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The functional assessment of patients with non-obstructive coronary artery disease: expert review from an international microcirculation working group

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KEYWORDS

- coronary interventions
- fractional flow reserve
- miscellaneous
- stable angina

Abstract

Symptomatic non-obstructive coronary artery disease (NOCAD) is an increasingly recognised entity that is associated with poor cardiovascular outcomes. Nearly half of those undergoing coronary angiography for appropriate indications, such as typical angina, or a positive stress test have no obstructive lesion. There are no guideline recommendations as to how to care properly for these patients. Physiologic assessment of the coronary arteries beyond two-dimensional angiography is not standardised, yet it can provide valuable information in patients presenting with typical angina in the setting of NOCAD. In this consensus document, we detail steps for the interventional cardiologist to evaluate the patient with symptomatic NOCAD in the cardiac catheterisation laboratory, first with the assessment of coronary flow reserve (CFR), and then with delineation of deficiencies in non-endothelium-dependent CFR (CFRne) versus endothelium-dependent CFR (CFRe) using provocative agents such as adenosine and acetylcholine, respectively, followed by the evaluation of smooth muscle function with nitroglycerine (NTG). Once the mechanism behind the anginal symptoms is established, one can identify the appropriate treatment strategies to address the physiologic deficiency that is present. Despite an established safety profile, a comprehensive assessment may be considered for selected patients which requires an understanding of the appropriate invasive evaluation by the practising interventional cardiologist when evaluating not only patients with obstructive CAD but also those with NOCAD.

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Abbreviations

CAD coronary artery disease
CFR coronary flow reserve
CFRe coronary flow reserve, endothelium-dependent
CFRne coronary flow reserve, non-endothelium-dependent
CVD cardiovascular disease
NOCAD non-obstructive coronary artery disease
NTG nitroglycerine

Introduction

Conventional angiography visually and subjectively assesses only approximately 5-10% of the coronary vascular resistance[1]. The remaining 90-95% of the coronary vasculature requires a detailed and systematic approach for successful evaluation of patients with typical symptoms of cardiac angina without obvious obstructive coronary artery disease (CAD). In the visible conduits, an atherosclerotic lesion greater than 70% has traditionally been treated with revascularisation – surgical or percutaneous – for the resolution of symptoms[2]. Non-obstructive CAD (NOCAD), a recently emerging term[3], is defined as less than 50% luminal diameter obstruction[4]. A plurality of patients undergoing coronary angiography for compelling symptoms or following a positive non-invasive ischaemia test have NOCAD[5]. Moreover, 50% of such patients who are referred for coronary angiography are found to have coronary endothelial or non-endothelial dysfunction independent of obstructive CAD[6]. Although there are no specific guideline recommendations, in cases where there is discordance between the patient's anginal symptoms, non-invasive stress testing, and the coronary luminogram, a comprehensive evaluation may be considered for selected patients with non-significant CAD on angiography or alternatively for research purposes to ascertain treatment and prognostic information.

Here we outline a step-by-step approach to the symptomatic patient with NOCAD by the interventional cardiologist in the cardiac catheterisation laboratory with an emphasis on the characteristics of patients presenting with NOCAD, methodological approaches to evaluate the structure and function of the coronary arteries, how these results should be interpreted/reported, and the resultant therapeutic options.

Physiologic basis of functional coronary assessment

The main role of epicardial coronary vessels is conductance; the key role of the microcirculation is to match blood supply to myocardial oxygen requirements (mostly through modulation of arteriolar tone) and to make possible the interchange of oxygen, nutrients and metabolites between myocytes and blood (performed in the capillary network). The mechanism for ischaemia generation in the epicardial vessels is conductance impairment, mostly caused by inward plaque growth during atherogenesis, intraluminal obstruction caused by thrombus, or coronary spasm. Conversely, microvascular disease results mostly from inadequate coronary arteriolar autoregulation and from structural remodelling of arterioles of the capillary bed, intraluminal plugging, or microvascular oedema/haemorrhage impairing the conductance of the microvasculature.

When assessing the ability of the epicardial vessels and microvasculature to meet myocardial demand, coronary flow reserve (CFR) is the fundamental measure of the ability of the entire coronary circulation to augment blood flow with stress measured as a ratio of maximal coronary blood flow (usually drug-induced) to baseline physiologic blood flow **(Figure 1)**. CFR assessment can be divided into an endothelium-independent component (CFRne) and endothelium-dependent coronary flow (CFRe), described later in the text. Testing for dysfunctional, endothelium-independent coronary microvascular function (CFRne) is accomplished by obtaining a ratio of blood flow or velocities at rest to that at maximal hyperaemia – usually achieved with intracoronary adenosine. In addition, measurements of microcirculatory resistance during hyperaemia may inform the clinician as to the cause of impaired microcirculatory conductance **(Figure 1)**.

