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Post-spastic flow recovery time to document vasospasm induced ischemia during acetylcholine provocation testing

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ABSTRACT

Background: Intracoronary acetylcholine (ACh) provocation is an established method for diagnosing epicardial and microvascular vasospasm in contemporary clinical practice. We hypothesize that ACh-induced vasospasm is followed by post-spastic reactive hyperemia (PSRH), which is measured as an increased flow-recovery time.
Objectives: To assess flow-recovery time, indicative of ischemia, among the diagnostic endotypes that follow ACh provocation testing.
Methods: Patients with angina and non-obstructive coronary artery disease on angiography who underwent ACh provocation testing were included in this analysis. Doppler flow was continuously measured during the procedure and used to determine the flow-recovery time, which was calculated as time between cessation of ACh infusion and the point of flow recovery.
Results: Conventional provocation testing according to the COVADIS criteria diagnosed vasospasm in 63%(77/123), an equivocal result in 22%(27/123) and a negative result in 15%(19/123) of patients. In reaction to the highest-dose of ACh, flow-recovery time was significantly extended and similar in the epicardial, microvascular and equivocal test results compared to the negative result (all $p < 0.001$) indicative of PSRH.
Conclusion: Flow-recovery time in patients with an equivocal result is similar to patients with vasospasm, which indicates the occurrence of myocardial ischemia and therefore, these patients may benefit from medical treatment.

1. Introduction

Coronary artery vasospasm, either due to epicardial and/or microvascular spasm, is an established cardiac condition that causes anginal complaints in the majority of patients without obstructive coronary artery disease (ANOCA) [1]. Diagnosis and appropriate treatment have been shown to improve anginal burden and prognosis at long term follow up [2–3]. Invasive coronary function testing (ICFT) using Acetylcholine (ACh) is recommended for the diagnosis of coronary vasospasm in contemporary clinical practice in patients with ANOCA. According to the Coronary Vasomotion Disorders International Study Group (COVADIS) criteria, epicardial spasm is defined as (i) reproduction of the previously reported chest pain and (ii) the induction of ischaemic electrocardiograph (ECG) changes to objectify the occurrence

Abbreviations: ACh, acetylcholine; ANOCA, angina and no obstructive coronary artery disease; APV, average peak velocity; CAS, coronary artery spasm; COVADIS, Coronary Vasomotor Disorders International Study Group; ECG, Electrocardiograph; CFT, invasive coronary vasomotor function testing; PSRH, post-spastic reactive hyperemia.

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of ischaemia and (iii) >90% epicardial vasoconstriction. Microvascular spasm is defined as the first two criteria mentioned above in absence of epicardial vasoconstriction of >90% [4–7]. However, about one quarter of the patients undergoing ACh provocation testing only meet one of these COVADIS criteria, the so-called equivocal results, which may lead to diagnostic and therapeutic uncertainty [8–10]. Since equivocal test results are a frequent finding it is important to further characterize these patients in view of the potential clinical implications. Hyperemic flow after ischemia is a well-known physiological response that is documented extensively in the setting of coronary balloon occlusion [11]. We hypothesize that the flow-recovery time, which is the time it takes for the flow to return to baseline after cessation of Ach infusion, is a marker of ischemia that can be used to characterize the different endotypes [12]. The aim of this study is therefore to evaluate flow-recovery time of the equivocal test result compared to the epicardial and microvascular endotypes.

2. Methods

2.1. Study design

Between November 2016 and July 2020, ANOCA patients who underwent clinically indicated invasive coronary function testing (ICFT) at the Amsterdam UMC – location AMC (Amsterdam, The Netherland) were included in this retrospective cohort study. Ethical approval for this study was waived by the Medical Ethics Review Committee of the Academic Medical Centre because there were no alterations to routine clinical care.

2.2. Procedure and data acquisition

Prior to ICFT, beta blockers were discontinued for at least 72 h and all other vasoactive medication for at least 24 h prior to ICFT, to reduce the influence of vasoactive medication. First, diagnostic coronary angiography was performed using routine techniques, but without the use of radial cocktail or intracoronary nitrates prior to ACh infusions, to exclude significant obstructive coronary artery disease. Absence of obstructive CAD was defined as <50% diameter stenosis in the epicardial coronary arteries and/or instantaneous wave-free ratio >0.89. Afterwards, a 0.014-inch guidewire equipped with both a pressure sensor and a Doppler crystal (ComboWire XT, Philips Volcano Corporation, San Diego, CA) was advanced into the proximal or mid left anterior descending coronary artery to allow continuous registration of average peak flow velocity (APV), during ACh provocation testing. In part of the cases, a microcatheter was used to stabilize the Doppler flow signal. When a microcatheter was used, the ComboWire was first equalized at the tip of the guiding catheter and subsequently reintroduced through the microcatheter. ACh testing consisted of incremental three minute infusion of ACh concentrations of 0.2, 2.1, 21.1 and 211 μg/ml (i.e. 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} mmol/L) infused at 82 ml/h using a mechanical pump. [6] When angina occurred the infusion was halted prematurely. An angiography was performed after each ACh infusion and 200 mg of nitroglycerine was routinely administered intracoronary after the COVADIS criteria were met or after the fourth dose of ACh. The 12-lead ECG was continuously recorded using Mac-Lab (GE, United States). After Ach provocation testing coronary flow reserve and resistance measurements were performed using intracoronary bolus administration of adenosine (100mcg) as part of the ICFT, but this is beyond the reach of this study. All ICFT in this study were performed by one experienced operator (JJP).

