infarction with NOCA who underwent ACh provocation testing were enrolled. The primary end point was the incidence of major adverse cardiovascular and cerebrovascular events at follow-up. Co-primary end points were angina recurrence and quality of life assessed by 12-month Seattle Angina Questionnaire (SAQ) summary score.

RESULTS: A total of 519 patients (mean age, 61.4±12.1 years; 275 [53.0%] women and 244 [47%] men) were enrolled: 346 (66.7%) with ischemia with NOCA and 173 (33.3%) with myocardial infarction with NOCA. A positive ACh test was observed in 274 (52.8%) patients, with a lower prevalence of epicardial spasm (82 [56.2%] versus 106 [82.8%]) and a higher prevalence of microvascular spasm (64 [43.8%] versus 22 [17.2%]) in women compared with men (*P*>0.001). After a median 22-month follow-up, major adverse cardiovascular and cerebrovascular events occurred in 53 (10.2%) patients, without significant sex differences (*P*>0.05). Men with a positive ACh test had a significantly higher rate of major adverse cardiovascular and cerebrovascular events (22 [17.2%] versus 5 [4.3%], *P*=0.002) compared with those with a negative test; no difference was observed in women (*P*>0.05) (*P* for interaction=0.003). Women with a positive test experienced a higher rate of angina recurrence (61 [41.8%] versus 32 [24.8%], *P*=0.005) and a lower SAQ summary score (82 [interquartile range, 72–90] versus 86 [interquartile range, 78–100], *P*<0.001) compared with those with a negative result; no difference was observed in men (*P*>0.05).

CONCLUSIONS: This study revealed the importance of recognizing sex-specific differences in the prognostic value of ACh testing for proper management of coronary vasomotor disorders.

Key Words: acetylcholine ■ INOCA ■ MINOCA ■ prognosis ■ sex

Despite a decrease in annual mortality rates over the past decades, coronary heart disease remains the leading cause of death globally, posing a significant global health burden.[1] Remarkably, nearly half of the patients undergoing coronary angiography for suspected angina or suspected or confirmed myocardial ischemia do not demonstrate obstructive coronary artery disease.[2,3] Coronary vasomotor disorders, occurring in epicardial arteries or in coronary microcirculation, have been proven to significantly contribute

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CLINICAL PERSPECTIVE

What Is New?

- This study reveals significant sex-specific differences in the prognostic implications of acetylcholine provocation testing, with men showing a higher risk of major adverse cardiovascular and cerebrovascular events associated with epicardial spasms and women experiencing recurrent angina and reduced quality of life attributable to microvascular spasms, highlighting the importance of considering sex-specific responses in coronary vasomotor dysfunction.

What Are the Clinical Implications?

- This study underscores the need for sex-specific strategies in managing coronary vasomotor disorders, with targeted interventions for men to prevent adverse cardiovascular events and symptom-focused approaches for women to improve quality of life, ultimately promoting personalized care for patients with ischemia with nonobstructed coronary arteries and those with myocardial infarction with nonobstructed coronary arteries.

Nonstandard Abbreviations and Acronyms

ACh	acetylcholine
INOCA	ischemia with nonobstructed coronary arteries
MACCE	major adverse cardiovascular and cerebrovascular events
MINOCA	myocardial infarction with nonobstructed coronary arteries
NOCA	nonobstructed coronary arteries
SAQ	Seattle Angina Questionnaire
UA	unstable angina

to precipitating myocardial ischemia in a substantial proportion of these cases.[4,5]

Intracoronary provocation testing with administration of acetylcholine (ACh) has emerged as a valuable diagnostic tool capable of eliciting epicardial coronary spasm or microvascular spasm in susceptible individuals.[6] This testing not only aids in the diagnosis of underlying vasomotor conditions but also holds important prognostic implications.[7–9] Indeed, patients with a positive test response face an elevated risk of cardiovascular events compared with those with a negative response.[10–12]

In recent years, a growing body of evidence has highlighted differences in the prevalence, predictors, and clinical correlates associated with positive ACh provocation testing among men and women, particularly regarding the prevalence of epicardial compared with microvascular spasm.[13–17] However, whether such disparities affect the prognostic value of a positive ACh test remains scarce and poorly investigated.[18]

In this context, the aim of this study was to elucidate and explore potential sex-related disparities on the prognostic significance of ACh provocative testing in patients with ischemia with nonobstructed coronary arteries (INOCA) or myocardial infarction with nonobstructed coronary arteries (MINOCA). By examining the differential impact of a positive ACh test on clinical outcomes between male and female patients, this study aims to provide insights that may inform more tailored risk assessment and management strategies in this heterogeneous patient population.

METHODS

Study Population

We prospectively enrolled consecutive patients admitted to the Department of Cardiovascular Sciences of Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, who underwent clinically indicated coronary angiography for suspected myocardial ischemia or myocardial infarction (MI) with angiographic evidence of nonobstructive coronary artery disease (defined as angiographically normal coronary arteries or diffuse atherosclerosis with stenosis <50% with or without fractional flow reserve >0.80), and underwent intracoronary provocation testing with ACh from September 2015 to September 2022. We enrolled both patients admitted with suspected INOCA and MINOCA diagnosed according to the most recent European Society of Cardiology guidelines.[19,20] Among patients presenting with suspected MINOCA, we excluded those with obvious causes of MI other than suspected coronary vasomotor abnormalities. Clinical, laboratory, echocardiographic, and angiographic characteristics of all of the included patients were extracted from the electronic medical records of all patients by the Gemelli Generator Real World Data Facility. To obtain structural information from unstructured texts (ie, clinical diary and radiology reports), natural language processing algorithms were applied based on text mining procedures such as sentence/word tokenization, a rule-based approach supported by annotations defined by the clinical subject matter experts, and semantic/syntactic corrections where necessary[21] (see Data S1 for further details). The study protocol complied with the Declaration of Helsinki and the study was approved by our institutional review board (Comitato Etico Policlinico Gemelli; ID 5405). The authors declare that all supporting data are available within the article and its online supplementary files. All patients provided written informed consent for participation in the study.

