










Non-invasive Coronary Functional Tests in Patients with Angina and Non-obstructive Coronary Artery Disease

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Abstract

Background: Invasive coronary functional tests (I-CFTs) can identify the mechanism(s) of angina in patients with non-obstructed coronary arteries (ANOCA). In this study, we assessed whether non-invasive coronary functional tests (NI-CFTs) can also produce reliable results when assessing these mechanisms. **Methods:** We performed NI-CFTs by recording coronary blood flow velocity (CBFV) in the left anterior descending coronary artery by transthoracic Doppler echocardiography in 18 patients with ANOCA who had undergone I-CFTs (an acetylcholine provocation test and an adenosine stress test) and 13 healthy controls. The NI-CFTs included hyperventilation, a dipyridamole stress test and a cold pressor test. **Results:** Acetylcholine induced epicardial or coronary microvascular spasm in 11 patients (61.1%), whereas adenosine coronary flow reserve (CFR) was reduced (<2.5) in seven (39%). Hyperventilation-induced coronary vasoconstriction produced a reduction in CBFV >10% compared to baseline in eight patients (44.4%) and none in the control group ($p=0.005$). Dipyridamole-CFR was lower in the patient group than in the control group (2.25 ± 0.49 versus 2.76 ± 0.49 ; $p=0.01$) and correlated with adenosine-CFR ($r=0.75$; $p<0.001$). Full agreement in coronary abnormalities detected during I-CFTs and NI-CFTs (hyperventilation/dipyridamole stress test) was found in 14 patients (77.8%). Furthermore, cold pressor test-CFR was lower in patients than in the control group (1.33 ± 0.18 versus 1.52 ± 0.22 ; $p=0.019$); cold pressor test-CFR <1.35 identified three patients who showed both normal I-CFTs and a normal response to hyperventilation and dipyridamole at NI-CFTs. **Conclusion:** In ANOCA patients, the results of NI-CFTs performed with transthoracic Doppler echocardiography of the left anterior descending coronary artery showed a high correlation with coronary function abnormalities detected using I-CFTs. Our data suggest that NI-CFTs deserve investigation in larger multicentre studies to assess their usefulness in guiding clinical management of ANOCA patients.

Keywords

Angina with non-obstructed coronary arteries, coronary functional tests, transthoracic Doppler echocardiography, hyperventilation test, dipyridamole stress test, cold pressor test

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Ethics: The study was approved by the Institutional Ethics Committee of Fondazione Policlinico Universitario A Gemelli IRCCS and was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Consent: All patients gave their written informed consent to participate in the study.

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Up to 60% of patients referred to the cath lab for chronic stable angina have non-obstructive coronary artery disease shown by angiography.¹ Several studies have consistently shown that a large proportion of these patients present with coronary microvascular abnormalities and/or epicardial coronary artery spasm (CAS).^{2–4} Studies have also suggested that treatment of these patients guided by the results of invasive coronary functional tests (I-CFTs) performed during coronary angiography, which aim to identify the mechanism(s) of angina chest pain, might improve the

symptomatic outcome of these patients with angina and non-obstructed coronary arteries (ANOCA).^{5,6}

However, performing I-CFTs may significantly prolong the invasive procedure, which is also associated with increased radiological exposure and dose of contrast medium, as well as a small increase in adverse events.^{7–9} Moreover, while the efficacy of chronic drug therapy on CAS can be predicted by its prevention by IV/intracoronary nitrates during coronary

angiography, the assessment of the effects of treatment on coronary microvascular abnormalities detected by I-CFTs is challenging and the invasive procedure would need to be repeated.¹⁰

Accordingly, assessing functional abnormalities of coronary circulation by non-invasive coronary functional tests (NI-CFTs) in ANOCA patients might be a good way to inform the clinical management and follow-up of these patients. Previous studies showed that several non-invasive methods, including PET, magnetic resonance and echocardiographic methods, are reliable in assessing coronary microvascular dilatation in these patients when compared with invasive results.^{11–13}

However, while several studies have shown that NI-CFTs are reliable in identifying patients with inducibility of epicardial CAS, mainly through the detection of electrocardiographic (ST-segment changes) and/or echocardiographic (new transient regional left ventricle wall motion abnormalities [RWMA]) ischaemic changes during provocative tests, but no previous study has investigated whether the detection of coronary vasoconstriction by NI-CFTs may also reflect a patient's susceptibility to CAS induction by I-CFTs.^{14–17} Further, no study has previously investigated whether an abnormal coronary microvascular constrictive response, demonstrated by I-CFTs, can also be predicted using non-invasive methods. Finally, to the best of our knowledge, there has been no comprehensive assessment of both vasodilator and vasoconstrictor coronary function by NI-CFTs in comparison with results from I-CFTs.

