State-of-the-Art Paper

Assessment of Vascular Dysfunction in Patients without Obstructive Coronary Artery Disease: Why, How and When

Running Title: Syndromes of Coronary Vascular Dysfunction

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# Key points

* Patients with symptoms and signs of ischemia and no obstructive coronary disease (INOCA) typically have underlying treatable disorders of coronary vasomotion including angina due to abnormalities of microvascular vasorelaxation and epicardial or microvascular vasoconstriction.
* Epicardial and microvascular vasospasm may be implicated in myocardial infarction (MI) with no obstructive coronary disease (MINOCA) and type 2 MI.
* An interventional diagnostic procedure (IDP) at the time of diagnostic angiography empowers cardiologists to exclude, diagnose and treat these conditions. Stratified medicine supports a proactive approach in the catheter laboratory.
* ‘Stratified medicine’ is a new paradigm that should transform the management and well-being of these patients, the majority of whom are women.

# Abstract

Ischemic heart disease (IHD) secondary to coronary vascular dysfunction causes angina and impairs quality of life and prognosis. In patients with symptoms and signs of ischemia, often chest pain, coronary angiography is performed to assess for coronary artery disease (CAD). However, around half of patients turn out not to have obstructive CAD and coronary vascular dysfunction may be an alternative mechanism of ischemia. Adjunctive tests of coronary vasomotion include guidewire-based techniques and acetylcholine reactivity testing, typically by intracoronary infusion of acetylcholine. The Coronary Microvascular Angina (CorMicA) trial provided evidence that routine management guided by an interventional diagnostic procedure (IDP) and stratified therapy improves angina and quality of life in patients with angina but no obstructive CAD.

In this article, we provide a comprehensive review of why, how and when coronary vascular dysfunction should be assessed invasively. We discuss the rationale through a shared understanding of vascular pathophysiology and clinical evidence. We summarize how an IDP is performed and focus on practical aspects. We discuss the clinical scenarios in patients with stable and acute coronary syndromes when measuring coronary vascular function should or may be considered consistent with recent Class IIA and Class IIB practice guideline recommendations, respectively.

**Key words:** ischemic heart disease, angina, microvascular angina, vasospastic angina, MINOCA, stratified medicine

# Introduction

Ischemic heart disease (IHD) is a leading global cause of premature disability (1) and death (2). The classic cause of IHD is coronary atherosclerosis but disorders of coronary vasomotion are increasingly recognized(3-5). Approximately half of patients undergoing coronary angiography for known or suspected angina are found to have non obstructed epicardial coronary arteries and a vasomotion disorder, including microvascular- and/or vasospastic angina may be relevant. Coronary angiography has very limited sensitivity for the detection of these disorders (Figure 1).

Epicardial artery spasm causes vasospastic angina, first described by Prinzmetal as ‘variant angina’ (6). Microvascular spasm and/or impaired coronary vasodilation cause microvascular angina, formerly known as Cardiac Syndrome X (5). Vasospastic disorders of the conduit arteries and microvessels are diagnosed by acetylcholine reactivity testing and often co-exist with coronary atherosclerosis. Moreover, coronary vascular dysfunction - whether epicardial or microvascular - can also cause myocardial ischemia in patients with obstructive coronary artery disease (CAD) (3-5).

Coronary vasomotion disorders cause a relative supply: demand mismatch of myocardial blood flow and nutrients relative to their requirements inducing myocardial ischemia that may be transient, recurrent and/or chronic. Ischemia with no obstructive CAD (INOCA) is typically a chronic health problem(7,8). ESC guidelines(9) have revised nomenclature (‘Chronic Coronary Syndromes’) in part reflecting the importance of patients with signs and symptoms of ischaemia without obstructive coronary artery disease—INOCA.(4,10) When studied using specific tests, microvascular angina and vasospastic angina are common findings; up to 4 in 5 patients with INOCA may be affected (11-13). They are mostly female and prognosis (14-18) and quality of life (7,19-21) are impaired. Vasospasm may also be a primary cause of myocardial infarction (MI) with no obstructive coronary disease (MINOCA) and type 2 MI. Although rarely used in daily practice, adjunctive tests of coronary function are supported by emerging clinical trial evidence and European Society of Cardiology Guidelines now support their use (9,11,13,22). Coronary functional disorders also occur among patients with obstructive CAD (3) but current diagnostic testing is limited with an upstream obstructive lesion so this review will focus on patients without epicardial obstruction.

In this article, we describe why, how and when coronary vascular function should be measured in selected patients in the cardiac catheter laboratory. Interventional cardiologists work at the critical point in the care pathway for the diagnosis and therapy of individual patients with INOCA, hence, interventional cardiologists are the target audience for this article. We outline the rationale for why invasive measurements of coronary function are clinically relevant, in line with emerging results from recent trials. We describe how coronary vasomotion assessment with an ‘*Interventional Diagnostic Procedure* (IDP)’ is performed, giving a focus to practical considerations and tips and tricks in the catheter laboratory. We then describe the clinical indications for when an adjunctive IDP should be performed in daily practice. Finally, we consider future directions.

Why measure coronary vascular function?

The rationale for adjunctive testing of coronary vascular function during invasive angiography is threefold. First, a normal angiogram does not exclude a disorder of coronary vascular function. In a symptomatic patient with INOCA, coronary angiography may be considered incomplete without adjunctive diagnostic tests of coronary vascular dysfunction (Table 1) (9,23,24). Other methods, such as intravascular imaging, are informative for myocardial bridging but not for vascular dysfunction. Second, in an undifferentiated population of patients undergoing invasive management during daily practice, an IDP empowers the cardiologist to make the correct diagnosis with linked therapy (Figure 1). Stratified medicine is the identification of key subgroups of patients (endotypes) within an undifferentiated, heterogeneous population, these endotypes (MVA, VSA, both or none) being distinguishable by distinct mechanisms of disease and/or responses to linked therapy (Figure 1) (25). The tests empower the clinician to include or exclude coronary vascular dysfunction in affected patients and discrimination of angina due vasospasm and/or impaired vasodilator reserve (functional disorder) from increased microvascular resistance (structural disorder) permits specific and distinct treatments outlined in practice guidelines (9). Third, demonstration of coronary vascular dysfunction as a mechanism/cause of myocardial ischemia provides new prognostic information empowering the patient and clinician to adopt optimal guideline-directed preventive therapy and enhancing treatment satisfaction (14,16).