2.3. Clinical characteristics

At baseline, data regarding cardiovascular risk factors, medical history and medication use was collected. In addition, symptom specific data was obtained, including (i) type of symptoms (rest angina, effort angina, dyspnea, or a combination), (ii) type of angina: typical angina (defined as constricting discomfort in the chest, neck, jaw, shoulder or arm, precipitated by exercise, relieved by rest or nitrates), (iii) atypical angina (meets two of the typical angina criteria), non-anginal chest pain (meets one or none of the criteria of typical angina). Furthermore, during follow-up data on medication use and angina status were collected. Follow-up was collected at 12 months and/or until optimal medical therapy was achieved (defined as the moment patients found their complaints acceptable) using patients medical records, and a telephone survey. Symptom improvement was defined as a decrease in angina frequency. Frequency of angina complaints was documented on a scale consisting of complaints: (i) 4 or more times a day, (ii) 1–3 times a day (iii) 3 or more times a week, (iv) 1–2 times a week, (v) less than once a week, (vi), no complaints.

2.4. Diagnostic criteria

Definitions and diagnostic criteria for coronary vasospasm were defined according to the COVADIS criteria for coronary spasm [5,7]. Epicardial vasospasm was defined as; (i) a reproduction of the previously reported angina symptoms, (ii) the induction of ischemic ECG changes (ST-segment deviation or new U-waves), and (iii) > 90% epicardial vasoconstriction by visual assessment in response to ACh. Microvascular vasospasm was diagnosed when the first two criteria mentioned above were met in absence of epicardial vasoconstriction of >90% [7]. The test result was considered equivocal when only angina or ECG changes occurred and the test was considered negative when neither recognizable angina nor ECG changes occurred.

2.5. Angiography and haemodynamic signal analysis

Hemodynamic data was collected from the digital archive (ComboMap, Philips-Volcano, San Diego, CA) and analyzed offline using custom software written in MATLAB(Mathworks, Inc, Natick, MA). Flow-recovery time analyses were performed by two independent analyst blinded to ACh provocation test results. First, a plot was obtained for APV averaged over one R-R interval after cessation of ACh infusion. Cessation of ACh infusion was identified by a bookmark attached to the hemodynamic data during the ACh provocation testing. A derivative function of a polynomial curve of the one-beat averaged APV trace was plotted to estimate the point of flow-recovery, which was considered when this function intersected with baseline. Flow recovery time (seconds) was calculated as time between cessation of ACh infusion and the point of flow recovery.

The reference interval of post-spastic reactive hyperemia PSRH was calculated from patients with epicardial and microvascular vasospasm using Reference Value Advisor V2.1 software. This software detects possible outliers according to the Tukay and Dixon-Reeds methods and computes a reference interval by the nonparametric method when n > 40 and provides 90% confidence interval for these two limits. The upper limit of normal was used as a reference value to detect abnormal flow-recovery indicative PSRH and consequently used as an alternative objective marker of ischemia to ECG changes to reclassify test result.

2.6. Statistical analysis

All statistical analyses were performed using SPSS version 27 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD) or median (first, third quartile [Q1, Q3]). Two-group comparisons of continuous variables were tested using a Student's T-test, or Wilcoxon signed-rank test in case of a non-normal distribution. Three-group comparisons were performed using a one-way ANOVA, or Kruskal-Wallis test in case of a non-normal distribution, followed by a pairwise comparison with Tukey correction. Categorical variables were compared using a chi-squared test, or Fisher's exact test in case of group sizes below 5 and Bonferroni correction was

used in case of multiple comparisons. A two-sided P value < 0.05 was considered statistically significant. A raincloud plot was made with an open access script on MATLAB [13].

3. Results

3.1. Study population

A total of 132 consecutive patients underwent clinically indicated intracoronary ACh vasospasm provocation testing with complete hemodynamic data available for the purpose of this analysis. We excluded 9 patients from the analysis for the following reasons that precluded assessment of flow-recovery time for the purpose of this analysis: in 3 patients the Doppler wire was repositioned during flow recovery period, 1 patient developed atrial fibrillation after ACh infusion, in 2 patients the procedure was terminated before flow recovery had occurred and in 3 patients the overall quality of the Doppler flow signal was insufficient for the purpose of the analysis. The final study population comprised 123 patients with a mean age of 56.3 ± 10.6 years and 85% of patients were female. Fifteen patients had vasospasm at low dose ACh infusion and 108 patients received all four doses of ACh. Fifteen percent (19/123) of patients had a negative test result, i.e. absence of angina symptoms and no ischemic ECG changes during ACh infusion. A further 36% (44/123) tested positive for epicardial vasospasm, 27% (33/123) for microvascular vasospasm and 22% (27/123) of patients had an equivocal test result. In the equivocal group, 26 patients had symptoms of angina without ECG changes and 1 patient had ECG changes without angina symptoms. Demographic and clinical data of the total population are summarized in Table 1. There were no significant differences in the baseline demographics among the different diagnostic endotypes.

Follow-up was available in 112 patients, 11 patients were lost to follow-up. Table 2 presents the medical treatment and anginal status after a 12 month follow-up and after optimal medical therapy. At 12 months follow-up, there was no significant difference in medical treatment between the epicardial, microvascular and equivocal test result group (Table 2). Symptom improvement was similar between epicardial spasm (72%), microvascular spasm (71%) and equivocal test result patients (67%) ($p = 0.839$) at 12 months follow-up.