Coronary Angiography and Invasive Provocation Testing Protocol

Coronary angiography was performed using either a radial or femoral artery approach. To fully expose all segments of the coronary arteries, at least 2 perpendicular projections for the right coronary artery and 4 projections for the left coronary artery were taken. Intracoronary ACh provocation testing was performed immediately following coronary angiography as previously described. A slow rate of manual infusion of ACh over a period of 3 minutes using a "stepwise approach," with increasing ACh doses infused at 2- to 3-minute intervals between injections (20 to 50 to 100 μg in the left coronary artery and 20 to 50 μg in the right coronary artery). The decision to proceed with testing the left coronary artery with a 200-μg dose was left to the operator's discretion.[10,22–25] A 12-lead ECG was continuously recorded throughout the test. Coronary angiography was performed 1 minute after each injection or when chest pain or ischemic ECG shifts were observed. The test was considered positive for epicardial coronary spasm in the presence of focal or diffuse epicardial coronary diameter reduction ≥90% in comparison with baseline, associated with the reproduction of the patient's symptoms (eg, typical chest pain) and ischemic ECG shifts.[26] Microvascular spasm was diagnosed when typical ischemic ST-segment changes associated with the reproduction of the patient's symptoms developed in the absence of epicardial coronary constriction (<90% diameter reduction).[27]

Study End Points

All patients received a clinical follow-up by telephonic interview with or without clinical visit at 6, 12, 24, 36, 48, and 60 months. The primary end point was the incidence of major adverse cardiovascular and cerebrovascular events (MACCE). MACCE were defined as the composite of cardiovascular death, nonfatal MI, hospitalization due to unstable angina (UA), and stroke/transient ischemic attack (TIA)[28] (see Data S1 for further details). We counted only the number of patients with the first occurrence of MACCE during the follow-up period. Co-primary end points were the recurrence of angina episodes (with or without hospitalization) during the follow-up period and the quality of life assessed by Seattle Angina Questionnaire (SAQ) summary score at 12 months. The SAQ is a validated measure of health-related quality of life in patients with coronary artery disease, where higher scores indicate better quality of life, fewer symptoms, and greater physical functioning.[29]

Statistical Analysis

Data distribution for continuous variables was assessed according to the Kolmogorov–Smirnov test, and variables are expressed as mean±SD or median and interquartile range (IQR) for normally and nonnormally distributed data, respectively. Continuous variables were compared using Student *t* test or Mann–Whitney *U* test, as appropriate. Categorical data were evaluated using χ^2 test or Fisher exact test. A significance level of P<0.05 was considered statistically significant. For categorical variables, no missing data were present due to the binary output capability of our natural language processing algorithms (indicating the presence or absence of a factor). For continuous variables, the approach taken for handling missing data was to exclude them from the analysis. This decision was based on the proportion of missing data and the potential impact on the study's outcomes. Indeed, given the relatively low percentage of missing data for most variables, excluding these values was deemed unlikely to significantly affect the overall analysis. Univariable Cox regression analysis was conducted to assess the relationship between individual variables and MACCE stratified by sex. Variables with a P value ≤0.05 in univariable analysis were included in the multivariable Cox regression model to identify independent predictors of MACCE. Survival curves for MACCE were plotted using the Kaplan–Meier method and were compared using log-rank test. A 2-tailed analysis was performed and a P value <0.05 was considered statistically significant. As there were no prior studies on this specific topic, a formal a priori sample size calculation was not possible. All analyses were performed using SPSS version 21 (IBM) and R software version 4.3.1 (The R Foundation for Statistical Computing).

RESULTS

Baseline Characteristics of Study Population

A total of 519 patients (mean age, 61.4±12.1 years; 275 [53.0%] women and 244 [47%] men) with myocardial ischemia and nonobstructed coronary arteries undergoing ACh provocation test were enrolled. Among them, 346 (66.7%) patients presented with INOCA and 173 (33.3%) with MINOCA. A positive response to ACh test was observed in 274 patients (52.8%). Among patients with a positive ACh test, 188 (68.6%) developed an epicardial spasm, whereas 86 (31.4%) developed a microvascular spasm.

Female patients, compared with male patients, were older (62.6±12.0 years versus 60.1±12.0 years, P=0.019) and had a lower prevalence of cardiovascular history (43 [15.6%] versus 57 [23.4%], P=0.03), lower hemoglobin (13.1±1.3 versus 13.7±1.5 g/dL, P<0.001) and serum creatinine (0.79 mg/dL [IQR, 0.67–0.92 mg/dL] versus 0.87 mg/dL [IQR, 0.74–1.06 mg/dL], *P*<0.001) levels at admission, and a lower prevalence of epicardial spasm (82 [56.2%] versus 106 [82.8%]), with a higher prevalence of microvascular spasm (64 [43.8%] versus 22 [17.2%]) (*P*<0.001).

Clinical, ECG, echocardiographic, and angiographic features of the overall population and according to sex are shown in Table 1.

Clinical Outcomes According to Sex in the Overall Population and in the ACh-Positive Population

In the overall population, at a median follow-up of 22 months (IQR, 13–30 months), MACCE occurred in 53 (10.2%) patients. There were no statistically significant differences in the incidence of MACCE (26 [9.5%] versus 27 [11.1%], *P*=0.49) or in the individual incidence of cardiovascular death (3 [1.1%] versus 1 [0.4%], *P*=0.38), nonfatal MI (3 [1.1%] versus 2 [2.0%], *P*=0.36), hospitalization for UA (20 [7.3%] versus 19 [7.8%], *P*=0.75), and stroke/TIA (1 [0.4%] versus 3 [1.2%], *P*=0.24) between women and men. Similarly, there were no statistically significant differences in the rate of angina recurrence (93 [33.8%] versus 60 [24.6%], *P*=0.06), in the 12-month SAQ summary score (84 [IQR 75–94] versus 86 [IQR 76–100], *P*=0.29), or the median follow-up time (22 months [IQR, 13–30 months] versus 22 months [IQR, 13–31 months], *P*=0.86) (Table 2).

In the ACh-positive population, at a median follow-up of 21 months (IQR, 12–30 months), MACCE occurred in 36 (13.1%) patients. There were no statistically significant differences in the incidence of MACCE (14 [9.6%] versus 22 [17.2%], *P*=0.08) or in the individual incidence of cardiovascular death (1 [0.7%] versus 1 [0.8%], *P*=0.93), nonfatal MI (2 [1.4%] versus 4 [3.1%], *P*=0.27), hospitalization for UA (11 [7.5%] versus 15 [11.7%], *P*=0.27), or stroke/TIA (0 [0.0%] versus 3 [2.3%], *P*=0.06) among women and men. Similarly, there were no statistically significant differences in the rate of angina recurrence (61 [41.8%] versus 42 [32.8%], *P*=0.24), the 12-month SAQ summary score (82 [IQR, 72–90] versus 84 [IQR, 74–94], *P*=0.15), or the median follow-up time (20 months [IQR, 12–29.25 months] versus 22 [IQR, 13–31 months], *P*=0.56) (Table 3). Kaplan–Meier curve showed that MACCE-free survival was not statistically significantly different between male and female patients (*P*=0.08, Figure 1).

Clinical Outcomes According to Sex and ACh Test Response

Among female patients, there were no differences in the incidence of MACCE (14 [9.6%] versus 12 [9.3%], *P*=0.59) or in the individual incidences of cardiovascular death (1 [0.7%] versus 2 [1.6%], *P*=0.55), nonfatal MI (2 [1.4%] versus 1 [0.8%], *P*=0.64), hospitalization for UA (11 [7.5%] versus 9 [7.0%], *P*=0.50), and stroke/TIA (0 [0.0%] versus 1 [0.8%], *P*=0.32) among those with a positive and negative ACh test response. However, female patients with a positive ACh test had a higher incidence of angina recurrence (61 [41.8%] versus 32 [24.8%], *P*=0.005) and lower 12-month SAQ summary score (82 [IQR, 72–90] versus 86 [IQR, 78–100], *P*<0.001) compared with those with a negative result, without differences in the median follow-up time (22 months [IQR, 12–29.25 months] versus 24 months [IQR, 15–30 months], *P*=0.13) (Table 4).

