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

Koji Sato, MD; Koichi Kaikita, MD, PhD; Naoki Nakayama, MD, PhD; Eiji Horio, MD; Hiromi Yoshimura, MD; Takamichi Ono, MD, PhD; Keisuke Ohba, MD; Kenichi Tsujita, MD, PhD; Sunao Kojima, MD, PhD; Shinji Tayama, MD, PhD; Seiji Hokimoto, MD, PhD; Kunihiko Matsui, MD, MPH; Seigo Sugiyama, MD, PhD; Hiroshige Yamabe, MD, PhD; Hisao Ogawa, MD, PhD

Background—The aim of this study was to elucidate the correlation between angiographic coronary vasomotor responses to intracoronary acetylcholine (ACh) injection, clinical features, and long-term prognosis in patients with vasospastic angina (VSA).

Methods and Results—This is a retrospective, observational, single-center study of 1877 consecutive patients who underwent ACh-provocation test between January 1991 and December 2010. ACh-provoked coronary spasm was observed in 873 of 1637 patients included in the present analysis. ACh-positive patients were more likely to be older male smokers with dyslipidemia, to have a family history of ischemic heart disease, and to have a comorbidity of coronary epicardial stenosis than were ACh-negative patients. ACh-positive patients were divided into 2 groups: those with focal (total or subtotal obstruction, n=511) and those with diffuse (severe diffuse vasoconstriction, n=362) spasm patterns. Multivariable logistic regression analysis identified female sex and low comorbidity of coronary epicardial stenosis to correlate with the ACh-provoked diffuse spasm pattern in patients with VSA. Kaplan–Meier survival curve indicated better 5-year survival rates free from major adverse cardiovascular events in patients with diffuse spasm pattern compared with those with focal spasm pattern (*P*=0.019). Multivariable Cox hazard regression analysis identified diffuse spasm pattern as a negative predictor of major adverse cardiovascular events in patients with VSA.

Conclusions—ACh-induced diffuse coronary spasm was frequently observed in female VSA patients free of severe coronary epicardial stenosis and was associated with better prognosis than focal spasm. These results suggest the need to identify the ACh-provoked coronary spasm subtypes in patients with VSA. (*J Am Heart Assoc.* 2013;2:e000227 doi: 10.1161/JAHA.113. 000227)

Key Words: acetylcholine-provocation test • coronary spasm • diffuse spasm • prognosis

Coronary artery spasm is an important pathogenic factor in variant angina and ischemic heart diseases (IHDs), as well as other forms of angina pectoris and acute coronary syndrome.[1–9] The precise mechanism responsible for coronary spasm remains largely unknown; however, evidence suggests that the pathogenesis of coronary spasm is in part different from that of coronary atherosclerosis–based stenosis. In fact, significant coronary stenosis is often absent angiographically in patients with vasospastic angina (VSA), especially in those with multivessel coronary spasm.[10]

Previous studies described different patterns of angiographic changes during the spasm provocation test, including focal and diffuse spasm.[5,11–13] The diffuse spasm pattern is reported to be more common in Japanese than in white patients (20% versus 7%),[5] whereas a recent study has reported that coronary spasm is also frequent in European patients without significant epicardial coronary stenosis.[14,15] The focal spasm pattern is associated with a thicker intima-media layer of the coronary artery than the diffuse spasm pattern[12] and is induced in a background of relatively advanced atherosclerotic lesions.[11,12] Sueda et al[13] reported that patients with VSA with

the diffuse spasm pattern show more tolerance to vasodilators than do those with focal spasm type.

Previous studies also identified old age, current smoking, high levels of high-sensitivity C-reactive protein (hs-CRP), and elevated serum levels of high remnant lipoprotein as significant risk factors for VSA.[16–19] Furthermore, a recent multicenter study by the Japanese Coronary Spasm Association reported that long-term prognosis of patients with VSA correlated with smoking, significant coronary epicardial stenosis, ST-elevation during spontaneous attack, history of out-of-hospital cardiac arrest, and provoked multivessel spasm.[20,21] However, to our knowledge, there is no information on the relation between angiographic patterns of acetylcholine (ACh)-induced coronary spasm, clinical features, and long-term outcomes of patients with VSA. In this study, we investigated the correlation between angiographic patterns of coronary vasomotor response induced by intracoronary injection of ACh, clinical features, and long-term prognosis of patients with VSA.

Methods

Study Population and Protocol

We analyzed retrospectively the angiographic coronary vaso-motor response induced by ACh injection in 1877 consecutive patients who had typical or atypical angina–like chest pain who were admitted to Kumamoto University Hospital between January 1991 and December 2010. The risk factors for coronary artery disease (CAD) were defined as current smoking (smoking within 1 year), hypertension (>140/90 mm Hg or taking antihypertensive medications), dyslipidemia (high-density lipoprotein [HDL] cholesterol <40 mg/dL, low-density lipoprotein [LDL] cholesterol ≥140 mg/dL, or triglycerides ≥150 mg/dL or taking medications for dyslipidemia), diabetes mellitus (symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dL, fasting plasma glucose concentration ≥126 mg/dL, 2-hour plasma glucose concentration ≥200 mg/dL during 75 g oral glucose tolerance test, or taking medications for diabetes mellitus), and family history of IHD, including obstructive CAD, VSA, or myocardial infarction.

The study protocol was approved by the Human Ethics Review Committee of Kumamoto University and a signed consent form was obtained from each subject.

Induction of Coronary Spasm

The ACh-provocation test was performed as described previously in the indication and procedure of the VSA Guideline by the Japanese Circulation Society.[22] Coronary spasm was defined as total or subtotal obstruction within the borders of 1 isolated coronary segment as defined by the American Heart Association[23] (focal spasm) or severe diffuse vasoconstriction

(90% stenosis defined by the American Heart Association[23] [76% to 90% narrowing of the luminal diameter]) observed in ≥2 adjacent coronary segments (diffuse spasm) of epicardial coronary arteries associated with transient myocardial ische-mia, as evidenced by ischemic ST-segment changes on the ECG. In the present study, we divided the patients positive for ACh-provocation test into 2 groups based on the pattern of coronary artery spasm on coronary angiography during ACh-provocation test: those with focal and those with nonfocal (diffuse) spasm patterns. Figure 1 shows coronary angio-graphic findings of representative cases of focal and diffuse spasm patterns. Patients who developed ACh-induced focal spasm with or without diffuse spasm in other coronary segments were included into the focal spasm group (Figure 1A through 1C), whereas patients who had only ACh-induced diffuse spasm were included in the diffuse spasm group (Figure 1D through 1F). In this study, ischemic ST-segment changes were defined as ST-segment elevation (>0.1 mV), ST-segment depression (>0.1 mV) from baseline level occur-ring at 60 to 80 ms after J point in at least 2 contiguous leads on the 12-lead ECG, or appearance of a new negative U wave on the ECG. Multivessel spasm was defined as ACh-induced spasm of ≥2 major epicardial arteries. Myocardial lactate production was evidenced by comparing serum lactate con-centrations at the root of the aorta and coronary sinus, sampled during myocardial ischemia induced by ACh-provocation.