# Why? – Diagnosis

Coronary angiography is the standard-of-care test for identifying obstructive CAD either by anatomical imaging using non-invasive computed tomographic coronary angiography (CTCA) or invasive coronary angiography (26,27). Although procedure numbers worldwide are uncertain, approximately 10 million invasive coronary angiograms are performed annually, including four million per annum in Europe and the United States (28,29). Invasive coronary angiography has a spatial resolution of approximately 0.5 mm and evaluation is determined by subjective visual interpretation. The limited spatial resolution of angiography does not allow visualization of the resistance arterioles (20 - 400 µm) that largely govern myocardial blood flow [Figure 1 (30)].

The non-invasive management of symptomatic patients has evolved in recent years. In Europe, practice guidelines for the management of symptomatic patients with a high (>85%) pre-test probability of a coronary artery stenosis support direct referral for invasive coronary angiography ± a functional assessment with either a non-hyperemic pressure ratio (NHPR) or fractional flow reserve (FFR) (9). Non-invasive CTCA is associated with high sensitivity for detection of epicardial CAD. In the United Kingdom, practice guidelines of the National Institute for Clinical Evidence (NICE clinical guideline 95) recommend CTCA as the first line diagnostic technique for patients with anginal chest pain and no prior history of CAD (31). Thus, an increasing proportion of patients who undergo invasive coronary angiography have not undergone functional stress testing, meaning that information on ischemia is often lacking at the time of anatomical testing with either invasive or non-invasive angiography. This gap presents new challenges for decision making in INOCA patients. Practice guidelines recommend (Class 1, Level of Evidence A) use of invasive measures of coronary disease severity to assess for flow-limiting coronary disease (32,33) but many consensus guidelines do not emphasize invasive testing of coronary vascular function in INOCA. This means that clinicians do not assess for ischemia caused by disorders of coronary vasomotion leading to diagnostic uncertainty.

Looking forward, coronary microvascular dysfunction presents an unmet therapeutic need and novel therapies, including implantable devices, are being actively pursued. Examples include the coronary sinus reducer stent for the treatment of refractory angina (ClinicalTrials.gov Identifier: NCT02710435) and pressure-controlled intermittent coronary sinus occlusion (PiCSO) in acute myocardial infarction (NCT03625869). The main objective of coronary sinus device therapy is to induce a controlled increase in coronary sinus blood pressure, thereby increasing retrograde myocardial perfusion to reduce the propensity to myocardial ischemia. Clinical evidence from randomized, controlled trials involving coronary sinus device therapy is awaited with great interest.

# Why? – Prognosis

Patients with undiagnosed chest pain (including those who have undergone cardiac investigations) are at increased risk of cardiovascular events for at least five years.(34) Women with angina appear to be particularly burdened by symptoms and morbidity even after a reassuringly ‘normal’ invasive coronary angiogram.(35)

There is evolving data from many large prospective studies on the independent prognostic impact of coronary microvascular disease (CMD) on major adverse cardiovascular events (MACE). Data from the NHLBI-sponsored WISE study suggests that there is a worse prognosis in INOCA: the 5-year annualized risk of MACE was 16.0% in women with non-obstructive CAD, 7.9% in women with normal coronary arteries, and 2.4% in an asymptomatic control group (p≤0.002 after adjustment for baseline cardiovascular risk)(36). At mean follow up of 5.4 years, the time to event analysis confirmed that low CFR was a robust independent predictor of MACE (HR 1.20; 1.05 to 1.38; p=0.008). Similarly, a large Danish cohort study of 11,223 patients found an increased risk of MACE for angina patients with diffuse non-obstructive CAD and those with normal coronaries (adjusted hazard ratio of 1.85 and 1.52 respectively), compared with a reference population. Taqueti *et al* recently produced a provocative study showing that MACE risk in women is driven by reduced CFR and not obstructive CAD – with CFR an important predictor of events even in those without obstructive CAD (adjusted HR 1.69; 1.04 – 2.76; p=0.03).(14) The adverse prognostic importance of impaired coronary vasomotion has also been identified in a meta-analysis of 6 studies including 1192 subjects who experienced 243 cardiovascular events during a follow-up period of 3.8–9.7 years. The overall relative risk (95% confidence interval) was 2.38 (1.74–3.25) and the risk (2.49) was even higher in 1048 patients (n=209 events) who had undergone acetylcholine reactivity testing (37).

# Why? – Treatment

Historically, there was no randomized evidence that a diagnostic strategy linked to therapy improves patient well-being. The Coronary Microvascular Angina (CorMicA) trial was undertaken to address this evidence gap (11,38). INOCA patients were randomized 1:1 to the intervention group (stratified medical therapy, interventional/functional diagnostic procedure (IDP) disclosed) or the control group (standard care, IDP performed, results not disclosed). The diagnosis of a clinical endotype (microvascular angina, vasospastic angina, both, none) was linked to guideline-based management (10). After disclosure of the IDP result, over half of treating clinicians changed the initial diagnosis and treatment based on angiography alone. The intervention was associated with a mean improvement of 11.7 units in the Seattle Angina Questionnaire Summary Score [46] at 6 months (95% CI: 5.0 - 18.4; p=0.001) (primary endpoint) associated with improvements in quality of life (EQ5D index 0.10 units; 0.01 – 0.18; p=0.024)). Longer term follow-up to one year has confirmed these benefits are maintained(39). In summary, the CorMicA study provides clinical evidence of better quality of life for angina patients without obstructive CAD when management is guided by invasive tests of coronary vascular function.

