Supplemental Material

Data S1.

Supplemental Methods

Invasive provocative test protocol

ACh was administered in a stepwise manner into the left coronary artery (LCA) (20–200 μg) or into the right coronary artery (RCA) (20–50 μg) over a period of 3 min with a 2–3 min interval between injections. Coronary angiography was performed 1 min after each injection of these agents and/or when chest pain and/or ischaemic ECG shifts were observed. The decision of testing with provocative test LCA or RCA as first was left to the discretion of the physicians; both LCA and RCA were tested if the first test was negative. In patients with myocardial infarction and non-obstructive coronary arteries (MINOCA), the provocative test was performed during the same procedure of coronary angiography in the acute phase (within 48 hours from admission). In MINOCA patients taking vasoactive drugs, the provocation tests were performed after a washout period of at least 24 h for calcium channel blockers (CCBs) and nitrates. In stable patients taking vasoactive drugs (calcium-channel blockers and nitrates), the provocation tests were performed after a wash-out period for these drugs of at 48 h. A fasting period (including caffeine consumption) >12 h was requested in all stable patients. Finally, in patients with coronary stenosis of 50%, assessment of FFR, preceded by intracoronary nitroglycerine administration, was performed after the provocative vasoreactivity test. Angiographic responses during the provocative test were assessed in multiple orthogonal views in order to detect the most severe narrowing and/or analysed by using computerized quantitative coronary angiography (QCA-CMS, Version 6.0, Medis-Software, Leiden, The Netherlands). Occurrence of bradyarrhythmias (defined as bradycardia with heart rate < 50 bpm or second- or third-degree AV block lasting more than 3 s), atrial fibrillation and ventricular tachycardia (defined as three or more consecutive premature ventricular complexes) during the provocative test were also recorded.

Echocardiographic assessment

All patients underwent a comprehensive echocardiographic evaluation during hospital admission using a standard ultrasound machine (Artida, Toshiba Medical System, Japan) and all images were digitally saved in raw data format to magneto optical discs for offline analysis performed by an experienced echocardiographer. Left ventricle (LV) and left atrial dimensions were obtained by M-mode and two-dimensional (2D) images whereas LV end-diastolic and end-systolic volumes and LVEF were calculated using the modified Simpson's biplane method.

Table S1. Clinical, echocardiographic and angiographic features in the overall population and according to the response at invasive provocative test.

Characteristics	Overall population (n= 310)	Positive Ach test (n= 183)	Negative Ach test (n = 127)	p value
Clinical characteristics				
Age [median (IQR)]	60.6 ± 11.9	59.4 ± 12.5	61.4 ± 12.6	0.151
Male sex [n, (%)]	136 (43.9)	78 (42.6)	58 (45.7)	0.595
Hypertension [n, (%)]	206 (66.5)	118 (64.5)	88 (69.3)	0.378
Diabetes [n, (%)]	61 (19.7)	35 (19.1)	26 (20.5)	0.769
Smoking habit [n, (%)]	105 (33.9)	60 (32.8)	45 (35.4)	0.628
Dyslipidaemia [n, (%)]	158 (51.0)	86 (47.0)	72 (56.7)	0.093
Obesity [n, (%)]	24 (7.7)	16 (8.7)	8 (6.3)	0.428
Family history of CAD [n, (%)]	94 (30.3)	52 (28.4)	42 (33.1)	0.380
Clinical presentation [n, (%)]				0.001
MINOCA [n, (%)]	141 (45.5)	98 (53.6)	43 (33.9)	
Stable angina [n, (%)]	169 (54.5)	85 (46.4)	84 (66.1)	
Previous CV history [n, (%)]	27 (8.7)	14 (7.7)	13 (10.2)	0.427
Laboratory data				
Hb (g/dL) [median (IQR)]	13.2 [12.4; 14.2]	13.2 [12.4; 14.2]	13.1 [12.2; 14.1]	0.452
WBC ($x10^3$/L) [median (IQR)]	7.1 [6.1; 7.9]	6.8 [5.9 7.9]	7.2 [6.2; 8.1]	0.152
Serum creatinine on admission (mg/dL) [median (IQR)]	0.83 [0.71; 0.96]	0.83 [0.71; 0.94]	0.85 [0.70; 1.01]	0.387
Troponin T peak (ng/mL) [median (IQR)]	0.01 [0.01; 0.19]	0.01 [0.01; 0.17]	0.01 [0.01; 0.28]	0.394
CRP (mg/L) [median (IQR)]	0.05 [0.05; 0.5]	0.05 [0.05; 0.50]	0.05 [0.05; 2.50]	0.092
Echocardiographic data				
EF on admission (%) [median (IQR)]	61 [58; 64]	61 [58; 64]	61 [58; 63]	0.435
EF on admission < 50% [n, (%)]	20 (6.5)	11 (6.0)	9 (7.1)	0.705
Diastolic dysfunction [n, (%)]	191 (61.6)	119 (65.0)	72 (56.7)	0.138
Angiographic data				

