Novel Insights into the Pathophysiology and Diagnosis of Patients with Angina and Non-obstructive Coronary Arteries

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List of Abbreviations

%MLD = percent minimal luminal diameter

ACE = angiotensin-converting enzyme

ACh = acetylcholine

ACS = acute coronary syndrome

ANOCA = angina with non-obstructive coronary arteries

ANOVA = analysis of variance

B-IPQ = Brief Illness Perception Questionnaire

CAD = coronary artery disease

CAS = coronary artery spasm

CBF = coronary blood flow

CCB = calcium channel blocker

CCTA = coronary computed tomography angiography

CFR = coronary flow reserve

CFT = coronary function testing

CMD = coronary micro-vascular dysfunction

COVADIS = Coronary Vasomotion Disorders International Study Group

CVDys = coronary vasomotor dysfunction

DC = direct current

ECG = electrocardiogram

EQ5D-5L = EuroQol 5-Dimension 5-Level

ESC = European Society of Cardiology

FFR = fractional flow reserve

Hs-CRP = high-sensitivity C-reactive protein

ICA = invasive coronary angiography

IMR = index of micro-circulatory resistance

IQR = interquartile range

JCS = Japanese Circulation Society

LAD = left anterior descending artery

LCA = left coronary artery

LCx = left circumflex artery

MACE = major adverse cardiovascular events

MBS = myosin-binding subunit

MVA = micro-vascular angina

NO = nitric oxide

NTG = nitroglycerine

Pa_{HYP} = proximal coronary pressure during hyperaemia

Pd = distal coronary pressure

PHQ-4 = Patient Health Questionnaire-4

QCA = quantitative coronary angiography

RCA = right coronary artery

RFR = resting full-cycle ratio

ROC = receiver operating characteristic

SAQ = Seattle Angina Questionnaire

SAQSS = Seattle Angina Questionnaire Summary Score

Tmn = mean transit time

TSQM-9 = Treatment Satisfaction Questionnaire

TTP = temporary transvenous pacemaker

VSA = vasospastic angina

VSMC = vascular smooth muscle cell

WISE = Women's Ischaemia Syndrome Evaluation

Chapter One: General Introduction

1.1. Introduction to angina with non-obstructive coronary arteries

Angina pectoris is the most common symptom of coronary artery disease (CAD) and is a leading cause of mortality in Australia. Contrary to common belief, a significant proportion of patients (up to 70%) undergoing coronary angiography have angina with non-obstructive coronary arteries (ANOCA) [1]. ANOCA encompasses a heterogeneous group of pathologies, including abnormalities of coronary microvasculature and vasospastic abnormalities of the coronary circulation.

Unfortunately, several patients with ANOCA, up to 50,000 Australians annually (according to the Coronary Angiogram Database of South Australia), continue to experience debilitating, unexplained symptoms. These patients often face recurrent angina, poor quality of life, repeated hospitalisations and adverse cardiovascular outcomes [2]. The prevalence of ANOCA is higher in women (50%–70%) than in men (30%–50%), predominantly affecting youngsters [3] with few conventional cardiac risk factors. Many of these patients have undergone multiple invasive coronary angiograms owing to persistent symptoms, as well as other non-cardiac investigations, often without a conclusive diagnosis. Unlike obstructive CAD, evidence-based guidelines to inform optimal diagnostic and therapeutic strategies for patients with ANOCA are lacking, underscoring the need for further research and clinical focus.

1.2. Clinical presentation

Patients with ANOCA often present with a broad spectrum of symptoms, which are frequently misattributed to non-cardiac causes, leading to under-recognition, insufficient investigation and suboptimal treatment. Most (>60%) patients report chest pain triggered by physical exertion and/or

emotional stress, which typically subsides with rest. The remaining sub-set experiences angina sporadically at rest, which may be accompanied by angina equivalents such as dyspnoea. Additional symptoms may include back pain, indigestion, nausea, profound fatigue, weakness, vomiting and sleep disturbance. Symptoms tend to be stable, although they may fluctuate and are usually present for at least 3 months without other identifiable causes. Initial presentation may occur in the primary care setting or during recurrent urgent visits to the emergency department. Patients frequently experience symptoms for years before an accurate diagnosis is made, during which time their functional capacity for physical activity often deteriorates [4]. The prolonged diagnostic journey, combined with the challenges of detecting ANOCA via conventional non-invasive testing, can substantially affect both physical and mental health. Moreover, this delay contributes to immense healthcare utilisation, imposing a considerable economic burden [5].

1.3. ANOCA endotypes

Various pathophysiological mechanisms underlie ANOCA, each contributing to distinct clinical presentations and therapeutic challenges. These mechanisms primarily involve coronary microvascular dysfunction (CMD) and coronary artery spasm (CAS). These conditions are characterised by abnormal coronary vasomotor function despite the absence of significant epicardial coronary stenosis. CMD is linked to structural and functional abnormalities in the coronary microcirculation, whereas CAS involves reversible vasoconstriction of the epicardial or micro-vascular coronary arteries. Comprehending these distinct endotypes is crucial for accurate diagnosis and tailored management strategies in this patient cohort.

1.3.1. Coronary microvascular dysfunction

The coronary arterial bed contains three structurally and functionally distinct compartments. The large epicardial coronary arteries (500 μm–5mm in diameter) function as conduit vessels that offer little resistance to coronary blood flow (CBF) in the absence of obstructive atheroma [6-8]. On the contrary, the epicardial pre-arterioles (500–100 μm) account for most resistance and respond to flow-related stimuli. While the proximal component is more responsive to shifts in flow, the distal one is more sensitive to pressure variations [6-8]. The intra-myocardial arterioles (<100 μm) exhibit the highest resistance and are responsible for the metabolic regulation of CBF in response to myocardial oxygen demand (autoregulation). Lastly, the capillaries (<10 μm) function as exchange vessels, given their large surface area and relatively high permeability [6-8]. Coronary microcirculation refers to the epicardial pre-arterioles, intra-myocardial arterioles and capillaries.

CMD is characterised by structural and functional remodelling of the microcirculation, leading to impaired CBF autoregulation [7]. Potential mechanisms comprise dysfunctional coronary vasodilator capacity and/or enhanced reactivity to microvascular vasoconstriction. The endothelium regulates CBF by modulating vasorelaxant substances, including nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarising factors [9,10]. Dysfunctional endothelium results from pathological vasoconstrictive substances (such as endothelin-1, superoxide, hydrogen peroxide and thromboxane), overhauling the vascular steady state [11,12]. In addition, microvascular spasm involves the impairment of vasomotion (physiological rhythmical contractions) and is closely associated with endothelial dysfunction [13,14]. Its complex pathophysiology is marked by vascular smooth muscle cell (VSMC) dysfunction and a predominance of vasoconstrictive metabolites in enhanced Rho-kinase activity [15-17]. Up-regulated Rho-kinase activity results in excessive myosin light chain phosphorylation by inhibiting the myosin-binding submit (MBS), leading to hyper-contractility [18].

The primary structural alterations in CMD include luminal narrowing of the intra-mural arterioles and capillaries, perivascular fibrosis and capillary rarefaction [7]. These pathological changes often occur in the context of left ventricular hypertrophy. Furthermore, conditions such as hypertrophic cardiomyopathy and hypertensive heart disease result in the thickening of medial and intimal vessel walls, leading to impaired CBF [19]. Such remodelling of the microcirculation is related to various comorbidities, including diabetes, hypertension, renal impairment and diffuse epicardial atherosclerosis [12]. Figure 1.1 summarises the pathogenic abnormalities that characterise CMD.

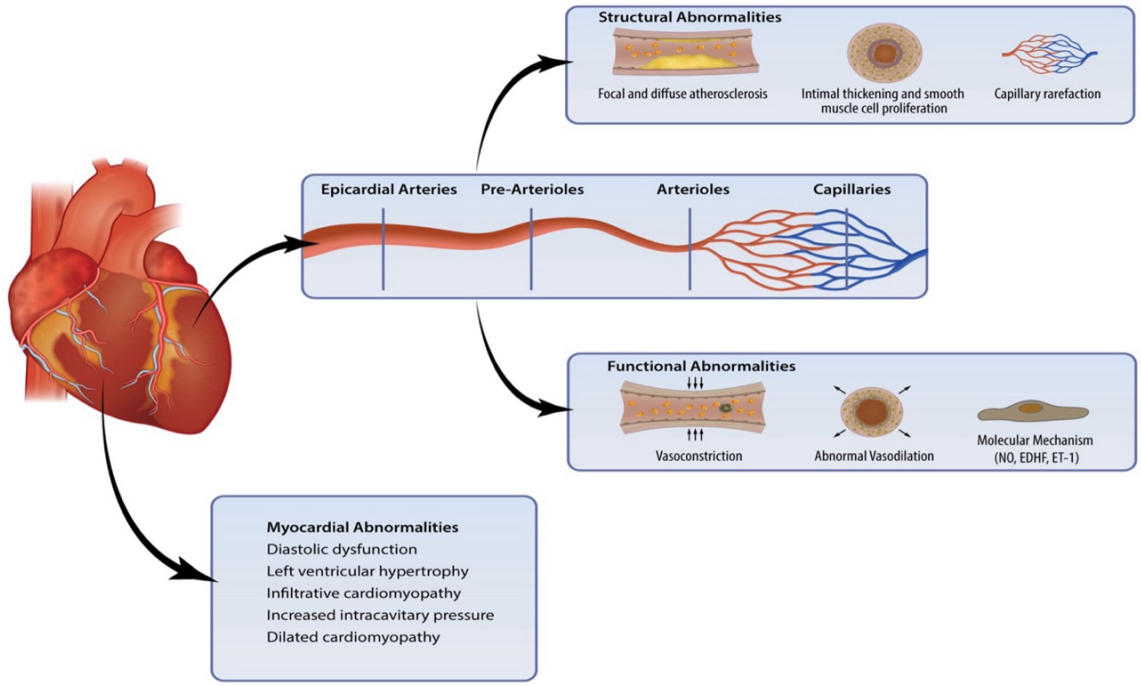


Figure 1.1: Complex pathophysiology of coronary microvascular dysfunction

1.3.2. Coronary artery spasm

CAS is characterised by transient and reversible vasoconstriction of the coronary vasculature, leading to myocardial ischaemia. This condition was initially described in a landmark report by Prinzmetal et al. in 1959 [20]. They reported 32 cases with a variant type of angina that occurred at rest, with transient ST-segment elevation on electrocardiogram (ECG) [20]. Animal models established that this variant was

a distinct functional disorder caused by intermittent hypertonic occlusion of the coronary arteries. Later, Yasue et al. recorded epicardial CAS provoked by methacholine or exercise on coronary arteriography [21,22]. These early discoveries sparked interest among the wider cardiovascular community. CAS is a heterogeneous phenomenon that can occur in patients with or without atherosclerosis and affects the epicardial coronary arteries or micro-vasculature. CAS is an under-appreciated cause of stable angina, acute coronary syndromes, malignant arrhythmias and sudden cardiac death [23,24].

The pathophysiology of CAS involves the impairment of vasomotion (physiological rhythmical contractions) in one or more coronary arteries . Moreover, VSMC dysfunction coupled with a predominance of vasoconstrictive metabolites plays an adjunctive role [18,25]. A localised vasomotion abnormality may produce a focal (segmental) spasm in a coronary artery, whereas a more diffuse abnormality may result in a multi-vessel spasm [15,18]. The critical pathophysiological mechanisms implicated in CAS are summarised in Figure 1.2.

Vascular Smooth Muscle Hyper-Contractility: The Rho-kinase enzyme in the VSMC is a crucial regulator of hyper-contractility. Enhanced Rho-kinase activity results in excessive myosin light chain phosphorylation by inhibiting the MBS, leading to a state of hyper-contractility [18,25]. In addition, several additional pathways involving NO, phospholipase C and KATP channels are linked to VSMC hyper-contractility [16,17,26]. These mechanistic pathways are primary contributors to CAS attacks.

Endothelial Dysfunction: The endothelium within the coronary vasculature is responsible for producing NO, a potent vasodilator [27]. It suppresses vasoconstrictive metabolites such as angiotensin II and endothelium I [27]. Endothelial dysfunction results in endogenous NO deficiency, leading to a proliferation of vasoconstrictive metabolites. This mechanistic pathway explains why several endothelium-dependent vasodilators (e.g. acetylcholine [ACh], ergonovine, histamine and serotonin) trigger vasoconstriction in patients with CAS [28].

Chronic Inflammation: Several inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6 and adhesion molecules, have been implicated in the role of chronic low-grade inflammation in CAS [29]. This notion has been reinforced by numerous animal and cellular models [30,31]. Unsurprisingly, persistent tobacco exposure is also associated with chronic inflammation [32]. Furthermore, adventitial and perivascular adipose tissue inflammation has been observed by Waters et al. in this population [33]. These findings confirm the significance of chronic inflammation in CAS.

Autonomic Nervous System: Overactivity of the sympathetic or parasympathetic nervous system can contribute to CAS via sensitisation of the VSMC and increased levels of effector molecules, such as ACh and adrenaline. The nocturnal predominance, together with the capability of ACh to induce CAS attacks, highlights the role of the parasympathetic system [34]. On the contrary, increased catecholamine levels and adrenergic activity following an episode of CAS represent the sympathetic system [35,36].

Ethnicity: Japanese and other Asian cohorts may show a higher prevalence and a more severe coronary artery involvement in CAS than White cohorts, which might be attributed to lifestyle and genetic differences [37,38]. Sueda et al. questioned these findings and documented fewer differences between Japanese and European cohorts [38]. A recent comparative international study identified that among patients with CAS, Caucasians had significantly lower survival than Japanese (76.6 vs 86.7%) despite exhibiting similar patterns of coronary spasm on provocative testing [39].

Genetic Polymorphisms: Genetic mutations involving NO synthase, adrenergic receptors, serotoninergic receptors, angiotensin-converting enzyme (ACE), paraoxonase I and RNF213 locus have been implicated in the pathogenesis of CAS [40-44]. Moreover, many studies have shown polymorphisms in genes encoding aldehyde dehydrogenase, NADH/NADPH oxidase and IL-6 [40,45,46]. Mainly, circulating microRNAs contribute to the pathogenesis of endothelial NO synthase and may act as novel biomarkers for diagnosing CAS [47].

Magnesium Deficiency: Magnesium is a key naturally occurring antagonist of intracellular calcium. Up to 45% of patients with CAS may display magnesium deficiency, which may be an essential contributor to the condition in certain patients [48,49].

Atherosclerosis and Thrombosis: Atherosclerosis and CAS are two discrete lesions that often coexist and share several risk factors [50]. The progression of one entity can aggravate the other. Coronary spasms can occur in both angiographically normal vasculature and atherosclerotic arteries [51,52]. These have a predisposition for branch points in coronary vessels and segments distal to stented lesions [53]. Given their complex pathophysiology, most researchers believe that lesions in coronary spasms are unlike those in conventional lipid-laded atherosclerosis [51,52]. Another noteworthy point is the relationship between CAS and thrombosis. Increased levels of plasminogen activator inhibitor 1 and fibrinopeptide A have been observed in post-CAS attacks [54]. Furthermore, platelets are activated after CAS attacks but not after stable angina [55]. These findings suggest that prolonged attacks of spam can trigger coronary thrombosis.

Acute Coronary Syndrome: CAS has a crucial etiologic role in the pathogenesis of acute coronary syndrome (ACS). The CASPER study demonstrated that one-third of patients presenting with non-ST elevation ACS and no obstructive lesions had evidence of CAS [56]. The mechanisms involved include prolonged CAS leading to vessel occlusion and subsequent myocardial ischaemia, coronary plaque progression and rupture of vulnerable plaques and acute thrombus formation [57-60]. Kobayashi et al. used intra-vascular optical coherence tomography to examine the culprit lesion in patients with CAS and ACS. The findings indicated reduced luminal area with vascular contraction and thrombus formation without atherosclerotic plaque disruption [61].

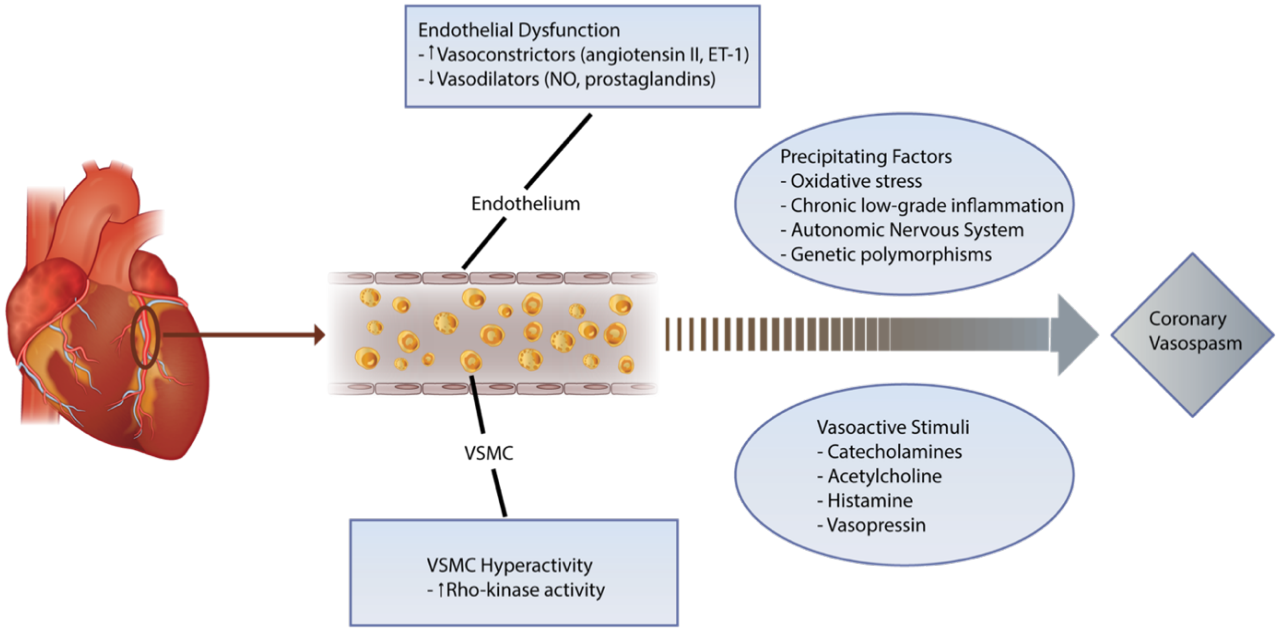


Figure 1.2: Complex pathophysiology of coronary artery spasm

VSMC – vascular smooth muscle cells, ET-1 – endothelin-1, NO – nitric oxide

1.4. Invasive diagnostic testing

Although invasive coronary angiography is considered the gold standard for diagnosing epicardial CAD, its limitations in evaluating ANOCA are well recognised. As a two-dimensional (2D) imaging modality, coronary angiography primarily assesses the presence of significant luminal obstruction but cannot evaluate coronary microvascular function or detect subtle vasomotor abnormalities. In contrast, invasive coronary function testing (CFT) offers a more comprehensive assessment by integrating guidewire-based micro-circulatory measurements with coronary vasoreactivity testing, most commonly using ACh provocation. This approach provides insights into microvascular and epicardial endothelial function, permitting the diagnosis of coronary vasomotor disorders. The European Society of Cardiology (ESC) guidelines have endorsed this method with a Class Ib

recommendation for evaluating patients with ANOCA, highlighting its clinical utility in uncovering the underlying mechanisms of ischaemia in these patients [62].

1.4.1. Coronary physiology assessment

Coronary physiology assessment can be performed using coronary thermodilution with a pressure–temperature sensor guidewire (PressureWire XTM, Abbott Vascular, Santa Clara, CA, USA) or a Doppler technique (ComboWire XT or Flowire, Philips Volcano Corporation, San Diego, CA, USA). The left anterior descending (LAD) coronary artery is the preferred pre-specified target vessel owing to its subtended myocardial mass and coronary dominance [63].

Given the simplicity and wide availability, the thermodilution technique has gained popularity in measuring these indices. Coronary flow reserve (CFR) is defined as the ratio of maximal coronary flow to resting coronary flow in a given perfusion territory [64]. CFR can be calculated using the following formula, assuming that the coronary vessel volume remains constant:

$$\frac{Hyperaemic\ Q}{Rest\ Q} = \frac{Hyperaemic\ (\frac{V}{Tmn})}{Rest\ (\frac{V}{Tmn})} = \frac{Rest\ Tmn}{Hyperaemic\ Tmn}$$

Where Q = flow, V = vascular volume between the injection site and sensor and Tmn = mean transit time.

CFR assesses the entire coronary vascular bed without distinguishing between the epicardial and microvascular circulation. In the absence of epicardial stenosis, CBF is predominantly regulated by the microvasculature, which includes the pre-arterioles and arterioles. These smaller vessels

dynamically modulate their luminal diameter and total coronary resistance in response to myogenic and vasomotor stimuli to balance myocardial oxygen supply and demand. CFR uniquely evaluates the functional reserve of the coronary circulation, including the microvasculature, and serves as a key indicator of microvascular function in patients with angiographically normal arteries . A thermodilution-derived CFR value of <2.5 is extensively employed as a diagnostic criterion for CMD and is a key prognostic marker [65]. In a meta-analysis of 11 studies involving 6,631 patients with non-obstructive coronary arteries, abnormal CFR was a strong predictor of mortality (OR = 3.93, P < 0.001) and major adverse cardiovascular events (MACE) (OR = 5.16, P < 0.001). Similarly, a meta-analysis of 16 studies involving 10,848 patients confirmed abnormal CFR as a robust predictor of mortality and MACE in individuals with non-obstructive CAD [66].

The index of micro-circulatory resistance (IMR) was initially developed to specifically determine the coronary microcirculation. IMR can be estimated using specialised guidewires with pressure and temperature sensors simultaneously measuring distal coronary pressure (Pd) and mean transit time (Tmn) using the thermodilution technique. IMR, defined as an index of the lowest achievable resistance in the microcirculation, can be calculated as follows:

$$IMR = \left(\frac{Pd_{Hyp}}{\left(\frac{1}{Tmn_{Hyp}} \right)} \right) = \left(Pd_{Hyp} x\, Tmn_{Hyp} \right)$$

As with other coronary physiology indices, the IMR is a continuous variable requiring a dichotomous cut-off for clinical use. Numerous studies have attempted to define the normal reference range for IMR by assessing the values in patients with CAD and healthy controls [67-70]. Based on these investigations, a cut-off value of ≥25 has been recommended by expert guidelines to indicate an

abnormal IMR [5]. Given its specificity for determining coronary microcirculation, IMR is a valuable tool for evaluating patients with ANOCA. In a study involving 139 patients with ANOCA, approximately 20% had an IMR of ≥25, implicating microvascular dysfunction as the underlying cause of their symptoms. Furthermore, IMR was strongly correlated with the Duke treadmill score ($r = -0.74$, $P < 0.001$) in the ANOCA cohort. These findings highlight the significant link between exercise limitation and the severity of microvascular dysfunction [68].