3.2. Flow-recovery time

Fig. 1 shows the flow-recovery time per diagnostic endotype for each dose of ACh in 108 patients whom received all four doses of ACh. In reaction to the first, second and third dose flow-recovery times were all normally distributed and similar between all diagnostic endotypes as determined by one-way ANOVA ($p = 0.528$, $p = 0.415$ and $p = 0.635$, respectively) and were 40 ± 9 s for dose 1, 38 ± 10 s for dose 2 and 46 ± 28 s for dose 3. In response to the fourth dose flow-recovery time in the negative group showed a skewed distribution in contrast to all other diagnostic endotypes (Fig. 2). Furthermore, the flow-recovery time was extended in the epicardial vasospasm group (268 [205 – 315] sec), microvascular vasospasm group (291 [193 – 371] sec) and equivocal test result (250 [217 – 322] sec) compared to the negative (61 [31–181] sec) test result (all $p < 0.001$) indicative of impaired post-spastic flow recovery (Fig. 1 and Fig. 2). Fig. 3 shows the flow-recovery time per diagnostic endotype during the highest dose of ACh in all 123 included patients. Similarly, in line with the analysis depicted in Fig. 1, flow recovery time during the highest dose of ACh was extended in the

Table 1
Baseline characteristics.

	Total (n = 123)	Negative (n = 19)	Epicardial spasm (n = 44)	Microvascular spasm (n = 33)	Equivocal (n = 27)	p-value
Age, Years	56.3 ± 10.6	55.8 ± 12.6	59.3 ± 9.3	55.0 ± 10.8	56.3 ± 10.2	0.101
Female, n(%)	105 (85)	18 (95)	35 (80)	31 (94)	21 (78)	0.098
Height, cm	170.0 ± 8.9	166.5 ± 8.1	170.1 ± 9.5	170.0 ± 7.5	171.9 ± 9.6	0.240
Weight, kg	75.1 ± 13.2	70.4 ± 11.0	77.5 ± 14.7	74.2 ± 11.0	75.6 ± 14.3	0.263
BMI, kg/m^2	25.9 ± 3.9	25.5 ± 4.4	26.5 ± 3.9	25.6 ± 3.1	25.6 ± 4.4	0.718
Coronary risk factors						
Hypertension, n(%)	59 (48)	7 (37)	25 (57)	16 (49)	11 (41)	0.409
Hypercholesterolemia, n(%)	46 (37)	8 (42)	22 (50)	10 (31)	6 (22)	0.066
Diabetes, n(%)	7 (6)	0 (0)	2 (5)	4 (12)	1 (4)	0.211
Current or past smoking, n(%)	42 (34)	5 (26)	15 (34)	12 (36)	10 (37)	0.836
Current smoking, n(%)	9 (7)	1 (5)	4 (9)	1 (3)	3 (11)	0.835
Family history of CVD, n(%)	87 (71)	14 (74)	29 (66)	24 (73)	20 (74)	0.861
Normal left ventricular ejection fraction (>55%), n(%)	114/118 (97)	17/18 (94)	40/41 (98)	31/32 (97)	26/27 (96)	0.941
CAG & heamodynamic parameters						
Previous PCI n(%)	15 (13)	1 (5)	10 (23)	1 (3)	3 (12)	0.072
Heart rate at baseline	81.7 ± 13.8	82.0 ± 15.6	80.8 ± 14.0	84.8 ± 14.1	79.2 ± 11.4	0.484
Systolic blood pressure at baseline	145.3 ± 22.0	143.1 ± 26.0	147.2 ± 21.4	149.0 ± 21.6	139.1 ± 20.2	0.355
Diastolic blood pressure at baseline	81.5 ± 10.1	77.7 ± 10.2	81.5 ± 10.4	83.9 ± 9.8	81.0 ± 9.7	0.245
CFR	3.0 ± 0.7	3.0 ± 0.6	2.9 ± 0.7	3.1 ± 0.6	3.0 ± 0.7	0.602
HMR	1.9 ± 0.7	1.7 ± 0.8	2.0 ± 0.8	1.9 ± 0.5	1.9 ± 0.5	0.427
Baseline APV after nitrates	18.1 ± 7.5	20.2 ± 9.1	19.2 ± 8.5	16.5 ± 5.9	16.3 ± 5.0	0.189
Baseline Pa after nitrates	96.7 ± 16.1	91.8 ± 27.6	98.2 ± 10.8	98.2 ± 14.5	96.7 ± 13.0	0.534
Hyperaemic APV after nitrates	53.9 ± 18.2	60.6 ± 22.8	54.1 ± 18.8	52.1 ± 15.9	50.3 ± 14.6	0.290
Hyperaemic Pa after nitrates	91.9 ± 15.8	90.9 ± 26.5	92.3 ± 11.3	92.2 ± 14.3	91.5 ± 13.2	0.991
Symptoms and exercise testing before ICFT						
Rest angina n(%)	106 (86)	17 (90)	37 (84)	29 (88)	23 (85)	0.931
Effort angina n(%)	69 (56)	12 (63)	26 (59)	18 (55)	13 (48)	0.736
Dyspnoea n(%)	17 (14)	2 (11)	7 (16)	4 (12)	4 (15)	0.931
Exercise test performed n(%)	77 (68)	15 (83)	24 (56)	21 (75)	17 (68)	0.141
Significant ST deviation during ET n(%)	16 (21)	4 (27)	4 (17)	5 (24)	3 (18)	0.855
Vasospasm provocation						
Induction of recognisable angina		0 (0)	44 (100)	33 (100)	26 (96)	NA
ECG changes (COVADIS)		0 (0)	44 (100)	33 (100)	1 (4)	NA
Epicardial lumen reduction of ≥ 90%		0 (0)	44 (100)	0 (0)	14 (52)	NA

Values are mean ± SD or n (%). APV: average peak velocity, BMI: Body mass index, CAG: coronary angiography, CFR: coronary flow reserve, ET; exercise testing: HMR: hyperaemic microvascular resistance, NA: not applicable.

