Comparison of the Diagnostic Yield of Intracoronary Acetylcholine Infusion and Acetylcholine Bolus Injection Protocols During Invasive Coronary Function Testing

Janneke Woudstra, MD[a,*], Rutger G.T. Feenstra, MD[a], Caitlin E.M. Vink, MD[a], Koen M.J. Marques, MD, PhD[a], Coen K.M. Boerhout, MD[a], Elize A.M. de Jong, MSc[a,b], Guus A. de Waard, MD, PhD[c], Tim P. van de Hoef, MD, PhD[b], Steven A.J. Chamuleau, MD, PhD[a], Etto C. Eringa, PhD[d,e], Jan J. Piek, MD, PhD[a], Yolande Appelman, MD, PhD[a], and Marcel A.M. Beijk, MD, PhD[a]

Coronary endothelial dysfunction (CED) and coronary artery spasm (CAS) are causes of angina with no obstructive coronary arteries in patients. Both can be diagnosed by invasive coronary function testing (ICFT) using acetylcholine (ACh). This study aimed to evaluate the diagnostic yield of a 3-minute ACh infusion as compared with a 1-minute ACh bolus injection protocol in testing CED and CAS. We evaluated 220 consecutive patients with angina and no obstructive coronary arteries who underwent ICFT using continuous Doppler flow measurements. Per protocol, 110 patients were tested using 3-minute infusion, and thereafter 110 patients using 1-minute bolus injections, because of a protocol change. CED was defined as a <50% increase in coronary blood flow or any epicardial vasoconstriction in reaction to low-dose ACh and CAS according to the Coronary Vasomotor Disorders International Study Group (COVADIS) criteria, both with and without T-wave abnormalities, in reaction to high dose ACh. The prevalence of CED was equal in both protocols (78% vs 79%, p = 0.869). Regarding the endotypes of CAS according to COVADIS, the equivocal endotype was diagnosed less often in the 3 vs 1-minute protocol (24% vs 44%, p = 0.004). Including T-wave abnormalities in the COVADIS criteria resulted in a similar diagnostic yield of both protocols. Hemodynamic changes from baseline to the low or high ACh doses were comparable between the protocols for each endotype. In conclusion, ICFT using 3-minute infusion or 1-minute bolus injections of ACh showed a similar diagnostic yield of CED. When using the COVADIS criteria, a difference in the equivocal diagnosis was observed. Including T-wave abnormalities as a diagnostic criterion reclassified equivocal test results into CAS and decreased this difference. For clinical practice, we recommend the inclusion of T-wave abnormalities as a diagnostic criterion for CAS and the 1-minute bolus protocol for practicality. **(Am J Cardiol 2024;217:49−58)**

Keywords: acetylcholine, ANOCA, coronary artery spasm, coronary endothelial dysfunction

Coronary endothelial dysfunction (CED) and epicardial and/or microvascular coronary artery spasm (CAS) frequently represent the primary etiology of angina in patients with non-obstructive coronary artery disease (ANOCA). CED and CAS are both associated with adverse clinical events, and the identification of these endotypes to tailor treatment markedly enhances clinical outcomes.[1−4] The established method to identify CED and CAS is through invasive coronary function testing (ICFT). Specifically, both endotypes are assessed using incremental acetylcholine (ACh) infusions. Unfortunately, contemporary ICFT protocols often focus primarily or exclusively on one of these conditions. In this respect, it is important to note that the established protocol for the diagnosis of CED consists of 3-minute infusions of low-dose ACh. These protocols often omit high-level infusions needed for CAS provocation or the high levels are not established because of the slow infusion rate and short half-time of ACh. Therefore, CAS-centered protocols frequently imply faster bolus injections of 1 minute or less. Although these protocols are

[a]Department of Cardiology, Amsterdam UMC Heart Centre, Amsterdam, The Netherlands; [b]Department of Cardiology, University Medical Center Utrecht, The Netherlands; [c]Department of Cardiology, Radboud University Medical Center, Nijmegen, The Netherlands; [d]Department of Physiology, Amsterdam Cardiovascular Sciences, Amsterdam UMC, Amsterdam, The Netherlands; and [e]Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands. Manuscript received December 22, 2023; revised manuscript received and accepted January 19, 2024.

Dr. Woudstra and Dr. Feenstra contributed equally to this manuscript.

Appelman and Beijk are shared last authors.

Funding: none.

See page 56 for Declaration of Competing Interest.

*Corresponding author.

E-mail address: j.woudstra@amsterdamumc.nl (J. Woudstra).

https://doi.org/10.1016/j.amjcard.2024.01.038

Table 1
Comparison of the increasing intracoronary acetylcholine doses of the 3-minute infusion and 1-minute bolus protocols

	Acetylcholine blood concentration μg/min into the left coronary artery				
3-minute ACh infusion protocol	0,86 μg (0,29 μg/min)	8,63 μg (2,89 μg/min)	86,33 μg (28,83 μg/min)		863,26 μg (287,7 μg/min)
1-minute ACh bolus injection protocol		2 μg	20 μg	100 μg	200 μg
mol/L ACh		10^{-5}	10^{-4}		10^{-3}

ACh = acetylcholine.

increasingly used in clinical practice, it remains uncertain whether they offer equivalent diagnostic performance for identifying CED. Consequently, a consensus on an ICFT protocol is lacking, potentially leading to the underdiagnosis of CED or CAS in many patients. Therefore, this study aimed to investigate the distinction between the continuous 3-minute intracoronary ACh infusion protocol and the 1-minute intracoronary ACh bolus injection protocol, while also comparing the diagnostic yield between both protocols in relation to CED, the various endotypes of CAS, and their associated hemodynamic characteristics.

Methods

This retrospective cohort study included patients with chronic typical or atypical angina pectoris (defined as symptoms at least 2 times a month despite medical therapy) in the absence of obstructive epicardial coronary artery disease on coronary angiography (<50% epicardial diameter stenosis, fractional flow reserve >0.80 and/or instantaneous waves:free ratio >0.89), who were referred to the ANOCA clinic of the Amsterdam UMC (Amsterdam, The Netherlands), a tertiary referral center. All consecutive patients who underwent a clinically indicated comprehensive ICFT between November 2016 and June 2022 were included in this analysis. Per protocol, patients underwent ICFT using a continuous 3-minute infusion of intracoronary ACh between November 2016 and September 2020. Thereafter, because of a change in the protocol, patients underwent ICFT using a 1-minute bolus injection of intracoronary ACh.