Discordance between CFR and IMR can occur in patients with ANOCA. The characteristics and outcomes of IMR and CFR discordance in patients with ANOCA were investigated in a study by Lee et al. Their study classified 230 patients without obstructive epicardial CAD into four groups: normal IMR/normal CFR, abnormal IMR/normal CFR, normal IMR/abnormal CFR and abnormal IMR/abnormal CFR. The underlying mechanism driving discordance between IMR and CFR was ascribed to variations in resting flow, with patients in the normal IMR/abnormal CFR group exhibiting significantly lower mean resting Tmn (Tmn_{Rest}) (0.31s vs 1.20s, $P < 0.05$) than those in the abnormal IMR/normal CFR group. Patients in the discordant IMR and CFR groups did not display significantly worse outcomes than those in the normal IMR/normal CFR group, and only patients in the abnormal IMR/abnormal CFR group had an increased risk of MACE (HR = 5.6, P = 0.03). This study suggested that concordant abnormalities in both CFR and IMR were necessary to place patients with ANOCA patients at a higher risk of adverse clinical outcomes [71].

1.4.2. Coronary vasoreactivity testing

The most established approach for vasoreactivity testing is the intracoronary infusion of ACh [62,72,73], which is a vasoactive substance that triggers vasospasm via cholinergic receptors on VSMCs [74]. When ACh is administered as a low-dose infusion in patients with a healthy endothelium, a vasodilatory

response is caused owing to the endothelial release of NO [27]. In contrast, dysfunctional endothelium cannot release sufficient NO to overcome the stimulated vascular smooth muscle muscarinic receptors, leading to mild vasoconstriction, typically <30% [27]. In ACh provocative spasm testing, rapid bolus administration of high-dose ACh produces severe vasoconstriction (i.e. >90%) in patients predisposed to coronary vasomotor hyperreactivity. The adoption of these mechanistic considerations and an established safety track record [75] have made ACh provocation testing the most commonly used diagnostic test for CAS. Nevertheless, the heterogeneity in dosage and administration time has limited cardiologists' understanding and acceptance of a uniform protocol [Figure 1.3].

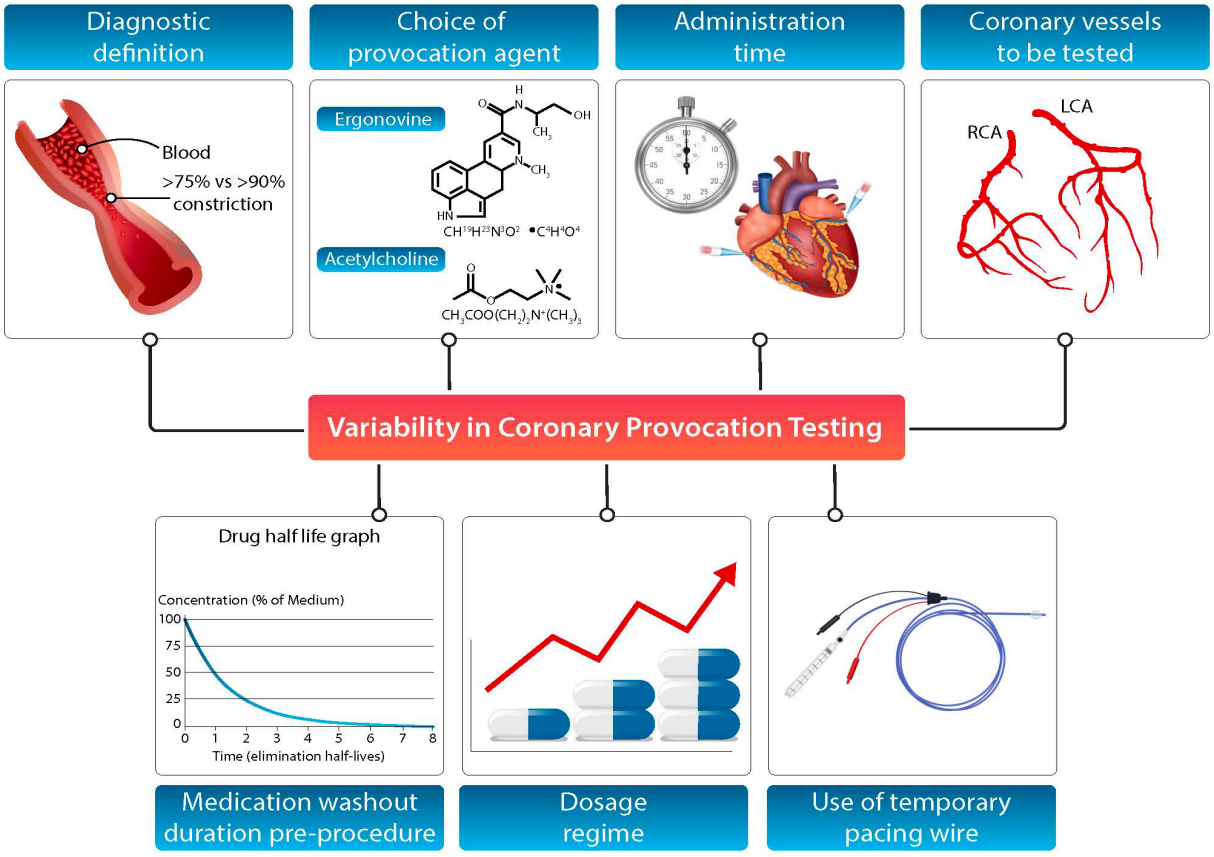


Figure 1.3: Key factors underlying the variability in coronary provocation testing

1.4.2.1. Dosage and coronary artery predominance

Doses vary for left (LCA) and right (RCA) coronary arteries but are uniformly higher for the left system [Table 1.1]. The maximum dosage for LCA is 200 μg and that for RCA is 80 μg. Sueda et al. suggested that a dose of 200 μg improved diagnostic utility, especially in patients with a high clinical suspicion of CAS and no provoked spasm with smaller doses [76]. However, the propensity for pseudo-positive reactions at such doses may reduce the diagnostic specificity. Alternatively, the Women's Ischaemia Syndrome Evaluation (WISE) study employed a protocol of sequential intracoronary reinfusions of incremental ACh doses (0.182–1.82–18.2 mg/mL), such as that of Tio and Sara et al. [77-79]. The CorMica trial emulated this protocol but added an ACh bolus of 100 μg (LCA) and 50 μg (RCA) [80]. Most protocols adopt an incremental dosing regimen and commence testing in the LCA. In contrast, others focus on testing the RCA initially and terminating the procedure later if a spasm is identified [81]. Saito et al. proposed omitting the 50 μg dose of ACh in the LCA if little coronary artery construction was caused by 20 μg, leading to reduced contrast volume and procedural time [82]. Interestingly, men and women exhibited different responses to ACh, with men demonstrating a minimal lumen diameter–dose relationship with doses of 200 μg and women showing little changes with doses >50 μg [83]. These findings suggested that an incremental sex-adjusted dosage regime could improve the diagnostic utility of provocation testing.

The possible predominance of coronary spasm within specific coronary arteries was explored by Sueda and Kohno [84]. In a retrospective analysis of 1,392 patients who had undergone ACh-based provocative testing, the proportion of spasm involving the left circumflex artery (LCx, 28.3%) was significantly lower ($P < 0.001$) than that involving the RCA (73.3%) and the LAD artery (72%) [84]. Subsequent studies from Korea confirmed these findings [85,86]. These results implied that the LCx might be less responsive to ACh-based provocative testing. Several studies have avoided provocative testing in the RCA altogether [77,83,86,87]. Despite reducing the procedural time and contrast

administration, this approach can neglect the presence of multi-vessel spasm, a known poor prognostic marker [57,58]. Furthermore, single-vessel testing may reduce the diagnostic yield.

1.4.2.2. Administration time

Okumura et al. administered 20, 50 and 100 µg in the LCA and 20 and 50 µg in the RCA as serial 20-s boluses in a study that validated the ACh provocative spasm protocol. In this landmark study, patients fulfilling the variant angina clinical criteria for spontaneous vasospastic episodes, along with a negative control cohort (i.e. patients with cardiomyopathy, arrhythmia, valvular disease, hypertension and congenital heart disease), were examined using this ACh administration protocol [88]. The findings confirmed that the protocol was highly sensitive (90%) and specific (99%), validating its use for diagnosing CAS. Although modifications of this protocol have been described, they have not been validated against the native disorder characterised by spontaneous spasm episodes.

The heterogeneity in ACh administration protocols has partly arisen from clinical research studies designed to assess functional endothelial integrity. These protocols utilise 2–3-min low-dose (<20 µg) ACh infusions and have been adopted in multiple studies [77-79,89-92]. Other protocols have described longer manual injections of 60 s [83,93,94]. Sueda and Kohno reported the results of a study that included 30 patients with ischaemic heart disease who initially received an intracoronary 20-s ACh injection, followed by a 3-min infusion. The researchers noted a positive spasm provocation in 73.3% and 33.3% ($P < 0.05$) patients, respectively [95]. These results implied that the administration time of intracoronary ACh may affect the results of provocative testing. The limitations of this study included a small sample size, with most patients being male smokers (83.3% and 76.7%, respectively) [95].

1.4.2.3. Temporary pacemaker insertion and procedural approach

The insertion of a temporary transvenous pacemaker (TTP) during provocative testing has also varied among international research groups. Many institutions advocate for the insertion of a TTP upfront [96-100]. Unsurprisingly, a back-up pacing rhythm was considerably higher during ACh administration into the RCA, especially during a rapid 20-s injection compared with a 3-min infusion (63.3% vs 23.3%; $P < 0.01$) [95]. Despite a marginal increase in procedural time, it may avoid serious complications. Ong et al. preferred to avoid a TTP and suggested that a transient atrioventricular block almost always resolves within seconds of reducing the speed of administration [91]. However, whether this approach, with a potentially slower administration of the provocation agent, will decrease the diagnostic yield is uncertain.

The optimal procedural approach site for both artery and vein (assuming insertion of a TTP) was investigated by Sueda and Kohno [101]. They retrospectively analysed the outcomes of 1,829 ACh-based provocative tests according to vascular access routes, including i) femoral artery and femoral vein (16%); ii) brachial artery and femoral vein (27.2%); iii) brachial artery and brachial vein (32.2%); iv) radial artery and brachial vein (13.8%) and v) radial artery and femoral vein (9.6%) [101]. These groups had no statistical difference in procedure-related major complications. However, the investigators observed that the radial artery and brachial vein combination provided the highest comfort for both operators and patients without compromising the diagnostic utility [101]. Current guidelines do not specify a preferred approach, although the use of intra-arterial vasodilators (e.g. nitroglycerine [NTG], verapamil) and operator experience are key influencing factors [102]. Intra-arterial vasodilators are routinely administered for trans-radial access to increase radial artery size and reduce radial spasm [103]. However, these agents may decrease the diagnostic yield of provocation testing when administered during the index procedure. Verapamil is generally avoided owing to its relatively long half-life. Nonetheless, NTG is used during concomitant coronary physiology testing. Despite its rapid half-life (approximately 3 min) [104], the residual effect of NTG on the sensitivity of

coronary provocation testing remains unclear. Current practice is based on expert consensus, but further studies are required to investigate the potential confounding effect of vasodilators on these provocation studies [72].

Table 1.1: Protocols for acetylcholine provocation testing

Authors	Year	Country	ACh dose LCA (ug)	ACh dose RCA (ug)	Administration time (s)	Medication washout duration	Epicardial spasm (%)	Micro-vascular spasm (%)	Positive spasm threshold
Bill et al.[89]	2021	Poland	25–50–100	25–50–75	180	48	46.9	34.1	>90%
Konst et al.[87]	2021	Netherlands	2–20–100–200	–	60–180	24–48	42.9	NR	>90%
Montone et al.[90]	2021	Italy	20–50–100–200	20–50	180	24–48	37.7	21.3	>90%
Pargaonkar et al.[83]	2020	United States	20–50–100–200	–	60	48	–	–	–
Probst et al.[92]	2020	Germany	2–20–100–200	80	180	–	26.1	42.2	>90%
Sara et al.[77]	2020	United States	0.182–1.82–18.2ug/ml	–	180	48	–	–	–
Sueda et al.[98]	2020	Japan	20–50–100–200	20–50–80	20	24	59.3	–	>90%
Ford et al.[80]	2019	UK	100	50	20	–	37.1	32.5	>90%
Deyama et al.[96]	2018	Japan	50–100	50	30	48	44.6	–	>90%
Tateishi et al.[100]	2018	Japan	20–50–100	20–50	20	48	48.8	–	–
Kim et al.[94]	2018	Korea	20–50–100	–	60	48			>90%
Lee et al.[86]	2017	Korea	20–50–100	–	–	48	13.9	29.5	>75%
Saito et al.[82]	2017	Japan	20–50–100	20–50	20	48	40.3	–	>90%
Hoshino et al.[81]	2016	Japan	20–50–100	20–50	60	24	30.9	–	>75%
Choi et al.[93]	2016	Korea	20–50–100	–	60	72	77.7	–	>70%
DiFiore et al.[105]	2015	Australia	25–50–100	25–50	20	48	44.3	32	>90%
Ong et al.[91]	2014	Germany	2–20–100–200	80	180	48	33.4	24.2	>75%
Sato et al.[97]	2013	Japan	20–50–100	20–50	20	–	49.6	–	>90%
Takagi et al.[99]	2013	Japan	20–50–100	20–50	20	24	100[#]		>90%

Wei et al.[79]	2012	United States	0.182–1.82–18.2 µg/mL	–	180	24	7.3	–	>50/70%
Tio et al.[78]	2002	Netherlands	0.182–1.82–18.2 µg/mL	–	180	24–72	–	–	>50/90%
Okumura et al.[88]	1988	Japan	20–50–100	20–50	20	–	90	–	–

1.5. Non-invasive diagnostic testing

Non-invasive evaluation of patients with ANOCA is an evolving field, with several imaging modalities available to assess the vasodilator capacity of the coronary microvasculature and estimate myocardial blood flow. Transthoracic Doppler echocardiography of the LAD is a widely applicable technique for assessing coronary flow velocity reserve[106]. However, its use is limited by technical challenges, reduced spatial resolution, and operator dependency. Myocardial contrast echocardiography offers a more objective measure of vasodilator capacity, but its clinical adoption has been restricted by the absence of validation in large-scale studies [107].

Positron emission tomography (PET) and cardiac magnetic resonance imaging (CMR) are currently the most robust modalities for quantifying myocardial blood flow. PET allows automated calculation of absolute global myocardial blood flow (mL/min/g) at rest and during hyperaemia through tracer uptake in the left ventricular myocardium[108]. CMR, alternatively, derives myocardial perfusion reserve from rest–stress signal intensity changes and can provide quantitative absolute flow estimates[109]. While CMR offers superior spatial resolution and avoids ionising radiation, its widespread use is limited by cost and availability.

Non-invasive methods for diagnosing coronary artery spasm remain less developed, owing to limitations in efficacy and reproducibility. The JCS guidelines endorse hyperventilation and

exercise testing with ECG monitoring as non-pharmacological provocative approaches, targeting a respiratory rate of 25/min for up to six minutes, with termination in the event of angina or ischaemic ECG changes[72]. The COVADIS group similarly recognises hyperventilation but favours the cold pressor test as a reflexogenic stimulus, although these techniques have largely been superseded by pharmacological provocation[110]. More recently, large-scale ergonovine echocardiography data has emerged; in a cohort of 14,012 patients, CAS was detected in 15.3% through either ECG changes or reversible regional wall motion abnormalities[111]. Further studies are required to establish diagnostic accuracy and define their role in clinical practice.

1.6. Aims of the thesis

Despite advances in our understanding of patients with ANOCA, significant knowledge gaps remain in the evaluation and management of this heterogeneous condition. The overarching objective of this thesis is to refine diagnostic strategies, optimise procedural protocols, and enhance clinical outcomes in this complex patient population. The specific aims are as follows:

1. To determine the prevalence and predictors of coronary vasomotor dysfunction (CVDys) in a prospective cohort of patients with ANOCA. All patients underwent standardised CFT in at least one major epicardial vessel. Group comparisons and multivariable logistic regression were employed to identify independent clinical and angiographic predictors of CVDys.

2. To assess the diagnostic validity of current ACh provocation protocols, comparing conventional versus high-dose regimens in detecting CAS. This was evaluated in a diverse population comprising patients with suspected ANOCA and individuals undergoing coronary angiography for

non-coronary indications. Diagnostic performance was assessed by calculating sensitivity, specificity, and predictive values of both regimens.

3. To evaluate the impact of prior intracoronary NTG administration on the diagnostic yield of ACh provocation testing**.** Using a ACh rechallenge protocol, we assessed the re-inducibility of epicardial spasm at 5- and 10-minute intervals following intravascular NTG administration in patients with a confirmed initial positive response. This study aimed to clarify the temporal influence of NTG on coronary vasoreactivity and its implications for diagnosing CAS.

4. To quantify the incidence and identify predictors of TTP use during ACh provocation testing. Given the variability in procedural safety protocols across institutions, we prospectively examined the rate of TTP activation and associated clinical characteristics in patients with suspected CAS.

5. To evaluate the incremental diagnostic utility of multi-vessel versus single-vessel CFT in patients with ANOCA. In a prospective study design, comprehensive CFT was performed in all three major epicardial coronary arteries to determine whether multi-vessel assessment improves the detection of CVDys and enables more granular classification of ANOCA endotypes.

6. To determine whether multi-vessel CFT improves patient-reported outcomes compared with single-vessel testing. In a randomised controlled trial, patients were assigned to undergo either multi-vessel or single-vessel CFT. The primary outcome was angina severity at 6 months. Secondary outcomes included diagnostic reclassification, changes in health status, clinical utility, and procedural safety.

Chapter Two: Methods

2.1. Invasive coronary angiography

Invasive coronary angiography (ICA) was performed as the standard procedure according to local protocols. At the two tertiary hospitals (Concord Hospital and Royal Prince Alfred Hospital) where most studies were conducted, arterial access was gained via the radial or femoral artery. A 6-French guiding catheter was used to engage the coronary arteries, and multiple angiographic projections were performed to visually assess the epicardial coronary arteries. Imaging was typically performed at 15 frames per second with standard zoom to enhance and optimise vessel delineation.

2.2. Invasive coronary function testing

CFT was performed in the morning, and patients were requested to withhold vasoactive medications (e.g. calcium channel blockers [CCB] and long-acting nitrates) and methylxanthine-containing substances for >4 times the duration of the drug's half-life. A continuous 12-lead ECG was monitored throughout the procedure, and standard resuscitation equipment was available. Intra-arterial vasodilator drugs (e.g. NTG and CCB) were avoided in all patients before CFT.

2.3. Coronary vasoreactivity testing

Coronary vasoreactivity was assessed via ACh provocation. A TTP was inserted via the femoral vein to compensate for potential bradycardia. Activation of the TTP was contingent upon a pause of >5 s. A 6-French angioplasty guiding catheter without side holes was inserted into the LCA or RCA as per

the operator's clinical judgement. For the LCA assessment, incremental doses of 20, 50, 100 and 200 μg of ACh were injected over 20 s, with a 2-min gap between doses. After each injection, cine-images were obtained to evaluate the change in coronary diameter via quantitative coronary angiography. If CAS was induced, with reproducible symptoms and ST-segment alterations, the provocation test was terminated and concluded to be positive. A similar protocol was followed for the RCA, with incremental doses of 20, 50 and 80 μg of ACh. When coronary spasm was induced and did not resolve spontaneously within 3 min after completing ACh testing or in instances of haemodynamic instability, NTG was administered.

2.4. Coronary physiology protocol

Coronary physiology was evaluated using a pressure–temperature sensor guidewire (PressureWire X; Abbott Corporation, Chicago, IL). The guidewire was prepared by flushing the package coil with normal saline and switching on the PressureWire™ Transmitter and Receiving System.

The pressure guidewire was advanced until the sensor was located 1–2 mm distal to the guide catheter tip, at which point the guidewire pressure was equalised to that of the guide catheter pressure. Multiple measures were taken to minimise pressure signal drift and measurement error. As electrical and thermal instabilities are inherent in all sensors and occur linearly over time, measurements were performed rapidly to reduce the inevitable signal drift that occurs during lengthy procedures [112]. To avoid contrast adhering to the guidewire and affecting pressure wire measurements, the guide catheter was generously flushed with saline before equalisation and the vessel was wired with a minimal amount of contrast [113]. The needle introducer was always removed from the haemostasis valve during both equalisation and measurement to avoid pressure drift [112].

The guidewire was advanced to the distal third of the vessel. Intracoronary NTG was administered at 200 µg to produce maximal epicardial coronary vasodilation. Subsequently, the proximal coronary pressure during rest (Pa_{Rest}) and distal coronary pressure during rest (Pd_{Rest}) were recorded. Three boluses of 3 mL room temperature saline were injected into the coronary artery via the guiding catheter. The transit time of the saline injections was determined using the thermodilution technique, and the average of the three resting transit times was recorded as Tmn_{Rest}. An intravenous adenosine infusion was administered via a large-bore peripheral cannula or femoral venous sheath at 140 µg/kg/min for a minimum duration of 90 s to achieve maximal hyperaemia. The proximal coronary pressure during hyperaemia (Pa_{Hyp}) and distal coronary pressure during hyperaemia (Pd_{Hyp}) were recorded. Thermodilution curves were then constructed in the same manner as mentioned above to estimate the hyperaemic mean transit time (Tmn_{Hyp}) [114]. After the coronary physiology measurements were completed, the guidewire was pulled back to the guide catheter tip to check for pressure signal drift. If a signal drift of >3 mmHg occurred, the pressure wire was re-equalised and the physiology measurements were repeated. A signal drift of ≤3 mmHg was considered acceptable as repeat measurements were unlikely to change significantly at this drift level [115].