Myocardial bridging presence [n, (%)]	53 (17.1)	42 (23.0)	11 (8.7)	0.001
Myocardial bridging localization				0.651
LAD [n, (%)]	46 (14.8)	36 (19.7)	10 (7.9)	
LCx [n, (%)]	7 (2.2)	6 (3.3)	1 (0.8)	
RCA [n, (%)]	0 (0.0)	0 (0.0)	0 (0.0)	
Myocardial bridging segment				0.557
proximal [n, (%)]	4 (1.3)	4 (2.2)	0 (0.0)	
mid [n, (%)]	41 (13.2)	32 (17.5)	9 (7.1)	
distal [n, (%)]	8 (2.6)	6 (3.3)	2 (1.6)	
Myocardial bridging length, mm [mean± SD]	24.9 ± 7.3	26.3 ± 7.5	18.2 ± 4.0	0.001
Presence of non-obstructive atherosclerosis	150 (48.4)	90 (49.2)	60 (47.2)	0.737
Complications	28 (0.1)	18 (0.1)	10 (0.1)	0.553
AF / SVT [n, (%)]	8 (2.6)	6 (3.3)	2 (1.6)	0.352
Atrioventricular Block [n, (%)]	19 (6.1)	11 (6.0)	8 (6.3)	0.917
VT/ VF [n, (%)]	1 (0.3)	1 (0.01)	0 (0)	0.404
Therapy at discharge [n,(%)]				
Statin	182 (58.7)	115 (62.8)	67 (52.8)	0.076
Calcium channel blockers	206 (66.5)	175 (95.6)	31 (24.4)	0.005
β-blockers	93 (30)	0 (0)	93 (73.2)	<0.001
Nitrates	6 (1.9)	5 (2.7)	1 (0.8)	0.222
Cardioaspirin	143 (46.1)	91 (49.7)	52 (40.9)	0.127
ACEi/ARBs	216 (69.7)	(132 (72.1)	84 (66.1)	0.259

IQR: InterQuartile Range; CAD: Coronary Artery Disease; ACS: Acute Coronary Syndrome; CV: Cardiovascular History; Hb: Haemoglobin; WBC: White Blood Count; CRP: C Reactive Protein; EF: Ejection Fraction; LAD: Left Anterior Descending; LCx: Left Circumflex; RCA: Right Coronary Artery; Ach: Acetylcholine; AF: Atrial Fibrillation; SVT; SupraVentricular Tachycardia; VT: Ventricular Tachycardia; VF: Ventricular Fibrillation; ACEi: angiotensin converting enzymes inhibitors; ARBs: Angiotensin receptor blockers

Table S2. Predictors of ACh positive test in the overall population by univariate and multivariate logistic regression analysis.