CFRne can be measured with intracoronary Doppler techniques using blood cell velocity as an approximation for flow based on Poiseuille's law of fluid dynamics. A typical coronary Doppler tracing is shown in **Figure 2**. It assesses the perfusion capabilities of the macrocirculation and microcirculation. In normal individuals, CFRne is above three and can be as high as five indicating that the coronary circulation can normally increase perfusion up to five times basal levels if provoked by physiologic, pharmacologic, or pathologic stressors. However, CFRne can be reduced in a wide variety of diseases such as obstructive CAD, coronary vasospasm, hypertension, left ventricular hypertrophy, and even valvular heart disease.

Initial evaluation

The epidemiology and initial evaluation comprise a history and physical, lab evaluation, ECG, non-invasive imaging, and initial diagnostic catheterisation. This is detailed in **Supplementary Table 1**.

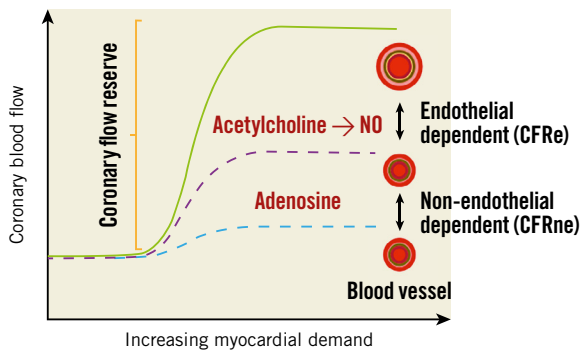

Figure 1. *Coronary blood flow response to increasing myocardial demand. Increasing myocardial demand leads to an increase in coronary blood flow (CBF). The ratio of CBF above baseline is referred to as coronary flow reserve (CFR) and is composed of an endothelium-independent component (CFRne) and endothelium-dependent component (CFRe).*

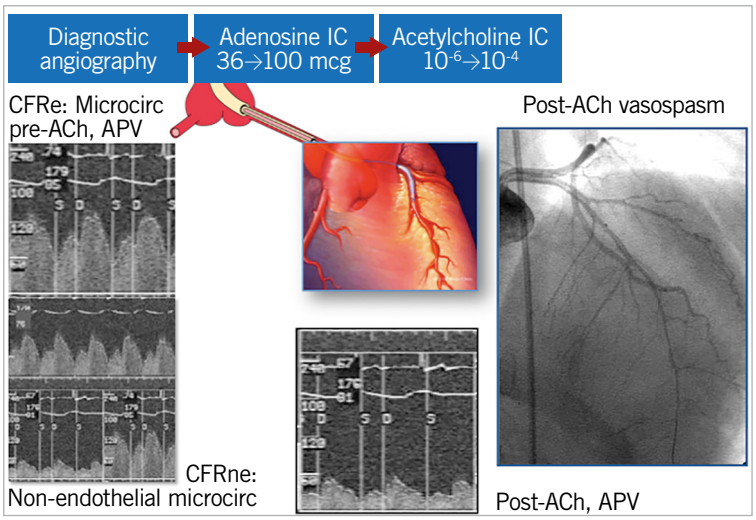

Figure 2. *Functional angiogram protocol. Typical functional angiography demonstrating coronary flow reserve (CFR) changes in an endothelium-dependent (CFRe) and endothelium-independent (CFRne) manner. The protocol, initiated with diagnostic angiography and moving on to adenosine and acetylcholine (ACh) infusion, is depicted on top. CFRne is assessed using blood flow velocity profiles (average peak velocity [APV]) at rest and after adenosine infusion (bottom middle). Assessment of CFRe is depicted on the far left with a normal pre-ACh reading consisting of a predominant diastolic component on the top. The lower left panel shows a marked reduction in APV after the infusion of ACh indicative of poor microvascular recruitment of blood flow seen in patients with microvascular disease. The angiogram (far right) shows an example of vasoconstriction after the administration of ACh.*

Protocol for functional coronary artery assessment

The functional assessment of the microcirculation has been extensively studied and described previously[6-9] **(Supplementary Table 2)**. Testing can be done *ad hoc* during the patient's initial coronary angiogram, or in a staged fashion after the initial angiogram if conditions are not favourable for proceeding with further invasive assessment (radial spasm, confounding intra-arterial vasoactive medications, ACS, etc.). Ideally, the majority of, if not all vasoactive drugs should be withheld prior to testing – particularly nitrates and calcium channel blockers. Other vasoactive substances such as nicotine and caffeine should also be withheld for 24 hours prior to testing. Furthermore, operators experienced in performing and interpreting these tests can be consulted in the study of these patients so that technical success is maximised in addition to arriving at a proper diagnosis.

VASCULAR ACCESS

In patients without obstructive CAD, as determined by FFR/iFR, where invasive physiologic assessment is indicated, one must carefully plan the procedure including the equipment used **(Figure 3, Figure 4)**, vessel studied, and order of assessments. Traditionally these cases are performed from a femoral approach. This is considered best for two main reasons. The first is to avoid the need for vasodilators routinely administered during radial access. Secondly, there is concern for radial artery spasm with the potential need for the administration of vasodilatory agents which may confound the results.