All measurements were recorded using the Coroflow system (Coroventis Research AB, Uppsala, Sweden). The coronary physiology indices were automatically calculated with computerised software using the following formulae:

$$FFR = \frac{Pd_{Hyp}}{Pa_{Hyp}}$$

$$CFR = \frac{Tmn_{Rest}}{Tmn_{Hyp}}$$

$$IMR_{App} = Pd_{Hyp}\ x\ Tmn_{Hyp}$$

$$IMR_{Calc} = Pa_{Hyp}\ x\ Tmn_{Hyp}\ x\ 1.35\ x\ (\frac{Pd_{Hyp}}{Pa_{Hyp}}) - 0.32$$

Figure 2.1: Example of different coronary physiology indices obtained in real-time using the Coroflow software

The blue and orange (green box) represent thermodilution curves produced from 3 mL injections of room temperature normal saline during rest and maximal hyperaemia, respectively. The mean Tmn_{Rest} and Tmn_{Hyp} and the values of their 3× individual measurements are highlighted by the orange and blue boxes, respectively. CFR = coronary flow reserve, FFR = fractional flow reserve, IMR = index of micro-circulatory resistance, Pa = proximal coronary pressure, Pd = distal coronary pressure, Tmn_{Hyp} = mean Tmn during hyperaemia, Tmn_{Rest} = mean Tmn during rest

2.5. Dobutamine-stress diastolic fractional flow reserve

Functional assessment of a myocardial bridge on coronary angiography was performed with dobutamine-stress diastolic fractional flow reserve (FFR). Intravenous dobutamine was given in increments of 10–20 μg/kg/min every 3 min until at least 85% of the maximal heart rate for age was achieved; alternatively, a maximal dose of 50 μg/kg/min with up to 1 mg of atropine was administered. A continuous Pd/Pa trace at peak dobutamine stress was recorded. Pd/Pa was measured using a digital calliper at peak dobutamine stress in diastole 3–5 times/beat and averaged using 2–3 beats to obtain the average diastolic FFR at peak dobutamine stress [116]. A dobutamine diastolic FFR of ≤0.76 was considered positive based on previous studies [117].

2.6. *Two-dimensional quantitative coronary angiography measurements*

2D quantitative coronary angiography (QCA) measurements were performed offline using standard commercial software in batches, with the operator blinded to the coronary physiology index values. Automated distance calibration was used to determine the pixel size. All measurements were acquired during the end-diastolic period, with the assistance of ECG-gated identification when available. Angiographic views that clearly depicted the measured stenoses and had the least foreshortening/overlapping were used. Edge detection correction was performed if necessary. The % diameter stenosis was measured twice and averaged.

Chapter Three: Prevalence of Coronary Vasomotor Disorders in Patients with Angina and Non-obstructive Coronary Arteries: A Sydney Experience

***Rehan R**, Wong CCY, Cooke C, Weaver J, Jain P, Adams M, Ng MKC, Yong ASC. Prevalence of Coronary Vasomotor Disorders in Patients with Angina and Nonobstructive Coronary Arteries: A Sydney Experience. Heart Lung Circ. 2024 Jun 25:S1443-9506(24)00161-6. doi: 10.1016/j.hlc.2024.02.020.*

3.1. Introduction

CAD, the single leading cause of mortality in Australia, is characterised by angina pectoris [118]. Approximately half of all patients undergoing coronary angiography for stable angina have non-obstructive coronary arteries [1]. CVDys, encompassing both CMD and CAS, is the underlying pathology in 60%–90% of this patient cohort [119,120]. The prevalence of ANOCA is higher in women (50%–70%) than in men (30%–50%), with a predilection for a younger patient cohort [3]. These patients experience an angina burden comparable to that of individuals with obstructive CAD in conjunction with a diminished quality of life, frequent hospitalisations and adverse cardiovascular outcomes [1,2,4,121].

Conventional diagnostic tests, including ICA and coronary computed tomography angiography (CCTA), often miss coronary vasomotor disorders. The American College of Cardiology/American Heart Association and the ESC guidelines recommend CFT to categorise patients with ANOCA into distinct endotypes, including epicardial vasospastic angina (VSA), microvascular angina (MVA) or a combination thereof [122,123]. This approach enables tailored therapy that addresses the underlying pathology, alleviating the angina burden and improving the quality of life [80].

Despite these recommendations, testing for coronary vasomotor disorders is available only in limited centres in Australia and New Zealand, leading to a lack of local prevalence data. This study aimed to determine the prevalence of coronary vasomotor disorders within an ANOCA population in Australia. Furthermore, it attempted to identify predictive factors associated with specific ANOCA endotypes in individuals undergoing CFT.

3.2. Methods

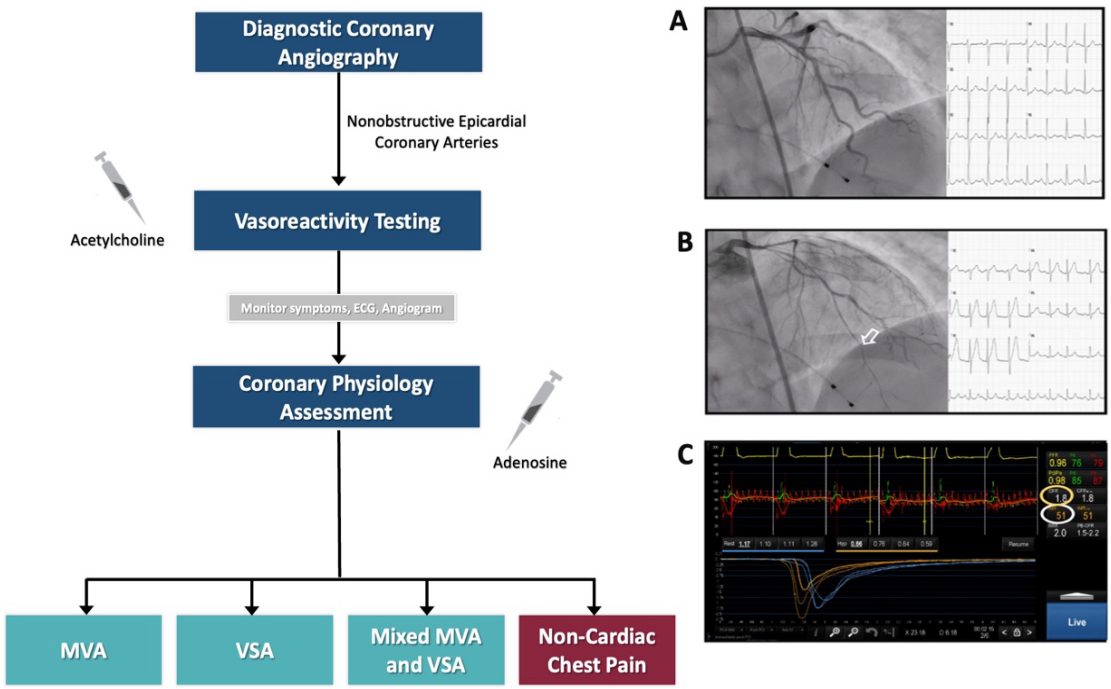
Consecutive patients with ANOCA who underwent CFT between June 2021 and June 2023 were included in this multi-centre, prospective observational study. These elective patients were referred to our centre due to a high suspicion of an ANOCA endotype, with the majority experiencing worsening or ongoing symptoms despite medical therapy. Before CFT, obstructive CAD was ruled out using ICA or CCTA. The patients (≥18 years) underwent ACh provocation and adenosine-mediated coronary physiology assessment in at least one epicardial artery, with additional testing of other arteries left to the operator's discretion. For safety reasons, CFT was not performed if the epicardial coronary artery was small, non-dominant or severely tortuous. Exclusion criteria included obstructive epicardial coronary arteries, non-coronary indication for invasive angiography (e.g. valve disease), technical factors precluding safe passage of the pressure guidewire, inability to receive adenosine, recent (within 3 weeks before cardiac catheterisation) ST-segment elevation myocardial infarction and/or inability to provide informed consent.

Comprehensive clinical data on patient characteristics, cardiac risk factors, and symptom profiles were systematically collected before CFT. Information on conventional cardiovascular risk factors and non-conventional variables linked to CVDys was also documented. Furthermore, patients were asked to provide details regarding their angina and quality of life by completing the Seattle Angina Questionnaire (SAQ), EuroQol 5-Dimension 5-Level Questionnaire (EQ5D-5L) and Patient Health Questionnaire-4 (PHQ-4). The study protocol adhered to the ethical principles outlined in the 1975 Declaration of Helsinki and received approval from the Human Research Ethics Review Board. Written informed consent was obtained from all participants, ensuring their voluntary participation. RR and ASCY had full access to all study data and were responsible for their integrity and analysis.

3.2.1. Invasive coronary function testing

CFT was performed in the morning, ensuring standardised timing across patients [Figure 3.1]. The patients were instructed to withhold vasoactive medications (e.g. CCBs and long-acting nitrates) and methylxanthine-containing substances for >4 times the duration of the drug's half-life. Throughout the procedure, continuous monitoring of a 12-lead ECG was implemented. At the same time, standard resuscitation equipment was readily available to ensure patient safety. ICA was performed according to standard institutional practice, utilising either the radial or femoral route. Administration of intra-arterial vasodilator drugs (e.g. NTG and CCB) was avoided before CFT. Diagnostic ICA was performed to confirm the absence of obstructive CAD, defined as a visual diameter stenosis of >50% in combination with a measured resting full-cycle ratio (RFR) of ≤0.89 and/or FFR of ≤0.80 [124]. If no obstructive CAD was present, CFT was performed.

Figure 3.1: Proposed diagnostic algorithm for coronary function testing



Following the exclusion of obstructive coronary artery disease via coronary angiography (A), the algorithm involves vasoreactivity testing (B) using intracoronary acetylcholine to detect coronary artery vasospasm. Diffuse left anterior descending artery spasm is denoted by the white arrow. Subsequently, coronary physiology assessment (C) with adenosine is employed to measure the coronary vasodilatory capacity and micro-circulatory resistance. An IMR valve of >25 (white circle) or a CFR value of <2.0 (yellow circle) denotes CMD.

Abbreviations: CFR = coronary flow reserve, CMD = coronary microvascular dysfunction, ECG = electrocardiogram, IMR = index of micro-circulatory resistance, MVA = microvascular angina, VSA = vasospastic angina

Coronary vasoreactivity was evaluated using ACh provocation. A TTP was introduced into the right ventricle via the femoral vein to compensate for potential bradycardia. Activation of the TTP was contingent upon a pause of >5 s. A 6-French angioplasty guiding catheter without side holes was inserted into the target epicardial coronary artery. For the LCA, incremental doses of ACh (20, 50, 100 and 200 μg) were injected over 20 s, with a 2-min interval between doses. After each injection, cine-images were obtained to assess the change in coronary diameter via QCA, as previously described [125]. If the provocation test induced CAS, reproducible symptoms and ST-segment alterations (see definitions below), the test was stopped and deemed positive. A similar protocol was adopted to evaluate the RCA, with incremental doses of ACh (20, 50 and 80 μg). Additional feedback was conveyed to the treating physician in instances of a positive CAS test triggered by higher doses (200 μg in the LCA and 80 μg in the RCA). Intracoronary NTG at a bolus dose of 200 μg was routinely administered to assess the basal coronary tone after CAS provocation.

Coronary physiology was evaluated using a pressure–temperature sensor guidewire (PressureWire X; Abbott Corporation, Chicago, IL). After equalising the pressure to that of the guide catheter, the guidewire was advanced to ensure that the sensor was positioned in the distal third of the vessel and at least 80 mm from the guiding catheter. If not already done, 200 μg of intracoronary NTG was

administered. The resting Pa and Pd were recorded. Three boluses of 3 mL room temperature saline were injected into the coronary artery via the guiding catheter. The transit time of the saline injections was determined using the thermodilution technique, and the average of the three resting transit times was recorded as the Tmn_{Rest}. Next, an intravenous adenosine infusion (140 μg/kg per min) was administered via a large-bore peripheral cannula or femoral venous sheath to induce a steady state of maximal hyperaemia. The hyperaemic mean proximal pressure and distal pressure were recorded. Thermodilution curves were similarly constructed to determine the Tmn_{Hyp}. The calculations for the respective coronary haemodynamic indices included (a) Pd/Pa during hyperaemia for FFR, (b) Tmn_{Rest} /Tmn_{Hyp} for CFR and (c) Pd × Tmn_{Hyp} for IMR. All measurements were recorded using the Coroflow system (Coroventis Research AB, Uppsala, Sweden).

In a subset of patients with myocardial bridge on coronary angiography, further functional assessment was performed using dobutamine-stress diastolic FFR. Intravenous dobutamine was given in increments of 10–20 μg/kg/min every 3 min until at least 85% of the maximal heart rate for age was achieved or a maximal dose of 50 μg/kg/min with up to 1 mg of atropine was administered. A continuous Pd/Pa trace at peak dobutamine stress was documented. Pd/Pa was measured using a digital calliper at peak dobutamine stress in diastole 3–5 times/beat and averaged using 2–3 beats to obtain the average diastolic FFR at peak dobutamine stress [116]. A dobutamine diastolic FFR of ≤0.76 was considered positive as per previous studies [117].

3.2.2. Definitions

CVDys was defined according to the underlying pathophysiological endotype based on the published international consensus [2,126]. Epicardial vasospasm was defined as a focal or diffuse epicardial coronary diameter reduction of >90% in response to ACh compared with the relaxed state after

intracoronary NTG infusion, with a reproduction of recognisable symptoms and ischaemic ECG changes. Microvascular spasm was diagnosed when recognisable symptoms were reproduced, with ischaemic ECG alterations in the absence of >90% epicardial diameter reduction in response to ACh. Ischaemic ECG changes were defined as transient ST-segment elevation or depression of >0.1 mV or ischaemic T-wave changes in at least two contiguous leads. Furthermore, CMD was confirmed if coronary physiology assessment showed a CFR of <2.0 and/or an IMR of ≥25, following current consensus documents [63]. Non-cardiac chest pain was diagnosed when there was no obstructive epicardial CAD (FFR > 0.80) and CFT was normal (CFR ≥ 2.0, IMR < 25 and negative ACh testing).

3.2.3. Statistical analysis

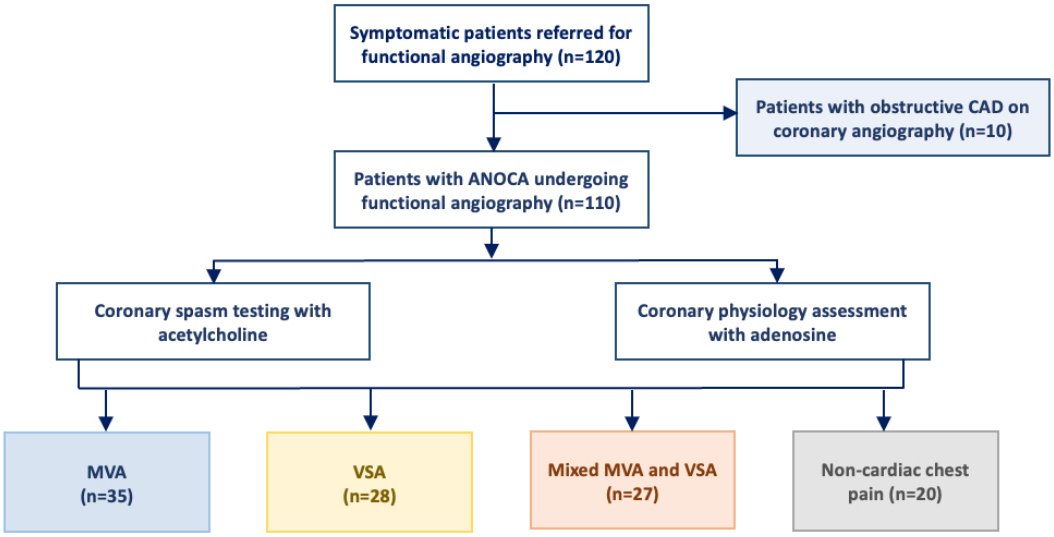
For continuous data, descriptive statistics were reported as either mean ± standard deviation (SD) or median with interquartile range (IQR) depending on the data distribution. Categorical data were presented as counts and percentages. Group differences were determined using appropriate statistical tests, including one-way analysis of variance (ANOVA), t-tests, chi-squared tests or Fisher's exact tests, as deemed appropriate for the specific comparisons. A significance threshold of $P < 0.05$ was used to determine statistical significance. Binary logistic regression analyses were performed using pre-specified variables of interest, with MVA and VSA as the outcomes. Receiver operating characteristic (ROC) curves were constructed to evaluate the predictive capacity of the variables for MVA and VSA. To assess discrimination of the regression model, Harrell's C-statistic corresponding to the area under the ROC curve was applied to estimate the goodness of fit and diagnostic accuracy. All statistical analyses were performed using R version 4.2.2 [127].

3.3. Results:

3.3.1. Clinical and procedural characteristics

This study was conducted at two tertiary referral institutions and included 120 patients from a geographically diverse New South Wales population [Table 3.1]. Ten patients who previously had non-obstructive coronary arteries on CCTA had obstructive CAD on ICA and were excluded [Figure 3.2]. The patient cohort had a mean age of 58 ± 13 years, with 63.6% of the participants being women. Clinical, laboratory and medication data are presented in Table 3.2. Patients with CVDys were more likely to be older (59 ± 11 vs 51 ± 15, P = 0.024), overweight (61.1% vs 15.0%, P ≤ 0.001) and receiving aspirin (72.2% vs 40.0%, P = 0.007) and beta-blocker therapy (33.3% vs 15.0%, P = 0.049). There was a statistically significant difference (P = 0.003) in the distribution of angina patterns between patients with CVDys and those without the condition. The CVDys group exhibited a significantly lower overall angina summary score (mean 53 vs 63 units; −10 [95% CI −15 to −2] P = 0.014), increased angina frequency (mean 63 vs 74 units; −11 [95% CI −21 to −1] P = 0.038), worse angina stability (mean 39 vs 53 units; −14 [95% CI −25 to −2] P = 0.026) and limitation (mean 63 vs 78 units; −15 [95% CI −26 to −5] P = 0.007) than those without CVDys. Additionally, the CVDys group reported a significantly lower quality of life (0.61 vs 0.67, P = 0.043), as assessed using EQ5D-5L. No significant differences in psychological comorbidities were observed between the groups, as evaluated using PHQ-4.

Figure 3.2: Study flowchart



Prevalence of coronary vasomotor disorder in patients with ANOCA

Abbreviations: ANOCA = angina with non-obstructive coronary arteries, CAD = coronary artery disease, MVA = micro-vascular angina, VSA = vasospastic angina

Table 3.1: Geographical distribution of patients undergoing coronary function testing

Health district	Number of patients
Sydney Local	24
Southwestern Sydney	24
Southeastern Sydney	17
Western Sydney	17
Northern Sydney	13
Western NSW	12
Nepean Blue Mountains	5
Hunter New England	3
Illawarra Shoalhaven	2
Centra Coast Local	1
Northern NSW	1

Table 3.2: Patient characteristics according to the presence or absence of coronary vasomotor dysfunction

	All patients (n = 110)	CVDys (n = 90)	No CVDys (n = 20)	P-value
Background				
Age, years	58 ± 13	59 ± 11	51.0 ± 15	0.024
Female	70 (63.6%)	55 (61.1%)	15 (75%)	0.528
BMI	27.2 (5.22)	27.5 (5.5)	25.5 (3.6)	0.044
Ethnicity				0.496
Caucasian	75 (68.1%)	63 (70.0%)	12 (60%)	
Asian	14 (12.6%)	10 (11.1%)	4 (20%)	
Southeast Asian	6 (5.4%)	6 (6.7%)	0 (0.00%)	
Middle Eastern	14 (12.7%)	10 (11.1%)	4 (20%)	
Others	1 (0.9%)	1 (1.11%)	0 (0.00%)	
Risk factors				
Hypertension	54 (49.1%)	45 (50.0%)	9 (45.0%)	0.728
Hypercholesterolaemia	64 (58.2%)	53 (58.9%)	11 (55.5%)	0.766
Diabetes	21 (19.1%)	19 (21.1%)	2 (10.0%)	0.362
Overweight	58 (52.7%)	55 (61.1%)	3 (15.0%)	<0.001
Family history of CAD	37 (33.6%)	28 (31.1%)	9 (45.0%)	0.441
Current smoking	45 (40.9%)	39 (43.3%)	6 (30.0%)	0.377
Peripheral vascular disease	10 (9.0%)	10 (11.1%)	0 (0.00%)	0.197
CAD	14 (12.7%)	12 (13.3%)	2 (10.0%)	0.730
Cerebrovascular accident	6 (5.5%)	4 (4.4%)	2 (10.0%)	0.596
Obstructive sleep apnoea	12 (10.9%)	11 (12.2%)	1 (5.0%)	0.481
Excessive alcohol	8 (7.3%)	6 (6.7%)	2 (10.0%)	1.00
Excessive caffeine intake	13 (11.8%)	11 (12.2%)	2 (10.0%)	1.00
Migraine	18 (16.4%)	14 (15.5%)	4 (20%)	0.627
Chronic pain syndrome	29 (26.4%)	23 (25.5%)	6 (30%)	0.683
Anxiety	23 (20.9%)	18 (20%)	5 (25%)	0.613
Depression	29 (26.4%)	23 (27.7%)	4 (20%)	0.543