Follow-up Data

Follow-up data were obtained directly from the patients, their families, or their family physicians, in addition to the informa-tion available on the medical records, and the physicians blinded to the spasm patterns and medical details available in the medical records performed assessments of the events. The primary end point was major adverse cardiac events (MACE), defined as cardiac death, hospitalization for acute myocardial infarction, or unstable angina pectoris. The time frame in the survival analysis was defined as time from the date of diagnosis until the date of the first event or until December 2012. The secondary end point was all-cause mortality. Cardiac death was defined as sudden death or death associated with acute myocardial infarction. Acute myocardial infarction was defined by the presence of prolonged (>30 minutes) chest pain, associated with ST-segment changes and elevated cardiac enzyme levels. Unstable angina pectoris represented recur-rence or worsening of chest discomfort or pain, associated with ischemic ECG changes.

Statistical Analysis

Data for normally distributed continuous variables were expressed as mean±SD, whereas those with skewed
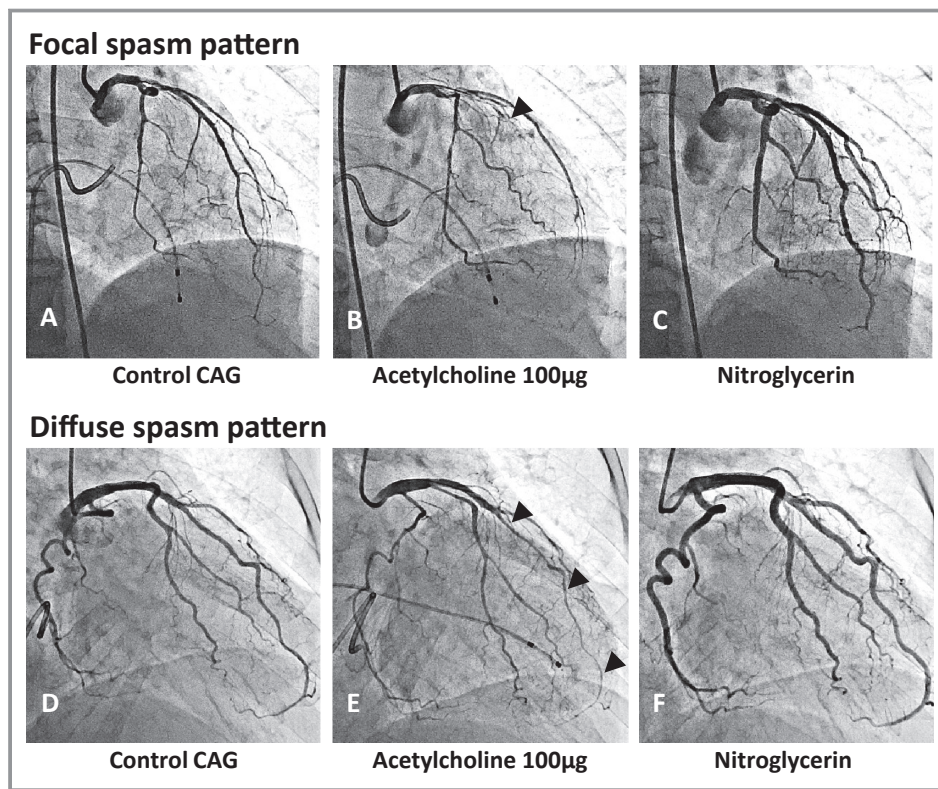
Figure 1. Acetylcholine (ACh)-induced focal and diffuse spasm patterns. A through C, Focal spasm pattern. B, Injection of 100 μg ACh into the left coronary artery induced focal spasm in the proximal site of the left anterior descending artery. D through F, Diffuse spasm pattern. E, Injection of 100 μg ACh into the left coronary artery induced diffuse spasm in the whole left coronary artery including the left circumflex artery. CAG indicates coronary angiography.

distribution were expressed as median values (interquartile range). Continuous variables were analyzed by the unpaired *t* test or Mann–Whitney *U* test, as appropriate. Categorical variables were presented by percentage values, and inter-group comparisons were analyzed by using the χ^2 test or Fisher's exact test as appropriate. Age, sex, and the relationships between the results of ACh-provocation test and other significant parameters in simple logistic analysis, between the angiographic type of coronary spasm and other significant parameters in simple logistic analysis, were entered into multivariable logistic regression analysis using the backward-selection entry method ($P<0.05$ for stay), and the Hosmer-Lemeshow goodness-of-fit statistic was calculated. Survival was analyzed by the Kaplan–Meier survival curve with log-rank test. The multivariable Cox hazard regression analysis was carried out for identification of predictors of outcomes. Significant variables according to univariate analysis and found to be involved in VSA outcome were subjected to the backward-selection entry method. A *P* value of <0.05 denoted statistical significance; all tests were 2-tailed. Statistical analyses were performed by using Statistical Package of the Social Science version 19.0 (SPSS).