Diagnostic coronary angiography was performed using routine techniques to exclude significant obstructive coronary artery disease but without the use of intracoronary nitrates. Beta-blockers were discontinued for at least 72 hours and all other vasoactive medication for at least 48 hours before the procedure. After diagnostic coronary angiography, a 0.014-in guidewire equipped with both a pressure sensor and a Doppler crystal (ComboWire XT, Philips-Volcano, San Diego, California) was advanced into the left anterior descending (LAD) for continuous measurement of the average peak velocity (APV). When quality of flow signals in the LAD was insufficient, the Doppler wire was placed in the ramus circumflex (Cx) or right coronary artery. In some cases, a microcatheter was used to stabilize the tip of the Doppler wire to obtain good-quality Doppler flow signals. Aortic pressure (Pa) was continuously registered through the guiding catheter according to standard clinical protocol.

ACh was administered through the guiding catheter in both protocols. In the 3-minute infusion protocol, ACh administration was performed using a mechanical infusion pump, consisting of incremental ACh concentrations of 0.29, 2.88, 28.8, and 288 μg/min infused over 3 minutes (3-minute infusion protocol) (Table 1). In the 1-minute bolus injection protocol, ACh administration was performed through manual injection within 1 minute, consisting of incremental ACh doses of 2, 20, 100, and 200 μg (1-minute bolus protocol) (Table 1). Incremental ACh infusions or injections were halted when epicardial vasospasm (>90% lumen narrowing) was documented. Coronary angiography was performed after each incremental dose in similar projections. At the end of ACh testing, intracoronary nitroglycerine (200 μg) was administered. After the APV signal had stabilized, a bolus of intracoronary adenosine (150 μg) was injected to induce hyperemia for measurement of coronary flow reserve (CFR) and hyperemic microvascular resistance (HMR). Surface electrocardiogram (ECG) and all hemodynamic data were recorded on a dedicated console (ComboMap, Volcano Corporation, San Diego, California), and the 12-lead ECG was continuously recorded using MacLab (GE Healthcare).

For the purpose of this study, only the results of ACh administration were analyzed.

The following diagnostic criteria of CED and CAS were used. CED was defined as a <50% increase in volumetric coronary blood flow (vCBF), and/or any epicardial vasoconstriction in reaction to 28.8 μg/min when the 3-minute infusion protocol was used and in reaction to 20 μg when the 1-minute bolus protocol was used compared with baseline (Table 1).[5,6] In this study, 2 diagnostic criteria were used for the diagnosis of CAS. First, according to the diagnostic criteria used by the Coronary Vasomotor Disorders International Study Group (COVADIS) working group.[7] Epicardial coronary spasm was diagnosed when ACh induced (i) recognizable angina; (ii) ECG changes suggestive of ischemia, which included ST-deviation of 0.1 mV in at least 2 contiguous leads; and (iii) >90% epicardial vasoconstriction by visual assessment at coronary angiography. Microvascular coronary spasm was diagnosed when the first 2 criteria previously mentioned were met, in the absence of epicardial vasoconstriction of >90%.[8] An equivocal test result was defined as recognizable angina, but without fulfilling the other criteria for epicardial or microvascular coronary spasm (with or without >90% epicardial spasm) or ECG changes alone, whereas the absence of any of the criterion was considered a negative test result. Second, according to the diagnostic criteria used by COVADIS working group previously mentioned, but with the inclusion of new-onset T-wave abnormalities on the surface ECG as a criterion for ischemic ECG changes.[9]

Quantitative coronary angiography analysis was performed offline from a singular angiogram at the end-diastolic phase to determine the coronary minimal lumen diameter (MLD) 5 mm from the tip of the Doppler crystal

by 2 researchers independently and blinded to the hemodynamic data. When discrepancies were less than 15%, the 2 measurements were averaged. Discrepancies of >15% were discussed and a final decision was reached by a senior operator MB or YA. Hemodynamic data were obtained from the digital archive of the ComboMap (Philips-Volcano, San Diego, California) and analyzed in Matlab (Mathworks, Inc, Natick, Massachusetts) with custom software. Five-beat averages of the APV and Pa were determined at baseline and at the end of each ACh infusion or bolus. The vCBF was calculated using the following formula (π*Ø/2) 2 × (APV/2), where Ø reflects the coronary diameter at the flow sensing position. Coronary vascular resistance was defined as the ratio of Pa to vCBF. CFR was defined as the ratio of the maximal hyperemic APV induced by adenosine to APV at rest and HMR was defined as the ratio of the hyperemic Pa to hyperemic APV during adenosine-induced peak hyperemia.[10] Table 1 lists the equivalent doses for the 3-minute infusion and 1-minute bolus protocols. Three doses from both protocols were comparable and used in the hemodynamic analysis. Hemodynamic changes in MLD, vCBF, and coronary vascular resistance from baseline to these 3 equivocal doses were compared between the 2 protocols. Only patients receiving all 3 doses were included in this analysis.

Continuous variables are presented as mean ± SD or median (first, third quartile [Q1, Q3]), and were compared with Student *t* test or Mann-Whitney *U* test, as appropriate. Categorical variables are presented as counts and percentages and were compared using chi-square test. Normality and homogeneity of variances were tested using Shapiro-Wilk and Levene tests. The data depicting hemodynamic changes were normally distributed and are presented as mean ± SD in text and as mean ± SEM in figures. Statistical analyses were performed using SPSS version 28 (IBM Corp., Armonk, New York). A $p < 0.05$ was considered statistically significant.

Results

A total of 220 patients with ANOCA who underwent ICFT using ACh were included, 110 patients received the 3-minute ACh infusion protocol and 110 consecutive patients received the 1-minute ACh bolus injection protocol. The overall mean age was 57.0 ± 10.3 years and 82% of patients were female. Baseline characteristics, including cardiovascular risk factors, medical history, and medication use before ICFT, did not exhibit significant differences between patients who were tested with the 3-minute infusion protocol and those tested with the 1-minute bolus protocol (Table 2). Likewise, the CFR and HMR were similar for both protocols (Table 2). Observed side effects during ICFT using ACh were low and similar between both protocols. ACh-induced transient bradycardia occurred in 11 patients in the 3-minute infusion protocol and in 12 patients in the 1-minute bolus protocol (10.0% vs 10.9%, p = 0.826). Atrial fibrillation occurred in 4 patients (3.6% vs 3.6%, p = 1.000). A coronary artery dissection occurred in 1 patient in the 1-minute bolus protocol (p = 0.316), no other side effects were observed.

The results of the ICFT are listed in Table 3. In the entire study population, CED was diagnosed in 173 (78.6%) patients, and no significant difference was observed between the 3-minute infusion and 1-minute bolus protocol (78.2% vs 79.1%, p = 0.869; Figure 1).