In this study, we investigated whether NI-CFTs can reliably identify the different functional coronary abnormalities detectable by I-CFTs in patients with ANOCA by performing provocative tests during transthoracic Doppler echocardiography (TTDE) of the left anterior descending coronary artery (LAD-CA).

Methods Participants

We enrolled patients who fulfilled the following inclusion criteria: 1) coronary angiography performed within the previous 12 months at the catheterisation laboratory of our hospital (Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy) because of typical stable angina chest pain, showing no significant coronary obstructions (i.e., no stenosis >50% or fractional flow reserve <0.80); and 2) I-CFTs, including both adenosine and acetylcholine testing, performed during the procedure.

Patients with any of the following clinical conditions were excluded: referral for acute coronary syndrome; evidence of typical variant angina (recurrent episodes of chest pain at rest associated with documented transient ST segment elevation at the ECG); previous interventions of coronary revascularisation; asthma or known adverse reactions to adenosine/dipyridamole; serious medical conditions, including renal failure defined by an estimated glomerular filtration rate (eGFR) <30 ml/min, liver diseases, malignancies, acute or chronic inflammatory autoimmune diseases; pregnancy; any condition that the investigators judged would have made it difficult for the patient to complete the study protocol.

Our study complies with the Declaration of Helsinki. All patients gave written informed consent to participate in the study, which was approved by our Institutional Ethics Committee.

Invasive Coronary Functional Tests

An acetylcholine provocation test and an adenosine stress test were

performed during invasive coronary angiography in patients with ANOCA. Coronary flow reserve (CFR) was measured with the intracoronary thermodilution method as the ratio between basal transit time and hyperaemic transit time during IV adenosine infusion (0.14 mg/kg/min) of an intracoronary saline bolus and considered abnormal when lower than 2.5.¹⁸ Susceptibility to epicardial or microvascular coronary spasm was assessed with the administration of incremental intracoronary boluses of 20, 50 and 100 µg of acetylcholine in the left coronary artery. Epicardial coronary artery spasm (CAS) was defined as the induction of total or subtotal (≥90%) vessel occlusion associated with angina and ischaemic ECG changes.¹⁹ Coronary microvascular spasm (CMVS) was defined as the induction of typical angina symptoms associated with ischaemic ECG changes during acetylcholine administration in the absence of CAS.¹⁸

Non-invasive Coronary Functional Tests

NI-CFTs were performed by the same cardiologist (NC) skilled in echocardiography and coronary blood flow velocity (CBFV) recording in the LAD-CA, who was blinded to the I-CFT results. Patients on β-blockers or calcium channel-blocker therapy were asked to discontinue the drug for 48 hours before the study. Patients were also asked to abstain from food and drinks containing xanthine, such as coffee, tea and chocolate, on the day of the tests.

After a rest period of 10 minutes, a complete baseline echocardiographic examination was obtained using an E95 echocardiographic machine (GE Healthcare), with assessment of global and regional wall motion contractility.

Then, the mid-distal portion of the LAD-CA was visualised by colour Doppler and peak diastolic CBFV was measured by pulsed-wave Doppler. After obtaining the baseline values, the following functional tests were performed sequentially, with 15-minute intervals after returning to the CBFV baseline, under continuous 12-lead ECG monitoring, regular assessment of blood pressure and monitoring of peripheral blood oxygen saturation (SpO₂):

- Hyperventilation test (HVT), where the participant was asked to breathe deeply and rapidly at a rate of 30 respirations per minute for 5 minutes.
- The cold pressor test (CPT), where the patient was asked to immerse their right hand in ice water for 2 minutes.
- The dipyridamole stress test (DST), which involves an IV infusion of 0.84 mg/kg of dipyridamole over 6 minutes.