###  Therapeutic nihilism and sex bias?

Some clinicians may take the view that patient benefits can be achieved assessing coronary function (40). A simpler, pragmatic approach may be to administer a trial of medical therapy as a matter of routine in all symptomatic patients and assess the patient’s response over time representing a trial of therapy. An angiography-guided approach avoids prolonging the procedure (around 15 minutes) and cost (guidewire, adenosine and acetylcholine) of the IDP. We contend that therapeutic nihilism is not in the best interests of patients and that precision medicine (right treatment to the right patient at the right time) is preferred (25). This may be especially relevant considering affected patients are often female (41). Practice guidelines give clear treatment protocols for these conditions (9) now supported by evidence from randomized, controlled trials. Further, avoiding unnecessary medicines and optimizing therapy when linked to the correct diagnosis will benefit patients, healthcare providers and the healthcare system (11).

## Coronary Physiology and Diagnosis of Vasomotor Disorders

Coronary vascular function reflects contributions from the epicardial conduit coronary arteries, its intramyocardial branches and the microcirculation. The key functional parameters are vascular tone, vasodilator reserve and resistance. Coronary resistance is mainly determined by intramural arterioles <400 µm diameter. Coronary flow reserve (CFR) reflects the vasodilator capacity of the coronary circulation. CFR is a global measure of vasodilator capacity that may be impaired by abnormalities of the conduit coronary arteries, the microcirculation through to the capillaries, or both compartments. CFR may also be limited if basal flow is high, if diastolic time is reduced or intramyocardial pressure is increased (42).

## Pathophysiologic basis of coronary vasomotor disorders

A disorder of coronary vascular function can be caused by structural and/or functional abnormalities (3-5), and the vasodilator response to hyperemic stimulants, such as pharmacological stress (43,44) or exercise (45), may be impaired. Coronary microvascular dysfunction (increased resistance) may result from remodeling of the vascular wall, inflammation, alterations in the composition and volume of the extravascular (interstitial) matrix (46) and systemic changes including capillary rarefaction (47) and arteriolar dysfunction [for reviews – (48-50)].

Vascular function may vary amongst different coronary artery territories and a normal global CFR may mask impaired vasodilator reserve in a single major artery (42). Regional differences and variations in resting flow support the rationale for estimation of the coronary flow capacity (%) by PET (49) and for assessing multiple coronary arteries during invasive management, when clinically appropriate.

Coronary artery spasm represents acute, flow-limiting vasoconstriction(51). Kaski *et al* showed that coronary hyperreactivity is mainly responsible for focal rather than diffuse epicardial vasospasm (52). Coronary artery spasm is caused by hyperreactivity of vascular smooth muscle cells (VSMC) and a triggering stimulus. The cause of the VSMC hyperreactivity is incompletely understood. Endothelial dysfunction is associated with coronary artery spasm, enhancing its likelihood and severity, but endothelial dysfunction is not the primary driver (51). Cardiovascular risk factors, inflammation, oxidative stress, genetic factors and ethnic differences are implicated. Coronary artery imaging using 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography CT (PET/CT) has identified localized inflammation in the coronary adventitia and perivascular adipose tissue of patients with vasospastic angina (53). Rho-kinase mediates epicardial coronary spasm and microvascular spasm, especially in patients with microvascular dysfunction(54). Autonomic imbalance, hyperventilation and platelet activation are potential triggers. Ethnic differences in coronary spasm, such as in Japanese patients (53), reflect an expansion of the personalized medicine concept.

Endothelial dysfunction typically precedes and causes atherosclerosis. Endothelium-derived nitric oxide mainly mediates vasodilatation of the conduit epicardial coronary arteries whereas endothelium-derived hyperpolarizing (EDH) factor(s) -mediated responses determine endothelium-dependent vasodilatation of resistance arteries (e.g. coronary microvessels) [for review(55)]. Endothelial dysfunction is associated with vascular risk factors, including diabetes mellitus and circulating inhibitors of nitric oxide synthase, as reflected by serum concentrations of asymmetric dimethylarginine (ADMA) (13,56), and low endothelial shear stress(57). The pathophysiology of endothelial dysfunction e.g. redox imbalance, is distinct from vasospasm (rho-kinase-induced myosin light chain phosphorylation) (55). Coronary endothelial dysfunction is therapeutic target for lifestyle and pharmacological interventions, notably statins and ACE inhibitors.

Endothelial function of the coronary artery may be defined according to the method used. If assessed using coronary angiography, endothelial dysfunction is defined as a decrease in one or more segments of an epicardial coronary artery luminal diameter of >20% after intracoronary infusion of low doses of acetylcholine(58). Normal endothelial function may be defined as normal [%Δ coronary artery diameter (acetylcholine) >20%], mild endothelial dysfunction [%Δ diameter (acetylcholine) 20% to −20%], and severe endothelial dysfunction [%Δ coronary diameter (acetylcholine) <−20%](59). Endothelium-dependent epicardial vasomotion can also be assessed by calculating the percentage in coronary cross-sectional area (CSA) change in response to intracoronary acetylcholine (change in epicardial CSA >0% is considered normal)(16).

Endothelial dysfunction may also be described according to changes in coronary blood flow (CBF) in response to infusion of acetylcholine (16,59). Normal coronary endothelium-dependent function is defined as a Doppler-derived increase in CBF of ≥50%, i.e., a ratio of >1.5 in response to acetylcholine, calculated by dividing the CBF after 10−4 mol/L acetylcholine (18.2 μg/mL) by the baseline. Endothelial dysfunction can be further classified as mild (0 - <50% change in CBF) or severe (<0% change in CBF). Impaired coronary endothelium-independent function can be defined as a ratio of flow velocity to adenosine with cutoffs varying from ≤ 2.0 to 2.5.(16,60).

Coronary endothelial dysfunction revealed by acetylcholine reactivity testing in the catheter laboratory is associated with inducible myocardial ischemia determined by injection of 99mTc sestamibi and SPECT imaging (61). In a study of 299 patients undergoing coronary angiography and endothelial function testing, 60 patients had normal endothelial function and 239 had abnormal endothelial function. When stratifying patients by the presence or absence of endothelial dysfunction, in those with preserved endothelial function, troponin I concentrations were higher in patients who developed major adverse cardiovascular events during 7.0 ± 0.3 years follow-up when compared to patients who did not (1.35 ng/L [IQR, 1.1-2.1 ng/L] vs 0.7 ng/L [IQR, 0.7-1.1 ng/L]; P=.02) (62). These findings are important because coronary endothelial dysfunction is a modifiable, therapeutic target for lifestyle interventions and medical therapy (statin, ACE-I), and a clinical strategy based on endothelial function testing may improve quality of life (22). Two trials of endothelin (ET)-1 receptor antagonists in patients with microvascular angina reported favorable results (63,64). The potential for patient benefits with ET receptor antagonist therapy is currently being evaluated in a precision medicine trial of zibotentan in microvascular angina (NCT04097314).