The prevalence of each diagnostic criterion for CAS is listed in Table 3. The occurrence of recognizable angina during ACh administration was similar between the 3-minute infusion and 1-minute bolus protocol (83.6% vs 90.0%, p = 0.231). A trend was observed indicating a higher occurrence of epicardial coronary spasm of >90% in the 3-minute infusion protocol (46.4% vs 34.5%, p = 0.074). Strict adherence to the COVADIS criteria for ischemic ECG changes revealed a significantly higher occurrence of ECG changes in patients receiving the 3-minute infusion protocol compared with the 1-minute bolus protocol (60.9% vs 46.4%, p = 0.031). Including new-onset T-wave abnormalities in the diagnostic criteria for ischemic ECG changes yielded no difference (62.7% vs 54.5%, p = 0.218).

The ICFT diagnosis of the various endotypes of CAS is also listed in Table 3. Strictly adhering to the COVADIS criteria, yielded no significant difference in the diagnosis of epicardial spasm (34.5% vs 23.6%, p = 0.075), microvascular spasm (25.5% vs 22.7%, p = 0.636) or a negative test (15.5% vs 10.0%, p = 0.312) between the 3-minute infusion protocol compared with the 1-minute bolus protocol (Figure 1). However, a trend was notable for the occurrence epicardial spasm. There was a significant difference in the occurrence of an equivocal diagnosis between the 3-minute infusion protocol and 1-minute bolus protocol (24.5% vs 43.6%, p = 0.004). From the patients with an equivocal diagnosis, most (n = 51; 68.0%) had only recognizable angina complaints. This phenomenon occurred significantly more in the 1-minute bolus protocol compared with the 3-minute infusion protocol (48.1% vs 79.2%, p = 0.006). In contrast, in the 3-minute infusion protocol, significantly more patients had >90% spasm plus angina pectoris (48.1% vs 20.8%, p = 0.014) (Supplementary Table 1).

When new-onset T-wave abnormalities were included as diagnostic criterion 11 patients with an equivocal test result reclassified as having CAS, 2 patients from the 3-minute infusion protocol, and 9 from the 1-minute bolus protocol. Subsequently, no significant differences were observed in the diagnosis of endotypes of CAS between the 3-minute infusion and 1-minute bolus protocols. However, a trend was notable in the equivocal endotype (22.7% vs 35.5%, p = 0.053). When new-onset T-wave abnormalities were included, 67 patients had epicardial spasm. Of them, 19 patients had focal epicardial spasm, with 15 in the LAD and 4 in the Cx. Focal spasm was observed in 13 patients in the 3-minute infusion protocol and 6 in the 1-minute bolus protocol. Additionally, 48 patients presented with diffuse epicardial spasm, including 18 in the LAD, 6 in the Cx, and 28 in both the LAD and Cx. Diffuse spasm was observed in 26 patients in the 3-minute infusion protocol and 22 in the 1-minute bolus protocol.

Baseline hemodynamic characteristics are listed in Table 2. In total, 170 (77.3%) patients were included in the hemodynamic analysis. Of the excluded 50 (22.7%) patients, 30 had CAS induction at a lower dose than the

Table 2
Baseline characteristics of patients undergoing ICFT

	Total (n=220)	ACh infusion (n=110)	ACh bolus (n=110)	p-value
Age	57.0 ± 10.3	56.5 ± 10.3	57.5 ± 10.4	0.446
Female	181 (82.3)	93 (84.5)	88 (80.0)	0.439
BMI	26.4 ± 4.3	25.8 ± 4.1	26.9 ± 4.5	0.060
Cardiovascular risk factors				
Hypertension	104 (47.3)	52 (47.3)	52 (47.3)	1.000
DM	17 (7.7)	6 (5.5)	11 (10.0)	0.207
Hypercholesterolemia	79 (35.9)	41 (37.3)	38 (34.5)	0.216
Former or current smoker	75 (34.1)	37 (33.6)	38 (34.5)	0.241
Current smoker	17 (7.7)	8 (7.3)	9 (8.2)	0.207
Family history of CVD	137 (68.2)	79 (71.8)	58 (62.4)	0.102
Prior PCI	33 (15.0)	16 (14.5)	17 (15.5)	0.595
Prior MI	31 (14.1)	18 (16.4)	13 (11.8)	0.333
Normal LVEF (>55%)	211 (96.8)	105 (97.2)	106 (96.4)	0.719
Timing of symptoms				
Rest angina	181 (82.3)	93 (84.5)	88 (80.0)	0.377
Effort angina	133 (60.5)	65 (59.1)	68 (61.8)	0.679
Laboratory results				
eGFR	80.6 ± 15.0	81.4 ± 17.8	80.2 ± 13.0	0.653
Hb (mmol/l)	8.5 ± 0.8	8.5 ± 0.7	8.5 ± 0.8	0.984
Medication use				
Calcium channel blocker	140 (63.6)	64 (58.2)	76 (69.1)	0.093
Long acting nitrates	80 (36.4)	38 (34.5)	42 (38.2)	0.575
Beta-blocker	53 (24.1)	25 (22.7)	28 (25.5)	0.636
Statin	94 (42.7)	45 (40.9)	49 (44.5)	0.506
Diuretics	23 (20.9)	10 (9.1)	13 (11.8)	0.479
Adenosine testing				
CFR	3.02 ± 0.8	3.06 ± 0.7	2.98 ± 0.9	0.474
HMR	1.88 ± 0.59	1.86 ± 0.57	1.92 ± 0.62	0.498
Procedural characteristics				
Heart rate	80.6 ± 15.5	81.5 ± 13.8	79.6 ± 17.1	0.385
Systolic BP	141.7 ± 20.6	144.3 ± 20.1	139.1 ± 20.9	0.064
Diastolic BP	81.3 ± 10.0	81.6 ± 10.1	81.0 ± 10.0	0.654
Baseline haemodynamic characteristics				
Study population	**n=170**	**n=88**	**n=82**	
Pa	107.6 ± 12.8	108.8 ± 13.1	106.3 ± 12.5	0.204
MLD	2.22 ± 0.52	2.28 ± 0.59	2.16 ± 0.42	0.121
APV	22.1 [17.2-27.8]	24.7 [19.4-29.4]	20.6 [16.1-26.0]	**0.004**
vCBF	40.0 [30.25-58.5]	45.2 [31.1-67.2]	35.1 [29.4-48.8]	**0.005**
Vascular resistance	2.57 [1.84-3.48]	2.40 [1.63-3.46]	2.78 [2.08-3.57]	0.060

ACh = acetylcholine APV = average peak velocity; BMI = body mass index; BP = blood pressure; CFR = coronary flow reserve; CVD = cardiovascular disease; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HMR = hyperemic microvascular resistance; ICFT = invasive coronary function testing; MLD = minimal lumen diameter; MI = myocardial infarction; Pa = aortic pressure; PCI = percutaneous coronary intervention; vCBF = volumetric coronary blood flow. Values are mean ± SD, n (%), or median [interquartile interval]. Differences between groups were assessed by an independent sample *t* test for continuous data with a normal distribution. Otherwise, the nonparametric Mann-Whitney *U* test was used. Categorical variables were compared using chi-square test.

maximum ACh dose (13 3-minute infusions, 17 1-minute boluses), whereas 20 patients had insufficient angiographic image quality for quantitative coronary angiography analysis or flow velocity signals (9 3-minute infusion, 11 1-minute ACh bolus). The baseline APV and vCBF were significantly higher for the 3-minute infusion protocol (p = 0.004 and p = 0.005, respectively).