Among male patients, those with a positive ACh test result had a statistically significant higher incidence of MACCE compared with those with a negative result (22 [17.2%] versus 5 [4.3%], *P*=0.002), mainly driven by a higher incidence of hospitalization for UA (15 [11.7%] versus 4 [3.4%], *P*=0.01) without differences in the incidence of cardiovascular death (1 [0.8%] versus 0 [0.0%], *P*=0.34), nonfatal MI (4 [3.1%] versus 1 [0.9%], *P*=0.22), and stroke/TIA (3 [2.3%] versus 0 [0.0%], *P*=0.11). Moreover, men with a positive ACh test had a higher incidence of angina recurrence compared with those with a negative test (42 [32.8%] versus 18 [15.5%], *P*=0.003), whereas no statistically significant differences were observed in the 12-month SAQ summary score (84 [IQR, 74–94] versus 86 [IQR, 78–100], *P*=0.06) and in the median follow-up time (22 months [IQR, 13–31 months] versus 22 months [IQR, 13–30.75 months], *P*=0.88) between the 2 groups (Table 5).

The interaction between sex and positive ACh test in relation to the incidence of MACCE at follow-up was statistically significant (*P* for interaction=0.003). Kaplan–Meier curves showed that MACCE-free survival was significantly different among patients with a positive or negative ACh test according to sex, with the worst MACCE-free survival for male patients with a positive ACh test and the best for male patients with a negative ACh test (*P*=0.007) (Figure 2).

Predictors of MACCE According to Sex

Among female patients, univariate Cox regression analysis identified MINOCA as the only predictor for the occurrence of MACCE at follow-up (hazard ratio [HR] 2.21 [95% CI, 1.01–4.84], *P*=0.04) (Table 6, Panel A).

Among male patients, multivariate Cox regression analysis identified cardiovascular history (HR, 2.80 [95% CI, 1.26–6.23], *P*=0.01) and a positive ACh provocation test (HR, 3.45 [95% CI, 1.16–10.26], *P*=0.03) as the only independent predictors for the occurrence of MACCE at follow-up (Table 6, Panel B).

Table 1. Clinical, Echocardiographic, and Angiographic Features in the Overall Population and According to Sex

Characteristics	Overall population (N=519)	Women (n=275)	Men (n=244)
Clinical characteristics			
Age, y	61.4±12.1	62.6±12.0	60.1±12.0
Hypertension	333 (64.2)	174 (63.3)	159 (65.2)
Diabetes	93 (17.9)	47 (17.1)	46 (18.9)
Smoking	147 (28.3)	81 (29.5)	66 (27.0)
Dyslipidemia	279 (53.8)	146 (53.1)	133 (54.5)
Obesity (BMI ≥30)	46 (8.9)	24 (8.7)	22 (9.0)
Family history of CAD	174 (33.5)	93 (33.8)	81 (33.2)
Clinical presentation			
MINOCA	173 (33.3)	91 (33.1)	82 (33.6)
INOCA	346 (66.7)	184 (66.9)	162 (66.4)
Cardiovascular history	100 (19.3)	43 (15.6)	57 (23.4)
10-year ASCVD risk, %	*6 (2.2–11.7)*	*4.4 (2.2–10.7)*	*6.5 (2.4–15.9)*
Laboratory data			
Hemoglobin, g/dL	13.4±1.4	13.1±1.3	13.7±1.5
WBC count, ×10^3/L	7.3±2.1	7.3±2.1	7.3±2.1
Serum creatinine on admission, mg/dL	0.83 (0.71–0.97)	0.79 (0.67–0.92)	0.87 (0.74–1.06)
hs-cTnI at admission, ng/mL	0.2 (0.01–4.00)	0.2 (0.01–4.0)	0.2 (0.01–4.0)
CRP, mg/L	0.5 (0.1–2.6)	0.5 (0.1–2.5)	0.05 (0.05–2.4)
Echocardiographic data			
LVEF on admission, %	59.5±5.8	60.0±5.7	59.0±5.9
Diastolic dysfunction	254 (48.9)	137 (49.8)	117 (48.0)
Angiographic data			
Presence of nonobstructive CAD	259 (49.9)	130 (47.3)	129 (52.9)
Provocative test			
Positive	274 (52.8)	146 (53.1)	128 (52.5)
Type of positive response			
Epicardial spasm	188 (68.6)	82 (56.2)	106 (82.8)
Microvascular spasm	86 (31.4)	64 (43.8)	22 (17.2)
Highest ACh dose (≥100 µg)	323 (62.2)	166 (60.4)	157 (64.3)
ACh maximum dose	100 (50–100)	100 (50–100)	100 (50–100)
Therapy at discharge			
Aspirin	256 (49.3)	126 (45.8)	130 (53.3)
Clopidogrel	56 (10.8)	28 (10.2)	28 (11.5)
Ticagrelor	5 (1.0)	4 (1.5)	1 (0.4)
Prasugrel	4 (0.8)	1 (0.4)	3 (1.2)
β-Blockers	178 (34.3)	99 (36.0)	79 (32.4)
CCBs	343 (66.1)	181 (65.8)	162 (66.4)
ACEi/ARBs	341 (65.7)	171 (62.2)	170 (69.7)
Statins	358 (69.0)	194 (70.5)	164 (67.2)
Diuretics	76 (14.6)	44 (16.0)	32 (13.1)
Nitrates	14 (2.7)	9 (3.3)	5 (2.0)
NOACs	47 (9.1)	27 (9.9)	20 (8.2)

Values are expressed as median (interquartile range), number (percentage), or mean±SD.

ACEi indicates angiotensin-converting enzyme inhibitor; ACh, acetylcholine; ARB, angiotensin receptor blocker; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blocker; CRP, C-reactive protein; hs-cTnI, high-sensitivity cardiac troponin I; INOCA, myocardial ischemia with nonobstructed coronary arteries; LVEF, left ventricular ejection fraction; MINOCA, myocardial infarction with nonobstructed coronary arteries; NOAC, novel oral anticoagulant drug; and WBC, white blood cell.

Table 2. Clinical Outcomes at Follow-Up in the Overall Population and According to Sex

Outcomes	Overall population (N=519)	Women (n=275)	Men (n=244)	*P* value
MACCE	53 (10.2)	26 (9.5)	27 (11.1)	0.49
Cardiovascular death	4 (0.8)	3 (1.1)	1 (0.4)	0.38
Nonfatal MI	8 (1.5)	3 (1.1)	5 (2.0)	0.36
Hospitalization for UA	39 (7.5)	20 (7.3)	19 (7.8)	0.75
Stroke/TIA	4 (0.8)	1 (0.4)	3 (1.2)	0.24
Angina recurrence	153 (29.5)	93 (33.8)	60 (24.6)	0.06
SAQ summary score	84 (75–100)	84 (75–94)	86 (76–100)	0.29
Follow-up time, mo	22 (13–30)	22 (13–30)	22 (13–31)	0.86

Values are expressed as number (percentage) or median (interquartile range).