At the end of DST, 70–240 mg of IV aminophylline over 3 minutes was given in case of side effects, such as chest pain, headache or palpitations.^{20–24}

The occurrence of symptoms, ECG changes and left ventricle RWMA were assessed during each test. At the peak of each test, CBFV in the LAD-CA was recorded and peak diastolic CBFV was measured as the average of three consecutive cardiac cycles. A preliminary assessment of intra-operator reproducibility showed <10% variability in measurements.

Control Group

The control group was made up of apparently healthy volunteers who did not have any symptoms or clinical history of heart disease. These subjects were enrolled from a group of totally asymptomatic adult subjects referred for a screening DST before starting non-competitive physical activity.

Control subjects underwent the same non-invasive tests following the same protocol as described for the ANOCA patients.

Definitions

The occurrence of coronary vasoconstriction during NI-CFTs was diagnosed when there was a reduction of CBFV >10% compared to the basal value (the ratio between CBFV at peak HVT and baseline CBFV <0.90). CFR assessed by dipyridamole infusion (DIP-CFR) was calculated as the ratio between maximal CBFV during the test and baseline CBFV and was considered reduced when lower than 2.0.^{18,24} Similarly, the coronary vasodilator response to CPT (CPT-CFR) was calculated as the ratio between maximal CBFV during the test and the basal CBFV. For purposes of this study, CPT-CFR was considered abnormal when it was lower than the tenth percentile value found in healthy controls.

Endpoints

The primary endpoint of the study was the correlation between coronary vasoconstriction detected by TTDE in response to HVT and the occurrence of a vasospastic response (either CAS or CMVS) during invasive acetylcholine test. Secondary endpoints of the study included: the correlation between non-invasive DIP-CFR and CFR measured invasively with adenosine, and the correlation between non-invasive CPT-CFR and both invasive adenosine CFR and non-invasive DIP-CFR.

Sample Size Calculation

We calculated that to have a statistical power of 80% to demonstrate as significant, at $p < 0.05$, a correlation Φ value of 0.70 or higher between a vasoconstrictor response to HVT and a vasospastic response to intracoronary acetylcholine (either CAS or CMVS), we needed to enrol 18 patients and 11 controls, while assuming a patient drop-out rate of 10%.

Statistical Analysis

The Kolmogorov–Smirnov test was applied to check for normal distribution of continuous variables. Comparisons of normally distributed variables between groups were done by independent t-test, whereas comparisons involving non-normally distributed variables were done with the Mann–Whitney U-test. Correlation analyses of normally distributed continuous variables were done by Pearson’s test, whereas correlations involving non-normally distributed variables were done by Spearman test. A Φ correlation test was applied to assess the relation between dichotomous variables. Fisher’s exact test was applied for comparisons of proportions. A receiver operating characteristic (ROC) curve was constructed to identify the non-invasive DIP-CFR value that best predicted an invasive adenosine CFR <2.5.

Continuous variables are summarised as mean and SD, while qualitative variables are shown as numbers and proportions. A p -value <0.05 was considered as statistically significant. All analyses were performed by the SPSS 28.0 statistical software.

Results

Patient Characteristics

Overall, 44 patients with ANOCA underwent I-CFTs for chronic angina chest pain at our centre between September 2023 and September 2024 and were eligible for the study. Five patients declined to participate and 19 patients were excluded due to the presence of relevant comorbidities, so 18 patients formed the final population of ANOCA patients in the study. In the control group, we enrolled 13 asymptomatic, apparently healthy subjects without any evidence of cardiovascular disease. The main clinical characteristics of the two groups are summarised in *Table 1*. There were

Table 1: Main Clinical Characteristics of Patients

	ANOCA (n=18)	Control (n=13)	p-value
Age (years)	57.1 ± 10	55.5 ± 9	0.67
Male sex	7 (38.9%)	8 (61.5%)	0.21
BMI (kg/m ²)	24.1 ± 2.7	25.2 ± 4.2	0.43
Cardiovascular risk factors			
Hypertension	7 (38.9%)	2 (15.4%)	0.16
Diabetes	4 (22.2%)	0 (0%)	0.07
Smoker	2 (11.1%)	1 (7.7%)	0.75
Dyslipidaemia	10 (55.6%)	2 (15.4%)	0.02
Drug therapy			
β-blocker	5 (27.8%)	1 (7.7%)	0.16
Calcium channel blocker	9 (50%)	1 (7.7%)	0.01
ACE inhibitor/ARB	10 (55.6%)	2 (15.4%)	0.02
Nitrate	2 (11.1%)	–	0.21
Ranolazine	3 (16.7%)	–	0.12
Ivabradine	1 (5.6%)	–	0.39
ASA	9 (50%)	–	0.002
Statin	10 (55.6%)	2 (15.4%)	0.02