Medications				
Aspirin	73 (66.4%)	65 (72.2%)	8 (40.0%)	0.007
P2Y12 Inhibitor	13 (11.8%)	13 (14.4%)	0 (0.00%)	0.131
Statin	69 (62.7%)	59 (65.6%)	10 (50.0%)	0.202
ACE-I/ARB	33 (29.7%)	26 (28.9%)	7 (35.0%)	0.973
Beta-blocker	33 (29.7%)	30 (33.3%)	3 (15.0%)	0.049
Calcium channel blockers	25 (22.7%)	20 (22.2%)	5 (25.0%)	0.755
Nicorandil	10 (9.0%)	9 (10.0%)	1 (5.0%)	0.681
Nitrates	22 (20.0%)	19 (21.1%)	3 (15.0%)	0.575
Laboratory				
Total cholesterol	3.91 (0.73)	3.94 (0.77)	3.75 (0.39)	0.303
LDL	1.86 (0.67)	1.90 (0.69)	1.68 (0.49)	0.275
HDL	1.34 (0.38)	1.38 (0.39)	1.18 (0.25)	0.083
Triglycerides	1.38 (0.56)	1.34 (0.51)	1.61 (0.80)	0.387
Creatinine	75.2 (15.9)	74.7 (16.3)	77.0 (15.7)	0.710
Hs-CRP ng/L	1.28 (1.01)	1.19 (0.96)	1.60 (1.16)	0.405
HsTnt, ng/L	7.80 (3.96)	7.98 (4.11)	6.75 (2.87)	0.315
CK-MB	3.47 (9.94)	3.89 (10.9)	1.46 (1.19)	0.214
Symptoms				
CCS angina class:				0.014
1	34 (30.9%)	22 (24.4%)	11 (55.0%)	
2	49 (44.5%)	41 (45.6%)	8 (40.0%)	
3	25 (22.7%)	24 (26.7%)	1 (5.0%)	
4	3 (2.7%)	3 (3.3%)	0 (0.00%)	
Angina pattern:				0.003
Predominately rest	28 (25.5%)	25 (27.8%)	3 (15.0%)	
Predominately exertion	40 (36.4%)	37 (41.1%)	3 (15.0%)	
Mixed	43 (39.1%)	28 (31.1%)	14 (70.0%)	
Circadian pattern:				0.030
Predominately daytime (7 am–7 pm)	31 (28.2%)	28 (31.1%)	3 (15.0%)	
Predominately nocturnal (7 pm–7 am)	21 (19.1%)	20 (22.2%)	1 (4.8%)	
Throughout the day	59 (53.6%)	42 (46.6%)	16 (80.0%)	

Functional cardiac assessment				
Normal	54 (49.1%)	12 (60.0%)	42 (46.7%)	0.280
Inconclusive	9 (8.2%)	2 (10.0%)	7 (7.8%)	0.743
Abnormal	38 (34.5%)	4 (20.0%)	34 (37.8%)	0.134
Seattle Angina Questionnaire				
Summary	55.4 (14.2)	53.4 (14.2)	62.6 (12.6)	0.014
Limitation	66.0 (20.8)	63.2 (20.0)	78.4 (19.8)	0.007
Stability	41.7 (19.9)	39.2 (18.5)	52.8 (22.5)	0.026
Frequency	65.3 (25.4)	63.3 (26.6)	74.1 (17.1)	0.038
Satisfaction	57.5 (25.4)	55.7 (24.7)	65.6 (27.5)	0.176
Angina QoL	41.5 (15.8)	41.4 (16.3)	42.1 (13.2)	0.843
Quality of life (EQ-5D-5L)				
Index score	0.63 (0.16)	0.61 (0.16)	0.67 (0.17)	0.043
VAS score	63.6 (13.8)	62.5 (13.9)	68.3 (12.4)	0.048
Psychological distress (PHQ-4)				
Total score	4.08 (3.27)	4.25 (3.32)	3.84 (3.69)	0.261
Anxiety score	2.02 (1.91)	2.15 (1.83)	1.93 (2.06)	0.319
Anxiety score ≥3 (suggesting anxiety)	41 (37.2%)	34 (37.7%)	7 (35%)	0.412
Depression score	1.99 (1.76)	2.10 (1.71)	1.91 (1.62)	0.291
Depression score ≥3 (suggesting depression)	39 (35.4%)	33 (36.6%)	6 (30%)	0.389

Abbreviations: ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BMI = body mass index, CAD = coronary artery disease, CCS = Canadian Cardiovascular Society, CK-MB = creatine kinase MB, CVDys = coronary vasomotor dysfunction HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, hsTnt = high-sensitivity troponin T, LDL = low-density lipoprotein, P2Y12 = purinergic receptor P2Y, G-protein coupled, 12 protein, QoL = quality of life, VAS = visual analogue score

*Data are shown as n (%) or mean ± SD (standard deviation)

Most ICAs were performed via radial arterial access (64.5%) without intra-arterial vasodilators. Three patients (4.2%) needed conversion to femoral access owing to radial spasm. Of the patients who initially underwent femoral access, 13% (5/39) had prior documented instances of radial spasm. Over half of the patients (54.5%) had angiographically normal coronary arteries. No fatal adverse events were reported. Nonetheless, one serious adverse event, a guiding catheter-induced coronary dissection that necessitated percutaneous coronary intervention, was observed (0.9%). Transient atrial fibrillation was noted in 12 patients (10.9%) during ACh provocation, of which two required direct current (DC) cardioversion and the remaining resolved spontaneously before discharge. Procedural characteristics are summarised in Table 3.3.

Table 3.3: Procedural characteristics

	All patients (n=110)
Final access site	
Radial	71 (64.5%)
Femoral	39 (35.5%)
Intra-procedural details	
Total procedure time (min)	51 (43–59)
Total contrast (mL)	135 (110–165)
Complications	
Atrial fibrillation	12 (10.9%)
Coronary dissection	1 (0.9%)

*Data are shown as n (%) or median and IQR (interquartile range)

3.3.2. Prevalence of coronary vasomotor disorders

In the cohort of 110 patients, CFT indicated CVDys in 81.8% (90/110) of the patients. Regarding specific ANOCA endotypes, MVA occurred in 31.8% (35/110), VSA in 25.5% (28/110) and a mixed presentation of MVA and VSA in 24.5% (27/110) of the patients [Table 3.4]. Functional assessment of the coronary microcirculation identified abnormalities in 57.3% (63/110) of the study cohort. In this group, 49.2% (31/63) had an elevated IMR, 31.7% (20/63) had a reduced CFR and the remaining 19.0% (12/63) had both abnormal IMR and CFR. ACh provocation testing revealed CAS in 57.3% (63/110) of the patients, with 50% (55/110) exhibiting epicardial vasospasm and 7.3% (8/110) displaying microvascular vasospasm. Of the patients with epicardial spasm, a diffuse pattern was evident in 59.2% (32/54), whereas 42.6% (23/54) exhibited focal spasm. When evaluating the dose–response association, most CAS occurred with a 100 µg dose in the LCA (53.8%) and a 50 µg dose in the RCA (50%) [Table 3.5]. Myocardial bridging was perceived in 12 patients in the study cohort. A formal assessment of the ischaemic potential of the tunnelled segment was performed in five patients with a dobutamine challenge, all of which were significant (dFFR 0.73 ± 0.03). The CFT results are listed in Table 3.6.

Table 3.4: Patient characteristics according to the ANOCA endotype

	MVA (n = 35)	**VSA (n = 28)**	**MVA/VSA (n = 27)**	**Non-cardiac (n = 20)**	**P-value**
Background					
Age	62 ± 9	55 ± 13	60 ± 12	51 ± 15	0.005
Female, n (%)	24 (68.6%)	18 (66.7%)	12 (44.4%)	15 (75.0%)	0.156
BMI	27.9 (3.8)	26.4 (4.4)	28.2 (7.9)	25.5 (3.6)	0.222
Ethnicity					0.425
Caucasian	26 (74.3%)	20 (74.1%)	16 (59.3%)	13 (65.0%)	
Asian	4 (11.4%)	1 (3.7%)	5 (18.5%)	4 (20.0%)	
Southeast Asian	2 (5.7%)	3 (11.1%)	1 (3.70%)	0 (0.00%)	

Middle Eastern	3 (8.6%)	2 (7.4%)	5 (18.5%)	4 (20.0%)	
Others	0 (0.00%)	1 (3.7%)	0 (0.00%)	0 (0.00%)	
Risk Factors					
Hypertension	22 (62.9%)	10 (37.0%)	13 (48.1%)	9 (45.0%)	0.207
Hypercholesterolaemia	20 (57.1%)	16 (59.3%)	17 (63.0%)	11 (55.0%)	0.903
Diabetes	6 (17.1%)	5 (18.5%)	8 (29.6%)	2 (10.0%)	0.389
Family history of CAD	13 (37.1%)	6 (22.2%)	9 (33.3%)	9 (45.0%)	0.463
Current smoking	3 (8.57%)	20 (74.1%)	16 (59.3%)	6 (30.0%)	<0.001
Peripheral vascular disease	9 (25.7%)	1 (3.7%)	0 (0.00%)	0 (0.00%)	0.001
CAD	3 (8.6%)	4 (14.8%)	5 (18.5%)	2 (10.0%)	0.626
Cerebrovascular accident	1 (2.9%)	2 (7.4%)	1 (3.7%)	2 (10.0%)	0.767
Obstructive sleep apnoea	5 (14.3%)	2 (7.4%)	3 (11.1%)	1 (5.0%)	0.696
Excessive alcohol	2 (5.71%)	2 (7.4%)	2 (7.4%)	2 (10.0%)	1.000
Excessive caffeine intake	4 (11.4%)	3 (11.1%)	4 (14.8%)	2 (10.0%)	0.953
Migraine	5 (14.3%)	6 (22.2%)	4 (14.8%)	4 (20.0%)	0.693
Chronic pain syndrome	7 (20.0%)	9 (32.1%)	7 (26.0%)	6 (30.0%)	0.783
Medications					
Aspirin	20 (57.1%)	21 (77.8%)	23 (85.2%)	8 (40.0%)	0.002
P2Y12 Inhibitor	5 (14.3%)	4 (14.8%)	3 (11.1%)	0 (0.00%)	0.363
Statin	22 (62.9%)	17 (63.0%)	20 (74.1%)	10 (50.0%)	0.316
ACE-I/ARB	16 (45.7%)	6 (22.2%)	11 (40.7%)	7 (35.0%)	0.266
Beta-blocker	10 (28.6%)	11 (40.7%)	9 (33.3%)	3 (15.0%)	0.248
Calcium channel blockers	7 (20.0%)	5 (18.5%)	8 (29.6%)	5 (25.0%)	0.795
Nicorandil	3 (8.57%)	3 (11.1%)	3 (11.1%)	1 (5.0%)	0.915
Nitrates	3 (8.57%)	7 (25.9%)	9 (33.3%)	3 (15.0%)	0.082
Laboratory					
Total cholesterol	3.99 (0.72)	3.75 (0.60)	3.99 (1.00)	3.75 (0.39)	0.707
LDL	2.06 (0.66)	1.54 (0.52)	1.97 (0.84)	1.68 (0.49)	0.127
HDL	1.39 (0.32)	1.39 (0.50)	1.32 (0.41)	1.18 (0.25)	0.581
Triglycerides	1.12 (0.45)	1.57 (0.55)	1.45 (0.44)	1.61 (0.80)	0.059
Creatinine	72.8 (16.9)	76.3 (11.8)	76.5 (19.3)	77.0 (15.7)	0.868
Hs-CRP ng/L	1.12 (1.16)	1.63 (0.91)	1.14 (0.67)	1.60 (1.16)	0.507

HsTnT, ng/L	7.37 (3.18)	8.80 (4.62)	8.29 (4.73)	6.75 (2.87)	0.598
CK-MB	5.42 (15.0)	1.56 (1.43)	2.49 (1.05)	1.46 (1.19)	0.761
Symptoms					
CCS angina class:					0.065
1	9 (25.7%)	5 (18.5%)	7 (25.9%)	12 (60.0%)	
2	15 (42.9%)	11 (40.7%)	15 (55.6%)	8 (40.0%)	
3	9 (25.7%)	10 (37.0%)	5 (18.5%)	1 (5.0%)	
4	2 (5.71%)	1 (3.70%)	0 (0.00%)	0 (0.00%)	
Angina pattern:					<0.001
Predominately rest	1 (2.86%)	16 (59.3%)	7 (25.9%)	3 (15.0%)	
Predominately exertion	26 (74.3%)	5 (18.5%)	6 (22.2%)	3 (15.0%)	
Mixed	8 (22.9%)	6 (22.2%)	14 (51.9%)	15 (75.0%)	
Circadian pattern:					<0.001
Predominately daytime (7 am–7 pm)	18 (51.4%)	6 (22.2%)	4 (14.8%)	3 (15.0%)	
Predominately nocturnal (7 pm–7 am)	3 (8.57%)	12 (44.4%)	5 (18.5%)	1 (5.0%)	
Throughout the day	14 (40.0%)	9 (33.3%)	18 (66.7%)	17 (85.0%)	
Functional cardiac assessment					
Normal	14 (40.0%)	16 (57.1%)	12 (44.4%)	12 (60.0%)	0.675
Inconclusive	4 (11.4%)	2 (7.1%)	1 (3.7%)	2 (10.0%)	0.285
Abnormal	13 (37.1%)	5 (17.9%)	11 (40.7%)	4 (20.0%)	0.103
Seattle Angina Questionnaire					
Summary	54.5 (15.0)	55.8 (13.5)	50.1 (12.9)	62.6 (12.6)	0.042
Limitation	65.1 (20.0)	64.4 (18.4)	59.4 (22.1)	78.4 (19.8)	0.028
Stability	41.2 (21.2)	36.9 (15.0)	37.8 (17.8)	52.8 (22.5)	0.052
Frequency	55.3 (23.9)	61.4 (23.5)	49.6 (26.0)	65.6 (27.5)	0.187
Satisfaction	65.2 (25.1)	60.5 (30.4)	61.4 (25.3)	74.1 (17.1)	0.330
Angina QoL	42.5 (17.4)	41.7 (14.2)	38.0 (15.7)	42.1 (13.2)	0.737
Quality of life (EQ-5D-5L)					
Index score	0.64 (0.19)	0.59 (0.21)	0.52 (0.16)	0.67 (0.17)	0.089
VAS score	64.8 (13.3)	61.3 (14.46)	60.7 (13.7)	68.3 (12.4)	0.071
Psychological distress (PHQ-4)					

Total score	3.91 (3.56)	4.35 (3.83)	4.13 (3.65)	3.84 (3.69)	0.236
Anxiety score	1.98 (1.76)	2.06 (1.98)	2.01 (1.87)	1.93 (2.06)	0.183
Depression score	1.93 (1.98)	2.29 (2.04)	2.12 (1.93)	1.91 (1.62)	0.197

Abbreviations: ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BMI = body mass index, CAD = coronary artery disease, CCS = Canadian Cardiovascular Society, CK-MB = creatine kinase MB, CVDys = coronary vasomotor dysfunction, HDL = high-density lipoprotein, hs-CRP = high-sensitivity C-reactive protein, hsTnt = high-sensitivity troponin T, LDL = low-density lipoprotein, MVA = micro-vascular angina QoL = quality of life, VSA = vasospastic angina

*Data are shown as n (%) or mean ± SD (standard deviation)

Table 3.5: Summary of acetylcholine dose response during coronary provocation testing

Left coronary artery (n = 52)		**Right coronary artery (n = 20)**	
Dosage (µg)	No. of CAS	Dosage (µg)	No. of CAS
20	5 (9.6%)	20	7 (35%)
50	15 (28.8%)	50	10 (50.0%)
100	28 (53.8%)	80	3 (15%)
200	4 (7.7%)		

Abbreviations: CAS = coronary artery spasm

*Data are shown as n (%)

Table 3.6: Coronary function testing results according to the ANOCA endotype

	MVA (n = 35)	**VSA (n = 28)**	**MVA/VSA (n = 27)**	**Non-cardiac (n = 20)**	**P-value**
Diagnostic angiography [6F JL and JR diagnostic]					

Angiographically normal vessels	19 (54.3%)	12 (44.4%)	14 (51.9%)	15 (75.0%)	0.141
Myocardial bridging*	2 (5.7%)	8 (29.6%)	2 (8.0%)	0 (0.00%)	0.094
Acetylcholine testing [6F EBU/XB ± JR guide or 6F Ikari guide]					
Epicardial spasm	0 (0.00%)	28 (100%)	27 (100%)	0 (0.00%)	<0.001
Diffuse	NA	18 (63.0%)	14 (51.9%)	NA	
Focal	NA	10 (37.0%)	13 (48.1%)	NA	
Micro-vascular spasm	8 (22.9%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0.010
#Coronary physiology assessment [6F EBU/XB ± JR guide or 6F Ikari guide]					
Mean resting transit time (s)	0.93 (0.63–1.29)	0.83 (0.61–1.20)	1.01 (0.73–1.26)	0.67 (0.48–0.77)	0.015
Resting Pd	89.0 (79.8–98.5)	90.5 (82.8–99.2)	87.5 (80.8–96.2)	90.0 (80.5–97.0)	0.971
Resting Pa	93.0 (84.5–104)	95.5 (90.0–108)	92.0 (85.8–100)	94.0 (85.5–101)	0.869
Mean hyperaemic transit time (s)	0.34 (0.24–0.46)	0.23 (0.15–0.28)	0.26 (0.22–0.36)	0.15 (0.11–0.17)	<0.001
RFR	0.92 (0.91–0.95)	0.92 (0.89–0.94)	0.92 (0.89–0.94)	0.93 (0.91–0.95)	0.129
FFR	0.91 (0.89–0.93)	0.90 (0.86–0.93)	0.91 (0.89–0.93)	0.90 (0.88–0.94)	0.549
IMR	26.0 (19.0–36.0)	14.0 (11.0–18.0)	25.0 (16.2–32.8)	12.0 (9.00–15.0)	<0.001
CFR	2.70 (2.02–3.80)	3.50 (2.70–5.80)	3.20 (2.42–4.38)	4.50 (3.10–8.00)	0.008

Abbreviations: ANOCA = angina with non-obstructive coronary arteries, CFR = coronary flow reserve, FFR = fractional flow reserve, IMR = index of micro-circulatory resistance, MVA = microvascular angina, , Pd = distal coronary artery pressure, Pa = aortic pressure, RFR = resting full-cycle ratio, VSA = vasospastic angina

#Coronary physiology assessment represents the left anterior descending artery

*Assessed via diagnostic angiography without specific intracoronary imaging

**Data are shown as n (%) or median and IQR (interquartile range)

3.3.3. Predictors of coronary vasomotor disorders

Being overweight (OR, 4.2 [95% CI 1.9–9.3]; P = 0.015) and ischaemia on stress testing (OR, 2.4 [95% CI 1.1–4.3]; P = 0.028) were the factors associated with MVA. Previously identified risk factors of

older age (OR, 1.22 [95% CI 1.01–1.48]; P = 0.055) and female sex (OR, 1.69 [95% CI 0.13–10.32]; P = 0.697) were positively linked to MVA, although the relationship was not statistically significant. Regarding VSA, smoking was positively correlated with the diagnosis (OR, 9.1 [95% CI 2.21–39.3]; P = 0.007). Exercise-induced angina was predominant in MVA (P = 0.023), whereas VSA was more commonly associated with rest angina (P = 0.001) [Table 3.7]. Angina burden, as evaluated using the SAQ, did not differ markedly between patients with MVA and those with VSA [Table 3.8]. In addition, a trend towards a higher angina burden was observed in patients with an elevated IMR (mean 52 vs 58; −6 [95% CI −13 to −3]; P = 0.056) or reduced CFR (mean 53 vs 60; −7 [95% CI −15 to −4]; P = 0.068), although not statistically significant [Tables 3.9 and 3.10].

Table 3.7: Differences in angina characteristics between patients with MVA and VSA

	MVA (n = 62)	**VSA (n = 54)**	**P-value**
Angina Pattern			
Predominately rest	8 (12.9%)	23 (42.6%)	0.001
Predominately exertion	32 (51.6%)	11 (20.4%)	0.023
Mixed	22 (35.5%)	20 (37.0%)	0.500

Abbreviations: MVA = micro-vascular angina, VSA = vasospastic angina, QoL = quality of life

Values are in n (%)

Table 3.8: Differences in angina and quality of life (as per SAQ) between patients with MVA and VSA

	MVA (n = 62)	**VSA (n = 54)**	**P-value**
Seattle Angina Questionnaire			
Summary	52.7 (19.4)	52.8 (17.3)	0.964

Limitation	62.8 (21.1)	61.7 (20.4)	0.800
Stability	39.8 (19.3)	37.4 (20.1)	0.500
Frequency	53.0 (24.3)	55.3 (22.7)	0.654
Satisfaction	63.7 (25.3)	61.0 (23.8)	0.614
Angina QoL	40.7 (15.7)	39.8 (16.2)	0.766

Abbreviations: MVA = microvascular angina, VSA = vasospastic angina, SAQ = Seattle Angina Questionnaire, QoL = quality of life

*Data are shown as n (%) or median and IQR (interquartile range)

Table 3.9: Differences in angina and quality of life (as per SAQ) between patients with normal and abnormal IMR

	IMR $\leq$ 25 (n = 67)	**IMR > 25 (n = 43)**	**P-value**
Seattle Angina Questionnaire			
Summary	57.9 (13.5)	52.2 (14.7)	0.056
Limitation	70.2 (19.3)	62.5 (21.5)	0.074
Stability	43.2 (20.1)	39.8 (19.8)	0.404
Frequency	60.2 (26.0)	54.1 (24.4)	0.239
Satisfaction	67.4 (24.7)	62.5 (26.2)	0.350
Angina QoL	42.1 (14.8)	40.8 (17.1)	0.685

Abbreviations: IMR = Index of micro-circulatory resistance, MVA = microvascular angina, VSA = vasospastic angina, SAQ = Seattle Angina Questionnaire, QoL = quality of life

*Data are shown as n (%) or median and IQR (interquartile range)

Table 3.10: Differences in angina and quality of life (as per SAQ) between patients with normal and abnormal CFR

	CFR $\geq$ 2.0 (n = 78)	CFR < 2.0 (n = 32)	P-value
Seattle Angina Questionnaire			
Summary	59.6 (14.2)	53.0 (14.4)	0.068
Limitation	69.3 (20.9)	60.2 (20.8)	0.089
Stability	43.2 (20.1)	37.9 (19.6)	0.235
Frequency	60.5 (24.7)	51.4 (26.3)	0.119
Satisfaction	67.0 (25.3)	60.2 (24.9)	0.228
Angina QoL	43.2 (17.0)	40.8 (15.4)	0.509

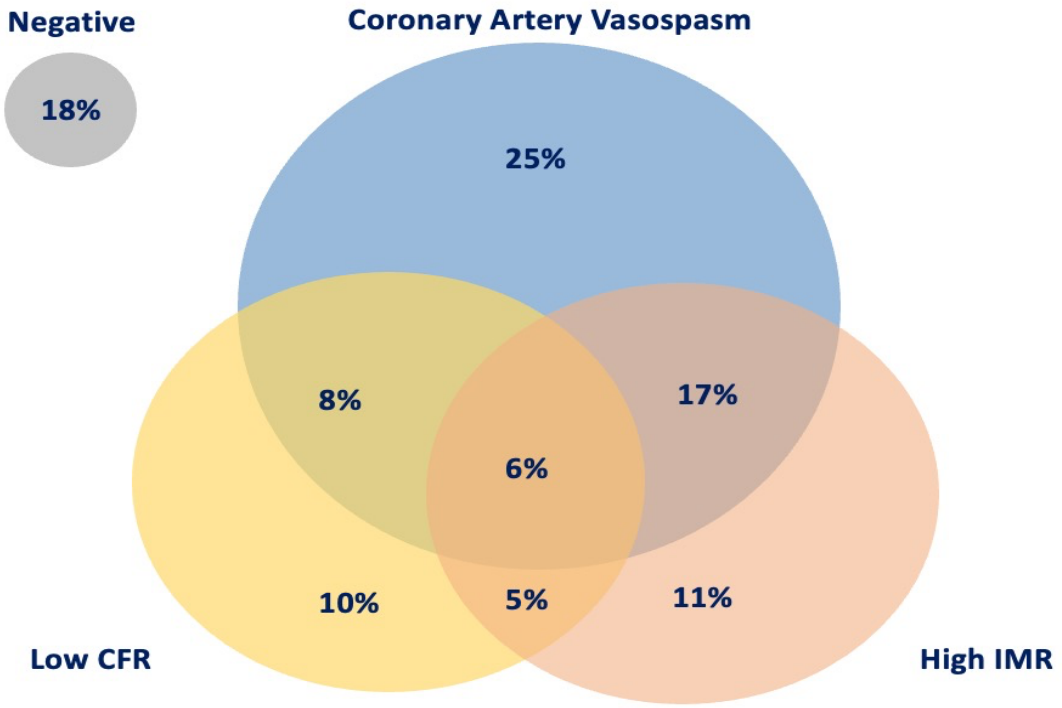
Abbreviations: CFR = Coronary flow reserve, MVA = microvascular angina, VSA = vasospastic angina, SAQ = Seattle Angina Questionnaire, QoL = quality of life

*Data are shown as n (%) or median and IQR (interquartile range)

3.4. Discussion

In this study of patients with ANOCA undergoing CFT, the following were the major findings: 1) Coronary vasomotor disorders were highly prevalent (>80%); 2) Functional coronary abnormalities such as enhanced coronary vasoreactivity, diminished coronary vasodilation and elevated micro-vascular resistance often overlapped each other [Figure 3.3]; 3) Risk factor profiles varied among ANOCA endotypes; 4) On objective evaluation, patients with CVDys had a significantly poorer quality of a life than those without such abnormalities. These results highlight the importance of CFT in elucidating the underlying aetiology of angina in this patient cohort. This approach enables clinicians to prescribe tailored therapy to improve the outcomes in patients with ANOCA.