Table 2
Follow up.

	Total (n = 112)	Negative (n = 19)	Epicardial spasm (n = 39)	Microvascular spasm (n = 30)	Equivocal (n = 24)	p-value
Baseline						
Typical angina n(%)	33 (30)	6 (32)	16 (41)	5 (17)	6 (32)	0.162
Atypical anginan (%)	62 (55)	10 (53)	15 (38)	21 (70)	16 (67)	0.038*
Non-anginal chest pain n(%)	17 (15)	3 (16)	8 (20)	4 (13)	2 (8)	0.594
CCB use n(%)	53 (47)	8 (42)	21 (54)	16 (53)	8 (33)	0.363
BB use n(%)	20 (18)	2 (11)	5 (13)	4 (13)	9 (38)	0.068
Nitrates use n(%)	35 (31)	3 (16)	14 (36)	10 (33)	8 (33)	0.455
Other vasodilator medication n(%)	5 (5)	0 (0)	3 (8)	2 (7)	0 (0)	0.171
No vasodilator treatment n(%)	38 (34)	8 (42)	12 (30)	9 (30)	9 (38)	0.781
Follow-up 12 months						
CCB use n(%)	74 (65)	3 (16)[a,b,c]	34 (88)[a]	19 (63)[b]	17 (71)[c]	<0.001
BB use n(%)	17 (15)	1 (5)	5 (12)	6 (20)	5 (21)	0.379
Nitrates use n(%)	42 (38)	3 (16)	17 (44)	10 (33)	12 (50)	0.100
Other vasodilator medication n(%)	11 (10)	0 (0)	7 (18)	1 (3)	3 (13)	0.039*
No vasodilator treatment n(%)	21 (19)	13 (68)[a,b,c]	0 (0)[a]	5 (17)[b]	3 (13)[c]	<0.001
Symptoms improved n(%)	76 (68)	11 (58)	28 (72)	20 (67)	17 (71)	0.739
Optimal medical therapy						
CCB use n(%)	75 (67)	4 (21)[a,b,c]	35 (90)[a]	19 (63)[b]	17 (71)[c]	<0.001
BB use n(%)	18 (16)	1 (5)	7 (18)	5 (17)	5 (21)	0.456
Nitrates use n(%)	39 (35)	3 (16)	14 (36)	11 (37)	11 (46)	0.233
Other vasodilator medication n(%)	11 (10)	0 (0)	6 (15)	1 (3)	4 (17)	0.045*
No vasodilator treatment n(%)	21 (19)	12 (63)[a,b,c]	0 (0)[a,d]	6 (20)[b,d]	3 (12)[c]	<0.001
Symptoms improved n(%)	86 (77)	11 (58)	31 (80)	26 (87)	18 (75)	0.130

BB: beta-blockers, CCB: Calcium channel blockers. Other vasodilator medication consisted of, ivabradine, molsodimide and bosentan. [a] Statistically significant after Bonferroni correction between negative and epicardial spasm. [b] Statistically significant after Bonferroni correction between negative and microvascular spasm. [c] Statistically significant after Bonferroni correction between negative and equivocal. [d] Statistical significant after Bonferroni correction between epicardial spasm and microvascular spasm. *none of the endotypes are statistically significant after Bonferonni correction.

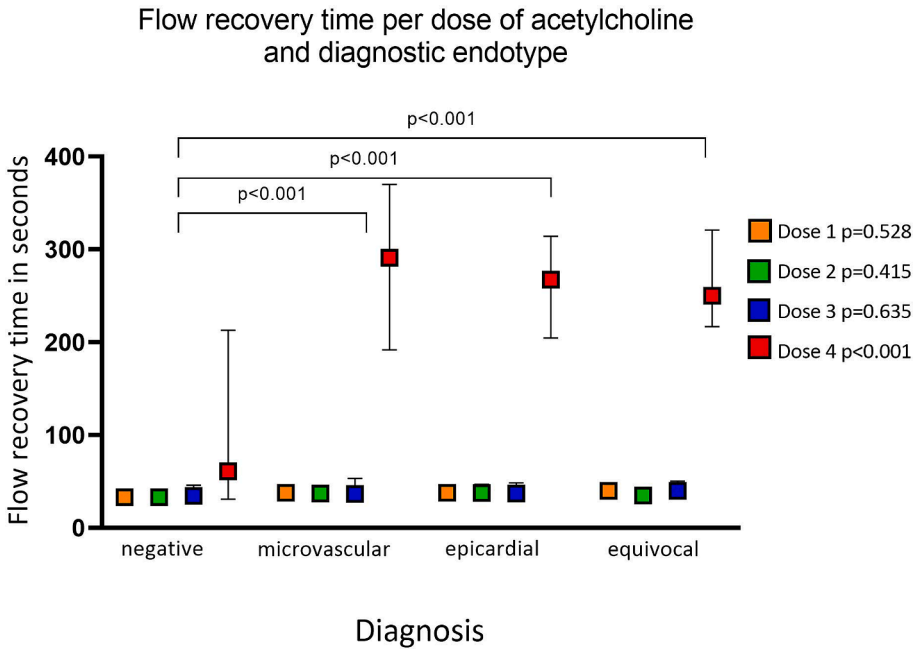

Fig. 1. Flow-recovery time in seconds per dose of acetylcholine among the diagnostic endotypes in patients receiving all four doses of acetylcholine. Data presented as median and interquartile range. Analysed using one-way ANOVA.

epicardial vasospasm group (266 [214 – 314] sec), microvascular vasospasm group (284 [193 – 369] sec) and equivocal test result (245 [206 – 322] sec) compared to the negative (61 [31–181] sec) test result (all $p < 0.001$). An illustrative example of the flow patterns of different endotypes during provocation testing is provided in Fig. 4.