How to assess coronary vascular dysfunction in the catheter laboratory?

### Set-up

The purpose of this section is to give practical guidance to clinicians on how to assess coronary vascular function in the catheter laboratory (Table 2) (65,66). A step-by-step guide is illustrated in Figure 2. Vasoactive medications should be withheld for at least 24 hours. Coronary vascular function can be assessed by a trained cardiologist using invasive techniques. Radial artery access generally works well. A cocktail of intra-arterial vasodilator drugs to prevent radial artery spasm may confound subsequent measurements of coronary function. We generally avoid use of intra-arterial calcium channel antagonists and longer acting nitrates (e.g. verapamil and isosorbide dinitrate). Glyceryl trinitrate has a short half-life and is preferred. Standard coronary catheters can be used although the benefits of a smaller arteriotomy and guide catheters (5 Fr) include reduced radial spasm and reduced risk of vascular injury.

### Coronary angiogram

The cardiologist visually assesses antegrade flow of contrast media during cine-angiography. Semi-quantitative analysis may be undertaken by calculating the Thrombolysis in Myocardial Infarction (TIMI) frame count [49]. In patients with unobstructed epicardial coronaries, a corrected TIMI frame count >27 (images acquired at 30 frames/sec) suggests microvascular angina due to impaired resting flow (coronary slow-flow phenomenon - CSFP) (67). Slow-flow points to an increase in vascular resistance under resting conditions and is typically seen in male smokers and may be implicated in propensity to acute coronary syndromes (68).

We typically use a diagnostic JR4 (Judkins right) catheter to perform angiography of the right coronary artery before crossing the aortic valve to measure left ventricular end-diastolic pressure. Elevated left ventricular end-diastolic pressure (LVEDP) may reflect heart failure which is one possible adverse sequela in the course of coronary vasomotor disorders (69). We then use a left coronary guiding catheter with reasonable support (e.g. JL3.5 or EBU3.5 – extra back up) which permits reproducible transit time injections and good intubation of the left main for acetylcholine infusions. In choosing a guiding catheter, we exercise caution to ensure co-axial coronary intubation and avoidance of pressure damping to avoid injury of the vascular wall.

## Interventional diagnostic procedure

The IDP is a combinatory technique involving direct invasive measurements of coronary vascular function initially with a diagnostic guidewire then acetylcholine reactivity testing (Tables 1 & 2; Figure 1). As a practical guide, following acquisition of the coronary angiogram, we recommend the IDP should initially focus on use of a diagnostic guidewire and then, where appropriate and feasible, acetylcholine vasoreactivity testing. There is no firm consensus on the approach [(9,60)]. We advocate this diagnostic sequence since, should vasospasm occur following intracoronary infusion of acetylcholine, the assessment of resting physiology becomes confounded by elevated sympathetic drive. An alternative approach sees vasospasm provocation first before assessment of CFR. This approach is advocated by some who have concerns about coronary vasospasm testing if short acting nitrate is initially administered e.g. to prevent vasospasm if using radial artery access, or to optimize coronary angiography. This point is diagnostically relevant in the case of a potential false negative test result.

### Diagnostic guidewire

The guidewire procedure is performed as an adjunct to coronary angiography. The IDP should be focused to a single major coronary artery to limit the duration of the procedure. Additional studies in a second coronary artery may be appropriate if the initial tests are negative and clinical suspicion is high.

The left anterior descending coronary artery is usually preferred as the pre-specified target vessel reflecting its subtended myocardial mass and coronary dominance (Table 2), and this artery is in our experience typically reactive to the effects of acetylcholine. If technical factors, such as tortuous coronary anatomy, preclude instrumentation of this artery then the circumflex or right coronary artery should be assessed. Intravenous heparin (50–70 U/kg) should be administered to achieve therapeutic anticoagulation (activated clotting time ~250 s) before coronary instrumentation. Diagnostic options include coronary thermodilution using a pressure-temperature sensor guidewire (PressureWire X™, Abbott Vascular, Santa Clara, CA) or a Doppler technique (ComboWire XT or Flowire, Philips Volcano Corporation, San Diego, CA). The ComboWire XT connects to the ComboMap system (Philips, Eindhoven).

Typically, intra-arterial glyceryl trinitrate (GTN) is given routinely during coronary angiography although we suggest using 200mcg or less. The half-life of GTN is around two minutes and thus after 10 minutes, only 3% of the medication is active, therefore unlikely to suppress a false positive test for epicardial vasospasm (70). The usual approach to inducing steady-state hyperemia is by use of intravenous adenosine (140 μg/kg/min) administered via a large peripheral vein. Intravenous adenosine activates vascular A2 receptors leading to predominantly non-endothelium dependent vasodilation although there may also be a lesser component of endothelial-dependent vasodilation (71). Intracoronary bolus injection of adenosine (up to 200 µg) or nicorandil (2 mg) is an alternative option to assess endothelium-independent vasodilatation [40]. The adenosine infusion is given for 2 – 3 minutes and while mild symptoms are common, it is generally well tolerated. Hemodynamic markers of coronary hyperemia are: (1) “ventricularization” of the distal pressure waveform, (2) disappearance of distal dicrotic pressure notch, and (3) separation of mean aortic and distal pressures(72). Changes in heart rate, blood pressure, and rate-pressure product are less reliable measures of coronary hyperemia (73).