Figure 3.3: Overlap of coronary vasomotor disorders in patients with ANOCA



Of the patients with ANOCA, 62 (56%) were diagnosed with coronary artery vasospasm. Additionally, 31 (27%) patients had a high IMR (IMR > 25), 20 (18%) patients had a low CFR (CFR < 2.0) and 12 (11%) patients had a combination of both. Notably, over half (56.5%) of the patients with coronary artery vasospasm demonstrated an overlap with microvascular functional abnormalities.

Abbreviations: ANOCA = angina and non-obstructive coronary arteries, CFR = coronary flow reserve, IMR = index of micro-circulatory resistance

3.4.1. Diagnostic yield of coronary function testing

The overall prevalence of CVDys was 81.8% in our study, consistent with previous cohorts that utilised CFT to diagnose CVDys [119-121]. In the current population, 57.3% of the patients exhibited an abnormal response on coronary physiology testing, which is higher than the 38%–44% reported in previous studies [80,120]. Considering specific endotypes, abnormal vasodilator capacity (CFR) was observed in 29.1% of the patients and elevated micro-vascular resistance (IMR) in 39.1%. The higher

overall prevalence of CMD observed in our study could be ascribed to increased multi-vessel testing based on clinical suspicion. This approach differs from previous studies, which focused on testing a single coronary vessel, often the LAD. These findings suggest that multi-vessel coronary physiology assessment may improve the identification of abnormalities in the coronary micro-vasculature.

In the current cohort, CAS was noted in over half of the patients during ACh provocation testing. These observations are aligned with a local study conducted by Di Fiore et al [105], which demonstrated inducible spasm (epicardial and microvascular) in 62% of the patients, and an investigation by Ford et al [121], which reported a prevalence of 54%. In contrast, studies performed in Japan have documented higher rates of CAS than our cohort [128]. Such patients have been described as having diffusely hyperactive coronary arteries, with a lower prevalence of significant CAD than Caucasian populations [38]. These variations may reflect the increased basal tone in this population, potentially attributable to lifestyle and genetic variations [37,38]. Furthermore, differences in the reported prevalence of CAS among studies may be due to the heterogeneity in current diagnostic protocols for invasive provocation testing, which differ in terms of administration time, dosage regimen and the specific vessels tested [129].

3.4.2. Clinical and angina characteristics of coronary vasomotion disorders

Our cohort's predominance of women (63.6%) is consistent with prior research emphasising the sex disparity within this patient population. The pivotal WISE study [130] established that symptomatic women with ANOCA had a worse prognosis, indicating that meticulous clinical evaluation and management are imperative in this patient population.

Our study further revealed that MVA was linked to being overweight and ischaemia on stress testing; in contrast, patients with VSA were more likely to be smokers. The female sex was positively associated with MVA, although it did not reach statistical significance, possibly attributed to the relatively small size of our patient cohort. Furthermore, variations were observed in angina characteristics, with MVA predominantly resulting in exercise-induced angina and VSA mainly causing resting angina. These findings are aligned with those from prior research in a similar patient population [39,91,131].

Serum biomarker levels, including troponin, creatine kinase-myocardial band and hs-CRP, did not differ significantly between ANOCA endotypes. These findings highlight the limitations of conventional biomarkers, which focus on atherosclerotic CAD and are weakly correlated with disorders of coronary vasomotion. This finding underscores the need for further research into novel biomarkers that can screen for specific ANOCA endotypes using non-invasive methods, offering valuable diagnostic and prognostic insights.

Patients with ANOCA with concomitant CVDys experienced a lower quality of life than those without the condition despite the lack of significant differences in psychological comorbidities. This is likely attributed to an increased angina burden, as indicated by the SAQ. Interestingly, the angina burden was not significantly different among the various ANOCA endotypes. This contrasts with the findings of Ford et al., who reported a more pronounced decline in the SAQ Summary Score (SAQSS) in patients with VSA than in those with MVA [121]. Our results might have been influenced by referral bias, geographic differences, and the relatively higher proportion of patients (24.5%) presenting with a mixed VSA and MVA endotype.

The diverse clinical characteristics observed in these patients underscore the presence of distinct phenotypes with varied pathophysiological mechanisms. The limited discriminative ability of MVA and VSA predictive models signifies that our understanding of these complex mechanistic pathways is incomplete. In addition, the lack of differences in the medical therapy among patients with ANOCA reinforces the need to classify this cohort into specific endotypes via CFT. Given the documented benefits of stratified medical therapy in this patient cohort, comprehensive evaluation and subsequent management are vital to optimise patient outcomes.

3.4.3. Limitations

First, this study included a select population referred specifically for CFT to ANOCA expertise centres by their treating physicians. This selective referral process might have led to the high rate of functional abnormalities observed in our study. Second, the number of epicardial coronary vessels tested was not uniform among the participants as the operators were allowed to decide on the number of vessels tested based on clinical suspicion. Third, although our ACh provocation protocol was based on clinical expertise and insights from prior clinical studies, significant heterogeneity exists in such protocols worldwide [129,132]. This diversity might have contributed to variations in the reported prevalence of CAS across institutions.

Lastly, owing to the recency of data, longitudinal assessment of symptom burden and quality of life was unavailable, limiting our capacity to draw definitive conclusions regarding the long-term clinical implications of CFT. Nevertheless, the CorMicA trial confirmed that CFT-guided stratified medical therapy improved the angina status and quality of life in a comparable patient cohort [80].

3.5. Conclusion

This study provides key insights into the clinical characteristics and prevalence of coronary vasomotor disorders among patients with ANOCA. The findings revealed that most of these patients exhibit coronary functional abnormalities, which compromise their quality of life. These results emphasise the importance of enhancing national awareness about this heterogeneous condition and establishing a standardised national protocol for CFT. Such initiatives will empower cardiologists to effectively diagnose and manage common causes of angina, ultimately leading to better patient care.

Chapter Four: Diagnostic Validity of Acetylcholine Provocation Protocols in the Evaluation of Vasospastic Angina in Patients with ANOCA

***Rehan R**, Khandur C, Wong CCY, Weaver J, Jain P, Adams M, Ng MKC, Tremmel JA, Yong ASC.* Diagnostic Validity of Acetylcholine Provocation Protocols in the Evaluation of Coronary Artery Spasm in Patients With ANOCA. *Circulation Cardiovasc Interventions*. 2025;18:e015339. doi: 10.1161/CIRCINTERVENTIONS.125.015339

4.1. Introduction

CAS, referring to the transient vasoconstriction of the coronary vasculature, is a common mechanism ANOCA. It's complex pathophysiology, driven by endothelial dysfunction and smooth muscle hypercontractility, leads to a spectrum of clinical manifestations ranging from recurrent angina to fatal arrhythmias. IC provocation testing with ACh remains the gold standard test for diagnosing CAS and carries a Class Ib recommendation for investigating patients with suspected ANOCA [62]. This diagnostic approach has gained popularity in recent years, allowing clinicians to tailor therapy, alleviating angina symptoms and improving the quality of life [80].

Nevertheless, significant heterogeneity in ACh provocation protocols worldwide has limited the widespread adoption and acceptance of this procedure among cardiologists [129]. Most researchers have used incremental ACh doses of up to 100 µg in the LCA and 50 µg in the RCA. This dose selection is based on a study performed in 1988 by Okumura et al., the only investigation of its kind, which reported high sensitivity (90%) and specificity (99%)[88]. However, some centres have recommended higher doses, including 200 µg in the LCA and 80 µg in the RCA [72,90,92,119,133]. While higher doses may improve the diagnostic yield, they exacerbate the risk of pseudo-positive reactions, potentially reducing the diagnostic specificity. The current study aimed to evaluate the diagnostic validity of current ACh provocation protocols, and also test the use of higher doses up to 200µg in the LCA and 80µg in the RCA.

4.2. Methods

This multicenter, prospective study assessed the diagnostic validity of ACh provocation testing. Over a two-year period between June 2022 to June 2024, consecutive patients who were referred to two tertiary referral centres for invasive coronary function testing for suspected ANOCA were included in the study. To include a negative control group, patients with no angina

referred for ICA due to non-coronary indications, such as cardiac arrhythmia and valvular heart disease, were also approached to be included in the study during this period.

Diagnostic ICA was performed to confirm the absence of obstructive CAD in all patients, defined as a visual stenosis of >50%. Patients (≥18 years) underwent ACh provocation testing in all three major epicardial arteries. However, this testing was avoided in cases where the coronary arteries were non-dominant. Patients were excluded if they had a recent (within 3 weeks before cardiac catheterisation) ACS, prior heart transplantation, coronary artery bypass grafting, serum creatinine >1.5 mg/dL and/or an inability to provide informed consent.

Clinical data on patient characteristics, cardiac risk factors and symptom profiles were acquired before the procedure. Information on conventional cardiovascular risk factors and non-conventional variables associated with vasomotor dysfunction was recorded. The study protocol adhered to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Human Research Ethics Review Board. Written informed consent was obtained from all participants. RR and ASCY had complete access to all study data and were responsible for data integrity and analysis.

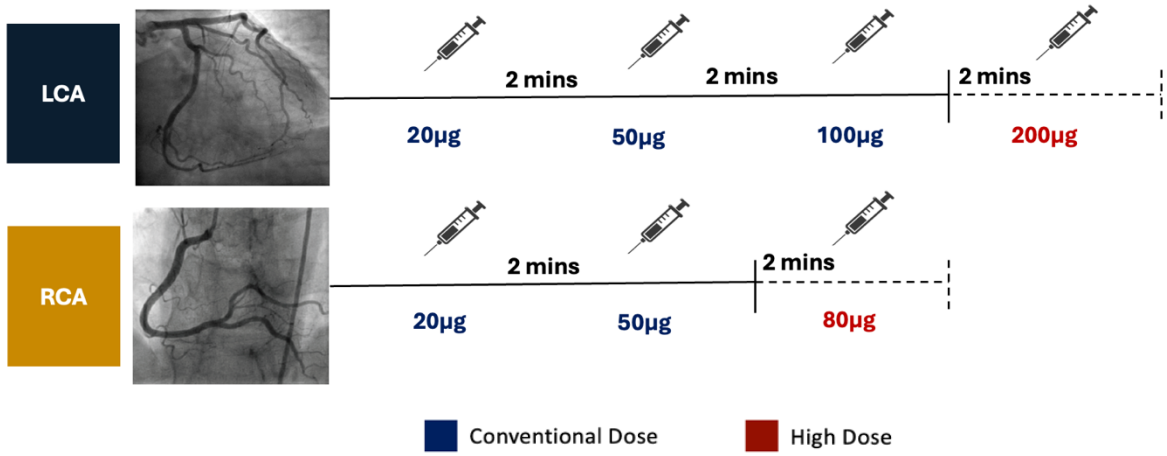
4.2.1. Acetylcholine provocation testing protocol

Patients were requested to withhold vasoactive medications (e.g. CCB and long-acting nitrates) and methylxanthine-containing substances for at least >4 times the duration of the drug half-life. Continuous 12-lead ECG monitoring with radiolucent leads was performed throughout the procedure. Diagnostic ICA was done according to standard institutional practice via the radial or femoral route. Administration of intra-arterial vasodilator drugs (e.g. NTG and CCB) was avoided

before ACh provocation testing in all patients. After confirming the absence of obstructive CAD, multi-vessel ACh provocation testing was conducted.

A TTP was inserted via the femoral vein to compensate for potential bradycardia. Activation of the TTP was contingent upon a pause of >5 s. A 6-French angioplasty guiding catheter without side holes was then positioned in either the LCA or the RCA, guided by clinical judgement. For the LCA assessment, incremental doses of 20, 50, 100 and 200 μg of ACh were injected over 20 s each time, with a 2-min gap between the doses [Figure 4.1]. After each injection, cine-images were obtained to assess the change in coronary diameter via QCA, as previously described[125]. If CAS was induced, with reproducible symptoms and ST-segment alterations (see definitions below), the provocation test was terminated and concluded to be positive. A similar protocol was followed for the RCA, with incremental doses of 20, 50 and 80 μg of ACh [Figure 4.1]. When coronary spasm was induced and did not resolve spontaneously within 3 min following the completion of ACh testing or in instances of haemodynamic instability or significant chest pain, NTG was administered. No further ACh provocation was performed subsequently. To evaluate basal coronary tone, intracoronary NTG was routinely administered using a bolus dose of 200 μg after completing ACh provocation.

Figure 4.1: Acetylcholine provocation protocol for the left and right coronary arteries. All doses were administered over 20 s, with a 2-min interval between consecutive doses



LCA = left coronary artery, RCA = right coronary artery

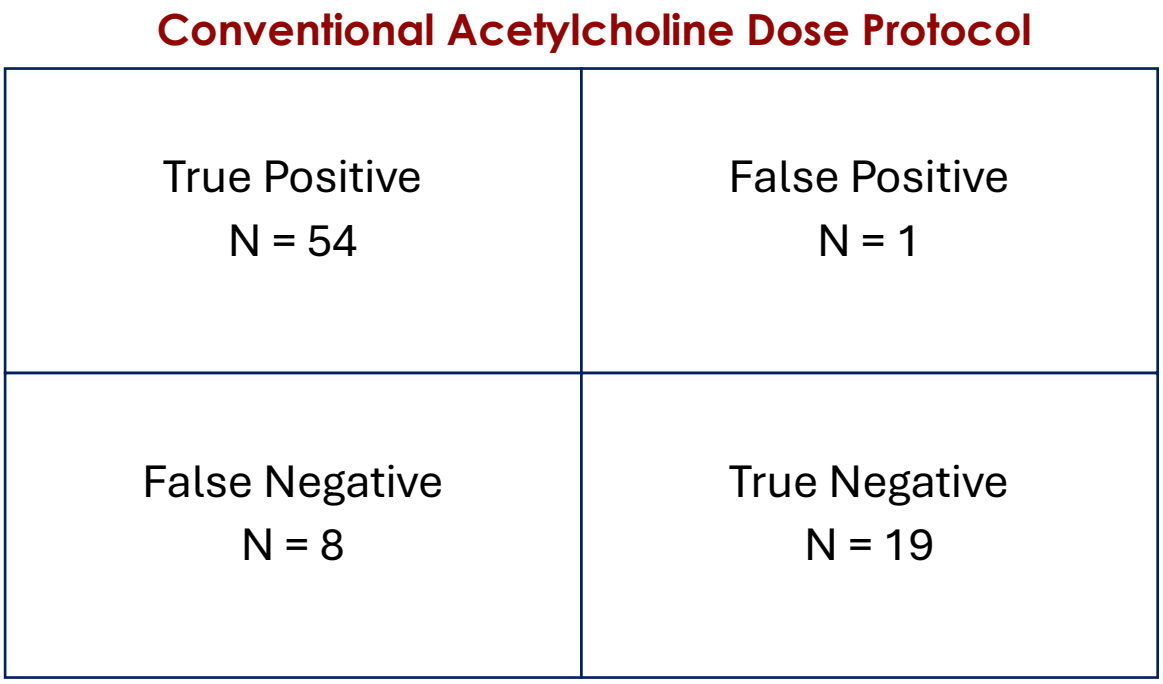
4.2.2. Definitions

A true positive was defined as a patient with typical symptoms of CAS who demonstrated a positive response to ACh provocation testing. Conversely, a false positive referred to an asymptomatic patient who demonstrated a positive response to ACh provocation testing. Epicardial CAS corresponded to a focal or diffuse epicardial coronary diameter reduction >90% in response to IC ACh compared with the relaxed state after intracoronary nitroglycerin, with reproduction of recognizable symptoms and ischemic ECG changes. Microvascular CAS was diagnosed when there was reproduction of recognizable symptoms with ischemic ECG changes in the absence of >90% epicardial diameter reduction in response to ACh. Ischemic ECG changes were defined as transient ST-segment elevation or depression of >0.1 mV, or ischemic T-wave changes, in at least two contiguous leads.

4.2.3. Statistical analysis

Continuous data are presented as mean ± standard deviation (SD), or median and interquartile interval, as appropriate. Categorical data are presented as counts and percentages. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated using standard contingency table methods [Figure 4.2]. Group differences were assessed using appropriate statistical tests, including one-way analysis of variance (ANOVA), t-tests, Chi-squared tests, or Fisher's exact tests, as deemed suitable for the specific comparisons. A significance threshold of $p < 0.05$ was used to determine statistical significance**.** All analyses were performed with R version 4.2.2. (Vienna, Austria) [127].

Figure 4.2: Comparison of contingency tables between conventional and high dose acetylcholine provocation protocols for diagnosing coronary artery spasm in patients with ANOCA.



High Acetylcholine Dose Protocol

True Positive N = 61	False Positive N = 4
False Negative N = 1	True Negative N = 16

4.3. Results

This study was conducted across two tertiary referral centres. Of 100 patients referred for CFT, 62 with typical symptoms of CAS were included. An additional 20 patients undergoing ICA for non-coronary indications served as controls. The overall cohort (n=82) had a 82 patients (62 with ANOCA and 20 controls). Clinical, laboratory, and medication data are detailed in Table 4.1. ANOCA patients were more likely to have undergone a previous coronary angiogram (46.8% vs. 20%, p = 0.02) and less likely to have angiographically normal arteries (47% vs. 80%, p = 0.009). Conversely, the negative control patients were significantly older (65.6 ± 9.9 years vs. 56.2 ± 10.2 years, p = 0.001) and less likely to be on anti-anginal therapy (20% vs. 64%, p = 0.001). The negative control group consisted of patients without angina who had a non-coronary indication for invasive angiography. Among these 20 patients, 10 (50%) had cardiomyopathy, 7 (35%) had valvular heart disease, and 3 (15%) had malignant arrhythmia.

Table 4.1: Patient characteristics

Characteristics	All patients (n=82)	ANOCA Group (n=62)	Negative Control Group (n=20)	p-value
Age, years	58.5 ± 10.9	56.2 ± 10.2	65.6 ± 9.9	0.001
Female	46 (56.1%)	38 (63.1%)	8 (40.0%)	0.159
Body mass index, kg/m^2	26.7 ± 3.6	26.5 ± 3.4	27.5 ± 4.2	0.323
Ethnicity				0.408
Caucasian	60 (73.2%)	43 (69.4%)	17 (85.0%)	
Asian	9 (11.0%)	8 (12.9%)	1 (5.00%)	
Southeast Asian	5 (6.10%)	4 (6.45%)	1 (5.00%)	
Middle Eastern	6 (7.32%)	6 (9.68%)	0 (0.00%)	
Other	2 (2.44%)	1 (1.61%)	1 (5.00%)	
Smoking Status, n (%)				0.382
Never	54 (65.9%)	39 (62.9%)	15 (75.0%)	
Former	24 (29.3%)	19 (30.6%)	5 (25.0%)	
Current	4 (4.88%)	4 (6.45%)	0 (0.00%)	
Hypertension	37 (45.1%)	28 (45.2%)	9 (45.0%)	1.000
Hypercholesterolemia	45 (54.9%)	36 (58.1%)	9 (45.0%)	0.446
Diabetes	14 (17.1%)	10 (16.1%)	4 (20.0%)	0.728
Overweight	44 (53.7%)	33 (53.2%)	11 (55.0%)	1.000
Family History of CAD	25 (30.5%)	21 (33.8%)	4 (20.0%)	0.472
Peripheral Vascular Disease	4 (4.88%)	2 (3.23%)	2 (10.0%)	0.249
CAD	11 (13.4%)	9 (14.5%)	2 (10.0%)	0.722
Previous Invasive Angiogram	33 (40.2%)	29 (46.8%)	4 (20.0%)	0.020
Preventative Therapy, n (%)				
Aspirin	44 (53.7%)	34 (54.8%)	10 (50.0%)	0.905
ACE-I/ARB	31 (37.8%)	17 (27.4%)	14 (70.0%)	0.002
Statin	41 (50.0%)	30 (48.4%)	11 (55.0%)	0.797
Angina Therapy, n (%)				
Beta-blocker	34 (41.5%)	27 (43.5%)	7 (35.0%)	0.679

Calcium Channel Blockers	25 (30.5%)	14 (22.6%)	11 (21.6%)	0.035
Nitrates	4 (4.88%)	4 (6.45%)	0 (0.00%)	0.569
Nicorandil	16 (19.5%)	16 (25.8%)	0 (0.00%)	0.016

ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease,

Values are mean ± or n (%)

4.3.1. Acetylcholine provocation testing

ACh provocation testing using a conventional dosage regimen of up to 100 µg in the LCA and 50 µg in the RCA revealed CAS in 67.1% (55/82) of patients. Epicardial spasm was observed in 63.4% (52/82), with 57.7% (30/52) exhibiting a diffuse pattern and 42.3% (22/52) a focal pattern. Microvascular spasm was observed in 3.7% (3/82). CAS was predominantly observed in the left anterior descending artery (LAD) in 60.0% (33/55, followed by the RCA in 25.5% (14/55) and the left circumflex artery (LCx) in 18.1% (10/55) of patients [Table 4.2].