Results

Prevalence of ACh-Provoked Coronary Spasm and Spasm Patterns

Figure 2 provides a flow chart of the patient recruitment process. Among 1877 participants, we excluded 117 patients for the following reasons: acute myocardial infarction (n=20), cardiomyopathy (n=75), Brugada syndrome (n=10), myocarditis (n=1), pulmonary thromboembolism (n=3), peripheral artery disease (n=1), secondary hypertension (n=2), chronic stable aortic dissection (n=1), vertigo (n=1), abdominal aortal aneurysm (n=1), and familial hypercholesterolemia (n=1). Therefore, data for 1760 patients (mean±SD age 63.0± 11.0 years) who had undergone selective ACh-provocation test were analyzed. A positive ACh-provocation test was recorded in 873 of 1760 patients. Of the remaining 887 patients who did not show epicardial coronary spasm after intracoronary injection of ACh, 123 were suspected to have developed coronary microvascular spasm or dysfunction because they had significant myocardial lactate production (although without epicardial coronary spasm) during the ACh-provocation test. Therefore, 764 patients were defined as the nonspasm group that was truly negative for ACh-provocation
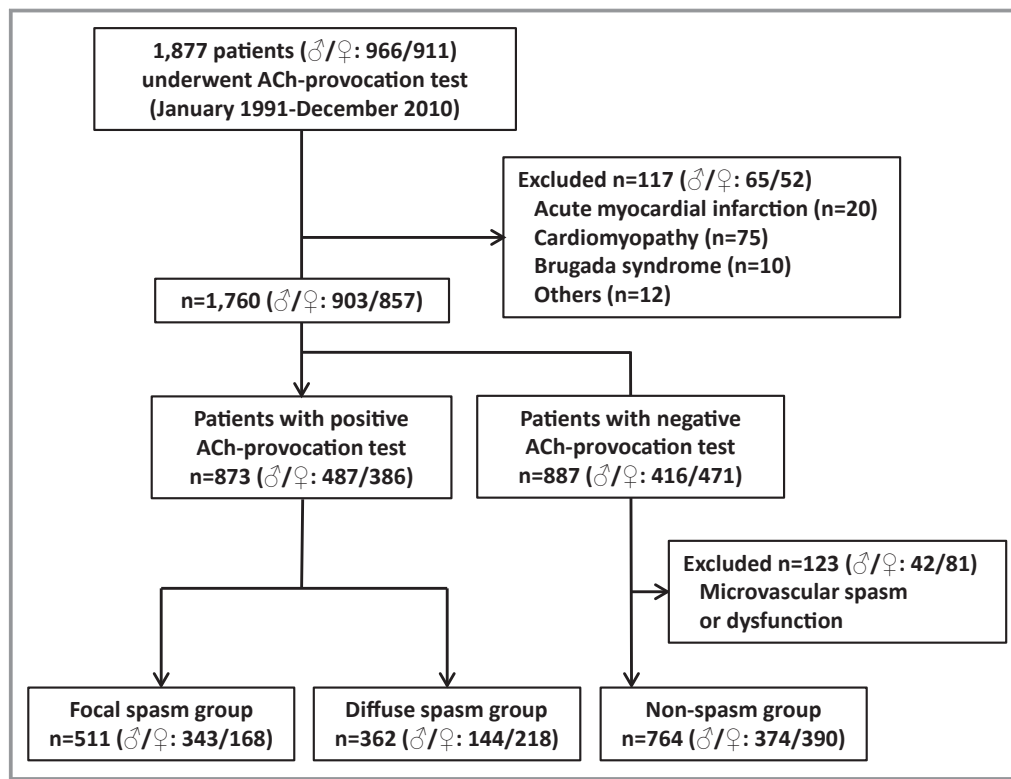
Figure 2. Flow chart of the study recruitment process. ACh indicates acetylcholine.

test. Among the 873 ACh-positive patients, 754, 418, and 299 patients were ACh-positive for left, right, and both coronary arteries, respectively. Among the 873 ACh-positive patients, 26 (3%) did not undergo ACh-provocation test in the right coronary artery because injection of ACh into the left coronary artery resulted in prolonged left coronary spasm or spasm that consequently induced ventricular tachycardia or fibrillation. The ACh-positive patients were divided into the focal (n=511) and diffuse spasm (n=362) groups based on the coronary angiographic spasm pattern during ACh-provocation test.

Clinical Characteristics of the Study Population

Table 1 shows the clinical characteristics of ACh-positive (n=873, 53%) and nonspasm (n=764, 47%) groups. Age, male sex, current smoking, dyslipidemia, family history of IHD, and comorbidity of coronary epicardial stenosis were significantly higher in the ACh-positive group than in the nonspasm group. On the other hand, serum hemoglobin A1c level, triglyceride level, and left ventricular ejection fraction (LVEF) measured with left ventriculography were significantly lower in the ACh-positive group than in the nonspasm group. Cardiac death and nonfatal myocardial infarction during the ACh-provocation test were not encountered in this study.

Predictive Factors of ACh-Provoked Coronary Spasm

Simple logistic regression analysis demonstrated that old age, male sex, current smoking, dyslipidemia, family history of IHD, and comorbidity of coronary epicardial stenosis correlated significantly with the ACh-provoked coronary spasm (Table 2). Multivariable logistic regression analysis identified old age (odds ratio [OR] 1.016, 95% CI 1.006 to 1.026; $P=0.001$), current smoking (OR 1.436, 95% CI 1.165 to 1.769; $P=0.001$), dyslipidemia (OR 1.473, 95% CI 1.200 to 1.807; $P<0.001$), family history of IHD (OR 1.791, 95% CI 1.316 to 2.437; $P<0.001$), and comorbidity of coronary epicardial stenosis (OR 1.857, 95% CI 1.425 to 2.420; $P<0.001$) as significant predictors of ACh-provoked coronary spasm (Table 2). Hosmer–Lemeshow goodness-of-fit $\chi2$ was 10.613 with a P value of 0.225.

ACh-Induced Coronary Spasm Patterns and Clinical Features

Table 3 details the clinical characteristics of the ACh-positive patients according to the angiographic pattern of ACh-induced coronary artery spasm. Age, male sex, current smoking, hypertension, dyslipidemia, family history of IHD, and comorbidity of coronary epicardial stenosis were significantly higher

Table 1. Patients' Characteristics

	ACh-Positive, n=873 (53%)	Nonspasm, n=764 (47%)	P Value
Age, mean y (SD)	63.8 (10.4)	62.2 (11.5)	0.002
Female sex, n (%)	386 (44)	390 (51)	0.006
Body mass index, mean kg/m^2 (SD)	23.7 (3.6)	23.6 (4)	0.594
Current smoking, n (%)	460 (53)	334 (44)	0.001
Diabetes mellitus, n (%)	179 (21)	137 (18)	0.183
Hypertension, n (%)	362 (42)	289 (38)	0.151
Dyslipidemia, n (%)	406 (47)	277 (36)	<0.001
Family history of IHD, n (%)	145 (17)	74 (10)	<0.001
Systolic blood pressure, mean mm Hg (SD)	127.6 (18.5)	129.4 (19.6)	0.055
Diastolic blood pressure, mean mm Hg (SD)	74.3 (11.5)	75.3 (11.9)	0.097
Fasting blood glucose, median mg/dL (IQR)	92 (85 to 100)	93 (85 to 107)	0.757
Hemoglobin A1c, median % (IQR)	5.8 (5.5 to 6.2)	6.0 (5.6 to 6.5)	<0.001
Total cholesterol, median mg/dL (IQR)	190 (169 to 215)	191 (161 to 219)	0.687
LDL cholesterol, median mg/dL (IQR)	109 (92 to 132)	109 (85 to 134)	0.424
HDL cholesterol, median mg/dL (IQR)	53 (43 to 65)	51 (42 to 63)	0.379
Triglycerides, median mg/dL (IQR)	106 (77 to 154)	115 (81 to 159)	0.021
hs-CRP, median mg/L (IQR)	0.6 (0.4 to 1.6)	0.8 (0.5 to 1.9)	0.046
eGFR, median mL/min per 1.73 m^2 (IQR)	72 (62 to 85)	71 (62 to 82)	0.224
Epicardial stenosis ≥75%, n (%)	232 (27)	107 (14)	<0.001
Left ventricular ejection fraction, mean % (SD)	71.1 (10.9)	73.1 (9.7)	<0.001