### Coronary thermodilution

The principle of coronary thermodilution is that transit time, derived from a bolus intracoronary injectate of normal saline administered at room temperature to mix with blood at body temperature, represents the inverse of coronary blood flow (74). From a practical perspective, the diagnostic guidewire connects wirelessly to transmit data to a personal computer using dedicated analysis software (Coroventis™, Uppsala, Sweden). The guidewire sensor tip is positioned at the tip of the guiding catheter and the pressure measurement from the wire is equalized with that of the guiding catheter. The guiding catheter should be co-axial with the long axis of the coronary artery to ensure effective delivery and mixing of saline. The sensor is then positioned in the distal third of the coronary artery followed by 3 intra-coronary injections of saline (3 ml) at room temperature. The mean transit time is measured with each bolus and averaged to calculate the resting mean transit time. When steady-state hyperemia is achieved by pharmacological stress testing, 3 additional injections of 3 mL of room temperature saline are performed. The transit time is automatically measured after each set of injections and averaged to calculate the hyperemic mean transit time. Simultaneous measurements of mean aortic pressure (by guiding catheter) and mean distal coronary pressure (by pressure wire) are also made during maximal hyperemia.

CFR is calculated using thermodilution as resting mean transit time divided by hyperemic mean transit time (abnormal CFR is defined as ≤2.0). (60,75) The index of microvascular resistance (IMR) is calculated as the product of distal coronary pressure at maximal hyperemia multiplied by the hyperemic mean transit time (76). IMR has a weak correlation with the subtended myocardial mass leading some to propose vessel specific cut offs. A guiding catheter must also be intubated well within the left main coronary artery to ensure reproducible coronary transit time estimates. The normal values for IMR and CFR have been challenging to define. The normal range of IMR is considered to be <25, based on three studies evaluating IMR in different populations.(77-80) The only truly ‘healthy’ population used to validate IMR was 20 subjects who underwent IMR testing prior to ablation for supraventricular rhythm disturbance. In this study, Solberg et al noted the upper limit of the estimated 95% percentile for IMR in 20 healthy controls to be 27 (95% CI 21 – 34). The authors stated that if a larger cohort of controls was used this upper limit would likely be reduced. More recently, an IMR ≥ 18 was identified as the optimal cut off for the prediction of MACE events in an Asian population of INOCA subjects (54).

Flow-limiting coronary disease may be calculated during the same setting of adenosine induced hyperemia simultaneously from the ratio of mean distal coronary pressure to mean aortic pressure at maximal hyperemia - abnormal FFR is defined as ≤0.80 (33) or a NHPR(81). We advocate a patient-centered approach to decision making. The binary thresholds of continuous data should be viewed within the context of the patient. A CFR between 2.0 – 2.5 reflects an impaired vasodilator reserve and may be considered a CFR ‘grey-zone’, as is also the case for FFR (0.75 – 0.82). CFR, IMR, NHPR/FFR have prognostic significance across the diagnostic range of their values. An accepted caveat of CFR measured by any modality is its inherent variability related to influence of resting hemodynamics. CFR is also affected by epicardial CAD so is not specific to microcirculatory pathology.

### Pressure- and flow measurements

The relative simplicity and accessibility of thermodilution derived CFR and IMR are attractive however, there are inherent limitations. The set-up conditions should be constant during the thermodilution measurements. Specifically, the guide catheter should be engaged without pressure damping and the position of the guidewire sensor should be constant to reduce variability in the saline transit times. Coronary vascular function can be assessed using a pressure-flow wire (Combowire XT Philips Volcano Corporation) or a Doppler wire to measure coronary flow velocity (Flowire; Philips Volcano Corporation) (66). CFR assessed using thermodilution (CFRtherm)(82) slightly overestimates flow reserve at higher levels compared to CFRDoppler. Doppler derived hyperemic microvascular resistance (HMR) may be a closer correlate of microvascular function assessed non-invasively using cardiac magnetic resonance (myocardial perfusion reserve).(83) Simultaneous measurement of coronary flow velocity reserve with pressure enables myocardial resistance (HMR) to be calculated (84).

Selective, intra-coronary infusion of acetylcholine using a dedicated microcatheter may be preferred rather than infusing the acetylcholine through a guiding catheter (66). The advantage of using a microcatheter is the sub-selective infusion of acetylcholine and potentially, avoidance of pan-coronary vasospasm. The disadvantage of this approach is the additional coronary instrumentation, related risks of vascular injury and expense. We think there are pros and cons for using a microcatheter or not. In the end, operator preference and the diagnostic circumstances of the procedure should guide the approach on an individual patient basis.

A Doppler wire may be used to measure coronary flow velocity during intra-coronary infusion of acetylcholine (22). When using Doppler, the infusion catheter is placed in the proximal segment of the target artery and the Doppler wire is sited in the mid- to distal segment. Since the Doppler wire is less flexible than a standard coronary guidewire, a ‘buddy wire’ or a microcatheter may be needed to safely advance the Doppler wire into the target artery. Coronary angiography is acquired to estimate the diameter of the coronary artery at baseline and after each infusion of acetylcholine. A projection without foreshortening is essential.

## Pharmacological coronary reactivity testing in the catheter laboratory:

Coronary vascular function is assessed by infusion of a vasoactive substance such as acetylcholine, substance P or ergonovine (65). The physiological alterations to vascular tone following intra-coronary infusion of these substances are determined by the relative functions of the endothelium and smooth muscle cells (85). Vasodilatation reflects a dominant response mediated by endothelial cells (vascular health) over the constrictor effects of vascular smooth muscle cells, whereas vasoconstriction reflects a dominant smooth muscle cell effect over impaired endothelial cell-mediated vasorelaxation (vascular dysfunction).

The most established approach for vasoreactivity testing is by intra-coronary infusion of acetylcholine (60). We support a pragmatic approach for coronary reactivity testing according to whichever protocol might work in individual centers. A standard approach involves sequential infusion of acetylcholine at concentrations approximating 0.182, 1.82, and 18.2 µg/mL (10-6, 10-5, and 10-4 mol/L, respectively) at 1 ml/min for 2 minutes via a mechanical pump. These doses were historically derived using experiments adopting sub-selective infusion through an intra-coronary catheter into the left anterior descending coronary artery assuming a resting flow rate of 80 ml/min. The effective concentration of acetylcholine at the tissue level was estimated at 10-8 to 10-6M. Alternative options to facilitate ease of adoption include manual infusion of 2, 20, 100 and 200 µg (86). Susceptibility to coronary vasospasm is assessed by manual infusion of 100 µg (5.5 ml 10-4M) or 200 µg (11 ml 10-4M) over 20 seconds into the left main(87). On a case by case basis, a dose of 200 µg may be infused to enhance sensitivity without adversely affecting specificity (86,88).