Data are presented as mean ± SD or n (%). ACE = angiotensin-converting enzyme; ARB = angiotensin-II receptor blocker; ASA = acetylsalicylic acid.

no significant differences between the two groups in terms of age, sex and cardiovascular risk factors, except for hypercholesterolaemia, which was more common among the ANOCA patients.

Invasive Coronary Functional Tests

Overall, the acetylcholine test induced a coronary spastic response in 11 of 18 patients (61.1%), five of whom (27.8%) developed CAS and six (33.3%) developed CMVS, while seven patients (38.9%) had a negative acetylcholine test. Adenosine CFR was 3.16 ± 1.23 and was reduced (<2.5) in seven patients (38.9%). Both a vasospastic response to acetylcholine and an impaired adenosine CFR were found in five patients (27.8%).

Non-invasive Coronary Functional Tests

The main results of NI-CFTs in patients and controls are summarised in *Table 2*. At baseline, there was no significant difference between the two groups in CBFV in the LAD-CA, heart rate and blood pressure. Global and regional left ventricle contractility were normal in all subjects.

Hyperventilation Test

Three ANOCA patients (16.7%) had angina chest pain during HVT, but no control subjects did. Neither ANOCA patients nor control subjects showed significant ST-segment changes or left ventricle RWMA during the test.

The ratio between CBFV at peak HVT and basal CBFV was 0.95 ± 0.17 versus 1.17 ± 0.20 in patients and controls, respectively ($p = 0.004$; *Figure 1*). A vasoconstrictor response (reduction of CBFV >10%) during HVT was found in eight ANOCA patients (44.4%), but in no control subjects ($p = 0.005$). Coronary vasoconstriction during HVT was significantly correlated with a positive acetylcholine test (either epicardial CAS or CMVS; Φ coefficient 0.71; $p = 0.002$). All six patients with acetylcholine-induced CMVS and two of five patients with acetylcholine-induced epicardial spasm (40%) had a vasoconstrictive response to HVT. All patients with a negative invasive acetylcholine test had negative HVT

Table 2: Results of Non-invasive Coronary Functional Tests

	ANOCA (n=18)	Control (n=13)	p-value
Basal values			
Heart rate (bpm)	70 ± 11	71 ± 11	0.83
Systolic BP (mmHg)	125 ± 19	122 ± 15	0.68
Diastolic BP (mmHg)	67 ± 6	70 ± 9	0.43
CBFV-LAD (cm/s)	30.7 ± 5.5	27.5 ± 6.2	0.14
Hyperventilation test			
Heart rate (bpm)	78 ± 15	75 ± 12	0.55
Systolic BP (mmHg)	121 ± 17	120 ± 18	0.89
Diastolic BP (mmHg)	71 ± 8	73 ± 9	0.62
CBFV-LAD (cm/s)	29.1 ± 6.8	32.1 ± 9.3	0.68
Ratio peak/basal CBFV-LAD	0.95 ± 0.20	1.17 ± 0.20	0.004
Coronary vasoconstriction	8 (44.4%)	0 (0%)	0.005
Cold pressor test			
Heart rate (bpm)	73 ± 11	76 ± 12	0.57
Systolic BP (mmHg)	137 ± 30	121 ± 17	0.08
Diastolic BP (mmHg)	73 ± 13	73 ± 8	0.96
CBFV-LAD (cm/s)	40.5 ± 7.4	41.8 ± 10	0.69
CFR-CPT	1.33 ± 0.18	1.52 ± 0.22	0.022
CFR-CPT <1.35	10 (55.6%)	1 (8.3%)	0.018
Dipyridamole stress test			
Heart rate (BPM)	91 ± 16	96 ± 13	0.25
Systolic BP (mmHg)	122 ± 24	115 ± 16	0.84
Diastolic BP (mmHg)	66 ± 11	66 ± 7	0.50
CBFV-LAD (cm/s)	67.8 ± 13	74.8 ± 14	0.17
CFR-DIP	2.25 ± 0.49	2.76 ± 0.49	0.007
CFR-DIP <2.0	6 (33.3%)	0 (0%)	0.028

Data are presented as mean ± SD or n (%). BP = blood pressure; CBFV-LAD = coronary blood flow velocity in the left anterior descending artery; CFR-CPT = coronary flow reserve in response to CPT; CFR-DIP = coronary flow reserve in response to dipyridamole.