ACh indicates acetylcholine; IHD, ischemic heart disease; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

in patients of the focal spasm group than in those of the nonspasm group, whereas serum hemoglobin A1c levels, serum hs-CRP levels, and LVEF were significantly lower in the focal spasm group than in the nonspasm group. On the other hand, old age, female sex, dyslipidemia, family history of IHD, and serum HDL cholesterol levels were significantly higher in the diffuse spasm group than in the nonspasm group, whereas diastolic blood pressure, serum hemoglobin A1c levels, serum

Table 2. Results of Simple and Multivariable Regression Analyses for ACh-Positive Findings in the Study Population

	Simple Regression Analysis			Multivariable Regression Analysis		
	OR	95% CI	P Value	OR	95% CI	P Value
Age	1.014	1.005 to 1.023	0.002	1.016	1.006 to 1.026	0.001
Female sex	0.76	0.626 to 0.924	0.006	Not selected		
Body mass index ≥25 kg/m^2	0.925	0.748 to 1.144	0.472			
Current smoking	1.411	1.160 to 1.716	0.001	1.436	1.165 to 1.769	0.001
Diabetes mellitus	1.183	0.924 to 1.515	0.183			
Hypertension	1.157	0.948 to 1.412	0.151			
Dyslipidemia	1.526	1.251 to 1.861	<0.001	1.473	1.200 to 1.807	<0.001
Family history of IHD	1.833	1.359 to 2.471	<0.001	1.791	1.316 to 2.437	<0.001
eGFR <60 mL/min per 1.73 m^2	1.065	0.823 to 1.378	0.634			
Epicardial stenosis ≥75%	2.222	1.725 to 2.864	<0.001	1.857	1.425 to 2.420	<0.001

ACh indicates acetylcholine; OR, odds ratio; IHD, ischemic heart disease; eGFR, estimated glomerular filtration rate.

Table 3. Clinical Characteristics of Patients With Diffuse and Focal Spasm Patterns

| | | ACh-Positive | | |
| | | FS | DS | |
	Nonspasm, n=764	n=511 (59%)	n=362 (41%)	P Value*
Age, mean y (SD)	62.2 (11.5)	63.6 (10.5)[†]	64.1 (10.1)[†]	0.488
Female sex, n (%)	390 (51)	168 (33)[†]	218 (60)[†]	<0.001
Body mass index, mean kg/m^2 (SD)	23.6 (3.5)	23.7 (3.2)	23.7 (4.0)	0.809
Current smoking, n (%)	334 (44)	313 (61)[†]	147 (41)	<0.001
Diabetes mellitus, n (%)	137 (18)	110 (22)	69 (19)	0.383
Hypertension, n (%)	289 (38)	227 (44)[†]	135 (37)	0.035
Dyslipidemia, n (%)	277 (36)	246 (48)[†]	160 (44)[†]	0.282
Family history of IHD, n (%)	74 (10)	79 (16)[†]	66 (18)[†]	0.276
Systolic blood pressure, mean mm Hg (SD)	129.4 (19.6)	127.7 (18.8)	127.4 (18.2)	0.865
Diastolic blood pressure, mean mm Hg (SD)	75.3 (11.9)	75.1 (11.3)	73.1 (11.6)[†]	0.011
Fasting blood glucose, median mg/dL (IQR)	93 (85 to 107)	93 (86 to 102)	90 (84 to 98)	0.07
Hemoglobin A1c, median % (IQR)	6.0 (5.6 to 6.5)	5.8 (5.6 to 6.2)[†]	5.7 (5.5 to 6.1)[†]	0.449
Total cholesterol, median mg/dL (IQR)	191 (161 to 219)	187 (165 to 213)	195 (178 to 219)	0.013
LDL cholesterol, median mg/dL (IQR)	109 (85 to 134)	108 (86 to 132)	111 (97 to 133)	0.14
HDL cholesterol, median mg/dL (IQR)	51 (42 to 63)	53 (42 to 63)	56 (45 to 69)[†]	0.002
Triglycerides, median mg/dL (IQR)	115 (81 to 159)	112 (77 to 156)	99 (76 to 153)[†]	0.044
hs-CRP, median mg/L (IQR)	0.8 (0.5 to 1.9)	0.6 (0.3 to 1.5)[†]	0.6 (0.4 to 1.6)[†]	0.68
eGFR, median mL/min per 1.73 m^2 (IQR)	71 (62 to 82)	72 (63 to 85)	72 (62 to 85)	0.769
Epicardial stenosis ≥75%, n (%)	107 (14)	170 (33)[†]	62 (17)	<0.001
Spasm at site of epicardial stenosis, n (%)	0 (0)	91/170 (54)	34/62 (55)	0.859
Left ventricular ejection fraction, mean % (SD)	73.1 (9.7)	70.1 (10.7)[†]	72.5 (11.0)	0.003
Multivessel spasm, n (%)	0 (0)	200 (39)	170 (47)	0.021
Myocardial lactate production, n (%)[‡]	0 (0)	183 (57)	169 (66)	0.049

ACh indicates acetylcholine; FS, focal spasm group; DS, diffuse spasm group; SD, standard deviation; IHD, ischemic heart disease; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.
*FS group vs DS group.
[†]P<0.05 vs nonspasm group.
[‡]Independent results of ACh-positive group in the left coronary artery.

triglyceride levels, and serum hs-CRP levels were significantly lower in the diffuse spasm group than in the nonspasm group. The percentage of female sex, serum total cholesterol levels, serum HDL cholesterol levels, LVEF, multivessel spasm, and myocardial lactate production were significantly higher in patients of the diffuse spasm group than in those of the focal spasm group, whereas current smoking, hypertension, diastolic blood pressure, serum triglyceride levels, and comorbidity of coronary epicardial stenosis were significantly lower in the diffuse spasm group than in the focal spasm group.

Table 4 compares the symptoms and ECG changes during spontaneous attack and ACh-provocation test in patients with VSA with diffuse and focal spasm patterns. ST-segment elevation during spontaneous attack was more frequently observed in the focal spasm group than in the diffuse spasm group (P=0.007). During ACh-provocation test, symptoms similar to those encountered during spontaneous attacks were more frequently observed in the focal spasm group than in the diffuse spasm group (P=0.004). Furthermore, ST-segment elevation and life-threatening arrhythmia were more frequently observed during ACh-provocation test in the focal spasm group than in the diffuse spasm group (P<0.001 and P=0.008, respectively).