Table 4.2: Summary of invasive acetylcholine provocation testing according to the coronary vessel

a) ANOCA group

	LAD (n=62)	**LCx (n=62)**	**RCA (n=60)**
Acetylcholine Testing			
Epicardial Spasm	34 (54.8%)	12 (19.4%)	13 (21.7%)
Diffuse Spasm	20 (32.3%)	8 (12.9%)	7 (11.6%)
Focal Spasm	14 (22.6%)	4 (6.5%)	6 (10.0%)
Microvascular Spasm	4 (6.5%)	0 (0%)	2 (3.3%)
Dose-related spasm			

20 µg	7 (11.3%)	2 (3.2%)	5 (8.3%)
50 µg	10 (16.1%)	4 (6.5%)	8 (13.3%)
*100 µg	14 (22.6%)	4 (6.5%)	2 (3.3%)
200 µg	6 (9.7%)	2 (2.0%)	NA

LAD = left anterior descending, LCx = left circumflex, RCA = right coronary artery

*(80 µg for RCA)

b) Negative control group

	LAD (n=20)	LCx (n=20)	RCA (n=20)
Acetylcholine Testing			
Epicardial Spasm	4 (20%)	1 (5.0%)	3 (15%)
Diffuse Spasm	4 (20%)	1 (5.0%)	3 (15%)
Focal Spasm	0 (0.0%)	0 (0.0%)	0 (0.0%)
Microvascular Spasm	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dose-related spasm			
20 µg	0 (0.0%)	0 (0.0%)	0 (0.0%)
50 µg	0 (0.0%)	0 (0.0%)	1 (5.0%)
*100 µg	1 (5.0%)	0 (0.0%)	2 (10%)
200 µg	3 (15%)	1 (5.0%)	NA

LAD = left anterior descending, LCx = left circumflex, RCA = right coronary artery

*(80 µg for RCA)

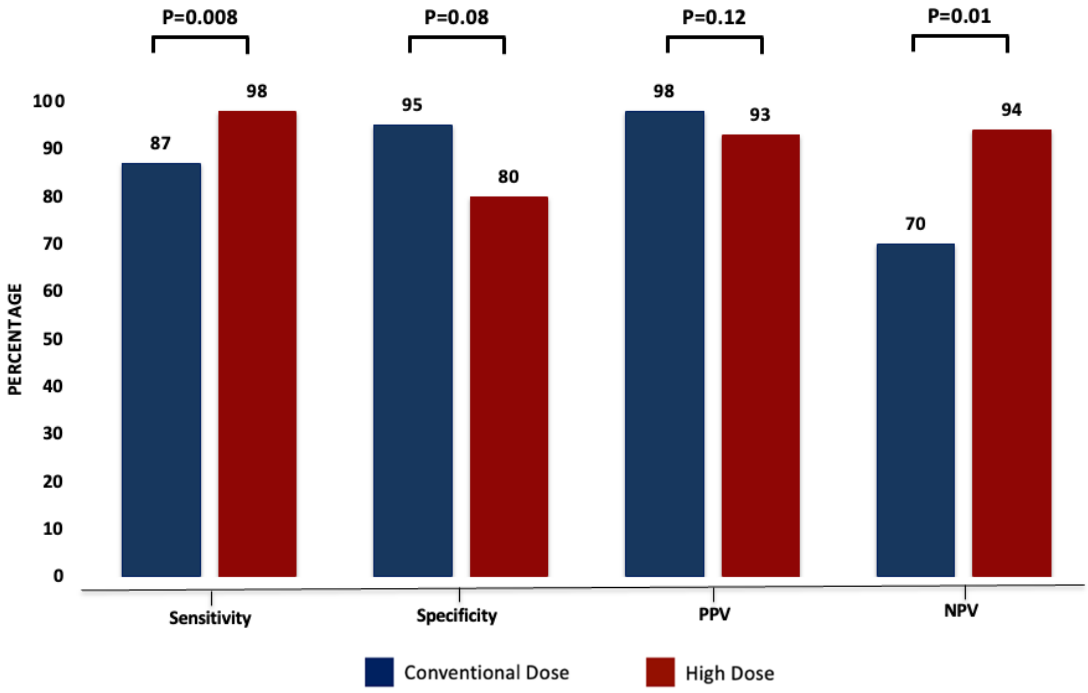
At a higher ACh dosage regimen of 200 µg in the LCA and 80 µg in the RCA, provocation testing revealed CAS in 79.3% (65/82) of patients. Epicardial spasm occurred in 74.4% (61/82), with 60.7% (37/61) showing a diffuse pattern and 39.3% (24/61) a focal pattern. Microvascular

spasm was identified in 4.9% (4/82). CAS was most frequently detected in LAD in 64.6% (42/65) of patients, followed by the RCA in 27.7% (18/65) and the LCx in 20.0% (13/65) of patients.

Among patients with inducible spasm on ACh provocation testing, 93.4% (61/65) had typical angina consistent with CAS. The conventional dosage regimen resulted in a diagnostic rate of 89.5% (54/61). The higher dosage regimen led to an increased detection of symptomatic CAS (n=61) but was associated with a higher incidence of false positives (n = 4) compared to the conventional doses (n = 1). Among the additional seven patients diagnosed with CAS at high ACh doses, 85.7% were men. Notably, one patient with typical symptoms of CAS did not demonstrate inducible spasm during intracoronary ACh provocation testing.

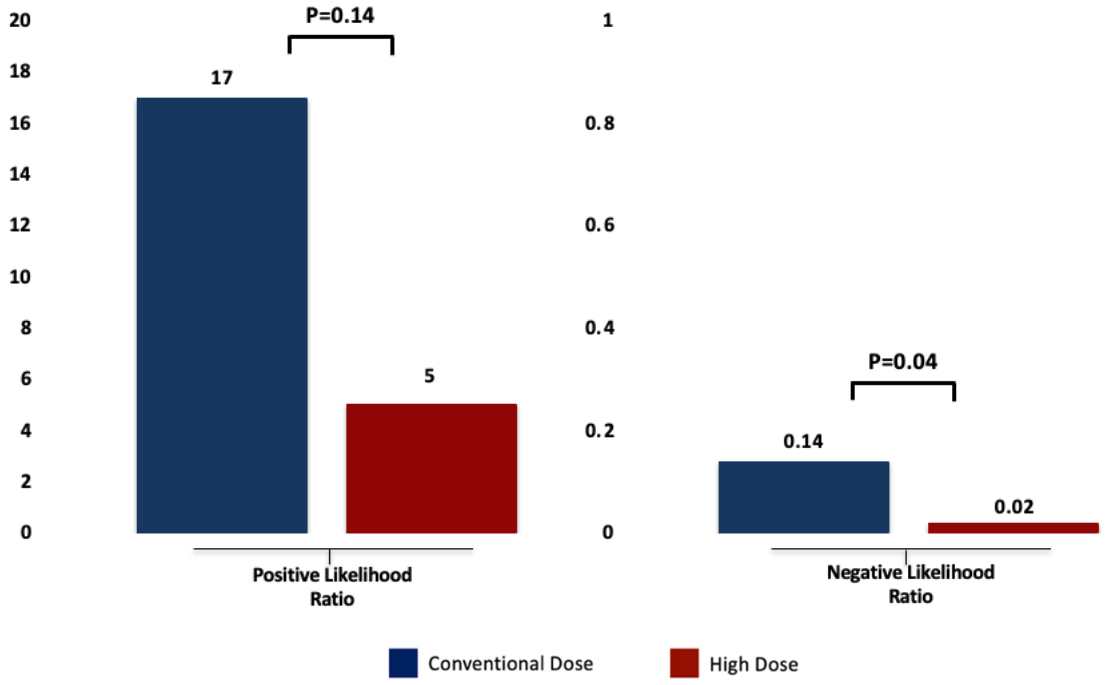
The high dose ACh regimen demonstrated significantly greater sensitivity compared to the conventional dose (98% vs. 87%, p = 0.008) [Figure 4.3]. However, this was accompanied by a non-significant trend toward reduced specificity (80% vs. 95%, p = 0.08). Additionally, the high dose regimen displayed a significantly higher NPV (94% vs. 70%, p = 0.01), while the PPV remained comparable between groups (93% vs. 98%, *p* = 0.12). The negative (NLR) and positive (PLR) likelihood ratios were calculated to account for disease prevalence, demonstrating consistent findings [Figure 4.4].

Figure 4.3: Comparison of diagnostic test performance between conventional and high dose acetylcholine provocation protocols for diagnosing coronary artery spasm in patients with ANOCA.



PPV = positive predictive value, NPV = negative predictive value

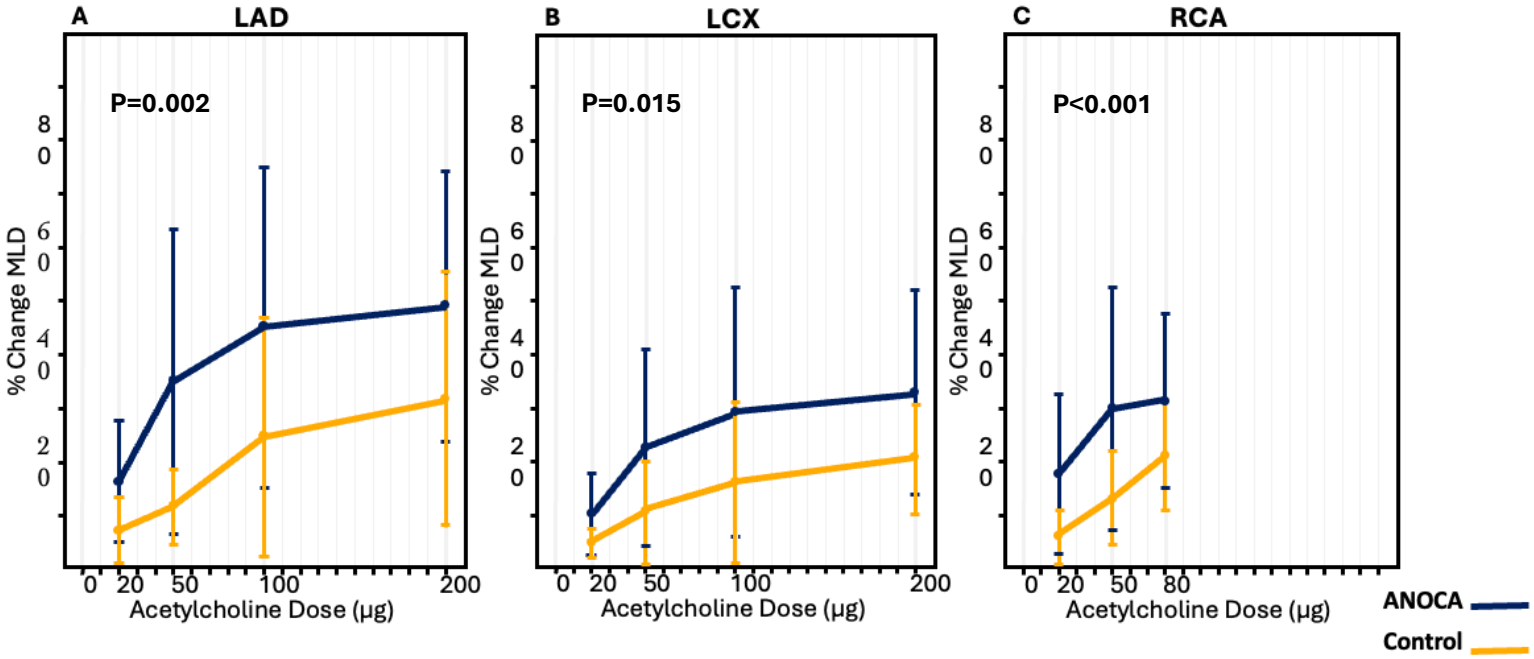
Figure 4.4: Comparison of likelihood ratios between conventional and high dose acetylcholine provocation protocols for diagnosing coronary artery spasm in patients with ANOCA.



4.3.2. Dose–response relationship between ACh and %MLD change

Figure 4.4 illustrates the dose-response relationship between ACh and the %MLD change across the three major epicardial vessels. ANOCA patients consistently demonstrated a more significant %MLD change in response to increasing ACh doses than the negative controls, a trend observed across the LAD, LCx, and RCA. Statistical analysis confirmed that the dose-response curves differed significantly between groups, with ANOCA patients showing a more pronounced vasoconstrictive response: LAD (P = 0.002,), LCx (P = 0.015), and RCA (P < 0.001). The divergence between ANOCA and control groups became more evident at higher doses, indicating a heightened susceptibility to ACh-mediated constriction in ANOCA patients [Table 4.5]. The variability in response, as indicated by the error bars, was notably higher in ANOCA patients. Conversely, the negative controls exhibited more consistent and narrower error bars, indicating more uniform responses across different ACh doses.

Figure 4.5: Dose–response relationship between intracoronary acetylcholine and minimal lumen diameter



ANOCA = Angina with non-obstructive coronary arteries, LAD = left anterior descending artery, LCx = left circumflex, RCA = right coronary artery

Table 4.3: Dose–response relationship between intracoronary acetylcholine and % minimal lumen diameter change

Coronary vessel	ANOCA group (n = 62)	Negative control group (n = 20)	P-value
LAD			
20 µg	16.0 ± 12.2	7.55 ± 7.28	<0.001
50 µg	35.5 ± 28.5	12.2 ± 7.61	<0.001
100 µg	44.9 ± 30.0	24.4 ± 19.4	0.001
200 µg	49.1 ± 26.9	33.7 ± 26.0	0.037
LCx			
20 µg	10.3 ± 7.65	4.75 ± 2.53	<0.001
50 µg	22.8 ± 19.6	10.9 ± 8.94	<0.001
100 µg	29.7 ± 21.3	17.9 ± 18.3	0.018
200 µg	31.7 ± 15.2	21.5 ± 9.79	0.002
RCA			
20 µg	18.1 ± 19.4	5.70 ± 5.79	<0.001
50 µg	30.0 ± 25.3	13.2 ± 7.95	<0.001
80 µg	31.2 ± 16.6	21.2 ± 9.60	0.001

LAD = left anterior descending, LCx = left circumflex

4.3.3. Procedural characteristics

Most ICAs were performed using radial arterial access (81.7%). All study participants underwent ACh provocation testing with a backup TTP in place. Over half of the patients

(54.8%) had angiographically normal coronary arteries. Right-dominant circulation was most common (71%), followed by left-dominant (21%) and co-dominant (8%). No fatal adverse events were reported. There was one serious adverse event (0.8%), specifically a guiding catheter-induced coronary dissection that required percutaneous coronary intervention. During ACh provocation testing, backup pacing was required in 14 patients (17.1%) during LCA assessment and 35 patients (58.3%) during RCA evaluation. Transient atrial fibrillation was observed in 9 patients (10.9%) during ACh provocation; one required direct current cardioversion, while the remaining cases resolved spontaneously before discharge. Procedural characteristics are summarised in Table 4.4.

Table 4.4: Procedural characteristics

Characteristics	All patients (n=82)	ANOCA Group (n=62)	Negative Control Group (n=20)
Final Access Site			
Radial	67 (81.7%)	49 (79.0%)	18 (90.0%)
Femoral	15 (18.3%)	13 (21.0%)	2 (10.0%)
Angiographic features			
Angiographically normal	45 (54.8%)	29 (46.8%)	16 (80.0%)
Complication			
Atrial Fibrillation	9 (11.0%)	7 (11.3%)	2 (10.0%)
Coronary Dissection	1 (0.8%)	1 (0.8%)	0 (0.00%)
Activation of TPW			
LCA	14 (17.1%)	11 (17.7%)	3 (15.0%)
RCA	35 (58.3%)	22 (35.5%)	13 (65%)

LCA = left coronary artery, RCA = right coronary artery, TTP = temporary transvenous pacemaker

Values are median and IQR or n (%)

4.4. Discussion

This study is the first to assess the diagnostic validity of contemporary ACh provocation testing protocols. Although prior studies have assessed the accuracy of doses up to 100μg in the LCA and 50μg in the RCA, they have yet to examine the diagnostic precision at higher doses. Our findings demonstrate that the high dose ACh regimen significantly improves sensitivity, highlighting its use in diagnosing CAS. However, the trend toward reduced specificity suggests a higher likelihood of false-positive results, necessitating cautious interpretation. The lack of difference in PPV (93% vs. 98%, p = 0.12) suggests both regimens perform similarly in confirming true positives.

Patients with ANOCA often face a considerable symptom burden, leading to impaired quality of life and recurrent hospitalisation [1,2,4]. Coronary vasomotor dysfunction is a common finding in these patients, with over half exhibiting evidence of VSA [134,135]. ACh provocation testing is a safe and effective approach for evaluating patients with ANOCA; however, testing protocols for administering this provocative agent vary widely across institutions [129].

In the study that validated ACh provocation testing, Okamura et al. administered serial boluses of 20, 50 and 100 μg in the LCA and 20 and 50 μg in the RCA over 20-s intervals [88]. This landmark research included patients who met the clinical criteria for variant angina with spontaneous vasospastic episodes. A control cohort comprising patients with cardiomyopathy, arrhythmia, valvular disease, hypertension, congenital heart disease and atypical chest pain was also included. The protocol demonstrated high sensitivity (90%) and specificity (99%) for diagnosing epicardial CAS, validating its clinical use. Notably, a proportion of patients in this study had moderate fixed

epicardial stenosis, indicating that the cohort did not exclusively represent patients with nonobstructive coronary arteries. In current clinical practice, ACh provocation testing is primarily performed in patients with nonobstructive coronary arteries, a criterion all participants in this study met. A key strength of our study is the inclusion of a subset patients undergoing ICA for non-coronary indications who had no history of angina. This group served as a negative control, enabling a rigorous evaluation of the diagnostic accuracy of ACh provocation testing in a contemporary cohort.

Sueda et al. explored the clinical utility of high-dose ACh provocation testing, reporting a heightened diagnostic yield when using a maximal dose of 200 μg in the LCA, with detection rates increasing from 19.3% to 40.9% [76]. Their cohort included patients with diverse anginal presentations, over 10% of whom had evidence of organic stenosis, likely contributing to the higher prevalence of observed epicardial CAS [136]. Among the ANOCA population in our study, the diagnostic yield for CAS increased by 8.5% with the high dose ACh dosage regimen. However, this was associated with increased false positives (n=4). Conversely, the false negative rate was higher with the conventional ACh regimen (9.8% vs 1.2%, p= 0.02). Sex-specific analysis revealed that false negatives were more prevalent in male patients (75%), while the false positive rate remained consistent between sexes at the higher ACh dose. These findings align with prior research indicating a dose-dependent ACh response in males at doses up to 200 μg, while females had minimal change beyond 50 μg [83].

Furthermore, our study confirmed that incremental doses of ACh elicited a significant %MLD change across all three epicardial vessels. The ANOCA group consistently exhibited a higher %MLD constriction than the control group at all ACh doses. This observation suggested more pronounced physiological responses indicative of heightened vascular reactivity. The %MLD constriction

decreased at higher doses, potentially because of a ceiling effect or receptor desensitisation. Considerable response variations were noted within the ANOCA group, indicating a heterogeneous reaction to ACh [Figure 4.5]. In contrast, the negative control group displayed more stable responses, likely due to the absence of endothelial dysfunction. These findings underscore the reliability of the ACh provocation testing in differentiating between normal and pathological states.

Higher doses of ACh may improve the diagnostic yield by uncovering epicardial CAS in patients with ANOCA who exhibit low disease activity. However, these doses may induce vasoconstriction in patients without prior symptoms, resulting in false positives. This phenomenon could be attributed to the fact that the higher concentration of ACh overwhelms the endothelial barrier, exerting a direct spasmogenic effect on the vascular smooth muscle [137]. The increased likelihood of false positives with high ACh doses highlights the importance of careful patient selection and consideration of appropriate dosing protocols. In populations with a lower pre-test probability of VSA, the risk of false positives could outweigh the benefits of increased sensitivity, potentially leading to overtreatment. Clinicians must balance the need for sensitivity in detecting epicardial CAS with the risk of reduced specificity owing to non-specific vasoconstriction. Optimising the dosing protocols and refining the patient selection criteria are pivotal for enhancing the accuracy and reliability of diagnosing VSA in the ANOCA population.

4.4.1. Limitations

First, although this study is the largest to examine the diagnostic accuracy of contemporary ACh protocols, the relatively small sample size, particularly in the control group, may limit the robustness of our conclusions, underscoring the need for validation in larger cohorts. Recruiting control patients was challenging as they had to undergo additional procedures of no clinical relevance to them. Second, our study did not include specific assessments for endothelial dysfunction using low-dose,

slow ACh infusion as the focus was on diagnostic testing for coronary spasm in the context of patients with ANOCA. Lastly, the absence of ACh rechallenge in our protocol might have limited the detection of coexisting epicardial and microvascular spasm, potentially underestimating the prevalence of this condition.

4.5. Conclusions

In patients with ANOCA, high dose ACh regimen increases the detection of CAS but may also increase risk of overdiagnosis. This approach should be reserved for patients with a high clinical suspicion, and results should be interpreted within the broader clinical context. Future research should focus on standardising ACh provocation protocols to optimise the balance between sensitivity and specificity for diagnosing CAS within the ANOCA population.