(sensitivity and specificity for positive acetylcholine test of HVT were 73% and 100%, respectively). An example of a normal and abnormal response to HVT is shown in Figure 2.

Cold Pressor Test

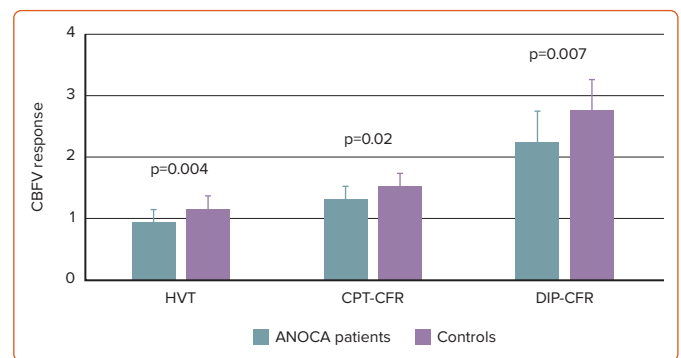
No patient or control subject developed angina, ischaemic ECG changes or left ventricle RWMA during the CPT. No vasoconstrictor response was observed in patients or controls during the test. CPT-CFR was significantly lower in ANOCA patients compared to controls (1.33 ± 0.18 versus 1.52 ± 0.22; p=0.022; Figure 1). The lowest tenth percentile value of CPT-CFR in control subjects was 1.35. A CPT-CFR <1.35 was found in one control subject (7.7%) and in 10 ANOCA patients (55.6%) (p=0.009).

Dipyridamole Stress Test

No patient or control subject developed angina, ischaemic ECG changes or left ventricle RWMA during DST. DIP-CFR was significantly lower in patients compared to controls (2.25 ± 0.49 versus 2.76 ± 0.49; p=0.007; Figure 1). A DIP-CFR <2.0 was found in six ANOCA patients (33.3%), but in no control subjects (p=0.07).

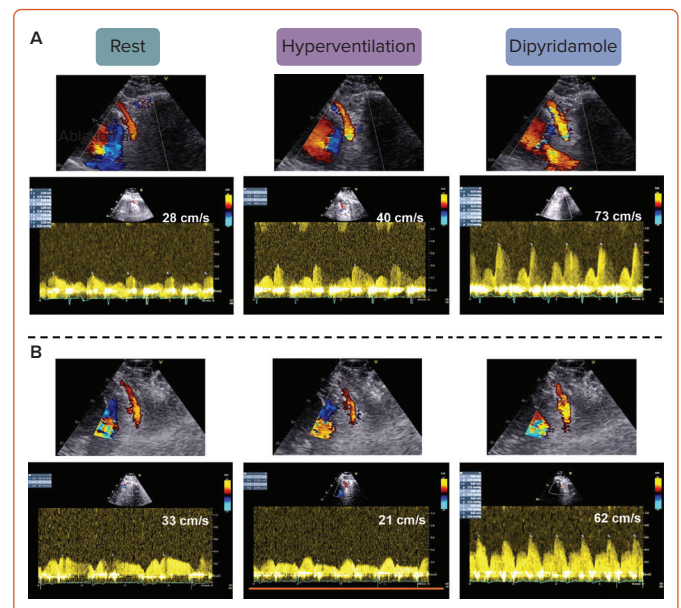
There was a strong correlation between DIP-CFR and invasive adenosine

Figure 1: Coronary Blood Flow Velocity Response to Non-invasive Coronary Functional Tests



ANOCA = angina and non-obstructed coronary arteries; CBFV = coronary blood flow velocity; CFR = coronary low reserve; CPT = cold pressor test; DIP = dipyridamole; HVT = hyperventilation test.