3.3. Identification of flow-recovery time cut-off for reclassification

For the purpose of reclassifying the diagnostic endotypes an upper limit of flow recovery time indicative of PSRH was derived from patients with a positive diagnosis (epicardial and microvascular vasospasm) according to diagnostic COVADIS criteria. The upper normal limit of PSRH was found to be 105 s (90% CI: 83 – 134).

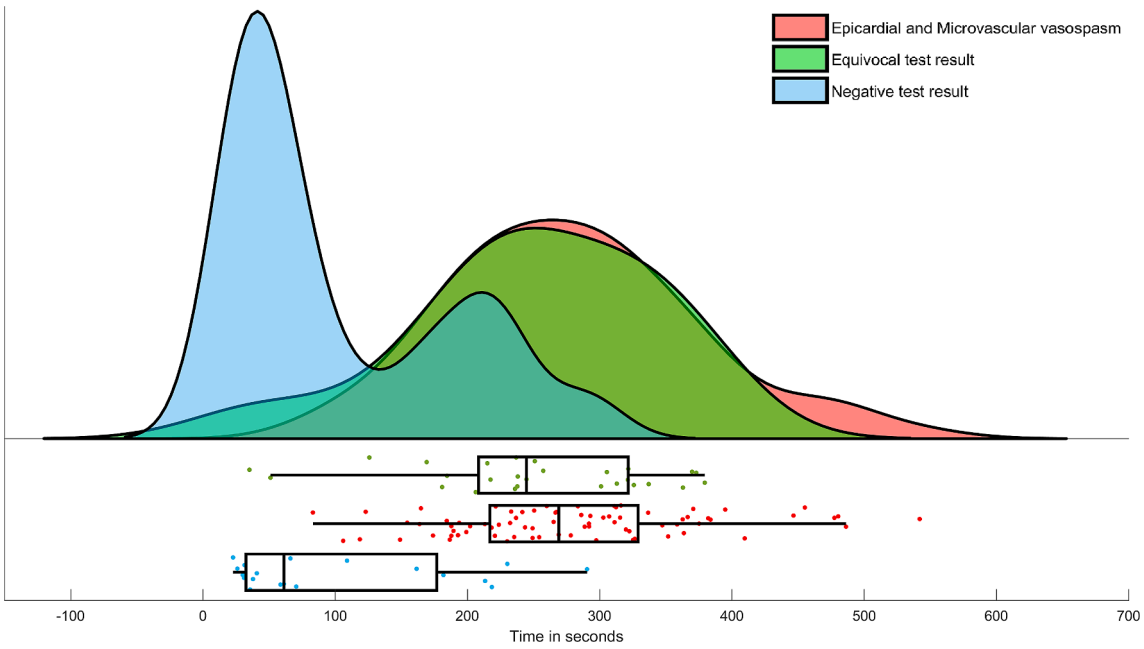

Fig. 2. Raincloud plot of flow recovery time after the highest dose of acetylcholine of patients in the negative group (blue), epicardial and microvascular vasospasm grouped together (pink) and from patients in the equivocal test results (yellow) comprising Gaussian kernel probability density, scatter and box-and-whisker plots.

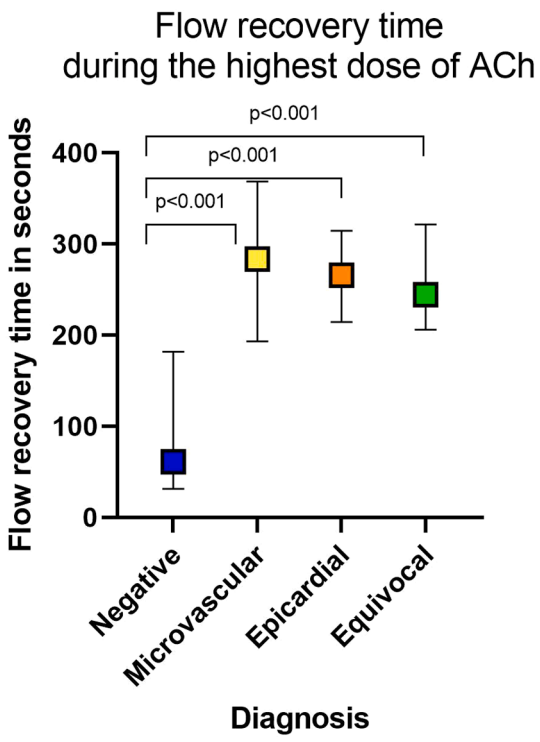

Fig. 3. Flow-recovery time in seconds among the diagnostic endotypes in all included patients during the highest dose of acetylcholine. Data presented as median and interquartile range. Analysed using one-way ANOVA. ACh. Acetylcholine.

When a flow-recovery time of >105 s, indicative of PSRH, was subsequently used as an alternative criterion of objective ischemia to ischemic ECG changes, 93% (25/27) patients out of the equivocal test result group transitioned into the epicardial or microvascular vasospasm group. As such, the diagnostic yield of the test increased by 20%; e.g. from 63% (77/123) to 83% (102/123), (Fig. 5). Furthermore, 37% (7/19) patients in the negative group had an extended flow recovery time, albeit without recognizable angina or ECG changes.