STUDY ARTERY

Unless otherwise indicated by regional abnormalities on a non-invasive stress test, the left anterior descending artery (LAD) is the conventional vessel of choice for these studies as this vessel has been most commonly studied. This vessel subtends the most myocardium, is typically very approachable with the equipment due to its large calibre, and can be assessed easily in terms of Doppler velocities and quantitative diameter assessments. The left circumflex or right coronary arteries can be studied if inferior or lateral

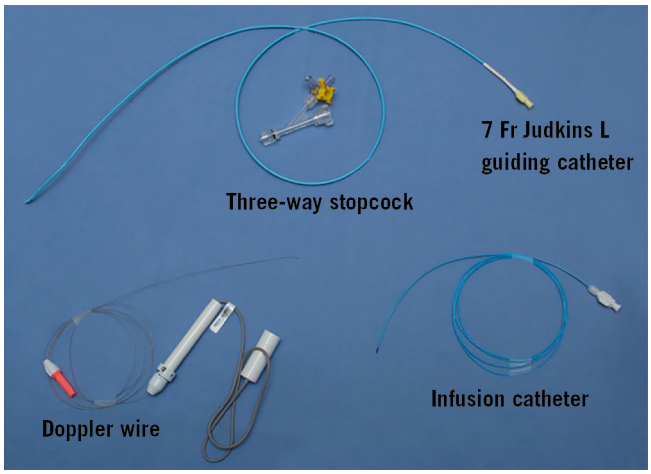

Figure 3. *Equipment used in functional angiography including guide catheter and second three-way stopcock (top), 3 Fr infusion catheter (bottom right), and Doppler wire (bottom left).*

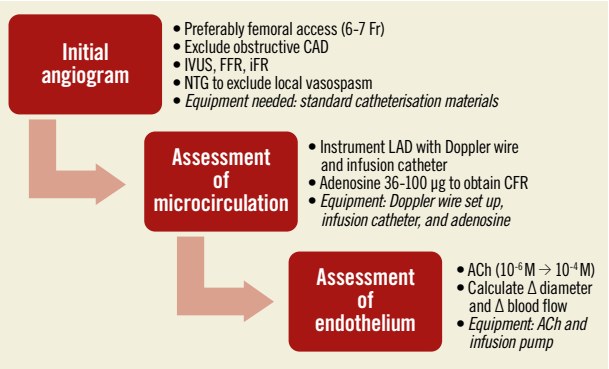

Figure 4. *Clinical protocol for NOCAD. Suggested order of catheterisation procedures and equipment required for each step in the process.*

changes are seen on the non-invasive test or if transient ischaemic mitral regurgitation secondary to posterior or lateral ischaemia is in question. If these arteries are to be studied, changes in guiding catheter selection may need to be made in addition to reductions in dosing of provocative medications.

EQUIPMENT

A 7 Fr guide is predominantly used. This is typically an extra backup (EBU/XB) or Judkins left shape depending on the anatomy **(Figure 3, Figure 4)**. Recently, the increasing popularity of the radial approach and the ability to utilise a 7.5 Fr sheathless guide or 7 Fr radial sheaths and guides has allowed these procedures to be performed using the radial approach. More data are needed to confirm that the radial approach is safe and effective to use. To avoid catheter or wire thrombosis, IV heparin should be administered at a standard dose (60-80 IU heparin/kg body weight) to ensure an activated clotting time (ACT) greater than 250 seconds. An additional safety measure is having a syringe for intra-arterial nitroglycerine (NTG) easily available should coronary spasm occur unexpectedly.

Once the 7 Fr guide is positioned within the ostium of the left main coronary artery, the Doppler wire and infusion catheter are positioned in the mid portion of the vessel. As the Doppler wire is not as manoeuvrable as a workhorse coronary wire, it is possible to use a soft-tipped coronary guidewire initially, over which the infusion catheter can be advanced to the appropriate position within the artery. The workhorse wire can then be exchanged for the Doppler wire, which should be positioned just distal to the tip of the infusion catheter required for concomitant intracoronary drug delivery. The infusion catheters typically used include the RapidTransit catheter 2.8 Fr (proximal), 2.3 Fr (distal), 150 cm length, internal diameter 0.021" (Codman Neuro, Johnson & Johnson, New Brunswick, NJ, USA) or Slip-Cath® Infusion Catheter 3.0 Fr, 150 cm length, internal diameter 0.025" (Cook Medical, Bloomington, IN, USA) **(Figure 3)**. The infusion catheter should be positioned at the proximal to middle portion of the vessel (if the vessel is large enough to accommodate it without creating ischaemia) to ensure adequate drug delivery to the distal portion of the artery with plenty of upstream vessel not directly affected by the drug infusion in order to test upstream flow-mediated vasodilation. The infusion catheter could still be used if the artery in question is the right coronary artery to evaluate upstream and downstream acetylcholine (ACh) effects on the artery. We recommend attaching, through a second three-way stopcock delivery system **(Figure 3)** set up outside of the body, an infusion pump able to deliver ACh at 1 ml/min in increasing concentrations, as shown in **Figure 2**. This proposed protocol **(Figure 4)** has demonstrated excellent safety in observational studies and randomised trials[6,10,11].