MACCE indicates major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction; SAQ, Seattle Angina Questionnaire; TIA, transient ischemic attack; and UA, unstable angina.

DISCUSSION

To the best of our knowledge, our study for the first time reveals significant sex-related prognostic differences in patients with coronary vasomotor disorders, underscoring the potential need for sex-specific approaches to these conditions.

The main results of our study can be summarized as follows: (1) women exhibited a higher incidence of microvascular spasm, whereas men demonstrated a higher incidence of epicardial spasm; (2) in the overall population, as well as in the ACh-positive population only, there were no differences in the rate of MACCE and angina recurrence at follow-up according to sex; (3) among female patients, a positive ACh test result was not associated with an increased rate of MACCE at follow-up but was associated with a higher rate of angina recurrence and a lower 12-month SAQ summary score; (4) among male patients, a positive ACh test result was associated with an increased rate of MACCE at follow-up, primarily driven by a higher incidence of hospitalization for UA, and a higher rate of angina recurrence; and (5) when stratified by sex and ACh test response, the poorest MACCE-free survival was observed among male patients with a positive ACh test, and the interaction between sex and positive ACh test in relation to the incidence of MACCE was statistically significant.

In line with prior research, we observed sex-specific disparities in the prevalence of coronary vasomotor disorders: female patients exhibited a higher prevalence of microvascular spasm, whereas male patients more frequently experienced epicardial coronary spasm.[13–17] These differences may partly arise from the distinct hormonal environments characterizing each sex, particularly the influence of estrogens. Although the majority of women in our cohort were likely postmenopausal based on their mean age (62±12 years), long-term exposure to estrogen before menopause may have lasting effects on coronary vasomotor function. Indeed, estrogens play a significant role in vascular health by affecting the vascular endothelium and smooth muscle cells, which are critical regulators of vasomotor function. Experimental studies indicate that estrogen-mediated modulation of nitric oxide plays a pivotal role in microvascular vasomotor function, potentially contributing to the observed sex-related differences.[30,31] Nevertheless, our findings are only hypothesis-generating, and further research, ideally stratifying by menopausal status and considering

Table 3. Clinical Outcomes at Follow-up in the ACh-Positive Population and According to Sex

Outcomes	ACh-positive population (n=274)	Women (n=146)	Men (n=128)	*P* value
MACCE	36 (13.1)	14 (9.6)	22 (17.2)	0.08
Cardiovascular death	2 (0.7)	1 (0.7)	1 (0.8)	0.93
Nonfatal MI	6 (2.2)	2 (1.4)	4 (3.1)	0.27
Hospitalization for UA	26 (9.5)	11 (7.5)	15 (11.7)	0.27
Stroke/TIA	3 (1.1)	0 (0.0)	3 (2.3)	0.06
Angina recurrence	103 (37.6)	61 (41.8)	42 (32.8)	0.24
SAQ summary score	82 (73–90)	82 (72–90)	84 (74–94)	0.15
Follow-up time, mo	21 (12–30)	20 (12–29.25)	22 (13–31)	0.56

Values are expressed as number (percentage) or median (interquartile range).

ACh indicates acetylcholine; MACCE, major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction; SAQ, Seattle Angina Questionnaire; TIA, transient ischemic attack; and UA, unstable angina.

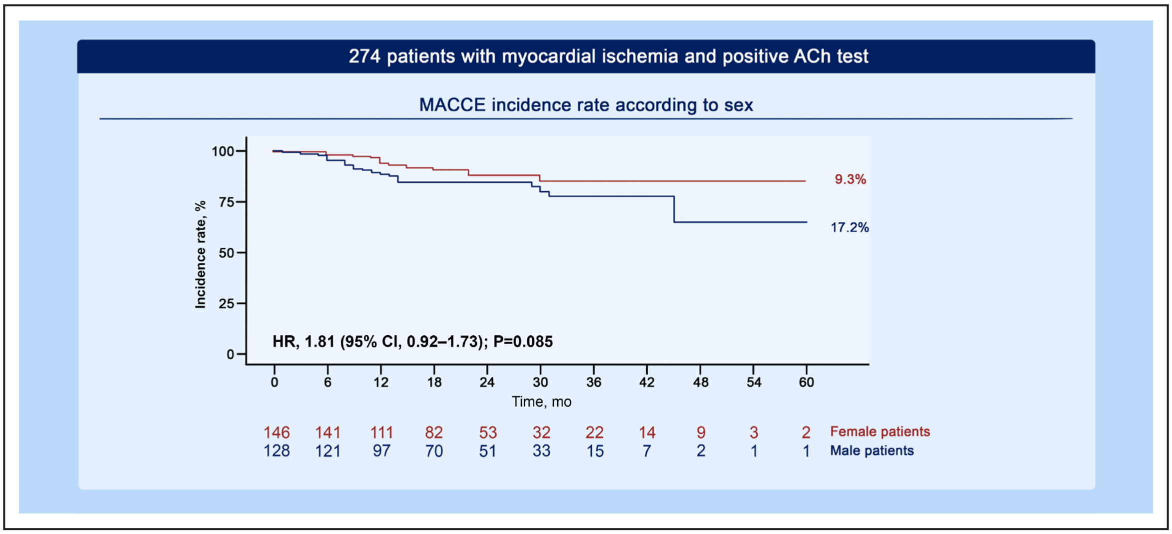

Figure 1. Incidence of composite of MACCE at follow-up in the ACh-positive population according to sex.
ACh indicates acetylcholine; and MACCE, major adverse cardiovascular and cerebrovascular events.

hormone replacement therapy use, is warranted to better understand the mechanisms underlying these sex-specific responses.

Moreover, regarding clinical outcomes, we found no sex-related differences in the rate of MACCE across the entire study cohort or in the subgroup with positive ACh test results. These findings align with previous research, such as the study by Park et al, which reported no significant differences in the rate of 5-year major adverse cardiac events and recurrent angina between sexes.[18] Notably, Park et al defined a positive test for coronary artery spasm as focal or diffuse vasoconstriction exceeding 70% of coronary arteries, a definition that does not differentiate between microvascular and epicardial spasm and does not adhere to the COVADIS criteria,[26,27] thus deviating from recommended clinical practice.[18] Other studies that focused solely on patients with vasospastic angina also reported no sex-related differences in long-term clinical outcomes.[16,32–34] Our study distinguishes itself from the existing literature by including a broader spectrum of patients, specifically those with INOCA and MINOCA, the latter of whom were not included in previous studies. Additionally, we conducted a comprehensive analysis of coronary vasomotor disorders, diagnosing both epicardial and microvascular spasm in accordance with the latest and current clinical practice guidelines. By integrating these critical distinctions, our study has provided robust evidence, for the first time, that among the overall INOCA and MINOCA population, clinical outcomes do not differ between men and women having a positive or a negative ACh test result.