4. Discussion

The current study is the first to objectify the occurrence of ACh induced ischemia by means of post-spastic flow recovery time. The major finding of this study is that the equivocal endotype by current diagnostic criteria is accompanied by post-spastic reactive hyperaemia similar to patients with epicardial and microvascular vasospasm in contrast to the negative diagnostic endotype. This finding builds further on the premise that the equivocal endotype is a form of vasospasm and that PSRH, measured as post-spastic flow recovery time, can be used in addition to the surface 12-lead ECG as an objective diagnostic criterion for ischemia in the diagnosis of coronary vasospasm during ACh vasospasm provocation testing.

4.1. COVADIS diagnostic criteria for coronary spasm

The diagnostic criteria published by the COVADIS working group state that ischemic ECG changes (ST-deviation of 0.1mmV or new U-waves in two contiguous lead on a continuously measured 12-lead ECG) are an objective measure of ischemia in addition to inducible recognizable angina in reaction to ACh. Furthermore, a distinction between epicardial and microvascular spasm is made by > 90% epicardial vasoconstriction evident on angiography [5,7]. When strictly adhering to the above mentioned criteria a significant proportion of patients meet one of these criteria but not both (recognizable angina and ECG changes) and are thus considered a negative test result. Previously Ong and colleagues described this group as the equivocal test result [10]. In the current study population 22% (27/123) of patients undergoing vasospasm provocation testing have an equivocal test result, in the vast majority due to recognizable angina without the necessary ECG changes (96%). The percentage of equivocal diagnostic endotypes in the current study is similar to that reported previously that used different protocols [6]. The equivocal test result was present in 29% of 1379 patients undergoing spasm provocation in the ACOVA study and among 20.1% in a Korean cohort of 4644 patients by Lee et al. [9–10]. In the ACOVA study equivocal study group was defined as angina without ECG changes or

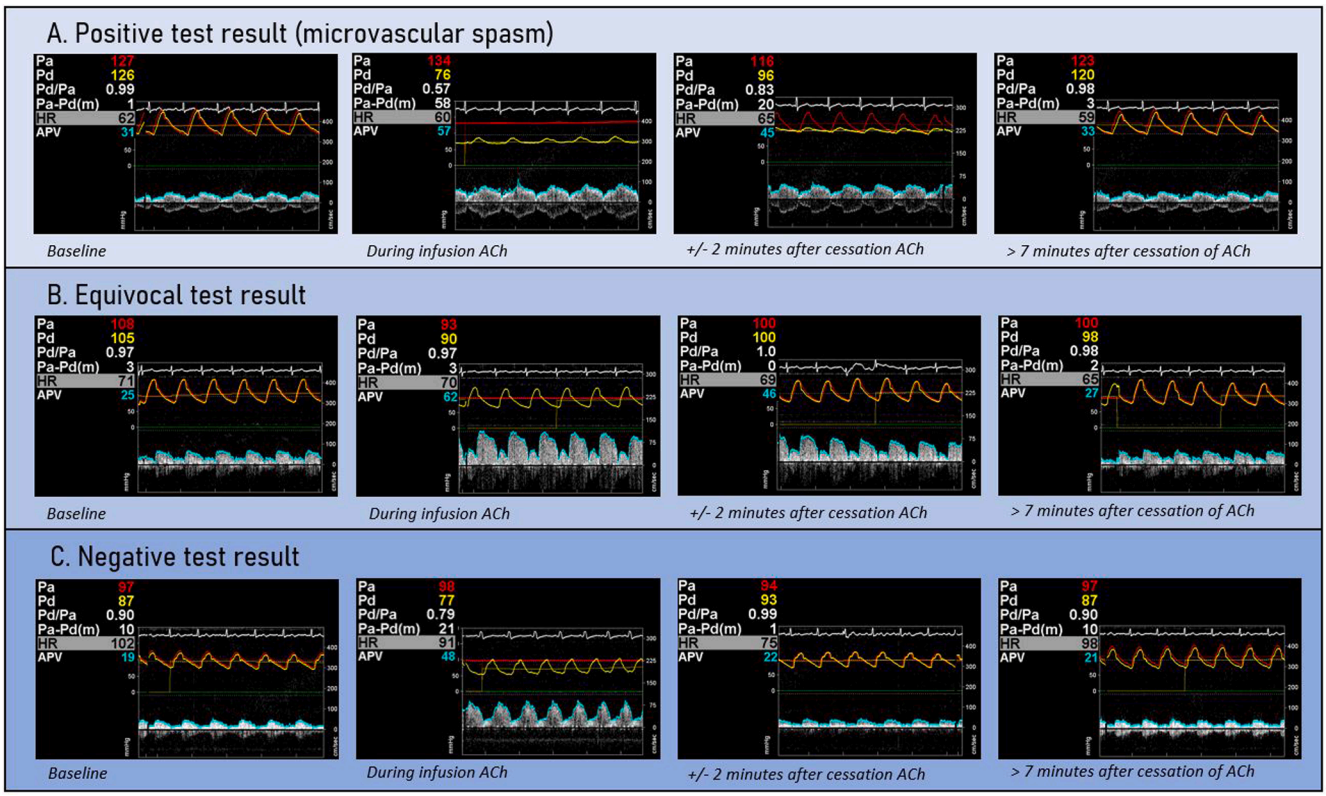

Fig. 4. An illustrative example of flow-patterns (blue line) expressed as average peak velocity (APV) of the different diagnostic endotypes during acetylcholine provocation testing. APV: average peak velocity, HR: heart rate, Pa: aortic pressure, Pd: distal pressure.