Table 5 shows the results of simple and multivariable logistic regression analyses for the diffuse spasm group. Simple logistic regression analysis demonstrated that female sex, current smoking, hypertension, comorbidity of coronary epicardial stenosis, and myocardial lactate production were associated with ACh-induced diffuse spasm pattern in ACh-positive

Table 4. Symptoms and ECG Changes During Spontaneous Attack and ACh-Provocation Test With Diffuse and Focal Spasm Patterns

| | | ACh-Positive | | |
| | | FS | DS | |
	Total (n=873)	n=511 (59%)	n=362 (41%)	P Value*
Condition at time of chest symptoms, n (%)				
Rest	644 (74)	385 (75)	259 (72)	0.209
Effort	146 (17)	79 (16)	67 (19)	0.234
Rest and effort	83 (10)	47 (9)	36 (10)	0.711
ST-segment change during spontaneous attack, n (%)				
ST elevation	89 (10)	64 (13)	25 (7)	0.007
ST depression	95 (11)	55 (11)	40 (11)	0.893
Life-threatening arrhythmia during spontaneous attack, n (%)	12 (1)	5 (1)	7 (2)	0.184
Symptom during ACh-provocation test				
Symptoms similar to spontaneous attack, yes, n (%)	583 (67)	361 (71)	222 (61)	0.004
ST-segment change during ACh-provocation test, n (%)				
ST elevation	279 (32)	217 (43)	62 (17)	<0.001
ST depression	594 (68)	294 (58)	300 (83)	<0.001
Life-threatening arrhythmia during ACh-provocation test, n (%)	9 (1)	9 (2)	0 (0)	0.008

ACh indicates acetylcholine; FS, focal spasm group; DS, diffuse spasm group.
*FS group vs DS group.

patients. Furthermore, multivariable logistic regression analysis identified female sex (OR 2.928, 95% CI 2.116 to 4.054; $P<0.001$) and comorbidity of coronary epicardial stenosis (OR 0.562, 95% CI 0.380 to 0.832; $P=0.004$) as significant predictors of the diffuse spasm pattern. Hosmer–Lemeshow goodness-of-fit χ^2 was 0.584 with a P value of 0.747.

Clinical Outcome of Patients With VSA Diagnosed by ACh-Provocation Test

During a mean follow-up period of 49 ± 19 months, MACE was registered in 43 patients (cardiac death, n=3; myocardial infarction, n=6; and unstable angina, n=34). Noncardiac deaths

Table 5. Results of Simple and Multivariable Regression Analyses for the Diffuse Spasm Pattern in ACh-Provocation–Positive Patients

| | Simple Regression Analysis | | | Multivariable Regression Analysis | | |
	OR	95% CI	P Value	OR	95% CI	P Value
Age	1.005	0.992 to 1.018	0.488	Not selected		
Female sex	3.091	2.336 to 4.089	<0.001	2.928	2.116 to 4.054	<0.001
Body mass index ≥25 kg/m^2	0.804	0.596 to 1.084	0.152			
Current smoking	0.432	0.328 to 0.569	<0.001	Not selected		
Diabetes mellitus	0.861	0.615 to 1.206	0.383			
Hypertension	0.744	0.565 to 0.980	0.035	Not selected		
Dyslipidemia	0.862	0.657 to 1.130	0.282			
Family history of IHD	1.221	0.853 to 1.747	0.276			
eGFR <60 mL/min per 1.73 m^2	0.951	0.669 to 1.353	0.782			
Epicardial stenosis $\geq75\%$	0.415	0.298 to 0.576	<0.001	0.562	0.380 to 0.832	0.004
Myocardial lactate production	1.503	1.101 to 2.051	0.01	Not selected		

ACh indicates acetylcholine; OR, odds ratio; IHD, ischemic heart disease; eGFR, estimated glomerular filtration rate.

Table 6. Clinical Features of Vasospastic Angina Patients With or Without MACE

	With MACE, n=43 (5%)	Without MACE, n=830 (95%)	P Value
Age, mean y (SD)	61.0 (11.7)	64.0 (10.3)	0.067
Female sex, n (%)	14 (33)	372 (45)	0.114
Body mass index, mean kg/m² (SD)	23.1 (3.2)	23.7 (3.6)	0.304
Current smoking, n (%)	29 (67)	432 (52)	0.049
Diabetes mellitus, n (%)	8 (19)	171 (21)	0.734
Hypertension, n (%)	19 (44)	343 (41)	0.71
Dyslipidemia, n (%)	20 (47)	386 (47)	0.989
Family history of IHD, n (%)	6 (14)	139 (17)	0.627
Systolic blood pressure, mean mm Hg (SD)	133.4 (20.4)	127.3 (18.4)	0.035
Diastolic blood pressure, mean mm Hg (SD)	76.3 (10.3)	74.2 (11.5)	0.254
Fasting blood glucose, median mg/dL (IQR)	93 (88 to 105)	92 (84 to 100)	0.453
Hemoglobin A1c, median % (IQR)	5.6 (5.4 to 5.9)	5.8 (5.5 to 6.2)	0.907
Total cholesterol, median mg/dL (IQR)	196 (172 to 213)	190 (169 to 215)	0.772
LDL cholesterol, median mg/dL (IQR)	116 (95 to 135)	109 (169 to 215)	0.971
HDL cholesterol, median mg/dL (IQR)	43 (39 to 49)	54 (44 to 65)	0.062
Triglycerides, median mg/dL (IQR)	164 (115 to 190)	103 (77 to 152)	0.251
hs-CRP, median mg/L (IQR)	0.5 (0.4 to 0.9)	0.6 (0.3 to 1.6)	0.424
eGFR, median mL/min per 1.73 m² (IQR)	87 (65 to 102)	72 (62 to 85)	0.909
Epicardial stenosis ≥75%, n (%)	19 (44)	213 (26)	0.007
Left ventricular ejection fraction, mean % (SD)	71.4 (10.7)	71.1 (10.9)	0.857
Multivessel spasm, n (%)	21 (49)	349 (42)	0.38
Diffuse spasm, n (%)	11 (26)	351 (42)	0.03
Variant angina, n (%)	9 (21)	80 (10)	0.017
Myocardial lactate production, n (%)	18 (60)	360 (56)	0.665

MACE indicates major adverse cardiovascular events; IHD, ischemic heart disease; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

were recorded in 27 patients. The complete follow-up rate was 91% for 2-year follow-up, 85% for 3-year follow-up, and 75% for 5-year follow-up. The median follow-up period of patients who did not have an event was 60 months (range 48 to 60 months). The frequencies of current smoking, organic stenosis, variant angina, and high systolic blood pressure were significantly higher in the patients with VSA with MACE than in those without MACE, whereas the frequencies of diffuse spasm pattern were significantly lower in the patients with VSA with MACE than those without MACE (Table 6). The 5-year survival rates free from MACE and all-cause death were 94.2% and 96.4%, respectively. Kaplan–Meier survival curve demonstrated significantly higher incidence of MACE in patients of the focal spasm group (P=0.019 by log-rank test) (Figure 3). Univariate Cox hazard analysis showed that smoking, comorbidity of coronary epicardial stenosis, diffuse spasm subtype, and variant angina, but not myocardial lactate production, were closely associated with the incidence of MACE. Multivariable

Cox hazard regression analysis identified diffuse spasm subtype as a significant negative predictor of MACE (hazard ratio 0.348, 95% CI 0.140 to 0.863; P=0.023) (Table 7).