Figure 2: Coronary Blood Flow Velocity Recording in the Left Anterior Descending Coronary Artery by Transthoracic Colour-Doppler Echocardiography in Two Patients with Angina and Non-Obstructed Coronary Arteries



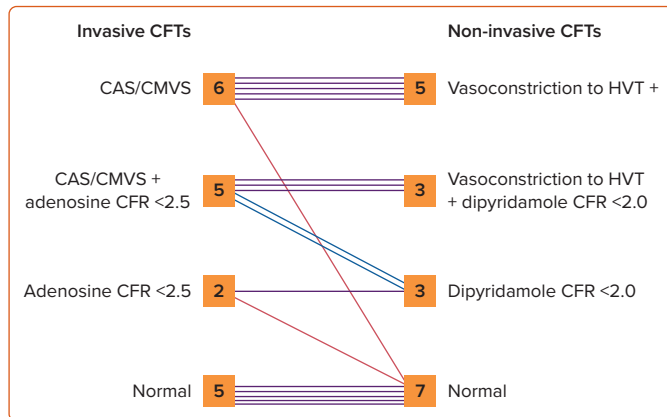
A: Patient with a normal CBFV response to both hyperventilation (peak/rest diastolic CBFV = 1.43) and dipyridamole (peak/rest diastolic CBFV = 2.61). B: Patient with a coronary vasoconstrictor response to hyperventilation (peak/rest diastolic CBFV = 0.43) and a reduced dilator response to dipyridamole (peak/rest diastolic CBFV = 1.88). CBFV = coronary blood flow velocity.

CFR (r=0.75; p<0.001). Among ANOCA patients, DIP-CFR value <2.0 had 86% sensitivity and 100% specificity for invasive adenosine CFR <2.5. Notably, at ROC curve analysis a DIP-CFR <2.0 was the best cut-off value for identification of patients with invasive adenosine CFR <2.5 (area under the curve = 0.864). Examples of normal and abnormal dilator responses to dipyridamole are shown in Figure 2.

Endotype Classification

The relation between the individual results of I-CFTs (acetylcholine and adenosine tests) and the corresponding NI-CFTs is summarised in Figure 3. Four possible types of response to CFTs were considered: a coronary vasoconstrictor response; an impaired coronary vasodilator response; mixed (vasoconstrictor and impaired vasodilator response); and negative CFTs.

Figure 3: Relation of the Individual Results of Invasive Coronary Functional Tests (Acetylcholine and Adenosine Tests) to the Corresponding Non-invasive Tests (Hyperventilation and Dipyridamole Tests)



CAS = coronary artery spasm; CFT = coronary functional test; CMVS = coronary microvascular spasm; CFR = coronary flow reserve; HVT = hyperventilation test.

As shown, there was full agreement in the type of abnormalities detected during I-CFTs and during NI-CFTs in 14 of the 18 patients (77.8%). Further, among 13 patients with any abnormality shown by I-CFTs, 11 (84.6%) had some abnormality also shown by NI-CFTs. Finally, all five patients with negative I-CFT also had negative corresponding NI-CFTs.

The addition of CPT to HVT and DST identified three further patients with a possible abnormality in endothelium-dependent coronary microvascular dilator function who had negative I-CFTs and normal HVT and DIP-CFR. CPT-CFR did not correlate significantly with invasive adenosine-CFR ($r=0.23$; $p=0.35$) and non-invasive DIP-CFR ($r=0.34$; $p=0.07$), thus confirming that this test might give additional information regarding coronary microvascular function compared to the other tests.

Discussion

To the best of our knowledge, this is the first study to investigate whether a non-invasive assessment of coronary vasoconstriction correlates with the induction of a vasospastic response (either CAS or CMVS) to an intracoronary acetylcholine test in patients with ANOCA. Our data show that a vasoconstrictor response to HVT as assessed by CBFV measurements in the LAD-CA using TTDE has a high correlation with a positive invasive acetylcholine test. Although HVT did not induce ischaemic ECG changes in any patients and chest pain in most, a coronary vasoconstriction was induced only in patients with positive acetylcholine test but in none of the control group, thus suggesting that this abnormal response may reliably identify patients with vasoconstrictor mechanisms of their angina syndrome.