Functional assessment protocol

Once the vessel has been successfully instrumented with the infusion catheter, one can carefully position the Doppler velocity wire through the infusion catheter into the vessel being studied. Two available 0.014" Doppler wires can be used – either the ComboWire® or the FloWire® (both from Philips Volcano, San Diego, CA, USA) **(Figure 3)**. Both wires have been independently tested and verified for safety and reliability in assessing coronary Doppler signals to obtain coronary velocity data[6-8,10,11]. The Doppler wire must be carefully placed and positioned within the coronary artery to avoid poor Doppler data quality. Subtle movements of the Doppler wire or infusion catheter to improve data quality must be made with extreme care in order not to damage the vessel. Repositioning of the equipment, if required, should be done using the workhorse wire to reposition the infusion catheter before re-inserting the Doppler wire.

Once the testing is ready to begin, the guide and infusion catheter should both be properly flushed with heparinised saline and equalised. The system should not be pre-treated with intracoronary NTG, as this will alter blood flow independent of the endothelium or microcirculation with the smooth muscle-directed vasodilation.

An alternative to an infusion catheter placed locally in the coronary artery is to advance the flow wire into the vessel without the use of an infusion catheter and to infuse the drug directly through the guide. This protocol variation can be considered if the clinical question can be answered by simply administering adenosine to measure Doppler velocities, obtaining a CFRne or testing for epicardial spasm with an ACh bolus. Whilst overall it is safe to perform, there is a risk of left main or proximal vessel dissection with this method, not to mention the possibility of vasospasm throughout the left-sided coronary arteries without placement of workable coronary wires in the distal vessels.

ENDOTHELIUM-INDEPENDENT MICROVASCULAR BLOOD FLOW – CFRne

It is recommended to begin by obtaining CFRne once the Doppler wire and infusion catheter are both in place. The administration of intracoronary adenosine (intravenous adenosine can produce unwanted and confounding systemic hypotension[12]) allows the measurement of maximal coronary endothelium-independent vasodilation. Depending on the artery of choice, a dose of 36 to

100 mcg is preferred **(Figure 2, Figure 4)**. The hyperaemic average peak velocity (APV) is compared to the resting APV, and a CFRne ratio is obtained **(Figure 2)**. This should be repeated two to three times until a stable maximal APV is obtained.

ENDOTHELIUM-DEPENDENT MICROVASCULAR BLOOD FLOW – CFRe

Once the assessment of the non-endothelium-dependent microcirculation has been ascertained via adenosine administration and the CFRne obtained, the next step in the workup of patients with NOCAD is to assess the endothelium-dependent epicardial diameter and microcirculation, CFRe (based on changes of APV) with the administration of the endothelium-dependent vasodilator ACh. This will assess the endothelium-dependent vasodilatory properties of the epicardial as well as the coronary microvasculature. Two measurements are needed to assess CFRe – the APV and the coronary artery luminal diameter.

Maintaining the stable positioning of the infusion catheter and with the Doppler wire in place, graded infusion of ACh administration should be initiated with increasing doses of ACh (10^{-6} M $\rightarrow$ 10^{-4} M; or equivalently, ACh 0.001 mmol $\rightarrow$ 0.1 mmol) for up to three minutes at 1 ml/min via an infusion pump. The infusion should be quickly stopped should symptoms or complications occur. During the infusion, concomitant measurement of epicardial diameter via quantitative coronary arteriography (QCA) using any angiographic imaging software and APV will allow calculation of coronary blood flow (CBF). The purpose of this testing is twofold: 1) assessment of coronary epicardial vasoconstriction, and 2) assessment of the ability of the endothelium of the coronary microcirculation to increase CBF appropriately. This particular protocol involves the measurement of coronary epicardial diameter at three sites along the artery five mm proximal, five mm distal to the infusion catheter for CBF calculations (usually mid vessel), and at the distal vessel as well as APV after three minutes of each ACh dose infusion. At the conclusion of the ACh infusions, or should ischaemic symptoms occur during ACh infusions, 100-200 mcg of intracoronary NTG should be delivered via the guide catheter. Haemodynamic data as well as patient symptoms should be assessed at baseline, the end of each three-minute period of infusion, and after the administration of the intracoronary NTG. Some operators will choose to administer ACh, then NTG, and finish with adenosine to obtain CFRne with the thought being to remove epicardial vessel tone with NTG prior to adenosine. Both protocols have been used and reported with comparable results[13].

Once the coronary diameter and APV have been recorded at each ACh dose, a large dose of ACh (100 mcg of 10^{-4} M) can be administered while measuring the degree of change in epicardial coronary diameter to rule out coronary vasospasm if there are no contraindications to the large intracoronary bolus of ACh. This approach is safer than the traditional means of assessing coronary vasospasm with methylergonovine[14]. There is also the risk of refractory[15] or multivessel spasm during this provocative test[16]. As there is a guiding catheter and coronary guidewire in place, intracoronary NTG or balloons can be delivered to resolve cases of severe spasm. An in-depth review of the medications used in these assessments is included in **Supplementary Appendix 1**.