Figures 2 and 3 show the changes in hemodynamic parameters per CAS endotype according to the COVADIS criteria. The changes in MLD, vCBF, and coronary vascular resistance were comparable between both protocols and within each diagnostic endotype from baseline to the CED testing dose (Figure 2) and from the CED testing dose to the highest ACh dose (Figure 2). Figure 3 shows the percentage change from baseline in hemodynamic parameters per dose per diagnostic endotype. Notably, in those patients with equivocal test results, there was a significant difference in vCBF and vascular resistance between the 3-minute infusion and 1-minute bolus protocol at the lowest dose (24.6% vs 59.8% change, p = 0.007 and −25.6% vs −37.2% change, p = 0.022, respectively). Conversely, the hemodynamic changes at the higher ACh doses were comparable. Supplementary Figures 1 and 2 depict identical figures with similar results when new-onset T-wave abnormalities were included as diagnostic criteria.

Table 3
ICFT diagnosis for coronary endothelial dysfunction and coronary artery spasm

	Total (n=220)	ACh infusion (n=110)	ACh bolus (n=110)	p-value
Endothelial function				
Coronary endothelial dysfunction	173 (78.6)	86 (78.2)	87 (79.1)	0.869
Diagnostic criteria coronary artery vasospasm				
Angina pectoris	191 (86.8)	92 (83.6)	99 (90.0)	0.231
Spasm >90%	89 (40.5)	51 (46.4)	38 (34.5)	0.074
ECG changes according to COVADIS	118 (53.6)	67 (60.9)	51 (46.4)	**0.031**
ECG changes including T-wave abnormalities	129 (58.6)	69 (62.7)	60 (54.5)	0.218
Endotypes of coronary artery spasm according to the COVADIS criteria				
Epicardial spasm	64 (29.1)	38 (34.5)	26 (23.6)	0.075
Microvascular spasm	53 (24.1)	28 (25.5)	25 (22.7)	0.636
Equivocal	75 (34.1)	27 (24.5)	48 (43.6)	**0.004**
Negative	28 (12.7)	17 (15.5)	11 (10.0)	0.312
Endotypes of coronary artery spasm when T-wave abnormalities are included as a diagnostic criterion				
Epicardial spasm	67 (30.5)	39 (35.5)	28 (25.5)	0.107
Microvascular spasm	61 (27.7)	29 (26.4)	32 (29.1)	0.651
Equivocal	64 (29.1)	25 (22.7)	39 (35.5)	0.053
Negative	28 (12.7)	17 (15.5)	11 (10.0)	0.312

ACh = acetylcholine; COVADIS = Coronary Vasomotor Disorders International Study Group; ECG = electrocardiogram; ICFT = invasive coronary function testing. Values are mean ± SD, n (%), or median (interquartile interval). Differences between groups were assessed by an independent sample *t* test for continuous data with a normal distribution. Otherwise, the nonparametric Mann-Whitney *U* test was used. Categorical variables were compared using chi-square test. Categorical variables were compared using chi-square test.

Discussion

To our knowledge, this is the first study to compare the diagnostic yield of ICFT using a 3-minute intracoronary ACh infusion protocol with a 1-minute ACh bolus injection protocol in patients with ANOCA. The main findings of this study are (a) the diagnostic yield of ICFT for the diagnosis of CED is similar between the 3-minute infusion or 1-minute bolus injection protocol; (b) the diagnostic yield of ICFT for the diagnosis of the endotypes of CAS according to the COVADIS criteria is different between the 3-minute infusion and 1-minute bolus protocol, mainly because of the difference in the occurrence of the equivocal endotype; (c) Including T-wave abnormalities as a diagnostic criterion reclassified 11 patients with an equivocal test result into CAS and markedly decreased the difference in equivocal test results, although a trend was still observed. These findings support the use of a 1-minute bolus protocol assessing both CED and CAS, which is more practical in daily practice and facilitates ad hoc ICFT.

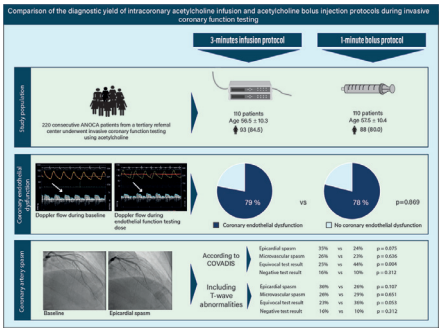

Figure 1. The Central illustration
ANOCA = angina with no obstructive coronary arteries.

In healthy vessels, low-dose ACh leads to endothelial-dependent vasodilatation and an impaired response indicates CED.[11] Consequently, ICFT with low-dose intracoronary ACh is used to assess CED. Ideally, the assessment of CED is performed together with the assessment of CAS, a combination that is currently rarely performed, as it has been shown that CED is also present in patients with ANOCA without CAS and these patients may benefit from tailored treatment.[5] However, it remains uncertain whether the 1-minute protocol yields the same diagnostic accuracy for CED as the 3-minute protocol, both with the use of the Doppler wire. Our study found that the overall prevalence of CED was 78%, agreeing with previous reports using comparable diagnostic criteria as in our study and validating our findings.[12,13] Some studies report a lower prevalence of 44% and 60% when stricter definitions of CED are used (reduction of coronary diameter >20% was considered abnormal).[14,15] Importantly, in this study, with the use of the Doppler wire, we found no difference in the prevalence across the different protocols and showed a similar diagnostic accuracy for the 1-minute bolus protocol as the 3-minute infusion protocol. This provides evidence for the possibility of combining CED and CAS testing, as CAS-centered protocols frequently imply faster bolus injections of 1-minute or less. In addition, using a 1-minute bolus protocol for both CED and CAS yields substantial time savings, enhancing practicality for daily practice and facilitating ad hoc ICFT using ACh. In ad hoc ICFT using ACh, manual ACh injections are preferred because they are easier to prepare and administer.