When microvascular spasm occurs, coronary flow transiently reduces or ceases in the absence of epicardial coronary artery spasm, i.e. the diameter of the coronary diameter is maintained in association with transient reduction of flow (TIMI flow grade ≤2) while the patient generally experiences chest pain in association with ischemic changes on the ECG. Prompt recovery is typical and i.e. nitrates can be administered if necessary. Epicardial coronary spasm is defined according to the COVADIS criteria requiring reproduction of chest pain and ischemic ECG changes in association with ≥90% vasoconstriction (89). In the case of severe epicardial spasm, it may not be possible to determine whether microvascular spasm co-exists. Reflecting the role of the right coronary artery to supply the sinus and atrio-ventricular nodes (or circumflex in left dominant anatomy), transient bradycardia commonly occurs. Given the propensity of acetylcholine to induce bradycardia, safety is ensured by administering a half dose i.e. 50 µg instead of 100 µg. Historically, a temporary implantable transvenous pacing line was used to balance this risk, however, this procedure is not without risk and in our view, not routinely needed, unless the right coronary artery is infused. The authors advocate proceeding without transvenous pacing and applying caution with testing the right coronary or left dominant circulations. Self-limiting atrial fibrillation is also common (8%), particularly during evaluation of right coronary vascular function (90). Patients undergoing clinically-indicated coronary angiography typically have risk factors for cardiovascular disease, including atrial fibrillation.

Ergonovine may induce coronary vasospasm via serotonin 1D receptors on vascular smooth muscle cells. Intracoronary ergonovine (20 – 60 µg) is an alternative to acetylcholine for the assessment of coronary vasospasm in some Asian countries (91). Acetylcholine is useful for assessing macrovascular and microvascular function, is safer, and more widely available.

### Intramyocardial Coronary Course – Invasive Pharmacological Assessment

Myocardial bridging is also prevalent in INOCA probably because of endothelial dysfunction within and distal to affected segments (92). Coronary reactivity testing in patients with a myocardial bridge may provoke transient spasm and chest pain that reproduces their symptoms. Furthermore, the ischemia-generating potential of myocardial bridges (MB) may have contributions from dynamic epicardial coronary obstruction. Despite a predominantly systolic effect of MB, it has been demonstrated that MB also affect diastolic flow, particularly under enhanced inotropism and tachycardia, both occurring during physical exercise. Inotropic challenge with pressure guidewire interrogation during dobutamine in addition to Ach has diagnostic value in such patients.(93)

### Non-pharmacological approaches to stress testing in the catheter laboratory

Atrial pacing has been used to increase coronary blood flow and shear stress in assessing vasoactive responses (94). However, this approach limited due to the achievable maximal tachycardia being limited by Wenckebach block and thus impacting on CFR determination. Supine exercise testing during coronary angiography with radial or brachial artery access is feasible and can provide clinically-relevant information on disease mechanisms (95). Raman et al measured coronary flow velocity and pressure under resting conditions, during intravenous adenosine-mediated hyperemia (140 µ/kg/min) and during bicycle exercise using a supine ergometer in the catheter laboratory. They found that in patients with angina without obstructive CAD (n=85), CFR but not microvascular resistance identified individuals with a maladaptive physiological response to exercise and subendocardial myocardial ischemia (n=55 - hyperemic subendocardial:subepicardial perfusion ratio <1.0 as revealed by stress perfusion CMR). The finding ties in CFR as an invasive functional correlate of impairments in exercise capacity and myocardial perfusion and lend support to the role of exercise testing in the catheter laboratory.

### Procedural safety

The risks of an IDP are those of coronary instrumentation with a guidewire and adverse physiological reactions. In CorMicA, an IDP was feasible with diagnostic information achieved in 99% of the study population. No serious adverse events occurred. Considering adverse effects of coronary reactivity testing, atrial fibrillation occurred in 1 in 20 patients. This was self-limiting in all but one patient in whom chemical cardioversion was achieved with intravenous amiodarone. Transient bradycardias reflect expected physiological responses which will resolve immediately after discontinuation of the acetylcholine infusion. A coughing maneuver may be helpful, and vasospasm is typically transient. The cardiac catheter laboratory environment facilitates patient safety. Multiple publications support the safety of coronary reactivity testing when administered in trained hands (44).

Coronary injury may occur secondary to the guiding catheter or diagnostic guidewire, typically at the start of the standard care procedure. These complications are more likely to occur at the start of the procedure in the hands of an inexperienced trainee when the guiding catheter is less compliant. Rarely, a dissection may be secondary to the diagnostic guidewire. For these reasons, the IDP should be performed by an experienced interventional cardiologist or by a trainee under direct supervision. Coronary dissections are not a consequence of the effects of acetylcholine.

### Complementary measurements

LVEDP is a clinically relevant parameter that is straightforward to measure, that provides information on fluid balance and left ventricular pump function. A low LVEDP i.e. < 3 mmHg, points to dehydration. An increased LVEDP may reflect volume overload (normal pressure-volume relationship), abnormal left ventricular filling or compliance (diastolic dysfunction), abnormality of left ventricular contractility (systolic dysfunction), or a combination of these factors. We recommend that LVEDP should be measured routinely during invasive diagnostic procedures. An indwelling LV catheter may also measure alterations in LVEDP during infusion of acetylcholine. When prior information on LV function is not already available then ventriculography should be considered. Further, depending on the results from left heart catheterization, occasionally, *ad hoc* right heart catheterization may be appropriate, e.g. to assess for intra-cardiac shunts, pulmonary hypertension, as an alternative cause of exercise impairment. Non-invasive imaging using echocardiography and cardiovascular magnetic resonance (CMR) imaging provide complementary diagnostic information, notably on left ventricular systolic and diastolic function, left ventricular mass, valve function and pulmonary artery systolic pressure.