Discussion

The coronary spasm provocation test, involving intracoronary injection of ACh or ergonovine, is used in clinical cardiovascular practice for the diagnosis of VSA.[24–28] However, the relationship between angiographic patterns of ACh-provoked coronary spasm and the clinical features of patients with VSA has not been thoroughly investigated. In the present study, we analyzed 1877 consecutive patients who had undergone the ACh-provocation test in a single center during the past 20 years. To the best of our knowledge, this is the first report that describes the relation between the pattern of ACh-provoked coronary spasm and the clinical characteristics of VSA in a large study population.
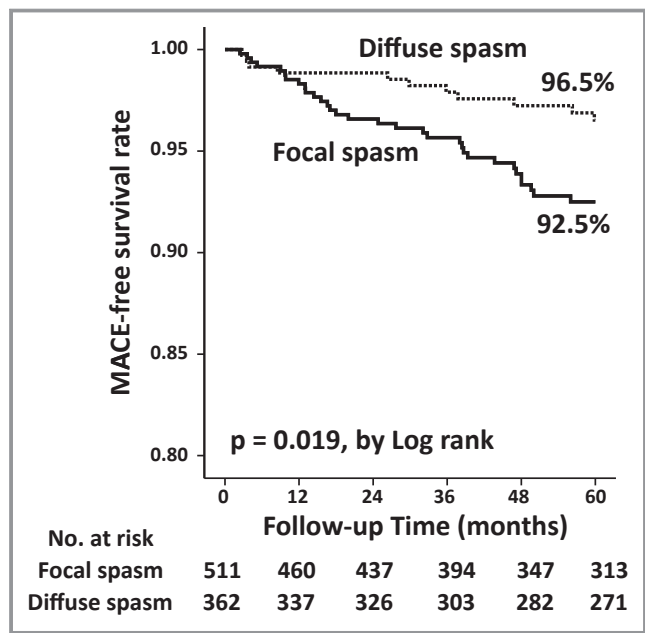
Figure 3. Kaplan–Meier curve for MACE-free survival in patients with VSA during the follow-up period according to diffuse or focal spasm pattern. MACE indicates major adverse cardiovascular events; VSA, vasospastic angina.

The present study demonstrated a positive ACh-provocation test in 53% of Japanese patients with history of angina-like chest pain, a rate higher than that described in a previous study.[27] One potential reason for the higher rate is

that the washout period for calcium channel blockers and nitrates was longer ($\approx$1 week in our study), which was set to enhance coronary vasomotor response to intracoronary ACh injection. Another reason could be related to the inclusion in our study of the diffuse spasm pattern as a criterion for a positive ACh-provoked coronary spasm.

In the present study, the factors that correlated with positive ACh-provocation test, such as age, current smoking, dyslipidemia, family history of IHD, and comorbidity of coronary epicardial stenosis, were similar to the general coronary risk factors of CAD. Furthermore, coronary spasm was provoked at a site similar to that of coronary epicardial stenosis in >50% of the patients with VSA with significant epicardial stenosis, a finding consistent with the report by MacAlpin et al.[29] However, further studies are needed to prove the relation between coronary spasm and progression of coronary atherosclerosis.

We divided ACh-positive patients into 2 groups according to the angiographic patterns of ACh-induced coronary artery spasm: focal and diffuse spasm patterns. The former was observed in patients with VSA with general coronary risk factors of CAD, while the latter was seen in female patients with VSA with less coronary risk factors and was more likely to be a multivessel spasm. Analysis of the data of the 2 groups indicated that the ACh-induced diffuse spasm pattern could represent the consequence of endothelial dysfunction rather than focal spasm because the sites of diffuse spasm were not likely to be accompanied by significant coronary

Table 7. Results of Univariate and Multivariable Cox Hazard Regression Analyses for MACE

	Univariate Analysis			Multivariable Analysis		
	OR	95% CI	P Value	OR	95% CI	P Value
Age	0.973	0.947 to 1.000	0.051	Not selected		
Female sex	0.560	0.296 to 1.061	0.075	Not selected		
Body mass index $\geq$25 kg/m^2	0.595	0.284 to 1.247	0.169			
Current smoking	2.096	1.107 to 3.966	0.023	2.206	0.997 to 4.881	0.051
Diabetes mellitus	0.902	0.419 to 1.945	0.793			
Hypertension	1.124	0.616 to 2.053	0.703			
Dyslipidemia	0.956	0.525 to 1.742	0.884			
Family history of IHDs	0.808	0.341 to 1.914	0.628			
eGFR <60 mL/min per 1.73 m^2	1.120	0.530 to 2.365	0.767			
Epicardial stenosis $\geq$75%	2.314	1.267 to 4.224	0.006	Not selected		
LVEF >50%	1.167	0.282 to 4.831	0.831			
Diffuse spasm	0.450	0.227 to 0.894	0.023	0.348	0.140 to 0.863	0.023
Multivessel spasm	1.239	0.681 to 2.254	0.482	2.027	0.970 to 4.233	0.060
Myocardial lactate production	1.135	0.547 to 2.357	0.733	Not selected		
Variant angina	2.424	1.163 to 5.054	0.018	Not selected		

MACE indicates major adverse cardiovascular events; OR, odds ratio; IHD, ischemic heart disease; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.

epicardial stenosis. Previous reports[30,31] identified severe coronary endothelial dysfunction as a predictor of cardiovascular events in patients with and without CAD.

The prevalence of coronary epicardial stenosis is reported to be higher in white patients with VSA than in their Japanese counterparts.[5,22] On the other hand, race-related differences in the prevalence of VSA between Japanese and whites have been reported.[5,26,27,32] The results of drug-induced coronary spasm provocation tests using ACh or ergonovine indicate higher incidence of coronary spasm in Japanese than in whites.[26,27,32] Pristipino et al[5] reported that coronary spasm was observed in 37% of whites and 80% of Japanese during the ACh-provocation test conducted early after the onset of myocardial infarction, indicating a high prevalence of coronary spasm in Japanese. On the other hand, a recent report has shown that coronary spasm is also frequent in European patients without significant coronary epicardial stenosis.[14,15] The ACOVA Study (Abnormal COronary VAsomotion in patients with stable angina and unobstructed coronary arteries)[15] reported that 62% of patients with stable angina and unobstructed CAD who had exercise-related anginal symptoms had a positive ACh-provocation test (45% epicardial and 55% microvascular spasm) and that those patients with evidence of abnormal coronary vascular tone were more likely to be females . Although it is difficult to compare directly the ACOVA and the present study because the inclusion criteria of the study population and the protocol of ACh-provocation test were different, functional abnormalities of the coronary arteries should be considered in the differential diagnosis in both Japanese and white patients with stable angina and unobstructed CAD. Based on these findings, an international collaborative study seems to be necessary to establish the criteria for the diagnosis of VSA and to compare the prevalence of VSA in a larger study population.