An alternative method to assess the coronary microcirculation, based on coronary thermodilution techniques, is the index of microcirculatory resistance (IMR). This technique uses distal coronary pressure divided by changes in intracoronary temperature measurements (proximal to distal) to approximate coronary resistance[17]. IMR has been shown to be independent of epicardial vascular function, reproducible, and has even been evaluated in STEMI patients, providing important prognostic information regarding ventricular function at three months[18]. Despite reasonable prediction of events and outcomes following percutaneous coronary intervention[19], as there are no established reference values for IMR there are no guideline-based recommendations for its regular utilisation in the catheterisation laboratory.

Standard for interpretation/reporting of the test results

To assess the coronary circulation with ACh administration, we propose the following parameters for the diagnosis of epicardial vs. microvascular dysfunction. Often the diagnosis of epicardial spasm can be made without specific calculations and with the combination of drastic angiographic vasospasm and reproduction of symptoms with ACh administration; however, one should be careful to calculate changes in CBF as changes in diameter might not alter distal microvascular flow. Coronary diameter can be measured via any standard catheterisation laboratory imaging software, and should be measured proximal to the infusion catheter, 5 mm distal to the site of ACh administration, and in the distal vessel. CBF can be calculated from measurements of coronary flow velocity and coronary cross-sectional area with the modified hydraulic equation CBF=0.5×velocity×area. Using the metrics obtained during testing, CBF=0.5×APV×(radius^2×π). Coronary radius (diameter/2) and CBF obtained at the site and distal to the site of ACh administration assess the direct endothelial response to ACh, while the diameter and CBF proximally assess the ability of the vessel to respond to increases in blood flow. This should also include vasodilation and increase in CBF. An increase in coronary diameter of greater than 20% and/or an increase in flow over 50% are considered normal **(Table 1)**[6-9].

The operator should communicate the presence of CAD and degree of atherosclerotic burden, the endothelium-dependent and non-endothelium-dependent behaviour of the microcirculation to respond to physiologic challenges, as well as notation describing the baseline APV in the vessel studied, maximum CFR obtained, the dosing of adenosine, and any symptoms **(Figure 5)**. Additionally, the report should also state whether spasm was present or not, and the degree of vasodilatory response of the vascular smooth muscle to NTG. Presenting the specific vascular pathology related to the symptoms is an important piece of information to pass along to the referring provider as it provides insight into targeted medical therapies.

Table 1. Recommended diagnostic parameters to assess endothelium-dependent and non-endothelium-dependent coronary circulation using adenosine, acetylcholine, and nitroglycerine (NTG) in terms of changes in coronary artery diameter (CAD) and coronary blood flow (CBF).

Medication	Non-endothelium-dependent function (CFRne)	Epicardial endothelial function	Microcirculatory endothelial function (CFRe)
Adenosine (microcirculation)	% Δ in ratio of hyperaemic to rest APV (i.e., CFR) >2.5	–	–
Acetylcholine (epicardial and microcirculation)	–	% Δ in CAD >20%	% Δ in CBF >50%
NTG (epicardial)	% Δ in CAD QCA >20%	–	–

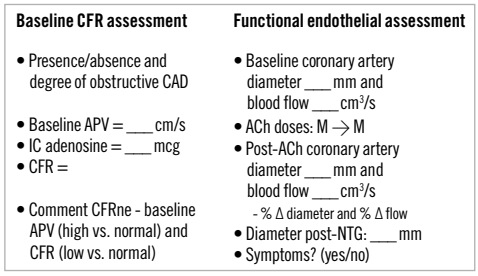

Figure 5. *Functional assessment report. Typical report with step-by-step interpretation of the data for coronary functional testing.*

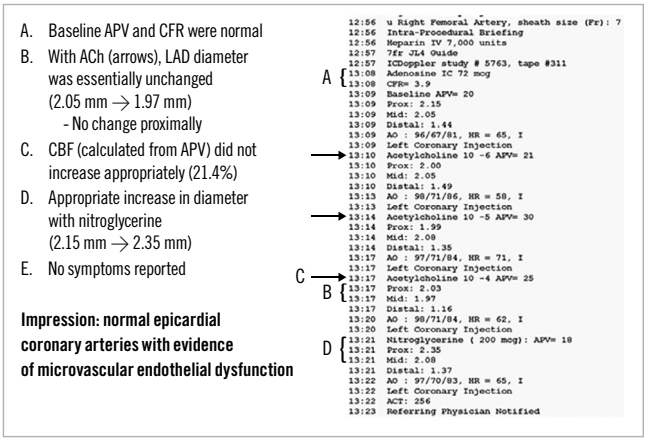

Figure 6. *Functional assessment report example. Proposed treatment log for non-obstructive CAD including vasodilators, both epicardial and microcirculatory, as well as non-vasodilatory therapies.*

The report should also include the percent changes in coronary diameter and CBF at the site of infusion as well as proximally with increasing ACh administration. One should report the maximum percent changes in diameter and CBF. This will allow commentary on the epicardial response to ACh in terms of vasospasm, as well as the CBF response, in order to report any endothelial microvascular dysfunction. Finally, the report should include any symptoms reported by the patient to see whether the provocative ACh testing reproduced any of the patient's angina symptoms.