When to measure coronary vascular function

There is a growing literature underpinning the rationale for clinical tests of coronary vascular function (Figure 3). The clinical indication during coronary angiography should be personalized and considered on a case by case basis. A benefit – risk ratio applies. Benefits to patients, healthcare providers and insurers relate to making the correct diagnosis with linked therapy (personalized medicine) and avoidance of inappropriate treatments and/or down-stream investigations. An adjunctive IDP carries theoretical risks, prolongs the duration of the procedure usually by 10 – 30 minutes. Staff training and experience can help to optimize patient flow through the catheter laboratory.

### Indications

The suggested clinical indications for an IDP are listed in Table 3. The indications align with the classification of microvascular angina by Crea and Camici (96). In patients with stable symptoms, CorMicA provides proof-of-concept evidence that stratified therapy guided by results from an adjunctive IDP may be beneficial to patients with INOCA. Larger scale studies will be needed to substantiate new practice guideline recommendations. Pharmacological and non-pharmacological measures together bring patient benefits (97).

Coronary vascular dysfunction is implicated in the pathogenesis of several forms of cardiac disease, notably stable IHD, acute MI, hypertension, diabetes, non-ischemic cardiomyopathies, and HFpEF. In patients with acute ST-segment elevation myocardial infarction (STEMI), multiple studies provide evidence supporting the prognostic value of IMR and CFR when measured at the end of PCI, and clinical trials for stratified medicine based on IMR are on-going [for review, (98)]. There is some evidence that coronary vascular dysfunction is implicated in the pathophysiology of MINOCA (99). More research on how vascular function testing may associate with treatment response within endotypes of INOCA and MINOCA is needed. Recent evidence supports a plausible role for targeted therapy (endothelin receptor A antagonists) modulating the endothelin-1 system in coronary microvascular dysfunction.(100)

In HFpEF, coronary microvascular dysfunction is implicated (69), notably in patients with cardiovascular risk factors such as hypertension. Presently, there are no evidence-based treatments for HFpEF, but coronary microvascular dysfunction could become a treatment target. Coronary microvascular dysfunction is also implicated in cardiac transplant vasculopathy and angiotensin converting enzyme inhibitor (ACE-I) may be beneficial (101).

Conclusions and future directions

Contemporary practice guidelines state that in patients with anginal symptoms and no obstructive coronary arteries, guidewire-based CFR and/or microcirculatory resistance measurements should be considered (Class IIA) and pharmacological tests may be considered (Class IIB) (9). In this article, we have described the available techniques, practical considerations and relevant clinical scenarios when an IDP may be useful. Adopting an IDP empowers the cardiologist to implement stratified medicine, championing personalized medicine for individual patients. Stratifying undifferentiated patients in the clinic will pave the way for new insights into vascular mechanisms and disease-modifying therapy.

Diagnostic advances are emerging, notably measurement of absolute myocardial resistance (102). The Achilles’ Heel of anatomical imaging with CTCA is the lack of information on vasomotor function. The extent and clinical significance of false negative results in patients with angina and no obstructive CAD is currently being investigated [CorCTCA NCT 03477890 (103)]. Technological advances are needed and non-invasive CMD strategies e.g. positron emission tomography (PET) and CMR are emerging. Developing evidence-based, disease-modifying therapy is a priority (54). To this end, randomized, controlled trials of novel and repurposed drugs, and precision medicine, hold future promise. Transferable relevance to the clinic will be determined by the results of trials, health economics, and education and training of clinicians and allied health professionals.

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## Table 1. Proposed standardized diagnostic criteria: coronary vascular dysfunction.

|  |  |
| --- | --- |
| **Diagnostic Group** | **Outcome definitions – disorders of coronary artery function** |
| Microvascular angina | 1. ***Abnormal microvascular resistance***
 | Index of microvascular resistance (IMR) ≥25Hyperemic microvascular resistance (HMR) >2.4 |
|  | 1. ***Impaired coronary vasorelaxation***
 | Coronary flow reserve by thermodilution (CFR) <2.0 |
|  | 1. ***Microvascular spasm***
 | Angina during intracoronary infusion of acetylcholine with typical ischemic ST-segment changes without epicardial coronary constriction (<90% reduction) in coronary artery diameter. NB. Microvascular spasm may occur earlier than diffuse, distal spasm of a conduit artery. |
| Vasospastic angina | ***Epicardial spasm*** | Reduction in coronary diameter >90% following intracoronary acetylcholine from baseline in any epicardial coronary artery segment together with symptoms and ST segment deviation on the ECG. NB. Prinzmetal vasospasm is typically focal. |
|  |  |
| Obstructive epicardial coronary disease |  | Fractional flow reserve (FFR) ≤ 0.80.Contrast FFR ≤0.83 Resting indices (i.e. iFR™, NHPR) ≤ 0.89 |
| Endothelial dysfunction |  | Impaired vasodilatation and/or impaired increase in coronary flow velocity in response to intra-coronary infusion of low-doses (1 – 30 µg) of acetylcholine |

iFR™ – instantaneous wave free ratio; NHPR – non-hyperemic pressure ratio, CFR: coronary flow reserve, FFR: fractional flow reserve, IMR: index of microcirculatory resistance, HMR: hyperemic microvascular resistance.