The present study demonstrated that there was a higher proportion of patients with VSA of the diffuse spasm group with myocardial lactate production during the ACh-provocation test compared with the focal spasm group. At present, the diffuse spasm pattern is not included as a positive criterion for ACh-provocation test in the current VSA guideline.[22] The results of the present study point to the need to include the diffuse spasm pattern in the criteria of a positive ACh-provocation test for the diagnosis of patients with VSA. Furthermore, measurement of serum lactate levels in the coronary circulation might be helpful as a supporting diagnostic test, in addition to ACh-provoked angiographic findings and ischemic ST-segment changes on the ECG. However, in the present study, ACh-provoked myocardial lactate production was not associated with the incidence of MACE. These results suggest that myocardial lactate production might have a diagnostic value for the evidence of ACh-provoked myocardium ischemia but not influence the prognosis of patients with VSA treated with coronary vasodilator agents.

In the present study, MACE was registered in 43 patients (5.8%) during a mean follow-up period of 49 ± 19 months. In this regard, a recent study from the Japanese Coronary Spasm Association[21] reported that 69 of 1244 patients (5.5%) with VSA developed MACE during a median follow-up period of 32 months, indicating that our results are almost identical to those of the above study.[21] It is possible that the low MACE rate in the 2 studies was due to medical treatment (eg, calcium channel blockers and long-acting nitrates) and the high rate of complete follow-up.

In the present study, Kaplan–Meier survival curve indicated a high incidence of MACE in the focal spasm group. Furthermore, multivariable Cox hazard regression analysis identified diffuse spasm pattern as a significant negative predictor of MACE in patients with VSA. The result of the correlation between angiographically confirmed ACh-induced coronary spasm subtype (focal and diffuse spasm) and long-term prognosis of patients with VSA is new and interesting information. In this regard, a recent study on 1244 patients with VSA conducted by the Japanese Coronary Spasm Association[21] reported that ACh- or ergonovine-provoked diffuse spasm did not correlate with the incidence of MACE during a median follow-up period of 32 months (the provocation test was performed with either ACh [57%] or ergonovine [40%] in their study). Furthermore, Sueda et al[33] reported that the site and pattern of coronary spasm provoked by ACh and ergonovine are different even in the same coronary artery of the same patients with VSA, suggesting the need to evaluate the relation between the angiographically confirmed drug-induced coronary spasm subtype and the prognosis of patients with VSA after unifying the drug-provocation test.

The present study has certain limitations. First, the study was retrospective study design. Therefore, we could not examine the MACE rate in nonspasm patients. Although we followed most of patients with VSA to treat with coronary vasodilator agents, it was difficult to follow the nonspasm patients because they did not regularly visit the hospital for the medical treatment. Second, the causal relationship between medications and pathological condition was not fully investigated. Third, we could not confirm whether diffuse spasm occurred at a site distal to focal occlusion. Fourth, we did not perform intravascular ultrasound or optical coherence tomography to investigate the relationship between the structure of the spasm site and ACh-induced spasm subtype. Fifth, outcome analysis had low statistical power due to the small number of events. A prospective multicenter study in a large study population to investigate the clinical features, structural analysis, and prognosis of patients with VSA with focal and diffuse spasm patterns should be conducted in the future.

In conclusion, although the factors that contribute to a positive ACh-provocation test were similar to the general coronary risk factors of CAD, patients with VSA with angiographically confirmed ACh-induced diffuse coronary spasm during the provocation test seem to have different clinical features and prognosis from those of the focal spasm pattern, highlighting the need to identify the ACh-provoked coronary spasm subtypes in patients with VSA.

Disclosures

None.

References

1. Yasue H, Omote S, Takizawa A, Nagao M. Coronary arterial spasm in ischemic heart disease and its pathogenesis. A review. *Circ Res*. 1983;52: I147–I152.

2. Bertrand ME, Lablanche JM, Tilmant PY, Thieuleux FA, Delforge MG, Chahine RA. The provocation of coronary arterial spasm in patients with recent transmural myocardial infarction. *Eur Heart J*. 1983;4:532–535.

3. Maseri A, L'Abbate A, Baroldi G, Chierchia S, Marzilli M, Ballestra AM, Severi S, Parodi O, Biagini A, Distante A, Pesola A. Coronary vasospasm as a possible cause of myocardial infarction. A conclusion derived from the study of "preinfarction" angina. *N Engl J Med*. 1978;299:1271–1277.

4. Oliva PB. The role of coronary artery spasm in acute myocardial infarction. *Cardiovasc Clin*. 1983;14:45–58.

5. Pristipino C, Beltrame JF, Finocchiaro ML, Hattori R, Fujita M, Mongiardo R, Cianflone D, Sanna T, Sasayama S, Maseri A. Major racial differences in coronary constrictor response between japanese and caucasians with recent myocardial infarction. *Circulation*. 2000;101:1102–1108.

6. Yasue H, Touyama M, Kato H, Tanaka S, Akiyama F. Prinzmetal's variant form of angina as a manifestation of alpha-adrenergic receptor-mediated coronary artery spasm: documentation by coronary arteriography. *Am Heart J*. 1976; 91:148–155.

7. Yasue H, Touyama M, Shimamoto M, Kato H, Tanaka S. Role of autonomic nervous system in the pathogenesis of Prinzmetal's variant form of angina. *Circulation*. 1974;50:534–539.

8. Ogawa H, Yasue H, Oshima S, Okumura K, Matsuyama K, Obata K. Circadian variation of plasma fibrinopeptide A level in patients with variant angina. *Circulation*. 1989;80:1617–1626.

9. Soejima H, Irie A, Miyamoto S, Kajiwara I, Kojima S, Hokamaki J, Sakamoto T, Tanaka T, Yoshimura M, Nishimura Y, Ogawa H. Preference toward a T-helper type 1 response in patients with coronary spastic angina. *Circulation*. 2003;107:2196–2200.

10. Okumura K, Yasue H, Horio Y, Takaoka K, Matsuyama K, Kugiyama K, Fujii H, Morikami Y. Multivessel coronary spasm in patients with variant angina: a study with intracoronary injection of acetylcholine. *Circulation*. 1988;77:535–542.