In patients with ANOCA who underwent ICFT, the prevalence of CAS varies because of differences in study populations, diagnostic criteria, and ICFT protocols used.[16] Our study, performed at a tertiary referral center, included patients scheduled for ICFT and demonstrated epicardial spasm in 31% and microvascular spasm in 28% of patients.

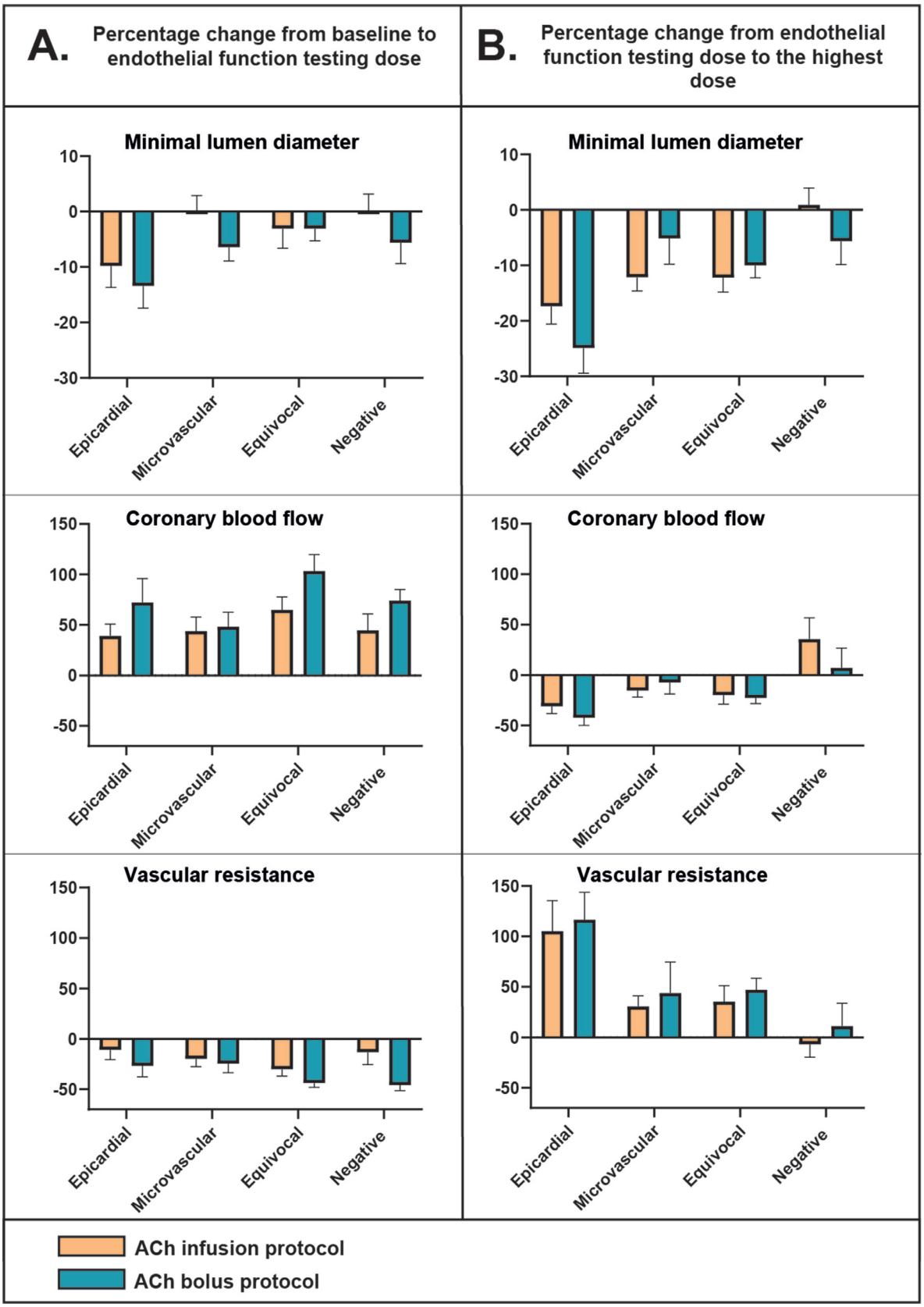

Figure 2. Percentage change from baseline in minimal lumen diameter, volumetric coronary blood flow, and vascular resistance for each diagnostic endotype according to the COVADIS criteria per ACh reactivity testing protocol in 170 patients receiving all 4 doses of ACh. No statistically significant differences between the protocols were observed.
ACh = acetylcholine.