Further, our data also show a highly significant correlation between non-invasive DIP-CFR and invasive adenosine CFR, thus confirming previous data that coronary microvascular dilator function can reliably be assessed by TTDE of the LAD-CA in ANOCA patients, although the threshold values for abnormality differed between the two types of tests, with invasive methods typically showing higher cut-off values.²⁵⁻²⁷ This is consistent with previous findings, however, and can in this case be attributed, at least in part, to methodological differences in the assessment of CFR (thermodilution versus coronary TTDE).²⁵⁻²⁷ The best cut-off value of DIP-CFR at TTDE to predict reduced CFR with an invasive adenosine test was 2.0, which is in line with present recommendations for non-invasive

diagnosis of impaired coronary microvascular dilatation.¹⁸ Indeed, a non-invasive $CFR < 2.0$ identified six out of seven patients (86%) with reduced invasive $CFR < 2.5$.

Importantly, the results of I-CFTs (acetylcholine and adenosine tests) and the corresponding NI-CFTs (HVT and DIP-CFR) identifying abnormal coronary constriction or endothelium-independent dilatation, or both or none, were fully concordant in 77.7% of patients, and the presence of any coronary functional abnormality was identified by both CFTs in 84.6% of patients.

The few discordances between the two types of tests were only related to the failure of NI-CFTs to detect some positive I-CFTs. A possible explanation for this discordance may reside in the fact that NI-CFTs were performed several weeks after the I-CFTs and dynamic changes in coronary vasomotor function might have occurred that affected the NI-CFTs results. Further, as far as vasoconstriction is concerned, HVT is a less effective stimulus compared to the increasing doses of intracoronary acetylcholine and might not have achieved adequate alkalosis, which is the effective vasoconstrictor stimulus, in a few patients.^{14,26}

Our data confirms the findings of previous studies by showing a lower coronary microvascular dilator response to CPT in ANOCA patients compared to healthy controls.^{23,28} This finding suggests that even an impairment of endothelium-mediated coronary microvascular dilator function can be assessed and identified by NI-CFTs. Indeed, CPT usually causes an increase in heart rate and blood pressure through adrenergic activation, which slightly increases myocardial work, determining an arteriolar metabolic dilatation and a flow-mediated, endothelium-dependent, pre-arteriolar dilatation.²⁹ An impaired CPT-CFR (< 1.35), defined as the lowest value of CPT-CFR detected in less than 10% of controls, was found in 55.6% of ANOCA patients. Notably, a low CPT-CFR detected three patients (16.7%) with coronary microvascular dysfunction who showed both normal acetylcholine/adenosine tests and HVT/DIP-CFR. Thus, these findings suggest that CPT (or another test assessing endothelium-dependent coronary microvascular function) should be recommended as an additional test to achieve a full assessment of coronary microvascular function in ANOCA patients, which is also in agreement with our previous data.²³

In summary, our data suggest that the very simple and widely available non-invasive method of TTDE could be used to identify the type of functional coronary alteration(s) present in ANOCA patients and guide treatment of these patients, as has already been suggested for invasive CFTs.

Of note, in this study, we chose HVT to assess vasoconstrictor reactivity in coronary circulation. HVT was consistently shown to be a valuable stimulus to trigger CAS in patients with typical Prinzmetal angina.²⁰⁻²² However, HVT is a notoriously less sensitive and standardised stimulus for CAS compared to the pharmacologic ergonovine provocative test that we previously found to induce coronary vasoconstriction (likely CMVS) using TTDE of the LAD-CA in a subset of patients with acute MI with non-obstructed coronary arteries.^{14,20,30} Future studies should evaluate whether ergonovine testing may further improve the detection of patients with increased vasoconstrictor reactivity among ANOCA patients.