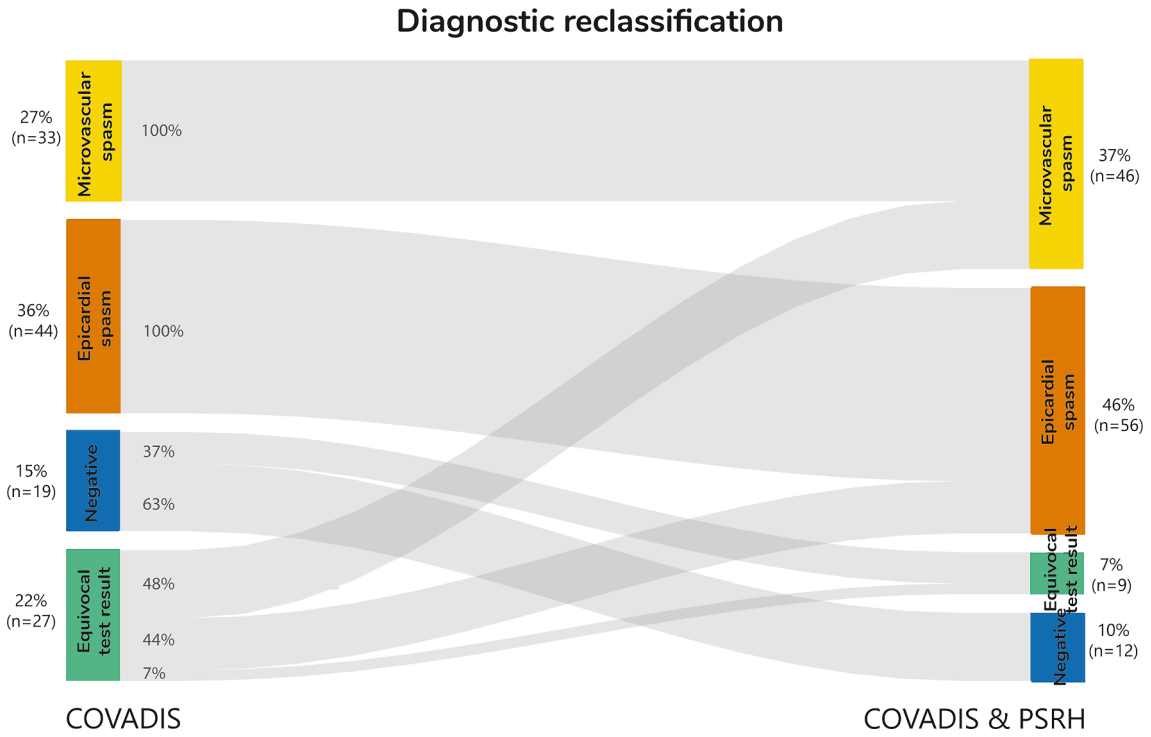

Fig. 5. A Sankey plot illustrating the reclassification of diagnostic endotypes when identification of post-spasm reactive hyperaemia is applied in addition to conventional provocation testing described by the COVADIS criteria. COVADIS: Coronary Vasomotion Disorders International Study Group, PSRH: Post-spastic reactive hyperemia.

ECG-changes or coronary vasospasm without angina, in the study of Lee et al it was defined as the latter.

4.2. The equivocal test result as an endotype of vasospasm

Our finding that the equivocal test result is a form of vasospasm is in line with previous studies. Previous studies have shown that the equivocal test result shares clinical features, angina status, medication use upon follow-up and hemodynamic changes during provocation testing with patients who test positive for vasospasm. For example, it was found in a large cohort of Korean patients. In this cohort, patients diagnosed with an equivocal endotype had similar features to epicardial vasospasm, such as predominance of male sex and a more frequently observed fixed lesion on baseline CAG as opposed to other diagnostic endotypes. Furthermore, Ong et al and Lee et al both reported a similar incidence of smoking in the equivocal and epicardial spasm group, which was increased compared to the normal group. A similar trend is seen in our baseline characteristics which did not reach statistical significance [2,9]. Likewise, in both studies, there was no difference in recurrent angina, Seattle angina score, and treatment regimen between the equivocal, epicardial and microvascular spasm group, in contrast to patients with a normal test result. This is in line with our findings, reporting no difference in medical treatment between equivocal, microvascular and epicardial spasm patients. In addition, our study demonstrated that symptom reduction was similar for equivocal, epicardial and microvascular spasm patients supporting that patients with an equivocal test result could benefit from tailored medical treatment.

Recently, we reported on the hemodynamic changes measured as coronary blood flow and vascular resistance among the diagnostic endotypes of vasospasm provocation testing. Herein, we demonstrated that in response to high-dose ACh the equivocal test result group has hemodynamic changes that suggest VSMC hyperreactivity similar to the microvascular vasospasm group, but with a preserved endothelial function, which is in contrast to the negative test result [14].

In addition to ischemic ECG changes, another objective measurement for ischemia is sinus lactate measurement, which has not yet been studied together with flow recovery time. A previous study has reported lactate production, which is indicative of ischemia, in patients undergoing ACh provocation testing not meeting the criteria for epicardial or microvascular spasm, suggesting that these patients are similar to patients in the equivocal group in our study and current guidelines fail to diagnose all coronary vasospasm patients [15].

4.3. Possible explanations for the absence of ECG changes

An equivocal test result in the current study is mainly caused by the absence of transient ST-deviations on the surface ECG. Therefore, relying solely on such changes on the 12-lead ECG changes as an obligatory criterion for diagnosing CAS during vasospasm provocation could be too stringent to adequately identify patients with ACh induced ischemia. There are several technical factors that could be at play, causing such equivocal test results.