Chapter 5: Impact of Nitroglycerin Administration on Acetylcholine Provocation Testing in ANOCA

Rehan R, *Wong CCY, Weaver J, Jain P, Adams M, Ng MKC, Tremmel JA, Yong ASC*. Impact of Nitroglycerin Administration on Acetylcholine Provocation Testing in Angina With Nonobstructive Coronary Arteries. *Journal of the Society for Cardiovascular Angiography & Interventions*. 2025;4. doi: 10.1016/j.jscai.2025.103668

5.1. Introduction

Nearly half of all patients undergoing coronary angiography for stable angina have non-obstructive coronary arteries [1]. Invasive CFT, using ACh to diagnose CAS and a pressure–temperature sensing guidewire to evaluate CMD, is considered the gold standard for evaluating patients with ANOCA [3,73]. These investigations carry a Class Ib recommendation in the ESC guidelines, supporting a tailored therapeutic approach proven to enhance angina control and improve quality of life [62,80].

The optimal sequence for CFT remains debatable, with several centres opting to perform ACh provocation testing as the initial step [95,138-140]. In this approach, NTG is administered after CAS testing, followed by inserting an intracoronary pressure wire and administering adenosine for CMD assessment. Conversely, other clinicians advocate that CMD testing be performed first as they believe that ACh-induced spasm may alter baseline micro-vascular resistance and overall coronary flow, potentially compromising the accuracy of the results [141,142]. This alternative approach requires NTG administration before ACh provocation, which can potentially lead to a false negative test for CAS [72,143]. Furthermore, the widespread adoption of radial access in most catheterisation laboratories, where intra-radial NTG and/or CCB are routinely administered to prevent radial artery spasm, may obscure accurate assessment of CAS. Although NTG is considered short-acting, its administration following radial access before ACh testing may further contribute to the under-diagnosis of CAS.

Given these divergent perspectives, the exact sequence of steps during CFT varies across centres, and a consensus on the optimal protocol is lacking. Therefore, this study aimed to evaluate the diagnostic impact of prior intra-vascular NTG on ACh provocation testing.

5.2. Methods

This multi-centre, prospective study assessed the effect of intra-vascular NTG on ACh provocation testing in patients with ANOCA from August 2022 to June 2024. All patients were referred by their treating cardiologist for suspected ANOCA. Diagnostic ICA was performed in all patients to rule out obstructive CAD, defined as a diameter stenosis of <50% via visual estimation. The patients underwent ACh provocation testing in the LCA and RCA. For safety reasons, ACh provocation testing was avoided if coronary arteries were non-dominant or severely tortuous. The patients were included in the study if they had evidence of epicardial CAS confirmed via ACh provocation testing. Exclusion criteria included recent (within 3 weeks before cardiac catheterisation) ACS, prior heart transplantation, coronary artery bypass grafting, serum creatinine >1.5 mg/dL and/or inability to provide informed consent.

Clinical data on patient characteristics, cardiac risk factors and symptom profiles were collected before the procedure. Both conventional cardiovascular risk factors and non-conventional variables linked to vasomotor disorders were documented. The study protocol adhered to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Human Research Ethics Review Board. Written informed consent was obtained from all participants. RR and ASCY had full access to all study data and were responsible for ensuring data integrity and performing the analysis.

5.2.1. Acetylcholine provocation testing protocol

All ACh provocation testing procedures were conducted in the morning to ensure standardised patient timing. The patients were requested to withhold vasoactive medications (e.g. CCB and long-acting nitrates) and substances containing methylxanthine for >4 times the duration of the drug's

half-life. Continuous 12-lead ECG monitoring was performed throughout the procedure. Diagnostic ICA was done as per standard institutional practice via the radial or femoral artery. The administration of intravascular vasodilator drugs (e.g. NTG and CCB) was avoided before ACh provocation testing in all patients. After confirming the absence of obstructive CAD, multi-vessel ACh provocation testing was conducted.

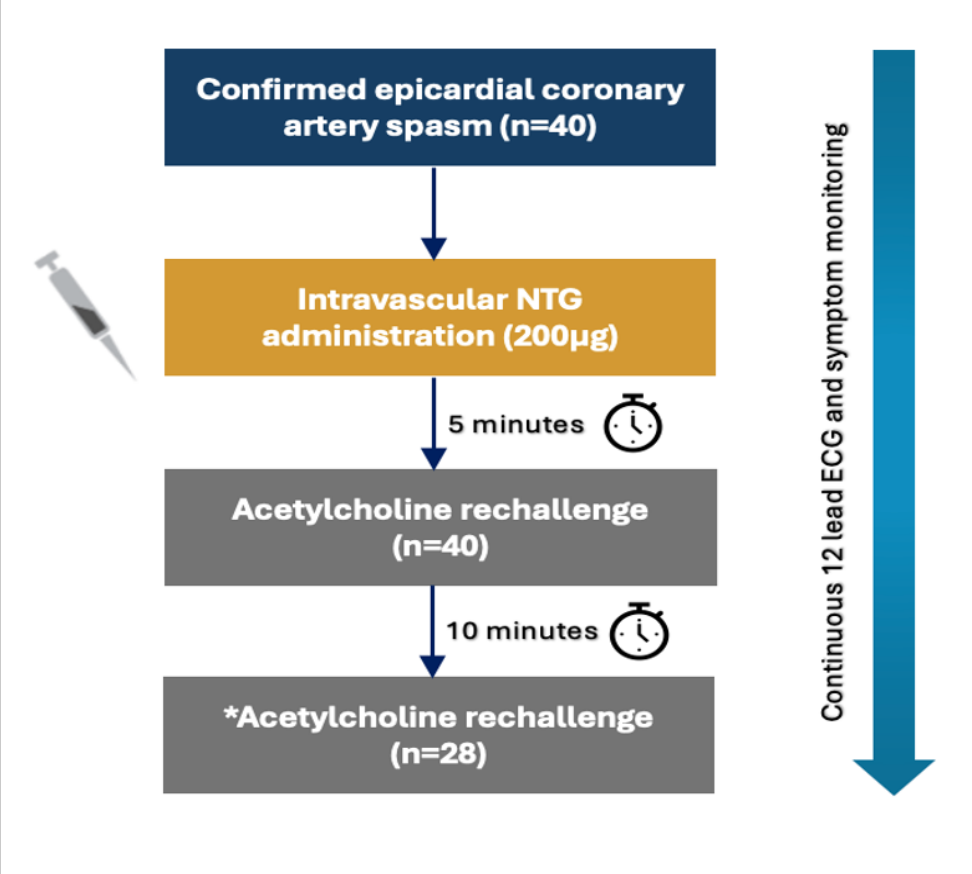
To compensate for potential bradycardia, a TTP was inserted via the femoral vein and was activated only if a pause of >5 s occurred. A 6-French angioplasty guiding catheter without side holes was then positioned in either the LCA or the RCA, guided by clinical judgement. For the LCA assessment, incremental doses of 20, 50, 100 and 200 μg of ACh were injected over 20 s, with a 2-min gap between doses. After each injection, cine-images were acquired to assess changes in coronary diameter via QCA, as previously described [125]. If CAS was induced, with reproducible symptoms and ST-segment changes (see definitions below), the provocation test was terminated and concluded to be positive. A similar protocol was followed for the RCA, with incremental doses of 20, 50 and 80 μg of ACh. Intracoronary NTG was administered to reverse the effects of ACh after the completion of testing.

5.2.2. Acetylcholine rechallenge protocol

To assess the re-inducibility of epicardial spasm, ACh rechallenge was performed in patients who tested positive by re-administering the spasm provocation dose into the affected coronary artery. Following the administration of intra-radial NTG (200 μg), ACh rechallenge was done at 5 min, with a second challenge at 10 min if no epicardial spasm was detected during the initial assessment [Figure 5.1]. The monitoring methods for patient symptoms, ischaemic ECG changes and coronary artery diameter reduction were similar to those of the initial assessment. Patients with an indeterminate

result during the ACh rechallenge, defined as symptom reproduction without either >90% vasoconstriction or ECG changes, were categorised as having no spasm.

Figure 5.1: Study flowchart. Protocol for acetylcholine (ACh) rechallenge in patients with confirmed epicardial coronary artery spasm (CAS)



ECG = electrocardiogram, NTG = nitroglycerine

*Only patients (n = 28) who did not demonstrate epicardial CAS at the 5-min ACh rechallenge proceeded to the 10-min ACh rechallenge.

5.2.3. Definitions

CAS was defined as per previously published international consensus [110]. Epicardial spasm was defined as a focal or diffuse epicardial coronary artery diameter reduction of >90% in response to ACh compared with the relaxed state, with a reproduction of recognisable symptoms and/or

ischaemic ECG changes. Microvascular spasm was diagnosed when recognisable symptoms were reproduced with ischaemic ECG changes in the absence of >90% diameter reduction in response to ACh. Ischaemic ECG alterations were defined as transient ST-segment elevation or depression of >0.1 mV or ischaemic T-wave changes in at least two contiguous leads.

5.2.4. Statistical analysis

Continuous data were presented as mean ± SD or median and interquartile interval, as appropriate. Categorical data were expressed as counts and percentages. The chi-square and Fisher's exact tests were used to compare categorical variables. The student's t-test and the Mann–Whitney U test were used to compare continuous variables, as appropriate. A significance threshold of $P < 0.05$ was used to determine statistical significance. All analyses were performed using R version 4.2.2. (Vienna, Austria) [127].

5.3. Results

This study was conducted across two tertiary referral centres and included 102 patients, 40 of whom were diagnosed with epicardial CAS and underwent ACh rechallenge. The patient cohort had a mean age of 59.3 ± 10.0 years, with 55% of the participants being women. The patients predominantly reported rest angina (55%), followed by mixed rest and exertional angina (32.5%), with the remaining presenting with exertional angina (12.5%). The patient cohort was diverse in terms of ethnicity, with most being Caucasian (67.5%). Clinical, laboratory and medication data are listed in Table 5.1.

Table 5.1: Patient characteristics

Characteristics	All patients (n = 40)
Age, years	59.3 ± 10.0
Female	22 (55%)
Body mass index, kg/m^2	26.2 ± 3.4
Ethnicity	
Caucasian	27 (67.5%)
Asian	4 (10.0%)
Southeast Asian	4 (10.0%)
Middle Eastern	5 (12.5%)
Smoking status, n (%)	
Never	27 (67.5%)
Former	9 (22.5%)
Current	4 (10.0%)
Hypertension	18 (45.0%)
Hypercholesterolaemia	25 (62.5%)
Diabetes	9 (22.5%)
Overweight	19 (47.5%)
Family history of CAD	12 (30.0%)
Peripheral vascular disease	1 (2.5%)
CAD	3 (7.5%)
Previous invasive coronary angiogram	18 (45.0%)
Cerebrovascular accident	1 (2.5%)
Obstructive sleep apnoea	3 (7.5%)
Migraine	10 (25.0%)
Chronic pain syndrome	8 (20.0%)
Preventative therapy, n (%)	
Aspirin	28 (70.0%)
ACE-I/ARB	16 (40.0%)
Statin	23 (57.5%)

Angina therapy, n (%)	
Beta-blocker	18 (45.0%)
Calcium channel blockers (withheld before testing)	10 (25.0%)
Nitrates (withheld before testing)	10 (25.0%)
Nicorandil (withheld before testing)	4 (10.0%)
Laboratory	
Total cholesterol	3.70 ± 0.62
LDL	1.85 ± 0.71
HDL	1.11 ± 0.41
Triglycerides	1.55 ± 0.65
Hba1c	5.55 ± 0.71
CCS angina class:	
1	10 (25.0%)
2	16 (40.0%)
3	11 (27.5%)
4	2 (5.0%)
Angina pattern:	
Predominately rest	22 (55.0%)
Predominately exertion	5 (12.5%)
Mixed	13 (32.5%)
Circadian pattern:	
Predominately daytime (7 am–7 pm)	12 (30.0%)
Predominately nocturnal (7 pm–7 am)	14 (35.0%)
Throughout the day	14 (35.0%)
Stress echocardiography	
Normal	22 (59.5%)
Inconclusive	1 (2.70%)
Abnormal	14 (37.8%)
Radionuclide myocardial perfusion	
Negative or inconclusive	2 (66.7%)

Abnormal	1 (33.3%)

ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease, CCS = Canadian cardiovascular society

Values are mean ± or n (%)

5.3.1. Procedural characteristics

All ICAs were attempted via radial access; however, 15% of the patients required conversion to femoral access owing to radial artery spasm and/or tortuosity. In all cases, NTG was administered via the initial radial access before the ACh rechallenge. All participants underwent ACh provocation testing, with a back-up TTP in place. More than half of the patients (55%) demonstrated angiographically normal coronary arteries. The most prevalent coronary circulation pattern was right-dominant (75%), followed by left-dominant (15%) and co-dominant (10%). There were no serious adverse events. During ACh provocation testing, back-up pacing with TTP was required in six (15%) and 21 patients (52.5%) during the LCA and RCA assessment, respectively. Transient atrial fibrillation occurred in four patients (10%), and all cases resolved spontaneously on the day of the procedure. The procedural characteristics are detailed in Table 5.2.

Table 5.2: Procedural characteristics

Characteristics	**All patients (n = 40)**
Final access site	
Radial	34 (85%)
Femoral	6 (15%)

Coronary dominance	
Right-dominant	30 (75%)
Left-dominant	6 (15%)
Co-dominant	4 (10%)
Complication	
Atrial fibrillation	4 (10%)
Activation of TTP	
LCA	6 (15%)
RCA	21 (52.5%)

LCA = left coronary artery, RCA = right coronary artery, TTP = temporary transvenous pacemaker

5.3.2. Acetylcholine provocation testing

In the study cohort, a diffuse spasm pattern was identified in 25 patients (62.5%), whereas the remaining 15 patients (37.5%) exhibited a focal spasm [Table 5.3]. Epicardial CAS was observed in the LAD in 90% (36/40) of the patients, followed by the LCx in 27.5% (11/40) and the RCA in 25% (10/40) of the patients. Multi-vessel spasm was present in 15 (37.5%) patients. When evaluating the dose–response relationship in the LCA (n = 37), CAS occurred in in 10.8% of the patients at 20 µg, 27% at 50 µg, 48.6% at 100 µg and 13.6% at 200 µg. For the RCA (n = 10), CAS occurred in 40% of the patients at 20 µg, 40% at 50 µg and 20% at 80 µg.

Table 5.3: Summary of invasive acetylcholine provocation testing

	All patients (n = 40)
Acetylcholine testing	
Epicardial spasm	40 (100%)

Diffuse spasm	25 (62.5%)
Focal spasm	15 (37.5%)
Location of the spasm	
LAD	36 (90.0%)
LCx	11 (27.5%)
RCA	10 (25.0%)
Multi-vessel	15 (37.5%)
Dose required to provoke spasm	
LCA (n = 37)	
20 µg	4 (10.8%)
50 µg	10 (27.0%)
100 µg	18 (48.6%)
200 µg	5 (13.6%)
RCA (n = 10)	
20 µg	4 (40.0%)
50 µg	4 (40.0%)
80 µg	2 (20.0%)
Acetylcholine rechallenge	
5 min	
Epicardial spasm	12 (30.0%)
Micro-vascular spasm	2 (5.00%)
No spasm	26 (65.0%)
*10 min	
Epicardial spasm	10 (25.0%)
Micro-vascular spasm	4 (10.0%)
No spasm	14 (35.0%)

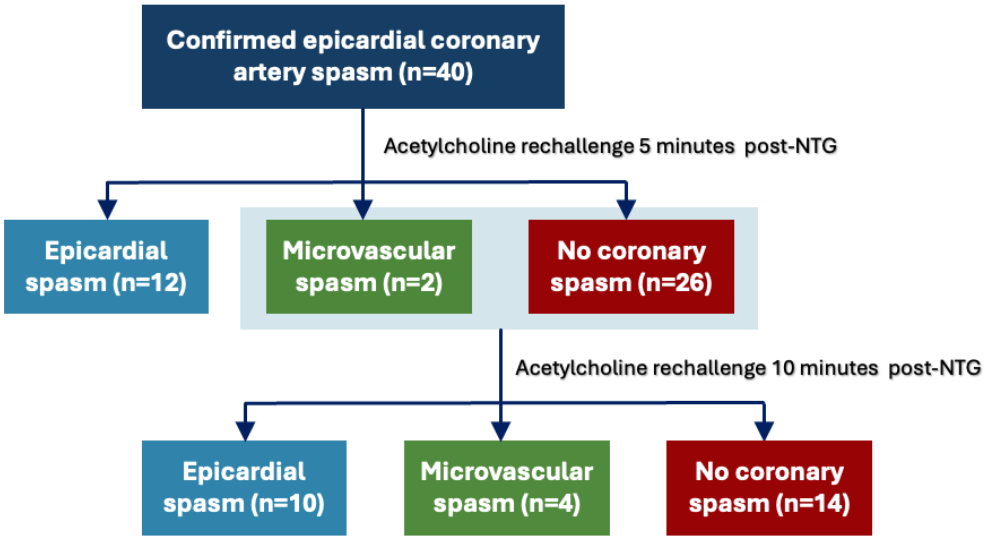
LAD = left anterior descending artery, LCA = left coronary artery, LCx = left circumflex artery, RCA = right coronary artery

*Only patients (n = 28) who did not demonstrate epicardial CAS at the 5-min ACh rechallenge proceeded to the 10-min ACh rechallenge.

5.3.3. Acetylcholine rechallenge characteristics

All patients underwent ACh rechallenge 5 min after intra-radial NTG administration. Epicardial spasm was re-induced in 12 patients (30%), microvascular spasm was observed in 2 patients (5%) and 26 patients (65%) showed no evidence of CAS. Subsequent ACh rechallenge at 10 min was performed in the latter 28 patients (70%) who did not demonstrate epicardial spasm during the initial rechallenge. During subsequent testing, epicardial spasm was re-induced in 10 patients (25%), microvascular spasm was noted in 4 patients (10%) and 14 patients (35%) did not show any evidence of spasm [Figure 5.2].

Figure 5.2: Results of acetylcholine rechallenge



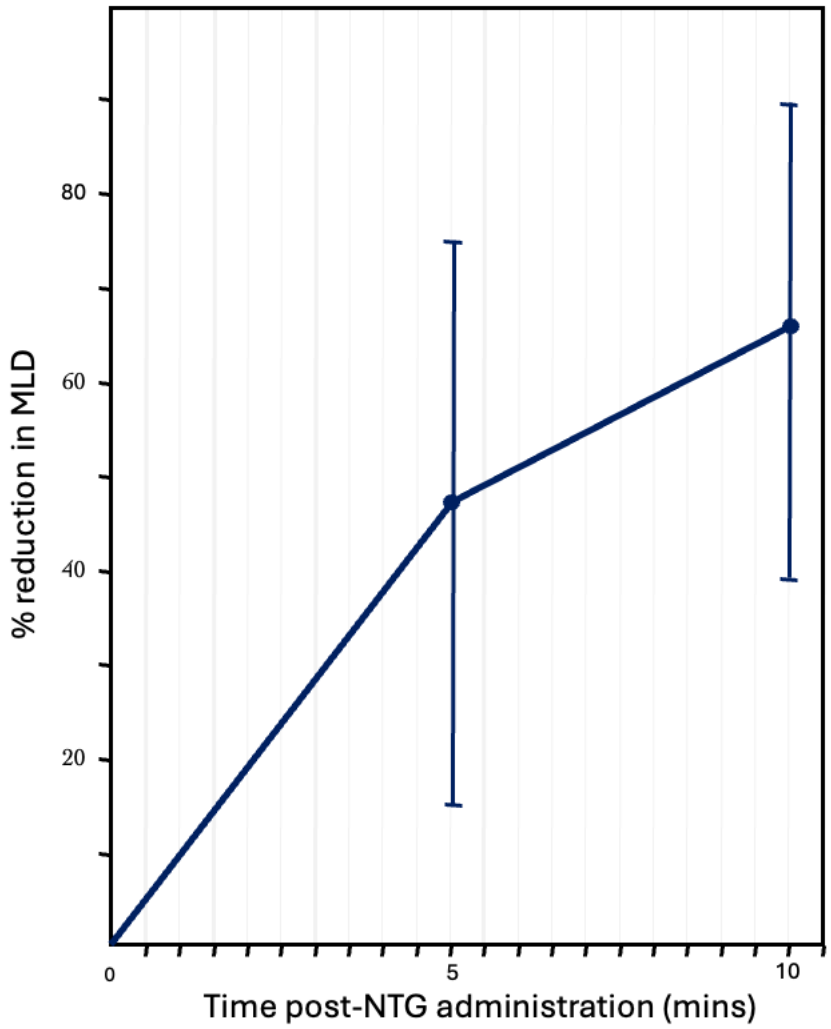
At 5 min, nitroglycerine was effective in preventing epicardial spasm in 28/40 (70%) patients. At 10 min, nitroglycerine was effective in preventing epicardial spasm in 18/40 (45%) patients.

NTG = nitroglycerine

Figure 5.3 demonstrates the time-dependent relationship between ACh and the %MLD change. At 5 min post-NTG administration, the average %MLD reduction was 47% (±0.30). This reduction

increased to 64% (±0.24) at the 10-min mark, indicating a progressive vasoconstrictive response over time.

Figure 5.3: Time–response relationship between intracoronary acetylcholine and % minimal lumen diameter change



MLD = minimal lumen diameter, NTG = nitroglycerine

5.4. Discussion

This study is the first to systematically evaluate the impact of intravascular NTG administration on downstream ACh provocation testing in patients with ANOCA. The major finding is that the sensitivity of ACh provocation testing is reduced to 30% and 55% at 5 min and 10 min post-NTG

administration, respectively. These results suggest that NTG administration decreases the diagnostic efficacy of ACh provocation testing for CAS.

Patients with ANOCA often experience severe and recurrent symptoms, resulting in considerably impaired quality of life, frequent hospitalisations for chest pain and repeated investigations [1,2,4]. Accurate identification of ANOCA endotypes via CFT is essential for tailoring optimal pharmacological therapy in this patient population [80]. The ESC and the American College of Cardiology/American Heart Association guidelines recommend CFT [62,144]. However, global variability in its implementation remains, particularly regarding the exact sequence of ACh provocation and CMD testing.