Suggested therapies

There is an absence of large, randomised clinical trials aimed at addressing therapeutic options for these patients; many of the trials focus either on symptom relief or on improvement in physiologic coronary blood flow. However, there are limited data demonstrating benefit in treating these patients[20]; upcoming studies do target such a population[21]. Therefore, most of the treatment strategies for NOCAD – similar to obstructive CAD – should focus on basic cardiovascular prevention principles such as weight loss, diet[22], and exercise[23]. In addition to lifestyle modification, there are limited data supporting pharmacologic agents as improving symptoms and physiology **(Figure 6)**, and individual agents as outlined specifically in previous publications[24,25]. Finally, consideration should be given to the establishment of a dedicated chest pain clinic to formalise procedures and protocols for the evaluation of chest pain, to establish best practices for ongoing and long-term treatment for symptoms, and to provide a consistent follow-up for these patients.

Conclusions

This document should serve as a guide for evaluating patients with symptomatic non-obstructive CAD. These comprehensive assessments are safe and have been intricately designed at certain centres for diagnostic or research consideration for selected patients found to have NOCAD on angiography. Beginning with the initial workup involving history and physical, laboratory, and potentially non-invasive imaging, the next step is the exclusion of obstructive CAD based on coronary angiographic, physiologic, and imaging assessment. Although there are no specific guideline recommendations to guide testing, once obstructive CAD is eliminated, the coronary microcirculation is evaluated in a standard way with appropriate protocols and equipment. This is accomplished with the administration of intracoronary adenosine, ACh, and NTG to obtain non-endothelial coronary function (CFRne), endothelium-dependent coronary function (CFRe), and to evaluate epicardial vasospasm and smooth muscle function. With these simple strategies, one can safely and effectively evaluate and care for those patients with NOCAD.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Appendix 1. Further background information.

Supplementary Table 1. Preliminary NOCAD workup prior to functional angiogram including history and physical, lab analysis, imaging, non-invasive testing, and diagnostic angiography.

Supplementary Table 2. Recommended protocol for coronary provocative testing in the cardiac catheterisation laboratory.

The supplementary data are published online at: http://www.pcronline.com/eurointervention/149th_issue/290

Supplementary data

Supplementary Appendix 1. Further background information

Up to one half of patients undergoing coronary angiography for compelling symptoms or positive non-invasive ischaemia testing have NOCAD[5]. Moreover, 50% of such patients who are referred for coronary angiography are found to have coronary endothelial or non-endothelial dysfunction independent of obstructive CAD[6]. When added to traditional risk models such as the Framingham score, the presence or absence of coronary epicardial endothelial dysfunction can correctly reclassify cardiovascular risk in nearly one fourth of patients[10]. These patients are at risk of developing obstructive CAD and have increased morbidity and mortality and poor quality of life[26] in addition to the high rates of downstream testing and the consumption of medical expenses[27], and thus deserve particular attention and further evaluation.

Workup

History and physical

The initial workup in symptomatic patients with NOCAD should include a good history and physical examination. The presence of risk factors for endothelium-dependent and endothelium-independent microvascular dysfunction including the presence of conventional risk factors, sex, smoking, hypertension, hyperlipidaemia, obstructive sleep apnoea, atrial fibrillation, or autoimmune or inflammatory diseases such as hypothyroidism and Raynaud's disease may provide hints suggestive of microvascular dysfunction[6] **(Supplementary Table**

1). Angina can include typical and atypical features, such as exertional dyspnoea, chest, neck, shoulder discomfort, or similar symptoms with certain triggers such as physical activity, anxiety, social situations, or emotional prompts[28]. Exercise-induced angina associated with shortness of breath is often seen with fixed microvascular disease, and episodes of sporadic chest pain relieved by nitroglycerine could be suggestive of endothelial dysfunction/coronary vasospasm in the absence of obstructive CAD causing ischaemia. Specifically in women, a history of polycystic ovaries[29] and toxaemia of pregnancy[30] can be associated with microvascular disease and/or endothelial dysfunction.

Labs and imaging

The laboratory assessment for NOCAD should include a complete blood count, electrolytes, renal function, lipid panel, inflammatory markers, and possibly cardiac biomarkers including troponin and potentially brain natriuretic peptides (NT-proBNP). Laboratory abnormalities are typically non-specific and usually can only confirm an ongoing, low-grade inflammatory process. However, abnormal thyroid function[31], uric acid levels[32], markers of inflammation such as lipoprotein-associated phospholipase A_2 ($LpPA_2$)[33] and C-reactive protein (CRP)[6], white blood cell count[34], and N-terminal brain natriuretic peptide (NT-proBNP) can be abnormal in these patients[35].