First, it has been frequently demonstrated that the occurrence of fulminant coronary vasospasm is dependent on the intracoronary ACh concentrations achieved at cellular level, which in turn, is influenced by different injection times and dosages of ACh [6]. For example, studies by Sueda et al. have demonstrated that by increasing the maximal dose of ACh from 100 to 200 μg, injected in 20 s, the number of positive tests will increase and multivessel spasm will be diagnosed more frequently [16–17]. Similarly, patients in whom a positive ACh provocation test was achieved using the 20 s protocol were re-tested with the same total dose of ACh injected over 3 min which revealed that spasm could be provoked more frequently using the 20 s injection compared to the 3 min infusion (73.3% vs. 33.3%, $p < 0.01$) [18]. Similarly, the occurrence of ECG changes during the test is most probably also affected by intracoronary ACh concentrations. Thus, patients with equivocal test results at sub-optimal intra-coronary achieved ACh concentrations may transition to a positive test result if the dose of ACh is increased or if the injection time in which the dose is administered is shortened.

Second, ischemic ECG changes might not occur at all, regardless of the aforementioned variations in vasospasm protocol. In a cohort of 84 patients undergoing elective percutaneous transluminal coronary angioplasty Ter Haar et al. described the ECG changes that occur in the hyperacute phase of ischemia during balloon occlusion of coronary epicardial arteries. The mean balloon occlusion time was 260 ± 76 s in this study and the authors found ST-segment elevation in only 55% of cases [19]. Furthermore, they showed that one minute of balloon-occlusion was required before ST-segment deviation of >0.1 mV occurred and after which the ST-segment deviation increased further [19]. These findings may also translate to ACh induced ischemia, since the short half-life of ACh could prevent the occurrence of ischemic ECG changes. As such, if a high enough intracoronary ACh concentration is achieved for a longer period of time, it would allow for ECG changes to develop.

Third, the absence of ischemic ECG changes in the majority of the equivocal group could also be explained by the fact that ECG changes can be minimal or absent in the case of ST-segment cancellation when concurrent ischemia of regions supplied by the Left Anterior Descending and Circumflex coronary arteries occurs. This can easily occur when ACh is administered in the Left Main coronary artery, which is most common in the majority of vasospasm provocation protocols [20]. In this case, a further increase in ACh dosages or duration of ACh injections would not increase the diagnostic yield of spasm provocation testing.

4.4. PSRH detection in addition to the 12-lead ECG

Reactive hyperemia after an period of ischemia is a well-known and frequently described mechanism and results from an interplay between physical and metabolic factors, including among others nitric oxide [21], prostaglandins [22] and adenosine [23]. The current study is the first study to describe hyperaemia in reaction to ACh induced ischemia, demonstrating comparable Doppler flow patterns as seen after balloon occlusion [11].

Based on the upper limit normal, the optimal cut-off point for a flow-recovery time that indicates PSRH in the current population with the protocol used in this study was found to be 105 s. In the current study population, the diagnostic yield increased by 20% when identification of PSRH was used as an alternative objective criterion of ischemia in the absence of ischemic ECG changes when recognizable angina occurred after ACh administration. Performing Doppler flow measurement during ACh vasospasm provocation for the purpose of PSRH measurements can be easily implemented, since the Doppler flow wire is already used to assess endothelial function in reaction to ACh and to assess vasodilator function in reaction to adenosine, most notably the coronary flow reserve, as part of a comprehensive ICFT.

Interestingly, 37% (7/19) of patients with a negative test result had an increased flow-recovery time indicative of PSRH that is represented by a second wave in the raincloud plot (Fig. 2). This is an interesting observation, showing a dual response within the negative test result group indicating that there are patients in this group with a prolonged flow recovery time, suggestive of myocardial ischemia. It is well known that two-thirds of episodes of vasospasm occur asymptomatically and are thought to be attributed to short episodes of CAS that may partly explain these findings in the negative test result group [24]. It also suggests that there are patients within the negative test result group that may benefit from medical treatment.

4.5. Clinical implications

When strictly adhering to the COVADIS criteria for coronary spasm, the equivocal diagnostic endotype is considered a negative test result in

vasospasm provocation testing, whilst in fact this group has a flow-recovery time that indicates the occurrence of PSRH similar to the epicardial and microvascular spasm endotypes.

This may have diagnostic and therapeutic consequences as patients with an equivocal test result will consequently not receive appropriate therapy, leading to persistent angina, and a reduced quality of life. [25–26] This patient group could profit from the treatment with anti-vasospastic medication, such as calcium channel blockers and nitrates. Using PSRH as an additional marker of myocardial ischemia transfers patients in the equivocal group to a positive test result. These patients may benefit from installed medical therapy, as shown in the present manuscript.

4.6. Limitations

Several limitations should be considered. First, this was a retrospective study, resulting in biases inherent to the study design. Second, no direct measure of ischemia, for example through coronary sinus sampling of lactate was performed in the equivocal group and post-ischemic recovery time has not been validated within an experimental model. Nonetheless, an increased hyperemia after ischemia is validated in a balloon angioplasty model [11]. Finally, the widespread use of a Doppler wire is limited in clinical practice, as it requires specific training with a learning curve and assessment of flow-recovery time is only possible using a Doppler wire.

4.7. Conclusion

This is the first study investigating PSRH in ANOCA patients undergoing ACh vasospasm provocation. We found that flow-recovery time after ACh administration is increased in the equivocal test result group similar to the epicardial and microvascular vasospasm groups indicating the occurrence of PSRH. These findings provide important additional evidence that the equivocal diagnostic endotypes should not be considered as a negative test result as current guidelines recommend.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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