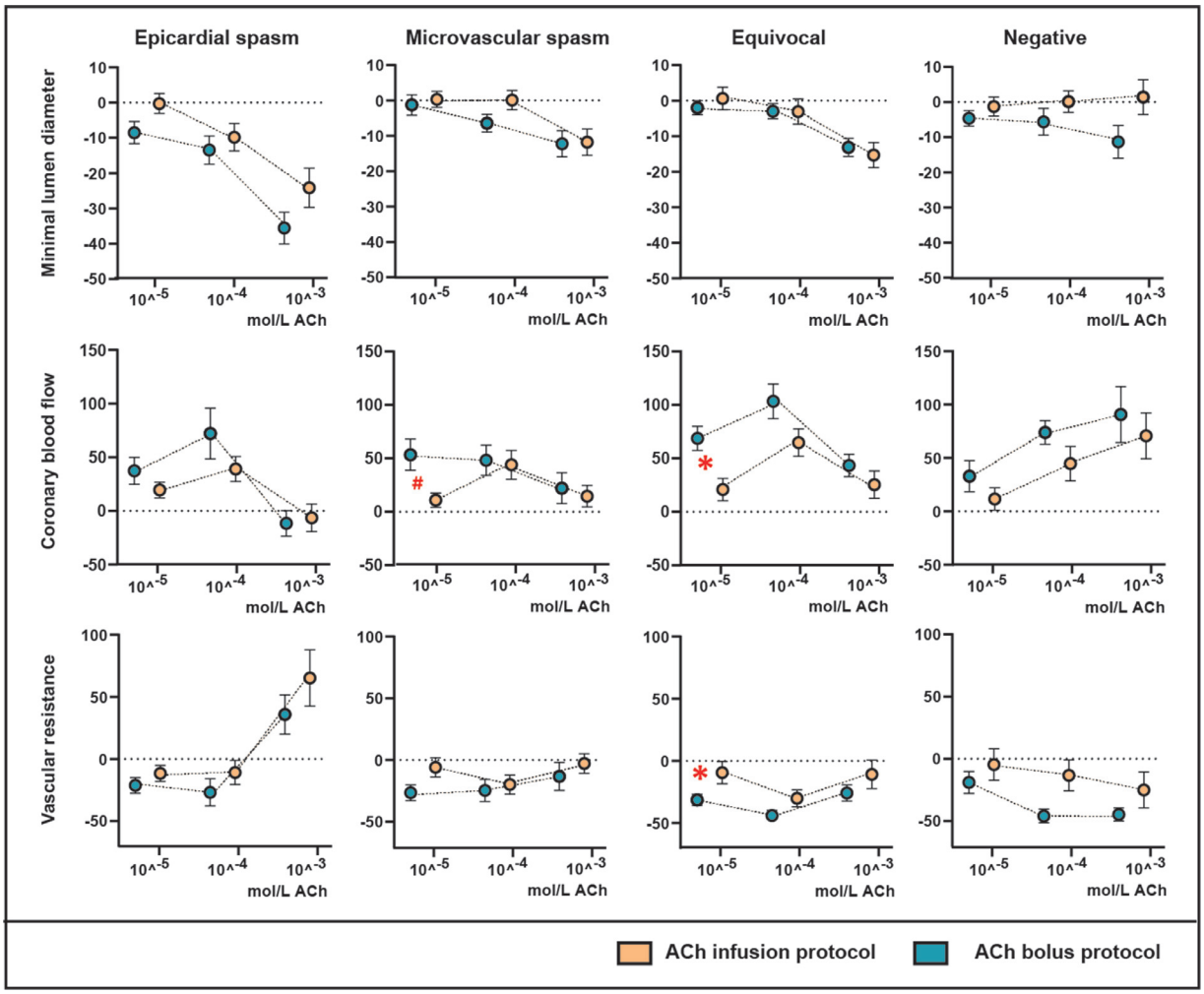

Figure 3. Percentage change from baseline in minimal lumen diameter at a fixed location, volumetric coronary blood flow, and vascular resistance per dose per diagnosis according to the COVADIS criteria in 170 patients receiving all 4 doses of ACh. Differences between groups were assessed by a Student *t* test or Mann-Whitney *U* test, as appropriate. Data are presented as mean ± SEM in figures. * Significant difference $p<0.05$. # $p = 0.050$. ACh = acetylcholine. 10 to 5 mol/L is equivalent to the 2.89 μg/min dose for the infusion protocol and the 2 μg dose for the bolus protocol. 10 to 4 mol/L is equivalent to the 28.8 μg/min dose for the infusion protocol and the 20 μg dose for the bolus protocol. 10 to 3 mol/L is equivalent to the 288 μg/min dose for the infusion protocol and the 200 μg dose for the bolus protocol.

These findings agree with contemporary literature.[17,18] Interestingly, our study indicates a difference in the diagnostic yield of the different ICFT protocols for CAS, when adhering to the COVADIS criteria. This difference was predominantly attributable to the difference in the occurrence of the equivocal endotype (25% in the 3-minute infusion protocol vs 44% in the 1-minute bolus protocol). There are several explanations for this phenomenon. In recent years, the ICFT has been increasingly utilized for the assessment of patients with ANOCA, which may have led to a lower threshold for referring patients in later years.[2] A selection bias may be present as the patients tested with the 3-minute infusion protocol were included in earlier years. In contrast, the 3-minute infusion and 1-minute bolus study populations had similar baseline characteristics and medication use before ICFT, and both protocols were performed in the same tertiary referral center. More likely, the difference in diagnostic yield is attributable to the differences in ACh concentrations reached by the difference in protocols. Sueda et al,[19,20] demonstrated that the number of provoked epicardial spasm increased when the ACh dose was increased from 100 to 200 ug injected in 20 seconds. Moreover, it has been demonstrated that the same amount of ACh provoked more epicardial spasm when infused in 20 seconds compared with 3 minutes.[21] Because of the short half-life of ACh, the ACh concentration will be lower when it is injected for a longer period of time. In this study, 288 μg ACh was infused in 1 minute in the 3-minute infusion protocol resulting in a total dose of 863.26 μg in 3 minutes, compared with 200 μg ACh over 1 minute in the 1-minute bolus protocol. This higher ACh dose, administered over 3 minutes, likely accounts for the difference in diagnostic yield. Interestingly, our data shows similar hemodynamic changes from baseline to the CED and CAS testing doses between both protocols. These findings provide evidence, that hemodynamic characteristics of CAS diagnostic

endotypes can be compared, which is important for interpreting studies using different protocols.

Furthermore, when strictly adhering to the COVADIS criteria, significantly fewer ischemic ECG changes were observed in the 1-minute bolus protocol. This lower prevalence might be explained by the duration of ACh infusion was longer in the infusion protocol (3 minutes), compared with the bolus injections in the bolus protocol (1 minute). As it has been previously shown, 1 minute of balloon occlusion is needed for 0.1 mV ST-segment deviation to occur.[22] Thus, it can be suggested that, in certain cases, the duration of CAS might be too short for the development of ECG changes in the 1-minute bolus protocol. To unify diagnostic criteria for CAS, the COVADIS working group has published diagnostic criteria.[7,8] However, numerous studies included new-onset T-wave abnormalities as an additional criterion for ischemic ECG changes.[4,23,24] Interestingly, in our study, including new-onset T-wave abnormalities led to a nonsignificant difference in ischemic ECG changes and a nonsignificant difference in the diagnostic yield of both protocols. Importantly, an increase in the diagnostic yield was seen, as 11 patients with an equivocal test result reclassified as CAS, more so in the 1-minute bolus protocol. These results support the incorporation of new-onset T-wave abnormalities as a criterion for ischemic ECG changes. However, even with the inclusion of T-wave abnormalities, an equivocal test result was found in 29% of patients, in line with previous studies reporting a prevalence of 20% to 30%.[25,26]

The equivocal test result poses a diagnostic dilemma for clinicians. Previous research conducted by our group showed that the post-spastic flow recovery time, indicative of ischemia, in patients with an equivocal test result is similar to those with CAS and significantly extended compared with the negative group.[27] Furthermore, our research group provided evidence that the hemodynamic characteristics of the equivocal group are comparable to those of patients with microvascular spasm.[28] This agrees with the present study, which showed that most patients with an equivocal test result did not exhibit >90% spasm, indicating a form of microvascular spasm. These studies, along with our clinical experience, suggest that the equivocal test result is a form of CAS and benefits from medical treatment for CAS.