These patients should also have inconclusive or likely abnormal non-invasive testing such as exercise ECG, stress imaging, CT, or MRI. Often, stress testing

reproduces symptoms, but there could be some other abnormal component such as poor exertional effort, cardiac output limitation on VO_2 testing, abnormal anaerobic thresholds, abnormal O_2 pulse rise, sudden increase in heart rate upon reaching anaerobic threshold, or other anginal symptoms[36]. Sometimes troubling to clinicians is the fact that non-invasive stress testing can reproduce symptoms in these patients; however, there is a discrepancy between non-invasive stress testing and both the epicardial and microvascular abnormalities found via invasive angiography[37].

Other non-invasive imaging such as coronary CT scanning can often rule out obstructive CAD; however, it has no functional testing component to assess the endothelium or microcirculation. Therefore, unless the question is whether the patient has obstructive CAD, and has no confounding coronary calcification, the utilisation of coronary CT may not provide the appropriate information on microvascular function.

Cardiac catheterisation

If clinically compelled by a history of typical, cardiac angina, a positive non-invasive stress test, or even elevated cardiac biomarkers, invasive cardiac catheterisation is performed in these patients only to reveal no obvious obstructive coronary lesion **(Supplementary Table 1)**. Of course, as a good interventionalist one must ensure that this is the correct diagnosis by inspecting angiography films thoroughly in orthogonal angles adequate to assure that ostial

branch disease, “flush” occlusions, coronary anomalies, myocardial bridging, or other angiographically significant lesions are present. In some instances, a stenotic lesion may be coronary vasospasm, and should not be stented. Intracoronary nitroglycerine can be used to reduce vascular tone and eliminate symptoms, as heightened coronary vascular tone can also cause typical angina without obstructive CAD. The physiologic significance of intermediate stenosis should be interrogated with fractional flow reserve (FFR), instantaneous wave-free ratio (iFR), and/or intravascular ultrasound (IVUS), and in selected cases optical coherence tomography (OCT) should be employed, typically in younger female patients, when considering the possibility of plaque erosion or spontaneous coronary artery dissection (SCAD) or unstable/vulnerable/eroding plaques that may bear smouldering culprit lesions. Evidence for moderate CAD and conditions such as anaemia, tachycardia, sepsis, etc., could cause a type 2 myocardial infarction, and should prompt a review for the underlying cause of the myocardial supply/demand mismatch, amelioration of the inciting cause, and potential adjustment of secondary prevention medications such as beta-blocker, antiplatelet, or vasodilator agents. Other invasive measures might also include an FFR value above 0.80[38], or iFR above 0.89[39,40], in patients with indeterminate (50-70%) luminal stenosis. Should the patient have typical angina symptoms with stress or exertion and be found to have no physiologically obstructive coronary lesion, proceeding with invasive coronary assessment of the microcirculation and endothelium is warranted for therapeutic and prognostic purposes.

Medications

Medications such as ACh and methylergometrine, a synthetic analogue of ergonovine, are used in the catheterisation lab to test the potential of the coronary arteries to vasospasm – providing an explanation for angina in a patient with NOCAD. Normal functioning endothelium should invoke smooth muscle relaxation in response to ACh administration; however, dysfunctional endothelium can cause paradoxical constriction of the vascular smooth muscle cells or potential spasm. Intracoronary nitrates should be immediately available when performing these tests to reverse any spasm that may occur.

Methylergometrine, or methergine, is a potent alpha-adrenergic, dopaminergic, and serotonergic agonist, that can also be used to produce coronary vasoconstriction in those prone to coronary vasospasm. Accordingly, this agent has largely fallen out of favour in testing for coronary spasm as the risk and danger of irreversible and fatal spasm during testing outweighs the benefit of making the diagnosis[16]. ACh has an advantage as it has a known mechanism of action with a shorter duration of action in addition to being extensively studied in clinical testing of endothelial function.

Adenosine

Intracoronary adenosine is released in response to low oxygen tension inducing mostly endothelium-independent microvascular vasodilation. This subsequent augmentation of flow – not dependent upon the release of nitric oxide (NO) from

the endothelium – is directly related to the velocity reading, i.e., the APV. While many of the large epicardial physiology clinical trials have been conducted using intravenous adenosine, for the purposes of these studies we recommend intracoronary adenosine to avoid the systemic side effects of bradycardia and hypotension which could complicate the haemodynamic assessment of the coronary circulation.

In order to assess CFRne, we recommend administering adenosine through the infusion catheter in a bolus of 36 μg. Measurement should not be made until plateau response of maximal APV. If CFR is thought to be submaximal, then a further administration of increasing adenosine doses of up to 100 ug can be given. If the testing is in the right coronary artery (only if the RCA is the suspected culprit), 36-72 μg of intracoronary adenosine is recommended. If there is a clinical need to administer adenosine IV to measure FFR, then one might also consider CFR testing as well as testing with ACh afterwards in order to ensure normal endothelial function.