Of importance, the novelty of our study relies on a deep analysis of sex-specific disparities among these patients. Specifically, when evaluating each sex separately, female patients showed no differences in the

Table 4. Clinical Outcomes at Follow-up in the Female Population and According to a Positive or Negative ACh Provocation Testing

Outcomes	Female population (n=275)	Positive ACh provocation testing (n=146)	Negative ACh provocation testing (n=129)	*P* value
MACCE	26 (9.5)	14 (9.6)	12 (9.3)	0.59
Cardiovascular death	3 (1.1)	1 (0.7)	2 (1.6)	0.55
Nonfatal MI	3 (1.1)	2 (1.4)	1 (0.8)	0.64
Hospitalization for UA	20 (7.3)	11 (7.5)	9 (7.0)	0.50
Stroke/TIA	1 (0.4)	0 (0.0)	1 (0.8)	0.32
Angina recurrence	93 (33.8)	61 (41.8)	32 (24.8)	0.005
SAQ summary score	84 (75–94)	82 (72–90)	86 (78–100)	<0.001
Follow-up time, mo	22 (13–30)	22 (12–29.25)	24 (15–30)	0.13

Values are expressed as number (percentage) or median (interquartile range).
ACh indicates acetylcholine; MACCE, major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction; SAQ, Seattle Angina Questionnaire; TIA, transient ischemic attack; and UA, unstable angina.

Table 5. Clinical Outcomes at Follow-up in the Male Population and According to a Positive or Negative ACh Provocation Testing

Outcomes	Male population (n=244)	Positive ACh provocation testing (n=128)	Negative ACh provocation testing (n=116)	*P* value
MACCE	27 (11.1)	22 (17.2)	5 (4.3)	0.002
Cardiovascular death	1 (0.4)	1 (0.8)	0 (0.0)	0.34
Nonfatal MI	5 (2.0)	4 (3.1)	1 (0.9)	0.22
Hospitalization for UA	19 (7.8)	15 (11.7)	4 (3.4)	0.01
Stroke/TIA	3 (1.2)	3 (2.3)	0 (0.0)	0.11
Angina recurrence	60 (24.6)	42 (32.8)	18 (15.5)	0.003
SAQ summary score	86 (76–100)	84 (74–94)	86 (78–100)	0.06
Follow-up time, mo	22 (13–31)	22 (13–31)	22 (13–30.75)	0.88

Values are expressed as number (percentage) or median (interquartile range).

ACh indicates acetylcholine; MACCE, major adverse cardiovascular and cerebrovascular events; MI, myocardial infarction; SAQ, Seattle Angina Questionnaire; TIA, transient ischemic attack; and UA, unstable angina.

rate of MACCE at follow-up, regardless of their ACh test results. However, women with a positive ACh test result experienced a higher rate of angina recurrence and lower scores on the 12-month SAQ summary score compared with those with a negative result. In contrast, men with a positive ACh test result exhibited a significantly higher rate of MACCE and angina recurrence at follow-up compared with those with a negative result. These sex-specific differences were further accentuated in the survival curves, which displayed the poorest outcomes in male patients with a positive ACh test. Although addressing the pathophysiological mechanisms underlying these differences was beyond the scope of this study, we might hypothesize that they may be related to the differing prevalences and outcomes associated with epicardial compared with microvascular spasm. Previous research has shown that epicardial spasm is linked to an increased risk of cardiovascular events, while microvascular spasm is more commonly associated with recurrent angina.[35,36] Further suggesting the different disease trajectory and weight of coronary vasomotor disorders in the prognosis of these patients, a positive ACh test was an independent predictor of MACCE in the male population but not in the female populations, where MINOCA as a clinical presentation was the only predictor of MACCE.

Taken together, our findings suggest that the clinical and prognostic implications of a positive ACh result for diagnosing vasomotor disorders may differ between the sexes, with specific within-sex (male or female) differences. Specifically, male patients more commonly present with epicardial spasm, and a positive ACh test in this group is associated with poorer clinical outcomes, especially regarding cardiovascular events. In contrast, female patients are more prone to microvascular spasm, with angina recurrence and deterioration of quality of life being the primary concerns following a positive ACh test. Considering these sex-specific differences may contribute to a more individualized approach to managing vasomotor disorders. For

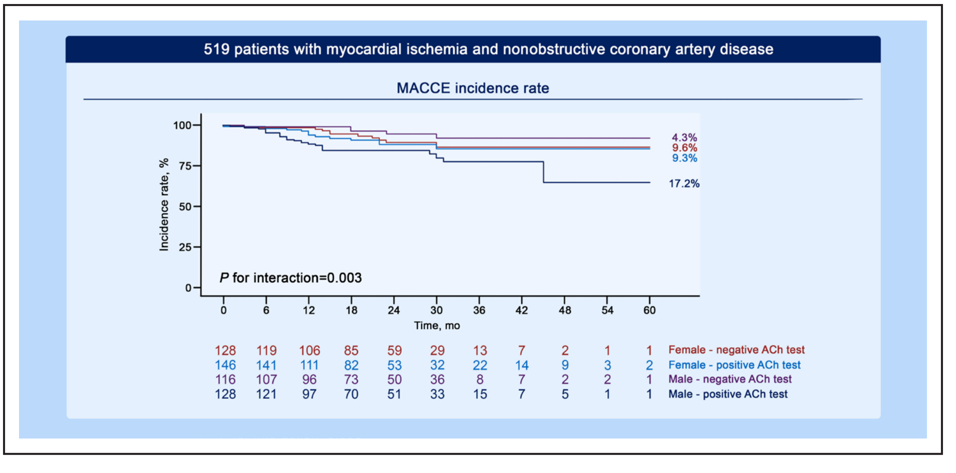

Figure 2. Incidence of composite of MACCE at follow-up according to a positive or negative ACh test and sex.
ACh indicates acetylcholine; MACCE, major adverse cardiovascular and cerebrovascular events.

Table 6. Predictors of MACCE According to Sex by Univariate and Multivariate Cox Regression Analysis

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Female population				
MINOCA as clinical presentation	2.21 (1.01–4.84)	0.04	-	-
Male population				
Cardiovascular history	2.33 (1.05–5.14)	0.04	2.80 (1.26–6.23)	0.01
Positive ACh provocation testing	4.21 (1.59–11.14)	0.004	3.45 (1.16–10.26)	0.03
β-Blockers therapy at discharge	0.22 (0.07–0.74)	0.01	0.30 (0.09–1.03)	0.06
CCB therapy at discharge	3.20 (1.11–9.28)	0.03	1.34 (0.41–4.38)	0.63

All characteristics shown in Table 1 have been tested to predict MACCE at follow-up, although only variables with a *P* value <0.05 are shown in this table. Variables that were significantly (*P*<0.05) related to MACCE at follow-up in the univariate Cox regression analysis were included in the multivariate Cox regression analysis.