Irrespective of the protocol used, CED and CAS are highly prevalent in patients with ANOCA who underwent ICFT, highlighting the importance of ICFT in patients with ANOCA.[18] Because multiple ICFT protocols are used worldwide, the implementation of a standardized ICFT protocol using ACh is important. Our study contributes 3 important findings. First, it provides evidence for incorporating new-onset T-wave abnormalities as a criterion for ischemic ECG changes, because this increases the diagnostic yield of ICFT. Second, CED assessment could be integrated into CAS provocation testing using the 1-minute bolus protocol, as both protocols demonstrate a similar diagnostic yield. Third, the 1-minute bolus protocol is easier to prepare and administer and can be performed ad hoc in less time than the 3-minute infusion protocol, making the 1-minute bolus protocol the clinically more feasible method. However, our study found a higher number of equivocal test results in the 1-minute bolus protocol when strictly adhering to the COVADIS criteria. This limitation is alleviated when the equivocal test results are classified as CAS, as it results in a comparable diagnostic yield for both protocols.

Several limitations should be acknowledged. First, this study was a retrospective cohort study, selection bias may be present inherent to the design. Second, as previously mentioned, patients who underwent ICFT using the continuous 3-minute infusion protocol were included before 2020. In the past few years, ICFT has been increasingly recognized and recommended by the European guidelines in patients with chronic coronary syndromes. This may have led to a lower threshold for referring patients for ICFT in later years which could have resulted in selection bias.[2] Third, conducting a head-to-head comparison between the 3-minute infusion and 1-minute bolus protocol within our dataset was not feasible. Fourth, the doses used for comparison in the hemodynamic analysis were not the same. Nonetheless, this analysis yields highly valuable information for daily clinical practice, as the ACh doses being compared were those used for the diagnosis of CED and CAS. Fifth, endothelial function assessment is currently complicated by the widespread use of the Doppler wire is limited in clinical practice.

In conclusion, in patients with ANOCA, the diagnostic yield of ICFT using ACh for the diagnosis of CED is similar between the 3-minute infusion and 1-minute bolus injection protocol. However, this study shows that the diagnostic yield of ICFT for the diagnosis of the endotypes of CAS when strictly adhering to the COVADIS criteria is different between the protocols, mainly because of a difference in the occurrence of the equivocal endotype. Including T-wave abnormalities as a diagnostic criterion reclassified patients with an equivocal test result into CAS and markedly decreased this difference. In daily clinical practice, we recommend the inclusion of new-onset T-wave abnormalities in the criteria for ischemic ECG changes in CAS diagnosis and the use of a 1-minute bolus protocol that facilitates ad hoc ICFT.

Declaration of competing interest

Dr. van de Hoef is a consultant and a speaker at educational events for Abbott and Philips and received institutional research grants from Philips and Abbott. Dr. Piek is a consultant for Philips. The remaining authors have no competing interests to declare.

CRediT authorship contribution statement

Janneke Woudstra: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rutger G.T. Feenstra:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Caitlin E.M. Vink:** Writing – review & editing, Data curation. **Koen M.J. Marques:** Writing – review & editing, Data curation. **Coen K.M. Boerhout:** Writing – review & editing. **Elize A.M. de Jong:** Writing – review & editing. **Guus A. de Waard:** Methodology, Conceptualization. **Tim P. van de Hoef:** Supervision, Methodology, Data curation, Conceptualization. **Steven A.J. Chamuleau:**

Writing – review & editing, Supervision, Methodology, Conceptualization. **Etto C. Eringa:** Writing – review & editing. **Jan J. Piek:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Yolande Appelman:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Marcel A.M. Beijk:** Writing – review & editing, Writing – original draft, Supervision, Methodology.

Acknowledgment

With support from the IMPRESS Consortium.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2024.01.038.