	Univariate analysis		Multivariable analysis	
	OR (95% C.I.)	**p**	**OR (95% C.I.)**	**p**
Presence of myocardial bridging	3.141 (1.55; 6.37)	0.002	2.569 (1.24; 5.33)	0.011
MINOCA presentation	2.385 (1.49; 3.82)	< 0.001	2.198 (1.35; 3.58)	0.002
CRP	1.058 (1.01; 1.11)	0.035	1.059 (1.01; 1.11)	0.028
Dyslipidaemia	1.597 (1.01; 2.52)	0.044		

MINOCA: myocardial infarction and non-obstructive coronary arteries; CRP: C-reactive protein; C.I.: Confidence Interval; OR: Odds Ratio.

Table S3. Predictors of MINOCA in the overall population by univariate and multivariate logistic regression analysis.

	Univariate analysis		Multivariate analysis	
	OR (95% C.I.)	**p**	**OR (95% C.I.)**	**p**
Presence of myocardial bridging	2.770 (1.490; 5.151)	0.001	2.386 (1.267; 4.494)	0.007
Positive ACh test	2.252 (1.410; 3.598)	0.001	2.022 (1.254; 3.261)	0.004

MINOCA: myocardial infarction with no obstructive coronary atherosclerosis ACh: acetylcholine; C.I.: Confidence Interval; OR: Odds Ratio.

Table S4. Predictors of MINOCA as clinical presentation in ACh positive test population by univariate and multivariate logistic regression analysis.

	Univariate analysis		Multivariable analysis	
	OR (95% C.I.)	**p**	**OR (95% C.I.)**	**p**
Presence of myocardial bridging	2.31 (1.09; 4.93)	0.030	2.908 (1.285; 6.578)	0.010
Obesity	0.92 (0.83; 0.98)	0.04		
Diastolic Dysfunction	1.950 (1.060; 3.589)	0.006	2.502 (1.302; 4.808)	0.005

C.I.: Confidence Interval; OR: Odds Ratio.

Table S5. Predictors of MACE in the overall population by univariate and multivariable cox regression analysis.

	Univariate analysis		Multivariable analysis	
	HR (95% C.I.)	**p**	**HR (95% C.I.)**	**p**
Presence of myocardial bridging	5.46 (2.48; 11.99)	<0.001	3.98 (1.78-8.93)	0.001
Positive ACh test	2.76 (1.03; 7.34)	0.043		
MINOCA as clinical presentation	5.48 (2.05-14.63)	0.001	4.23 (1.56-11.48)	0.005

MACE: Major Adverse Cardiovascular Event; MINOCA: myocardial infarction with non-obstructive coronary arteries; ACh: acetylcholine; C.I.: Confidence Interval; OR: Odds Ratio.

Table S6. Therapy at discharge according to presence or absence of MB and according to ACh response.

Therapy	Overall population (n= 310)	Presence of Myocardial bridging and positive ACh test (n= 42)	Presence of Myocardial bridging and negative ACh test (n=11)	Absence of Myocardial Bridging and positive ACh test (n=141)	Absence of Myocardial bridging and negative ACh test (n = 116)	p value
Statin [n, (%)]	182 (58.7)	26 (61.9)	6 (54.5)	81 (57.4)	69 (59.5)	0.945
CCB [n, (%)]	206 (66.5)	40 (90.9)	4 (36.3)	136 (96.4)	26 (22.4)	<0.001
β-blockers [n, (%)]	93 (30)	2 (4.8)	6 (54.5)	13 (9.2)	72 (62.1)	<0.001
Nitrates [n, (%)]	6 (1.9)	3 (7.1)	0 (0.0)	3 (2.1)	0 (0.0)	0.082
Cardioaspirin [n, (%)]	143 (46.1)	21 (50.0)	6 (54.5)	66 (46.8)	50 (43.1)	0.801
ACEi/ARBs [n, (%)]	216 (69.7)	34 (80.9)	7 (63.6)	99 (70.2)	76 (65.5)	0.205

ACh: Acetylcholine; ACEi: angiotensin converting enzymes inhibitors; ARBs: Angiotensin receptor blockers; CCB: calcium-channel blockers.