## Table 2. Practical considerations for invasive assessment of coronary vascular function.

|  |  |
| --- | --- |
| Procedure  | Practical points |
| Set-up |  |
|  | Acetylcholine may be pre-ordered, according to local arrangements |
|  | Informed consent |
|  | Team brief on indication and protocol |
|  | Heparin, 5000 IU (as per local standard care procedures) |
|  | Radial artery access - avoid administration of vasodilator drugs since they may confound measurement of coronary vascular functionShort acting intra-arterial GTN (avoid verapamil/GDN) 5F guide to reduce spasm in small radial arteries |
| Coronary angiography |  |
| Projection  | Choose an imaging projection that reveals the long axis of the target vessel i.e. no foreshortening, and with minimal vessel overlap  |
| TIMI frame count | Ensure a cine-acquisition is sufficiently long to assess for myocardial blush of contrast media |
|  |  |
| Diagnostic guidewire |  |
|  | A single target coronary artery may be sufficient for diagnosis and decision making; in general, select the left anterior descending coronary artery |
|  | If normal results are obtained and clinical suspicion remains high then consider undertaking the IDP in a second coronary artery. |
|  | Advance the guidewire into the distal 1/3rd of the target coronary artery, or as close to  |
| Combowire Doppler |  |
|  | Consider using a buddy wire to safely advance the Combowire |
|  |  |
|  |  |
| Coronary reactivity testing |  |
|  | Avoid a vasodilator cocktail in radial procedures |
|  | Retain the buddy wire *in situ* to facilitate direct intra-coronary testing |
|  | A dedicated intra-coronary catheter is generally not necessary (and may increase the risks of the procedure) – injection of acetylcholine is done through the guiding catheter into the lumen of the left main coronary artery. Prior to starting the infusion of acetylcholine, initially flush the lumen of the guide with ~2 ml of the infusate (depending on the French size of the catheter used) to replace the flushing saline in the shaft of the catheter. Once the acetylcholine solution has reached the tip of the catheter further injection is done slower and steadily over 20 seconds. The catheter is then slowly refilled with saline remembering that this procedure will lead to extrusion of acetylcholine at the tip of the catheter for at least as long until all the acetylcholine solution is replaced by saline.If infusing into a ‘dominant’ coronary artery, consider ‘half-dose’ of the acetylcholine to limit bradycardiaIn cases with normal coronary function or ‘negative’ test responses, if clinical suspicion persists, a dose of 200 µg may be infused into the left coronary artery increasing sensitivity without impairment of specificity. |
|  | Use isosorbide dinitrate since this has short acting effects unlike glyceryl trinitrate |

## Table 3. Indications for measuring coronary vascular function as an adjunct to clinically indicated coronary angiography.

|  |  |  |
| --- | --- | --- |
| Condition  | Invasive diagnostic management | Abbreviation |
| Current indications |  |  |
| Angina | No obstructive coronary disease  | INOCA |
| Myocardial infarction | Infarction without culprit stenosis where vasospastic angina is considered | MINOCA |
| Cardiac Arrest | In certain scenarios (ventricular arrhythmias/resuscitated cardiac arrest) where no clear cardiac cause can be found and patient is stabilized with normal LV function, no obstructive CAD, normal ECG. | VSA |
| Future possibilities |  |  |
| Angina | Suspected obstructive coronary artery disease | Pre-PCI |
|  | Post-percutaneous coronary intervention  | Post-PCI |
| Heart failure  | Preserved systolic function  | HFPEF |
|  | Post cardiac transplantation |  |
| Myocardial infarction | Stratification of risk and prognosis | STEMI, NSTEMI |
|  | No obstructive coronary disease  | MINOCA |

# Figure legends

## Central Illustration – Limited visualization of the coronary microvasculature with invasive coronary angiography.

A - This figure illustrates a typical normal coronary angiogram (left) with a smooth and well opacified left anterior descending coronary artery. The right image is a bismuth stereo angiogram from a cadaveric heart in work performed over fifty years ago by the late Prof S Fulton (with permission, Heart 1956) [91]. This image offers unsurpassed illustration of the coronary microcirculation contrasting starkly with the lack of microcirculatory information on the invasive coronary angiogram.(30)

B - This schematic illustrates compartmentalized physiological assessment according to the two probes acetycholine and adenosine. The metrics FFR/NHPRs are predominantly tests of epicardial coronary obstruction to blood flow whereas IMR/HMR are more specific to the microcirculatory function. Finally, CFR is a metric which can be influenced by any combination of epicardial, microvascular disease or changes in resting flow.

FFR: fractional flow reserve, NHPR: non-hyperemic pressure ratios (e.g. iFR, DPR, RFR, Pd/Pa), IMR: index of microcirculatory resistance, HMR: hyperemic microcirculatory resistance, CFR: coronary flow reserve.

## Figure 1 – Clinical utility of an interventional diagnostic procedure (IDP) in patients with symptoms and/or signs of ischemia but no obstructive coronary artery disease (INOCA).

Figure illustrating two cases with similar baseline angiograms and clinical presentations without obstructive epicardial coronary artery disease. Each case undergoes the IDP which reveals distinct diagnoses. The therapy of microvascular and vasospastic angina are distinct and should be guided by the IDP results. The yellow figure shoes a typical case of VSA with preserved microvascular function. The patient was previously on a beta-blocker and this was substituted for a calcium-channel blocker with smoking cessation counselling. The blue case has proven microvascular dysfunction but no severe vasospasm. There was abnormalities in both microcirculatory resistance (IMR) and coronary vasodilator reserve (CFR). The patient has a diagnosis of microvascular angina, they had cessation of long acting nitrate medication with uptitration of beta-blocker. The patient underwent cardiac rehabilitation classes to assist in weight loss and identify relevant lifestyle factors implicated in their condition. \*Some operators may prefer to do vasoreactivity testing first without the guidewire allowing acetylcholine challenge prior to short acting nitrate administration.

## Figure 2. Cardiac catheterization laboratory IDP protocol

Proposed step-by-step approach to guidewire-based assessment of coronary vascular function using thermodilution or Doppler and then vasoreactivity testing using acetylcholine. This simple approach focuses on thermodilution which is straightforward to include during daily practice. \* Some operators may prefer to do vasoreactivity testing first without the guidewire allowing acetylcholine challenge prior to any short acting nitrate administration. CFR: coronary flow reserve, IMR – index of microcirculatory resistance, HMR – hyperemia microvascular resistance, TT – transit time (for bolus of normal saline), Ach – acetylcholine. GTN – glyceryl trinitrate. LVEDP – left ventricular end-diastolic pressure, NHPR – non hyperemic pressure ratio, FFR - fractional flow reserve, iFR™ – instantaneous wave-free ratio

## Figure 3. Rising trend in article citations in human coronary vascular physiology.

A stacked area chart depicting the magnitude of change in citations between 1988 - 2018 and total values across this time-period. Citations of ‘coronary vascular dysfunction and human’ (<https://www.ncbi.nlm.nih.gov/pubmed/?term=coronary+vascular+dysfunction+human>; search date, February 2, 2020).

# Central Illustration.

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