ACh indicates acetylcholine; CCB, calcium channel blocker; HR, hazard ratio; MACCE, major adverse cardiovascular and cerebrovascular events; and MINOCA, myocardial infarction with nonobstructed coronary arteries.

male patients with a positive ACh test, clinical management could reasonably focus on the administration of high-dose calcium channel blockers and statins, which have demonstrated effectiveness in improving prognosis in prior studies.[25,37–40] Additionally, close follow-up to control cardiovascular risk factors remains essential as part of secondary prevention strategies. For female patients with a positive test, clinical management may emphasize effective control of angina symptoms and quality of life improvement, potentially incorporating therapies such as nicorandil,[41] ranolazine,[42] adenosine antagonists,[43] and tricyclics (able to modulate pain perception).[44,45] Although large randomized trials are lacking, these therapies have shown promise in managing angina symptoms. Psychological support may also play a valuable role in addressing the broader impacts of chronic angina.[46,47] In patients presenting with MINOCA, particularly women, appropriate management strategies are crucial, acknowledging the unique challenges of this condition.[48–51] Tailoring management strategies to sex-specific risks and responses, alongside guideline-directed treatments for secondary prevention and improvement in quality of life for all patients, holds the potential to enhance the overall management of patients with coronary vasomotor disorders, potentially reducing the burden of disease and improving patient outcomes. Nonetheless, given the lack of significant differences in overall MACCE outcomes between the sexes, these findings should be interpreted with caution, and further validation in larger, multicenter studies is warranted to confirm the clinical relevance of these sex-specific patterns.

LIMITATIONS

Some limitations of our study should be acknowledged. First, this is a single-center study with a relatively small sample size. Although a formal sample size calculation was not possible because of the lack of prior studies, we based our sample size on the available INOCA and MINOCA population, making this one of the largest studies on this subject. Nevertheless, larger, more rigorously powered studies are needed to confirm our results. Furthermore, the observational design of our study can only establish associations, not causality, and the findings may not be generalizable to all patient populations. This is particularly important as, along with sex-related differences, the presence of ethnic-related differences has been reported in patients with coronary vasomotor disorders.[52] Therefore, further studies ideally involving larger cohorts or multicenter trials are needed to validate our findings. Second, coronary blood flow and coronary flow reserve and resistance were not measured during the invasive study; thus, their potential relationship with the response to vasoconstrictor stimuli remains undetermined. Third, in patients with MINOCA taking vasoactive drugs, the provocation testing was not performed after a washout period for calcium channel blockers and nitrates, potentially interfering with the result of the test. Fourth, the choice to perform a provocation test, especially in patients presenting with stable angina, was left to the operator's discretion. This could have resulted in selection bias and could explain why the prevalence of MINOCA in our study population was higher compared with previous studies. Furthermore, the decision to administer the maximum ACh dose (200 μg) was left to the operator's discretion, which may have introduced false-negatives and potentially influenced the results. However, our protocol aligns with current clinical guideline recommendations.[19]

CONCLUSIONS

This study represents an important advancement in the understanding of the complexity of coronary vasomotor disorders, revealing that although clinical outcomes do not differ significantly between men and women, the character and trajectory of these disorders are distinctly influenced by sex. In the era of precision medicine, where treatments are increasingly tailored to individual characteristics, our study represents a significant step forward in integrating sex-specific considerations into the management of coronary vasomotor disorders. Recognizing these sex-related differences is not just critical for personalized patient care but also for guiding future research aimed at unraveling the complex interplay of sex-specific factors in cardiovascular health. Further studies are warranted to confirm our result and to better understand the pathophysiological mechanisms behind our observations.

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None.