1. Hokimoto S, Kaikita K, Yasuda S, Tsujita K, Ishihara M, Matoba T, Matsuzawa Y, Mitsutake Y, Mitani Y, Murohara T, Noda T, Node K, Noguchi T, Suzuki H, Takahashi J, Tanabe Y, Tanaka A, Tanaka N, Teragawa H, Yasu T, Yoshimura M, Asaumi Y, Godo S, Ikenaga H, Imanaka T, Ishibashi K, Ishii M, Ishihara T, Matsuura Y, Miura H, Nakano Y, Ogawa T, Shiroto T, Soejima H, Takagi R, Tanaka A, Tanaka A, Taruya A, Tsuda E, Wakabayashi K, Yokoi K, Minamino T, Nakagawa Y, Sueda S, Shimokawa H, Ogawa H, Japanese Circulation Society and Japanese Association of Cardiovascular Intervention and Therapeutics and Japanese College of Cardiology Joint Working Group. JCS/CVIT/JCC 2023 Guideline Focused Update on Diagnosis and Treatment of Vasospastic Angina (Coronary Spastic Angina) and Coronary Microvascular Dysfunction. *Circ J* 2023;87:879–936.
2. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, Prescott E, Storey RF, Deaton C, Cuisset T, Agewall S, Dickstein K, Edvardsen T, Escaned J, Gersh BJ, Svitil P, Gilard M, Hasdai D, Hatala R, Mahfoud F, Masip J, Muneretto C, Valgimigli M, Achenbach S, Bax JJ, ESC Scientific Document Group. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J* 2020;41:407–477.
3. Virani SS, Newby LK, Arnold SV, Bittner V, Brewer LC, Demeter SH, Dixon DL, Fearon WF, Hess B, Johnson HM, Kazi DS, Kolte D, Kumbhani DJ, LoFaso J, Mahtta D, Mark DB, Minissian M, Navar AM, Patel AR, Piano MR, Rodriguez F, Talbot AW, Taqueti VR, Thomas RJ, van Diepen S, Wiggins B, Williams MS. 2023 AHA/ACC/ACCP/ASPC/NLA/PCNA guideline for the management of patients with chronic coronary disease: a report of the American Heart Association/American College of Cardiology joint committee on clinical practice guidelines. *Circulation* 2023;148:e9–e119.
4. 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.
5. Feenstra RGT, Boerhout CKM, Woudstra J, Vink CEM, Wittekoek ME, de Waard GA, Appelman Y, Eringa EC, Marques KMJ, de Winter RJ, Beijk MAM, van de Hoef TP, Piek JJ. Presence of coronary endothelial dysfunction, coronary vasospasm, and adenosine-mediated vasodilatory disorders in patients with ischemia and nonobstructive coronary arteries. *Circ Cardiovasc Interv* 2022;15:e012017.
6. Hasdai D, Cannan CR, Mathew V, Holmes DR Jr, Lerman A. Evaluation of patients with minimally obstructive coronary artery disease and angina. *Int J Cardiol* 1996;53:203–208.
7. Beltrame JF, Crea F, Kaski JC, Ogawa H, Ong P, Sechtem U, Shimokawa H, Bairey Merz CN, Coronary Vasomotion Disorders International Study Group (COVADIS). International standardization of diagnostic criteria for vasospastic angina. *Eur Heart J* 2017;38:2565–2568.
8. Ong P, Camici PG, Beltrame JF, Crea F, Shimokawa H, Sechtem U, Kaski JC, Bairey Merz CN, Coronary Vasomotion Disorders International Study Group (COVADIS). International standardization of diagnostic criteria for microvascular angina. *Int J Cardiol* 2018;250:16–20.
9. Seitz A, Feenstra R, Konst RE, Martínez Pereyra V, Beck S, Beijk M, van de Hoef T, van Royen N, Bekeredjian R, Sechtem U, Damman P, Piek JJ, Ong P. Acetylcholine rechallenge: a first step toward tailored treatment in patients with coronary artery spasm. *JACC Cardiovasc Interv* 2022;15:65–75.
10. Feenstra RGT, Seitz A, Boerhout CKM, de Winter RJ, Ong P, Beijk MAM, Piek JJ, Sechtem U, van de Hoef TP. Reference values for intracoronary Doppler flow velocity-derived hyperaemic microvascular resistance index. *Int J Cardiol* 2023;371:16–20.
11. Ganz P, Vita JA. Testing endothelial vasomotor function: nitric oxide, a multipotent molecule. *Circulation* 2003;108:2049–2053.
12. Corban MT, Godo S, Burczak DR, Noseworthy PA, Toya T, Lewis BR, Lerman LO, Gulati R, Lerman A. Coronary endothelial dysfunction is associated with increased risk of incident atrial fibrillation. *J Am Heart Assoc* 2020;9:e014850.
13. Kumar S, Mehta PK, Eshtehardi P, Hung OY, Koh JS, Kumar A, Al-Badri A, Rabah R, D'Souza M, Gupta S, McDaniel M, Vaccarino V, Douglas J, Mavromatis K, Lee JM, Quyyumi A, Samady H. Functional coronary angiography in symptomatic patients with no obstructive coronary artery disease. *Catheter Cardiovasc Interv* 2021;98:827–835.
14. Lee BK, Lim HS, Fearon WF, Yong AS, Yamada R, Tanaka S, Lee DP, Yeung AC, Tremmel JA. Invasive evaluation of patients with angina in the absence of obstructive coronary artery disease. *Circulation* 2015;131:1054–1060.
15. Siasos G, Sara JD, Zaromytidou M, Park KH, Coskun AU, Lerman LO, Oikonomou E, Maynard CC, Fotiadis D, Stefanou K, Papafaklis M, Michalis L, Feldman C, Lerman A, Stone PH. Local low shear stress and endothelial dysfunction in patients with nonobstructive coronary atherosclerosis. *J Am Coll Cardiol* 2018;71:2092–2102.
16. Woudstra J, Vink CEM, Schipaanboord DJM, Eringa EC, den Ruijter HM, Feenstra RGT, Boerhout CKM, Beijk MAM, de Waard GA, Ong P, Seitz A, Sechtem U, Piek JJ, van de Hoef TP, Appelman Y. Meta-analysis and systematic review of coronary vasospasm in ANOCA patients: prevalence, clinical features and prognosis. *Front Cardiovasc Med* 2023;10:1129159.
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.
18. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd KG, Berry C. Stratified medical therapy using invasive coronary function testing in angina: the CorMicA trial. *J Am Coll Cardiol* 2018;72:2841–2855.
19. Sueda S, Fujimoto K, Sasaki Y, Sakaue T, Kohno H. Dose maximal acetylcholine dose into the left coronary artery affect the positive provoked spasm in the left circumflex artery? *Coron Artery Dis* 2019;30:547–548.
20. Sueda S, Kohno H, Miyoshi T, Sakaue T, Sasaki Y, Habara H. Maximal acetylcholine dose of 200 μg into the left coronary artery as a spasm provocation test: comparison with 100 μg of acetylcholine. *Heart Vessels* 2015;30:771–778.
21. Sueda S, Kohno H. The acetylcholine administration time plays the key role for provoked spasm in the spasm provocation test. *J Cardiol* 2017;70:141–146.
22. ter Haar CC, Maan AC, Warren SG, Ringborn M, Horáček BM, Schalij MJ, Swenne CA. Difference vectors to describe dynamics of the ST-segment and the ventricular gradient in acute ischemia. *J Electrocardiol* 2013;46:302–311.
23. Konst RE, Damman P, Pellegrini D, Hartzema-Meijer MJ, van Uden BJC, Jansen TPJ, Brandsma J, Vart P, Gehlmann H, Maas AHEM, van Royen N, Elias-Smale SE. Vasomotor dysfunction in patients with angina and nonobstructive coronary artery disease is dominated by vasospasm. *Int J Cardiol* 2021;333:14–20.
24. Lee DH, Park TK, Seong CS, Gwag HB, Lim AY, Oh MS, Cho SW, Yang JH, Song YB, Hahn JY, Choi JH, Lee SH, Gwon HC, Choi SH. Gender differences in long-term clinical outcomes and prognostic factors in patients with vasospastic angina. *Int J Cardiol* 2017;249:6–11.
25. Lee EM, Choi MH, Seo HS, Kim HK, Kim NH, Choi CU, Kim JW, Lim HE, Kim EJ, Rha SW, Park CG, Oh DJ. Impact of vasomotion type on

prognosis of coronary artery spasm induced by acetylcholine provocation test of left coronary artery. *Atherosclerosis* 2017;257:195–200.
26. Ong P, Athanasiadis A, Borgulya G, Vokshi I, Bastiaenen R, Kubik S, Hill S, Schäufele T, Mahrholdt H, Kaski JC, Sechtem U. Clinical usefulness, angiographic characteristics, and safety evaluation of intracoronary acetylcholine provocation testing among 921 consecutive white patients with unobstructed coronary arteries. *Circulation* 2014;129:1723–1730.
27. Feenstra RGT, Woudstra J, Bijloo I, Vink CEM, Boerhout CKM, de Waard GA, Wittekoek ME, de Winter RJ, Marques KMJ, Eringa EC, van de Hoef TP, Beijk MAM, Appelman Y, Piek JJ. Post-spastic flow recovery time to document vasospasm induced ischemia during acetylcholine provocation testing. *Int J Cardiol Heart Vasc* 2023;47:101220.
28. Feenstra RGT, Boerhout CKM, Vink CEM, Woudstra J, Wittekoek ME, de Waard GA, Appelman Y, Eringa EC, Marques KMJ, de Winter RJ, van de Hoef TP, Beijk MAM, Piek JJ. Haemodynamic characterisation of different endotypes in coronary artery vasospasm in reaction to acetylcholine. *Int J Cardiol Heart Vasc* 2022;42:101105.