11. Koizumi T, Yokoyama M, Namikawa S, Kuriyama N, Nameki M, Nakayama T, Kaneda H, Sudhir K, Yock PG, Komiyama N, Fitzgerald PJ. Location of focal vasospasm provoked by ergonovine maleate within coronary arteries in patients with vasospastic angina pectoris. *Am J Cardiol*. 2006;97:1322–1325.

12. Koyama J, Yamagishi M, Tamai J, Kawano S, Daikoku S, Miyatake K. Comparison of vessel wall morphologic appearance at sites of focal and diffuse coronary vasospasm by intravascular ultrasound. *Am Heart J*. 1995; 130:440–445.

13. Sueda S, Kohno H, Fukuda H, Watanabe K, Ochi N, Kawada H, Uraoka T. Limitations of medical therapy in patients with pure coronary spastic angina. *Chest*. 2003;123:380–386.

14. Ong P, Athanasiadis A, Hill S, Vogelsberg H, Voehringer M, Sechtem U. Coronary artery spasm as a frequent cause of acute coronary syndrome: the CASPAR (Coronary Artery Spasm in Patients With Acute Coronary Syndrome) Study. *J Am Coll Cardiol*. 2008;52:523–527.

15. Ong P, Athanasiadis A, Borgulya G, Mahrholdt H, Kaski JC, Sechtem U. High prevalence of a pathological response to acetylcholine testing in patients with stable angina pectoris and unobstructed coronary arteries. The ACOVA Study (Abnormal COronary VAsomotion in patients with stable angina and unobstructed coronary arteries). *J Am Coll Cardiol*. 2012;59: 655–662.

16. Hung MJ, Cherng WJ, Yang NI, Cheng CW, Li LF. Relation of high-sensitivity C-reactive protein level with coronary vasospastic angina pectoris in patients without hemodynamically significant coronary artery disease. *Am J Cardiol*. 2005;96:1484–1490.

17. Miwa K, Makita T, Ishii K, Okuda N, Taniguchi A. High remnant lipoprotein levels in patients with variant angina. *Clin Cardiol*. 2004;27:338–342.

18. Oi K, Shimokawa H, Hiroki J, Uwatoku T, Abe K, Matsumoto Y, Nakajima Y, Nakajima K, Takeichi S, Takeshita A. Remnant lipoproteins from patients with sudden cardiac death enhance coronary vasospastic activity through upregulation of Rho-kinase. *Arterioscler Thromb Vasc Biol*. 2004;24:918–922.

19. Sugiishi M, Takatsu F. Cigarette smoking is a major risk factor for coronary spasm. *Circulation*. 1993;87:76–79.

20. Takagi Y, Yasuda S, Tsunoda R, Ogata Y, Seki A, Sumiyoshi T, Matsui M, Goto T, Tanabe Y, Sueda S, Sato T, Ogawa S, Kubo N, Momomura S, Ogawa H, Shimokawa H. Clinical characteristics and long-term prognosis of vasospastic angina patients who survived out-of-hospital cardiac arrest: multicenter registry study of the Japanese Coronary Spasm Association. *Circ Arrhythm Electrophysiol*. 2011;4:295–302.

21. Takagi Y, Yasuda S, Takahashi J, Tsunoda R, Ogata Y, Seki A, Sumiyoshi T, Matsui M, Goto T, Tanabe Y, Sueda S, Sato T, Ogawa S, Kubo N, Momomura SI, Ogawa H, Shimokawa H. 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;34:258–267.

22. Ogawa H, Akasaka T, Hattori R, Kawashima S, Kawasuji M, Kimura K, Miwa K, Mizuno K, Mohri M, Murohara T, Node K, Okumura K, Saito S, Shimokawa H, Sueda S, Takeyama Y, Tanabe Y, Tsuchihashi K, Yamagishi M, Yoshimura M, Ibuki C, Inoue T, Kaikita K, Kawano H, Kojima S, Kosuge M, Nakayama M, Oshita A, Soejima H, Takarada S, Yasuda S, Haze K, Kishida H, Tomoike H, Yokoyama M. Guidelines for diagnosis and treatment of patients with vasospastic angina (coronary spastic angina) (JCS 2008): digest version. *Circ J*. 2010;74:1745–1762.

23. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, McGoon DC, Murphy ML, Roe BB. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation*. 1975;51:5–40.

24. Inobe Y, Kugiyama K, Morita E, Kawano H, Okumura K, Tomiguchi S, Tsuji A, Kojima A, Takahashi M, Yasue H. Role of adenosine in pathogenesis of syndrome X: assessment with coronary hemodynamic measurements and thallium-201 myocardial single-photon emission computed tomography. *J Am Coll Cardiol*. 1996;28:890–896.

25. Okumura K, Yasue H, Matsuyama K, Ogawa H, Kugiyama K, Ishizaka H, Sumida H, Fujii H, Matsunaga T, Tsunoda R. Diffuse disorder of coronary artery vasomotility in patients with coronary spastic angina. Hyperreactivity to the constrictor effects of acetylcholine and the dilator effects of nitroglycerin. *J Am Coll Cardiol*. 1996;27:45–52.

26. Sueda S, Kohno H, Fukuda H, Ochi N, Kawada H, Hayashi Y, Uraoka T. Frequency of provoked coronary spasms in patients undergoing coronary arteriography using a spasm provocation test via intracoronary administration of ergonovine. *Angiology*. 2004;55:403–411.

27. Sueda S, Ochi N, Kawada H, Matsuda S, Hayashi Y, Tsuruoka T, Uraoka T. Frequency of provoked coronary vasospasm in patients undergoing coronary arteriography with spasm provocation test of acetylcholine. *Am J Cardiol*. 1999;83:1186–1190.

28. Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, Kugiyama K, Obata K, Morikami Y, Kimura T. Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation*. 1986;74: 955–963.

29. MacAlpin RN. Early evolution of symptoms and long-term prognosis in variant angina: importance of the functional component of coronary arterial disease. *Am J Med*. 1988;85:19–28.

30. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954.

31. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. *Circulation*. 2002;106:653–658.

32. Bertrand ME, LaBlanche JM, Tilmant PY, Thieuleux FA, Delforge MR, Carre AG, Asseman P, Berzin B, Libersa C, Laurent JM. Frequency of provoked coronary arterial spasm in 1089 consecutive patients undergoing coronary arteriography. *Circulation*. 1982;65:1299–1306.

33. Sueda S, Kohno H, Fukuda H, Ochi N, Kawada H, Hayashi Y, Uraoka T. Induction of coronary artery spasm by two pharmacological agents: comparison between intracoronary injection of acetylcholine and ergonovine. *Coron Artery Dis*. 2003;14:451–457.