It should also be observed that the feasibility of full NI-CFTs by stress echocardiography was previously shown by Djordjevic Dikic et al. in a study involving 40 ANOCA patients, using HVT, an exercise stress test

(EST) and adenosine as provocative stimuli.³¹ The interpretation of their data, however, was limited by the lack of both validation from gold standard I-CFTs and comparison with results in healthy controls. In that study, HVT induced vasoconstriction – defined as any reduction of CBFV – in 52% of patients versus 44.4% of our study, in which vasoconstriction was defined in a more restrictive way as a >10% reduction of CBFV to avoid the bias of spontaneous and measurement variability. The adenosine test, however, was normal in all patients in the Djordjevic Dikic et al. study, which might have been related, at least in part, to the short duration of the administration of adenosine (1 minute only versus a recommended infusion of 5–6 minutes).^{24,31} Interestingly, exercise testing significantly increased the diagnostic yield for functional coronary abnormalities in that study, suggesting that it deserves further investigation and validation in future studies.

Limitations of the Study

The assessment of CBFV by TTDE was unable to establish whether the coronary vasoconstrictor response to HVT observed in ANOCA patients occurred in epicardial vessels or in small coronary artery resistance vessels. However, HVT induced coronary vasoconstriction in all patients with evidence of CMVS during the invasive acetylcholine test, suggesting that a coronary microvascular constriction was also induced by HVT in these patients. Further, CMVS was likely to have also been induced in the two patients with positive HVT who had CAS at the invasive acetylcholine test, since they showed a reduction in CBFV and clinical findings (no pain, no ECG changes, no RWMA at echocardiography) during HVT that was comparable to those of CMVS patients, while typical RWMA (akinesia or dyskinesia), together with transient ST-segment changes, might have been expected in cases of typical epicardial spasm.^{14,20,22}

However, the pharmacological treatment of patients would not change whatever the site of constriction (epicardial vessels or microcirculation) as vasodilator drugs (mainly calcium channel blockers) would be indicated as first-line therapy in both cases.^{5,6} Of note, recent data have cast doubts on the pathophysiological and clinical relevance of acetylcholine-induced CAS in ANOCA patients. In at least some cases, acetylcholine-induced CAS might merely reflect an increased coronary reactivity, rather than the causal mechanism of patients' symptoms as suggested by the lack of significant improvement of angina and quality of life in response to dilator therapy in recent trials.³² CMVS, which is known to be less responsive to calcium-channel blocker therapy and could be masked by CAS during an acetylcholine test, might in fact be the true mechanism responsible for angina pain in at least a group of these patients, again explaining the less satisfactory therapeutic response to vasodilator therapy.^{33–36}

A second limitation of our study is that an invasive assessment of endothelium-dependent coronary microvascular dilator function was not

performed in our patients which meant we could not establish whether, and by which entity, CPT data reflect an impairment of this type of vascular function established by more standardised invasive methods, such as an increase of invasive coronary blood flow $\geq 50\%$ during low-dose acetylcholine tests.³⁷ Accordingly, this point should be assessed in appropriately designed future studies.

Some limitations concerning the echocardiographic method used to perform NI-CFTs in this study should also be acknowledged, particularly including the need for adequate training to visualise the LAD-CA, the need for an adequate echocardiographic window to obtain valuable images of the vessel and the fact that assessment of CBF velocity changes are usually performed in the LAD-CA only, as was the case in our study, due to the difficulty in adequately visualising the other coronary arteries by transthoracic echocardiography in several patients.³⁸

Finally, our study included only a small population of patients and therefore our findings need to be confirmed by larger studies. We would stress, however, that the sample size was preventively calculated to have adequate statistical power to detect the presence of a significant correlation between a vasoconstrictor response to HVT and a positive acetylcholine test – the primary end point of the study. Further, our study was a single-centre study with TTDE of the LAD-CA performed by an experienced cardiologist and the possibility of a large application of the method and confirmation of data in multicentre studies needs to be adequately assessed before our non-invasive diagnostic method can be used in clinical practice.

Conclusion

In ANOCA patients, NI-CFTs performed with TTDE of the LAD-CA showed a high correlation with the results of I-CFTs. Future larger and multicentre studies should investigate whether NI-CFTs may be helpful for guiding clinical management of these patients. □

Clinical Perspective

- This study suggests that non-invasive transthoracic Doppler echocardiography of the left anterior descending coronary artery with stress tests (dipyridamole, hyperventilation and cold pressor test) can reliably be used to identify functional coronary abnormalities present in patients with angina chest pain and non-obstructed coronary arteries. In particular coronary microvascular dysfunction involving impaired coronary microvascular dilatation, increased coronary microvascular constriction, or both, and can be used to guide clinical management of these patients.

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