Supplemental Material

Data S1

REFERENCES

1. Martin SS, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, Baker-Smith CM, Barone Gibbs B, Beaton AZ, Boehme AK, et al. 2024 heart disease and stroke statistics: a report of US and global data from the American Heart Association. *Circulation*. 2024;149:E347–E913. doi: 10.1161/CIR.0000000000001209
2. SCOT-HEART investigators. CT coronary angiography in patients with suspected angina due to coronary heart disease (SCOT-HEART): an open-label, parallel-group, multicentre trial. *Lancet*. 2015; 385:2383–2391. doi: 10.1016/S0140-6736(15)60291-4
3. Jespersen L, Hvelplund A, Abildstrøm SZ, Pedersen F, Galatius S, Madsen JK, Jørgensen E, Kelbæk H, Prescott E. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J*. 2012;33:734–744. doi: 10.1093/eurheartj/ehr331
4. Del Buono MG, Montone RA, Camilli M, Carbone S, Narula J, Lavie CJ, Niccoli G, Crea F. Coronary microvascular dysfunction across the Spectrum of cardiovascular diseases: JACC state-of-the-art review. *J Am Coll Cardiol*. 2021;78:1352–1371. doi: 10.1016/j.jacc.2021.07.042
5. Ong P, Aziz A, Hansen HS, Prescott E, Athanasiadis A, Sechtem U. Structural and functional coronary artery abnormalities in patients with vasospastic angina pectoris. *Circ J*. 2015;79:1431–1438. doi: 10.1253/circj.CJ-15-0520
6. Montone RA, Meucci MC, De Vita A, Lanza GA, Niccoli G. Coronary provocative tests in the catheterization laboratory: pathophysiological bases, methodological considerations and clinical implications. *Atherosclerosis*. 2021;318:14–21. doi: 10.1016/j.atherosclerosis.2020.12.008
7. Rinaldi R, Salzillo C, Caffè A, Montone RA. Invasive functional Coronary assessment in myocardial ischemia with non-obstructive Coronary arteries: from pathophysiological mechanisms to clinical implications. *Rev Cardiovasc Med*. 2022;23:371. doi: 10.31083/j.rcm2311371
8. Probst S, Seitz A, Martínez Pereyra V, Hubert A, Becker A, Storm K, Bekeredjian R, Sechtem U, Ong P. Safety assessment and results of coronary spasm provocation testing in patients with myocardial infarction with unobstructed coronary arteries compared to patients with stable angina and unobstructed coronary arteries. *Eur Heart J Acute Cardiovasc Care*. 2020;10:2048872620932422.
9. Gurgoglione FL, Vignali L, Montone RA, Rinaldi R, Benatti G, Solinas E, Leone AM, Galante D, Campo G, Biscaglia S, et al. Coronary Spasm testing with acetylcholine: a powerful tool for a personalized therapy of Coronary vasomotor disorders. *Life (Basel)*. 2024;14:292. doi: 10.3390/life14030292
10. Montone RA, Rinaldi R, Del Buono MG, Gurgoglione F, La Vecchia G, Russo M, Caffè A, Burzotta F, Leone AM, Romagnoli E, et al. Safety and prognostic relevance of acetylcholine testing in patients with stable myocardial ischaemia or myocardial infarction and non-obstructive coronary arteries. *EuroIntervention*. 2022;18:E666–E676. doi: 10.4244/EIJ-D-21-00971
11. Montone RA, Niccoli G, Fracassi F, Russo M, Gurgoglione F, Cammà G, Lanza GA, Crea F. Patients with acute myocardial infarction and non-obstructive coronary arteries: safety and prognostic relevance of invasive coronary provocative tests. *Eur Heart J*. 2018;39:91–98. doi: 10.1093/eurheartj/ehx667
12. Shimokawa H, Suda A, Takahashi J, Berry C, Camici PG, Crea F, Escaned J, Ford T, Yii E, Kaski JC, et al. Clinical characteristics and prognosis of patients with microvascular angina: an international and prospective cohort study by the Coronary vasomotor disorders international study (COVADIS) group. *Eur Heart J*. 2021;42:4592–4600. doi: 10.1093/eurheartj/ehab282
13. Saito Y, Saito Y, Kato K, Kobayashi Y. Gender differences in factors associated with vasospastic angina. *Int J Cardiol*. 2022;349:7–11. doi: 10.1016/j.ijcard.2021.11.047
14. Ford TJ, Yii E, Sidik N, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, et al. Ischemia and no obstructive coronary artery disease: prevalence and correlates of coronary vasomotion disorders. *Circ Cardiovasc Interv*. 2019;12:e008126. doi: 10.1161/CIRCINTERVENTIONS.119.008126
15. Montone RA, Niccoli G, Russo M, Giaccari M, Del Buono MG, Meucci MC, Gurgoglione F, Vergallo R, D'Amario D, Buffon A, et al. Clinical, angiographic and echocardiographic correlates of epicardial and microvascular spasm in patients with myocardial ischaemia and non-obstructive coronary arteries. *Clin Res Cardiol*. 2020;109:435–443. doi: 10.1007/s00392-019-01523-w
16. Sueda S, Sakaue T. Sex-related differences in patients with positive coronary spasm as identified by acetylcholine testing. *Intern Med*. 2021;60:2357–2365. doi: 10.2169/internalmedicine.6630-20
17. Aziz A, Hansen HS, Sechtem U, Prescott E, Ong P. Sex-related differences in vasomotor function in patients with angina and unobstructed Coronary arteries. *J Am Coll Cardiol*. 2017;70:2349–2358. doi: 10.1016/j.jacc.2017.09.016
18. Park JY, Choi SY, Rha SW, Choi BG, Noh YK, Kim YH. Sex difference in coronary artery spasm tested by intracoronary acetylcholine provocation test in patients with nonobstructive coronary artery disease. *J Interv Cardiol*. 2022;2022:5289776. doi: 10.1155/2022/5289776
19. Vrints C, Andreotti F, Koskinas KC, Rossello X, Adamo M, Ainslie J, Banning AP, Budaj A, Buechel RR, Chiariello GA, et al. 2024 ESC guidelines for the management of chronic coronary syndromes. *Eur Heart J*. 2024;45:3415–3537. doi: 10.1093/eurheartj/ehae177
20. Byrne RA, Rossello X, Coughlan JJ, Barbato E, Berry C, Chieffo A, Claeys MJ, Dan GA, Dweck MR, Galbraith M, et al. 2023 ESC guidelines for the management of acute coronary syndromes. *Eur Heart J*. 2023;44:3720–3826. doi: 10.1093/eurheartj/ehad191