Acetylcholine

Under normal physiologic conditions, ACh stimulates the production and release of NO from the endothelium, which results in vasodilatation and increased coronary blood flow. In patients with epicardial or microvascular endothelial dysfunction, the bioavailability of endothelial NO is reduced, and its direct action

on smooth muscle cells causes vasoconstriction (called paradoxical vasoconstriction).

ACh should be infused through either (a) the infusion catheter (see above), or (b) the guide (only with careful bolusing). As mentioned previously, the doses of ACh are 10^{-6} M (0.001 mmol), 10^{-5} M (0.01 mmol), and 10^{-4} M (0.1 mmol) for endothelial function testing. The infusion rate for each dose should be 1 mL/min for three minutes. One should obtain an angiogram and APV between 2 and 2.5 minutes into the infusion. To assess for epicardial spasm one can, at the end of the case, infuse a single bolus dose of 100 µg of 10^{-4} M ACh delivered over 20-30 seconds. ACh can be given either as a bolus or as an infusion. An infusion allows measured assessment of the coronary endothelium, and large boluses up to 200 µg have been given to induce coronary vasospasm[41]; however, the most common administration described in the literature is the infusion technique. ACh infusion is safe when given according to this protocol, which has been well described in the literature[6,11]. There is no increase in the amount of bradycardia and backup pacing is not required. While any vessel can be studied with a Doppler wire and administration of vasoactive agents, because this is a NOCAD population, we support the clinical practice in this area by studying only the LAD. Finally, it is safe to escalate dosing of ACh from a total dose of 36 µg to 100 µg as long as there has not been any inducible spasm identified at the 36 µg dose.

At the completion of these tests, intracoronary nitroglycerine (an endothelium-independent epicardial vessel dilator) should be administered at a dose of 100-200 μg[42]. This dose will depend upon systemic blood pressure as, however, it has been noted that intracoronary nitroglycerine up to 200 μg has little effect on systemic blood pressure and should not affect the microcirculation. If patients have a systolic blood pressure over 100 mmHg, then most patients can tolerate nitroglycerine intracoronary but will still need to be warned about the potential for headache. APV should usually decline appropriately with increased arterial diameter in order to maintain CBF. If one still encounters vasoconstriction or slow flow, not relieved by nitroglycerine, adenosine[43], verapamil[44], or nitroprusside[45] can be administered, although there are limited data available on these agents outside of the ACS setting.

Safety

While not without risk, these additional invasive measures recorded in the cardiac catheterisation lab, including the use of intracoronary vascular reactivity testing[11] and IVUS[46], can be performed with very high fidelity and safety. Aside from the usual risks of invasive coronary angiography[47], there have only been three reports of coronary artery dissection (0.6%) with instrumentation of the artery with the infusion catheter or wire and even more rare reports of pathologic vasospasm[6,11]. With careful attention to safety and detail, these potential complications can be avoided or quickly reversed to avert serious patient harm.

Supplementary Table 1. Preliminary NOCAD workup prior to functional angiogram including history and physical, lab analysis, imaging, non-invasive testing, and diagnostic angiography.

History and physical	• Signs/symptoms of typical angina (dyspnoea or chest pain) exacerbated by exertion or stress • Conventional risk factors: female gender, current smoking, hypertension, hyperlipidaemia, atrial fibrillation, or sleep apnoea • Non-conventional risk factors: autoimmune/inflammatory disease, polycystic ovaries, toxaemia of pregnancy, erectile dysfunction, periodontitis, dementia, renal disease, metabolic syndrome
Labs and imaging	• CBC, electrolytes, Cr, lipids, TSH, (hs)-troponin, BNP, inflammatory markers (CRP, LpPA2, uric acid) • ECG and CXR show no abnormalities • Exercise stress test with/without imaging can be normal or show global functional impairment
Diagnostic angiogram	• If obstructive disease is ruled out (IVUS/FFR/iFR), then consider alternative diagnoses such as myo/pericarditis, stress-induced cardiomyopathy, fixed or dynamic LVOT obstruction, SCAD, type 2 myocardial infarction • If indicated, proceed to functional angiogram and physiologic assessment

Supplementary Table 2. Recommended protocol for coronary provocative testing in the cardiac catheterisation laboratory.

	Primary approach	**Alternative approach(es)**
Approach	Femoral	Radial*
Vessel	Left anterior descending artery	LCx/RCA (dose adjustments)
Guide	7 Fr EBU/Judkins	6 Fr, 7 Fr, 7.5 Fr sheathless (radial)
Wire	Workhorse wire leading infusion catheter and switch out for combo/flow wire	Doppler and pressure sensor fitted guidewires
Drug delivery	Infusion catheter	Directly through guide
CFRne measurement	Adenosine 36-100 mcg bolus through infusion catheter	Papaverine 30-65 mg through infusion catheter
CFRe measurement	ACh infusion in increasing doses (10^{-6} → 10^{-4})	
Epicardial spasm assessment	100 mcg ACh 10^{-4} bolus IC	Methylergonovine (10 mcg/min up to 50 mcg)

* The radial approach can be used if the patient can tolerate a 6-7.5 Fr radial guide without the need for spasmolytic cocktail which could obscure test results.