21. Damiani A, Masciocchi C, Lenkowicz J, Capocchiano ND, Boldrini L, Tagliaferri L, Cesario A, Sergi P, Marchetti A, Luraschi A, et al. Building an artificial intelligence laboratory based on real world data: the experience of Gemelli generator. *Front Comp Sci*. 2021;3:768266.
22. Montone RA, Gurgoglione FL, Del Buono MG, Rinaldi R, Meucci MC, Iannaccone G, La Vecchia G, Camilli M, D'Amario D, Leone AM, et al. Interplay between myocardial bridging and Coronary Spasm in patients with myocardial ischemia and non-obstructive Coronary arteries: pathogenic and prognostic implications. *J Am Heart Assoc*. 2021;10:e020535. doi: 10.1161/JAHA.120.020535
23. Rinaldi R, Colucci M, Torre I, Ausiello D, Bonanni A, Basile M, Salzillo C, Sanna T, Liuzzo G, Leone AM, et al. Predicting the response to acetylcholine in ischemia or infarction with non-obstructive coronary arteries: the ABCD score. *Atherosclerosis*. 2024;391:117503. doi: 10.1016/j.atherosclerosis.2024.117503
24. Ford TJ, Ong P, Sechtem U, Beltrame J, Camici PG, Crea F, Kaski JC, Bairey Merz CN, Pepine CJ, Shimokawa H, et al. Assessment of vascular dysfunction in patients without obstructive Coronary artery disease: why, how, and when. *JACC Cardiovasc Interv*. 2020;13:1847–1864. doi: 10.1016/j.jcin.2020.05.052
25. Kunadian V, Chieffo A, Camici PG, Berry C, Escaned J, Maas AHEM, Prescott E, Karam N, Appelman Y, Fraccaro C, et al. An EAPCI expert consensus document on ischaemia with non-obstructive coronary arteries in collaboration with European Society of Cardiology Working Group on Coronary Pathophysiology & Microcirculation Endorsed by Coronary vasomotor disorders international study group. *Eur Heart J*. 2020;41:3504–3520. doi: 10.1093/eurheartj/ehaa503
26. Beltrame JF, Crea F, Kaski JC, Ogawa H, Ong P, Sechtem U, Shimokawa H, Bairey Merz CN. International standardization of diagnostic criteria for vasospastic angina. *Eur Heart J*. 2017;38:2565–2568.
27. Ong P, Camici PG, Beltrame JF, Crea F, Shimokawa H, Sechtem U, Kaski JC, Bairey Merz CN. International standardization of diagnostic criteria for microvascular angina. *Int J Cardiol*. 2018;250:16–20. doi: 10.1016/j.ijcard.2017.08.068
28. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD. Fourth universal definition of myocardial infarction (2018). *Circulation*. 2018;138:e618–e651. doi: 10.1161/CIR.0000000000000617
29. Chan PS, Jones PG, Arnold SA, Spertus JA. Development and validation of a short version of the Seattle angina questionnaire. *Circ Cardiovasc Qual Outcomes*. 2014;7:640–647. doi: 10.1161/CIRCOUTCOMES.114.000967
30. Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation*. 2004;109:2518–2523. doi: 10.1161/01.CIR.0000128208.22378.E3
31. Crea F, Montone RA, Rinaldi R. Pathophysiology of coronary microvascular dysfunction. *Circ J*. 2022;86:1319–1328. doi: 10.1253/circj.CJ-21-0848
32. Kim HL, Jo SH, Kim HJ, Lee MH, Seo WW, Baek SH. Sex differences in clinical characteristics and long-term outcomes in patients with vasospastic angina: results from the VA-Korea registry, a prospective multi-center cohort. *Biol Sex Differ*. 2020;11:66. doi: 10.1186/s13293-020-00341-4
33. Lee DH, Park TK, Seong CS, Gwag HB, Lim AY, Oh MS, Cho SW, Yang JH, Song YB, Hahn JY, et al. Gender differences in long-term clinical outcomes and prognostic factors in patients with vasospastic angina. *Int J Cardiol*. 2017;249:6–11. doi: 10.1016/j.ijcard.2017.05.094
34. Kawana A, Takahashi J, Takagi Y, Yasuda S, Sakata Y, Tsunoda R, Ogata Y, Seki A, Sumiyoshi T, Matsui M, et al. Gender differences in the clinical characteristics and outcomes of patients with vasospastic angina. *Circ J*. 2013;77:1267–1274. doi: 10.1253/circj.CJ-12-1486
35. Schoenenberger AW, Adler E, Gujer S, Jamshidi P, Kobza R, Stuck AE, Resink TJ, Erne P. Prognostic value of an abnormal response to acetylcholine in patients with angina and non-obstructive coronary artery disease: long-term follow-up of the heart quest cohort. *Int J Cardiol*. 2016;221:539–545. doi: 10.1016/j.ijcard.2016.07.035
36. Seitz A, Gardezy J, Pirozzolo G, Probst S, Athanasiadis A, Hill S, Mahrholdt H, Bekeredjian R, Sechtem U, Ong P. Long-term follow-up in patients with stable angina and unobstructed Coronary arteries undergoing intracoronary acetylcholine testing. *JACC Cardiovasc Interv*. 2020;13:1865–1876. doi: 10.1016/j.jcin.2020.05.009
37. Chahine RA, Feldman RL, Giles TD, Nicod P, Raizner AE, Weiss RJ, Vanov SK. Randomized placebo-controlled trial of amlodipine in vasospastic angina. Amlodipine study 160 group. *J Am Coll Cardiol*. 1993;21:1365–1370. doi: 10.1016/0735-1097(93)90310-W
38. Nishigaki K, Inoue Y, Yamanouchi Y, Fukumoto Y, Yasuda S, Sueda S, Urata H, Shimokawa H, Minatoguchi S. Prognostic effects of calcium channel blockers in patients with vasospastic angina—a meta-analysis. *Circ J*. 2010;74:1943–1950.
39. Cannon RO, Watson RM, Rosing DR, Epstein SE. Efficacy of calcium channel blocker therapy for angina pectoris resulting from small-vessel coronary artery disease and abnormal vasodilator reserve. *Am J Cardiol*. 1985;56:242–246. doi: 10.1016/0002-9149(85)90842-2
40. Ishii M, Kaikita K, Sato K, Yamanaga K, Miyazaki T, Akasaka T, Tabata N, Arima Y, Sueta D, Sakamoto K, et al. Impact of statin therapy on clinical outcome in patients with coronary spasm. *J Am Heart Assoc*. 2016;5:e003426. doi: 10.1161/JAHA.116.003426
41. Chen JW, Lee WL, Hsu NW, Lin SJ, Ting CT, Wang SP, Chang MS. Effects of short-term treatment of Nicorandil on exercise-induced myocardial ischemia and abnormal cardiac autonomic activity in microvascular angina. *Am J Cardiol*. 1997;80:32–38. doi: 10.1016/S0002-9149(97)00279-8
42. Bairey Merz CN, Handberg EM, Shufelt CL, Mehta PK, Minissian MB, Wei J, Thomson LE, Berman DS, Shaw LJ, Petersen JW, et al. A randomized, placebo-controlled trial of late Na current inhibition (ranolazine) in coronary microvascular dysfunction (CMD): impact on angina and myocardial perfusion reserve. *Eur Heart J*. 2016;37:1504–1513. doi: 10.1093/eurheartj/ehv647
43. Elliott PM, Krzyzowska-Dickinson K, Calvino R, Hann C, Kaski JC. Effect of oral aminophylline in patients with angina and normal coronary arteriograms (cardiac syndrome X). *Heart*. 1997;77:523–526. doi: 10.1136/hrt.77.6.523
44. Cannon RO 3rd, Quyyumi AA, Mincemoyer R, Stine AM, Gracely RH, Smith WB, Geraci MF, Black BC, Uhde TW, Waclawiw MA, et al. Imipramine in patients with chest pain despite normal coronary angiograms. *N Engl J Med*. 1994;330:1411–1417. doi: 10.1056/NEJM199405193302003
45. Cox ID, Hann CM, Kaski JC. Low dose imipramine improves chest pain but not quality of life in patients with angina and normal coronary angiograms. *Eur Heart J*. 1998;19:250–254. doi: 10.1053/euhj.1997.0615
46. Montone RA, Rinaldi R, Niccoli G, Andò G, Gragnano F, Piccolo R, Pelliccia F, Moscarella E, Zimarino M, Fabris E, et al. Optimizing management of stable angina: a patient-centered approach integrating revascularization, medical therapy, and lifestyle interventions. *J Am Coll Cardiol*. 2024;84:744–760. doi: 10.1016/j.jacc.2024.06.015
47. Pasceri V, Lanza GA, Buffon A, Montenero AS, Crea F, Maseri A. Role of abnormal pain sensitivity and behavioral factors in determining chest pain in syndrome X. *J Am Coll Cardiol*. 1998;31:62–66. doi: 10.1016/S0735-1097(97)00421-X
48. Lindahl B, Baron T, Albertucci M, Prati F. Myocardial infarction with non-obstructive coronary artery disease. *EuroIntervention*. 2021;17:E875–E887. doi: 10.4244/EIJ-D-21-00426
49. Del Buono MG, La Vecchia G, Rinaldi R, Sanna T, Crea F, Montone RA. Myocardial infarction with nonobstructive coronary arteries: the need for precision medicine. *Curr Opin Cardiol*. 2022;37:481–487. doi: 10.1097/HCO.0000000000000998
50. Del Buono MG, Montone RA, Iannaccone G, Meucci MC, Rinaldi R, D'Amario D, Niccoli G. Diagnostic work-up and therapeutic implications in MINOCA: need for a personalized approach. *Future Cardiol*. 2021;17:149–154. doi: 10.2217/fca-2020-0052
51. Montone RA, Cosentino N, Graziani F, Gorla R, Del Buono MG, La Vecchia G, Rinaldi R, Marenzi G, Bartorelli AL, De Marco F, et al. Precision medicine versus standard of care for patients with myocardial infarction with non-obstructive coronary arteries (MINOCA): rationale and design of the multicentre, randomised PROMISE trial. *EuroIntervention*. 2022;18:e933–e939. doi: 10.4244/EIJ-D-22-00178
52. Ong P, Hubert A, Schwidder M, Beltrame JF. Coronary Spasm: ethnic and sex differences. *Eur Cardiol*. 2023;18:e43. doi: 10.15420/ecr.2023.13