Certain institutions advocate assessing the coronary microcirculation before ACh provocation owing to its potential confounding influence on subsequent physiological measurements. Previous studies have reported that resting mean transit time and baseline resistance index are elevated in patients with a positive ACh provocation test compared with those exhibiting a negative response [142,145]. These findings have been corroborated by studies using transthoracic Doppler echocardiography and intracoronary dual-sensor guidewires (measuring Doppler velocity and pressure) [146,147].

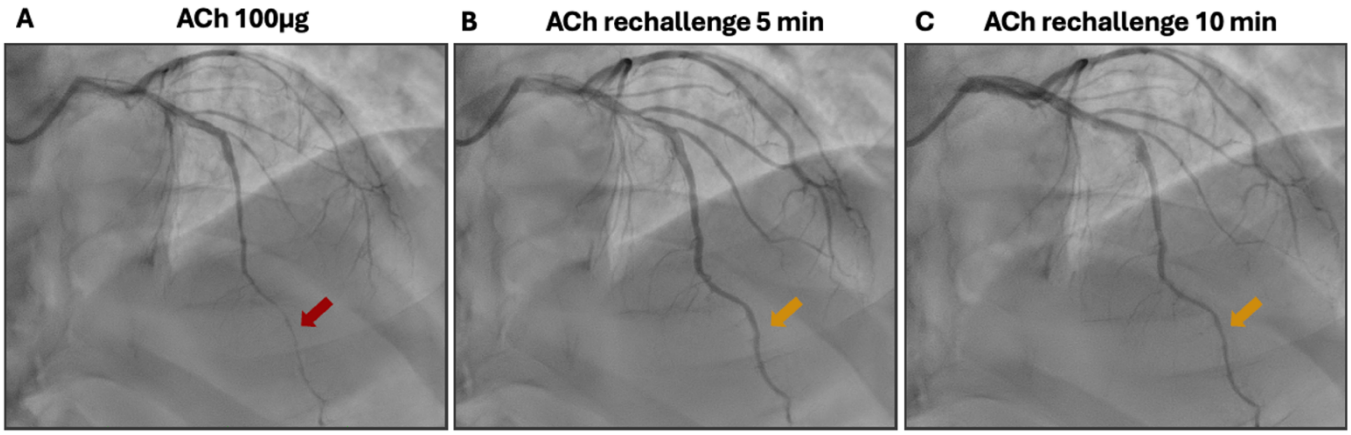
Conversely, although NTG administration is vital for achieving maximal vasodilation during CMD assessment, it may inadvertently result in the under-diagnosis of CAS [72,143]. NTG acts primarily on larger conduit coronary arteries via the NO pathway, with its vasodilatory effects diminishing as the vessel size decreases [148]. In addition, nitrates have been shown to exert their vasodilatory effects on epicardial coronary arteries for approximately 10–15 min [143]. Seitz et al. demonstrated that after the induction of significant epicardial spasm with a dose of ACh provocation, re-administration of the

same dose 3 min post-NTG failed to re-induce focal epicardial vasospasm and was highly effective in preventing diffuse epicardial spasm [149]. This finding implies that a 3-min interval is insufficient for accurate CAS diagnosis following NTG administration. The present study was designed to evaluate the effect of ACh administration after 5 and 10 min of intra-radial NTG, which is the approximate time taken to perform CMD assessment in an equipped CFT lab. Our results established that intracoronary NTG prevented the reproduction of epicardial CAS in 70% and 45% of the patients at the 5- and 10-min time points, respectively.

Furthermore, our study revealed a time-dependent reduction in MLD within the epicardial vessels following intra-vascular NTG administration. At 5 min post-NTG, the mean MLD reduction was 47% (±0.30), which increased to 64% (±0.24) at 10 min, indicating an elevated vasoconstrictive response with delayed re-exposure to ACh [Figure 5.4]. This finding suggests that at least a prolonged interval between NTG administration and subsequent ACh provocation should be considered, or alternatively, NTG should be avoided before ACh testing.

ACh rechallenge identified concomitant microvascular spasm in 15% (6/40) of the patients, consistent with previous research [149]. As noted by Sellke et al., NTG has reduced efficacy in preventing spasm within the coronary micro-circulation compared with its more potent effects on epicardial circulation [150]. The limited effect in smaller coronary microvessels may be attributed to the diminished biotransformation of NTG in these vessels. This observation highlights the heterogeneity and complexity of vasomotor disorders in patients with ANOCA. It is crucial for clinicians to consider additional diagnoses, such as microvascular spasm, as it has significant treatment implications [151,152].

Figure 5.4: Example of a patient who underwent acetylcholine rechallenge



An example of a patient with **(A)** diffuse epicardial spasm of the left anterior descending artery (red arrow) provoked by intracoronary acetylcholine (ACh) 100 µg; ACh rechallenge at 5 min **(B)** and 10 min **(C)** demonstrated a progressive vasoconstrictive response (gold arrows) without reaching the >90% diameter stenosis threshold for epicardial spasm.

5.4.1. Limitations

First, our sample size is relatively small, underscoring the need to validate our findings in a larger cohort. Second, the timing of the ACh rechallenge was limited to 5- and 10-min post-NTG administration. Although this protocol provided valuable data on the time-dependent response to ACh, it did not explore longer intervals, which might have offered further insights. Third, tachyphylaxis cannot be excluded as the mechanism for failed CAS provocation during the ACh rechallenge. Furthermore, the same dose of ACh that initially provoked epicardial CAS during the ACh rechallenge was used. Employing higher doses during the rechallenge might have potentially increased the detection rate of epicardial CAS. Lastly, our study did not assess the clinical implications of vasomotor responses, particularly whether patients with re-inducible spasms post-NTG administration were less responsive to conventional therapy.

5.5. Conclusions

NTG administration can decrease the effectiveness of ACh provocation testing in diagnosing CAS in patients with ANOCA. Our findings suggest that clinicians should avoid NTG administration before performing ACh provocation testing or delay the testing much longer than 10 min after its administration. Future studies should aim to define the appropriate sequential order for CFT in patients with ANOCA.

Chapter 6: Usefulness of Temporary Pacemaker During Acetylcholine Provocation Testing

***Rehan R**, Wong CCY, Weaver J, Jain P, Adams M, Tremmel JA, Ng MKC, Yong ASC. Usefulness of temporary pacemaker during acetylcholine provocation testing. Int J Cardiol Heart Vasc. 2024 Jun 12;53:101440. doi: 10.1016/j.ijcha.2024.101440. PMID: 38966805; PMCID: PMC11222927.*

6.1. Introduction

As part of CFT, ACh provocation testing has become an integral diagnostic tool when evaluating patients with ANOCA. ACh administration protocols vary from one institution to the other in terms of dose and speed of administration. Rapid ACh administration to specifically test for coronary spasm, such as 20-s injections, has been recommended by several large groups, including the Japanese Circulation Society (JCS) [72] and the landmark CorMICA study that established the benefit of CFT for evaluating patients with ANOCA [80]. Intracoronary ACh can result in transient sinus bradycardia and/or atrioventricular block, particularly with higher and faster administration. There is significant variability among international institutions regarding the routine use of a TTP in managing bradyarrhythmias during such procedures [73]. The current practice does not recommend the routine use of a prophylactic TTP unless the RCA is selected for testing [73]. This recommendation is based on expert consensus owing to a paucity of definitive evidence. Our objective was to assess the incidence of back-up pacing during ACh provocation testing using rapid administration and identify potential predictive factors.

6.2. Methods

In this multi-centre prospective observational study, multi-vessel testing was systematically performed in consecutive patients with ANOCA suspected of CAS. The patients were instructed to withhold vasoactive medications (e.g. CCBs and long-acting nitrates) and methylxanthine-containing substances for >4 times the duration of the drug's half-life. The administration of intra-arterial vasodilator drugs (e.g. NTG and CCB) was avoided before ACh provocation testing in all patients. Diagnostic ICA was performed to confirm the absence of obstructive CAD, defined as visual stenosis of >50% in combination with a measured RFR of ≤0.89 and/or FFR of ≤0.80.

Before ACh provocation testing, a TTP was inserted into the right ventricle via the femoral vein. Activation was contingent upon a prolonged sinus pause (>5 s) or a profound bradycardia (<30 bpm for >30 s). The testing involved incremental doses of ACh in the left (20–200 μg) and right (20–80 μg) coronary arteries injected manually through a 6-French guiding catheter without side holes over 20 s. After each injection, cine-images were acquired to assess the change in coronary diameter via QCA, as previously described [125]. If CAS was induced, with reproducible symptoms and ST-segment alterations (see definitions below), the provocation test was terminated and concluded to be positive. NTG was administered when spasm was induced and did not resolve spontaneously within 3 min after completing ACh testing or in instances of haemodynamic instability.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Sydney Local Health District Human Research Ethics Committee. Written informed consent was obtained from all participants. RR and ASCY had complete access to all study data and were responsible for their integrity and analysis.

6.2.1. Definitions

Epicardial spasm was defined as a focal or diffuse epicardial coronary diameter reduction of >90% in response to ACh compared with the relaxed state after intracoronary NTG, with a reproduction of recognisable symptoms and ischaemic ECG changes. Microvascular spasm was diagnosed when recognisable symptoms were reproduced with ischaemic ECG alterations in the absence of >90% epicardial diameter reduction in response to ACh. Ischaemic ECG changes were defined as transient ST-segment elevation or depression of >0.1 mV or ischaemic T-wave changes in at least two contiguous leads.

6.2.2. Statistical analysis

Continuous data were presented as mean ± SD or median and IQR, as appropriate, and analysed using an independent samples t-test or Mann–Whitney U test, depending on data distribution. Categorical data were expressed as count (%) and examined using chi-squared or Fisher's exact test, as appropriate. A P-value of <0.05 was considered statistically significant. All analyses were performed using R version 4.2.2. (Vienna, Austria) [127].

6.3. Results

This study was conducted across two tertiary referral institutions from 16th December 2021 to 13th October 2023 and included 196 vessels from 102 patients. The patient cohort had a mean age of 58.9 ± 11.5 years, with 55.9% of the participants being women. ACh provocation testing revealed CAS in 60.8% (62/102), with 50% (51/102) exhibiting epicardial spasm and 10.8% (11/102) showing evidence of microvascular spasm. Among patients with epicardial spasm, a diffuse pattern was observed in 56.9% (29/51), whereas 43.1% (22/51) had focal spasms. CAS was observed mainly in the LCA (80.6%) and to a lesser extent in the RCA (33.9%). In addition, multi-vessel spasm were evident in 30 patients (29.4%).

During ACh provocation of the LCA (n = 102), 25.5% (26/102) of the patients required back-up pacing. Dose-dependent analysis revealed no pacing requirement at 20 µg, 1% at 50 µg, 10.8% at 100 µg and 25.5% at 200 µg. When testing the RCA (n = 94), 61.7% (58/94) required back-up pacing, with rates of 20.2% at 20 µg, 48.9% at 50 µg and 61.7% at 80 µg. While assessing the LCA, patients with left coronary dominance were more likely to require back-up pacing (42.3% vs 11.8%, P = 0.001) [Table 6.1]. During RCA testing, back-up pacing was increased in patients with baseline sinus bradycardia compared with those without (25.9% vs 5.2%, P = 0.036). Conduction abnormalities on

baseline ECG were not significantly different between the groups (p = ns). No patients required conversion to a permanent pacemaker. Atrial fibrillation occurred in 9.8% (10/102) of the patients, of which two required DC cardioversion before discharge. The remaining cases of atrial fibrillation resolved spontaneously within 30 min, with no need for treatment or consequent thromboembolic events. No episodes of ventricular tachycardia or fibrillation were observed. No procedural complications were observed following TTP insertion.

Table 6.1: Patient and procedural characteristics

	ACh provocation – LCA (n = 102)			**ACh provocation – RCA (n = 94)**		
	Back-up pacing (n = 26)	**No back-up pacing (n = 76)**	**P-value**	**Back-up pacing (n = 58)**	**No back-up pacing (n = 36)**	**P-value**
Age, years	57.5 ± 10.9	59.4 ± 11.8	0.446	59.9 ± 11.2	57.2 ± 12.1	0.276
Women	17 (65.4%)	40 (52.7%)	0.402	27 (46.5%)	21 (58.3%)	0.266
Body mass index, kg/m^2	26.5 ± 3.1	27.4 ± 3.9	0.272	26.6 ± 3.5	28.2 ± 4.0	0.057
Coronary risk factors						
Hypertension	10 (38.5%)	40 (52.6%)	0.254	28 (48.2%)	17 (47.2%)	0.921
Hypercholesterolaemia	17 (65.3%)	48 (63.2%)	1.000	39 (67.2%)	19 (52.8%)	0.161
Diabetes	3 (11.5%)	14 (18.4%)	0.541	10 (17.2%)	5 (13.9%)	0.666
Family history of CAD	10 (38.5%)	20 (26.3%)	0.398	17 (29.3%)	10 (27.8%)	0.873
Current smoking	7 (26.9%)	15 (19.7%)	0.784	12 (20.1%)	9 (25%)	0.626
Previous coronary intervention	4 (15.4%)	9 (11.8%)	0.640	8 (13.8%)	5 (13.9%)	0.989
Cerebrovascular accident	1 (3.9%)	4 (5.3%)	0.812	4 (6.9%)	1 (2.8%)	0.743
Obstructive sleep apnoea	2 (7.7%)	9 (11.8%)	0.725	5 (8.6%)	4 (11.1%)	0.689
Angiographic features						
Minor CAD	14 (53.8%)	38 (50.0%)	0.739	28 (48.3%)	18 (50.0%)	0.871
Coronary dominance						
Right-dominant	13 (50.0%)	60 (78.9%)	0.005	44 (75.8%)	26 (72.2%)	0.694

Left-dominant	11 (42.3%)	9 (11.8%)	0.001	9 (15.5%)	6 (16.7%)	0.882
Co-dominant	2 (7.7%)	8 (10.5%)	0.970	5 (8.6%)	2 (11.1%)	0.884
Conduction abnormalities						
Sinus bradycardia	7 (26.9%)	13 (17.1%)	0.276	15 (25.9%)	3 (5.2%)	0.036
Left anterior fascicular block	5 (19.2%)	10 (13.2%)	0.450	8 (13.8%)	4 (11.1%)	0.705
Left posterior fascicular block	3 (11.5%)	14 (18.4%)	0.541	12 (20.7%)	3 (8.3%)	0.193
First-degree AV block	1 (3.8%)	5 (6.6%)	0.609	3 (5.2%)	2 (5.6%)	0.695
Second-degree AV block	1 (3.8%)	1 (1.3%)	0.987	1 (1.7%)	1 (2.8%)	0.696
Third-degree AV block	0 (0.00%)	0 (0.00%)	NA	0 (0.00%)	0 (0.00%)	NA
Right-bundle branch block	1 (3.8%)	1 (1.3%)	0.987	1 (1.7%)	1 (2.8%)	0.696
Left-bundle branch block	1 (3.8%)	2 (2.6%)	0.722	2 (3.4%)	1 (2.8%)	0.672

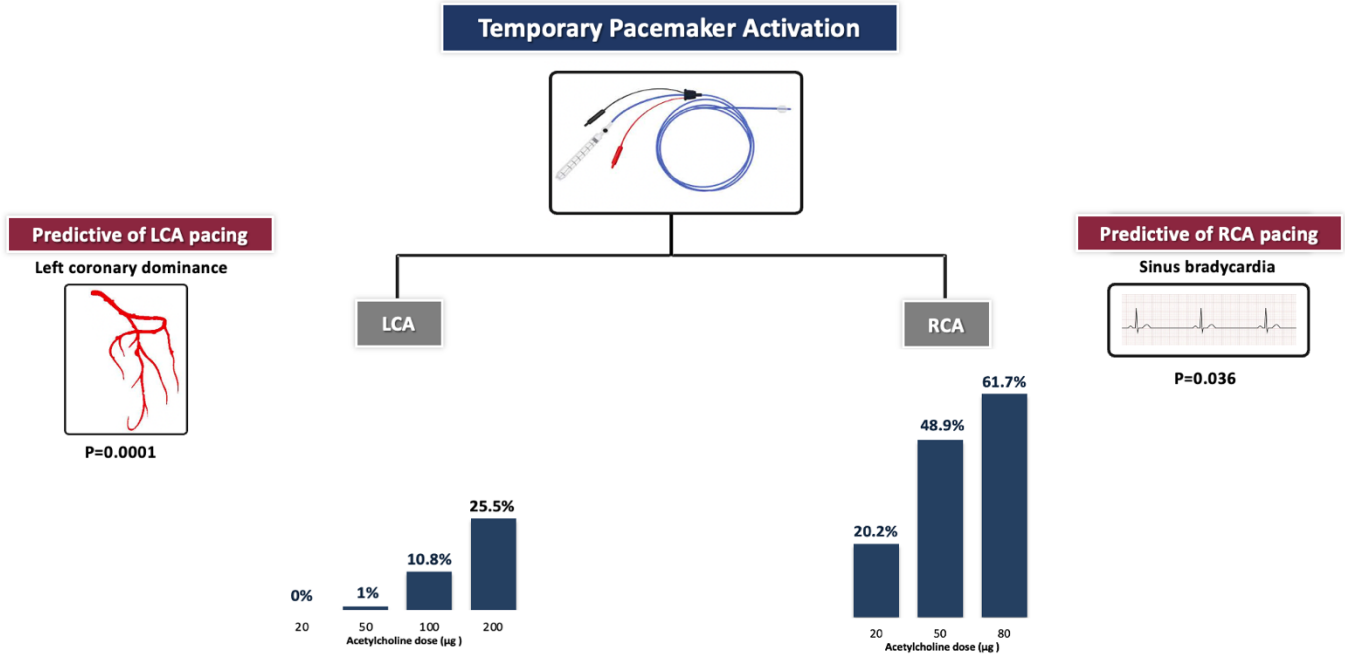
AV = atrioventricular, CAD = coronary artery disease

Values are mean ± or n (%)

6.4. Discussion

In this study of patients with ANOCA undergoing ACh provocation testing, the following were the significant findings: 1) back-up pacing in the LCA was observed in a quarter of the patients, particularly at high doses; 2) almost two-thirds of the patients required back-up pacing during RCA testing; 3) patients with left coronary dominance were more likely to need back-up pacing during LCA testing; 4) patients with baseline sinus bradycardia were more likely to require back-up pacing during RCA testing [Figure 6.1]

Figure 6.1: Predictive factors and acetylcholine dose-dependent relationship for temporary pacemaker activation



LCA = left coronary artery, RCA = right coronary artery

Contrary to conventional belief, which predominantly associates bradyarrhythmias with RCA testing, our findings revealed a high incidence during LCA testing. Coronary anatomical variations may be responsible for this observation. Despite most patients having sinoatrial and atrioventricular nodal arteries originating from the RCA, coronary arterial dominance may affect their origin, with some arising from the left circulation [153]. Such anatomical differences can expose these vessels to high doses of ACh and subsequent bradyarrhythmias. While some advocate for upfront TTP insertion, others lean towards its avoidance, contending that transient atrioventricular block self-resolves within seconds upon reducing the speed of ACh administration [133]. Although intentionally slowing

down ACh administration may alleviate transient bradyarrhythmias, such adjustments could potentially compromise the diagnostic yield and lead to a false negative result when testing for CAS [95].

While acknowledging the importance of our findings, a key limitation is the use of a rapid ACh administration time of 20 s, which inherently predisposes to a higher likelihood of back-up pacing. Nevertheless, this practice mirrors protocols employed by the JCS [72], the CorMICA study [80] and the Robert–Bosch–Krankenhaus institution [129].

6.5. Conclusions

Our study is the first to report the rates of significant bradycardia or pauses when employing rapid ACh injections for CFT and provides guidance in situations wherein TTP is required when performing these examinations. Our experience supports using a prophylactic TTP during ACh provocation testing of the RCA, particularly in patients with baseline sinus bradycardia. For LCA testing, clinicians should consider a TTP in cases of left coronary dominance and with the administration of a high ACh dose.

Chapter 7: Multi-vessel Coronary Function Testing Increases the Diagnostic Yield in Patients with Angina and Non-obstructive Coronary Arteries

***Rehan R**, Wong CCY, Weaver J, Chan W, Tremmel JA, Fearon WF, Ng MKC, Yong ASC. Multivessel Coronary Function Testing Increases Diagnostic Yield in Patients with Angina and Nonobstructive Coronary Arteries. JACC Cardiovasc Interv. 2024 May 13;17(9):1091-1102. doi: 10.1016/j.jcin.2024.03.007. PMID: 38749588.*

7.1. Introduction

Nearly half of all patients who undergo coronary angiography for stable angina have non-obstructive coronary arteries [1]. CVD, including CAS (epicardial and micro-vascular) and/or CMD, is the underlying pathophysiology in 60%–90% of these patients [119,154]. CVDys is characterised by a relative mismatch between coronary artery blood flow and oxygen demand, resulting in angina pectoris and myocardial ischaemia. This condition is associated with high rates of morbidity, including impaired quality of life and recurrent hospitalisations, leading to the considerable utilisation of healthcare resources [1,2,4].

Although ICA remains the gold standard for diagnosing obstructive CAD, its diagnostic utility is limited in cases of ANOCA. Invasive CFT, using ACh to diagnose coronary (epicardial and micro-vascular) spasms and adenosine to evaluate the structural and functional integrity of the micro-circulation, is the gold standard for assessing patients with ANOCA. The ESC and American College of Cardiology/American Heart Association guidelines advocate using CFT to categorise patients with ANOCA into distinct endotypes [123,144]. This classification allows clinicians to tailor therapy to individual patients, an approach that has been shown to improve the angina status and quality of life [80].

CFT is routinely performed on a single coronary vessel. Testing the left coronary circulation, specifically the LAD, is recommended as it typically subtends the largest myocardial territory [63]. In cases where technical factors, such as tortuous coronary anatomy, preclude instrumentation of the LAD, testing of the LCx and RCA may be considered [63]. While single-vessel testing assumes that disorders of coronary vasomotion are pan-coronary, previous studies have suggested the presence of regional discrepancies across different coronary territories . The current practice is based on

consensus, and whether testing multiple coronary territories would augment the diagnostic yield is unclear. Hence, this study aimed to evaluate the diagnostic efficacy of multi-vessel CFT in patients with ANOCA.

7.2. Methods

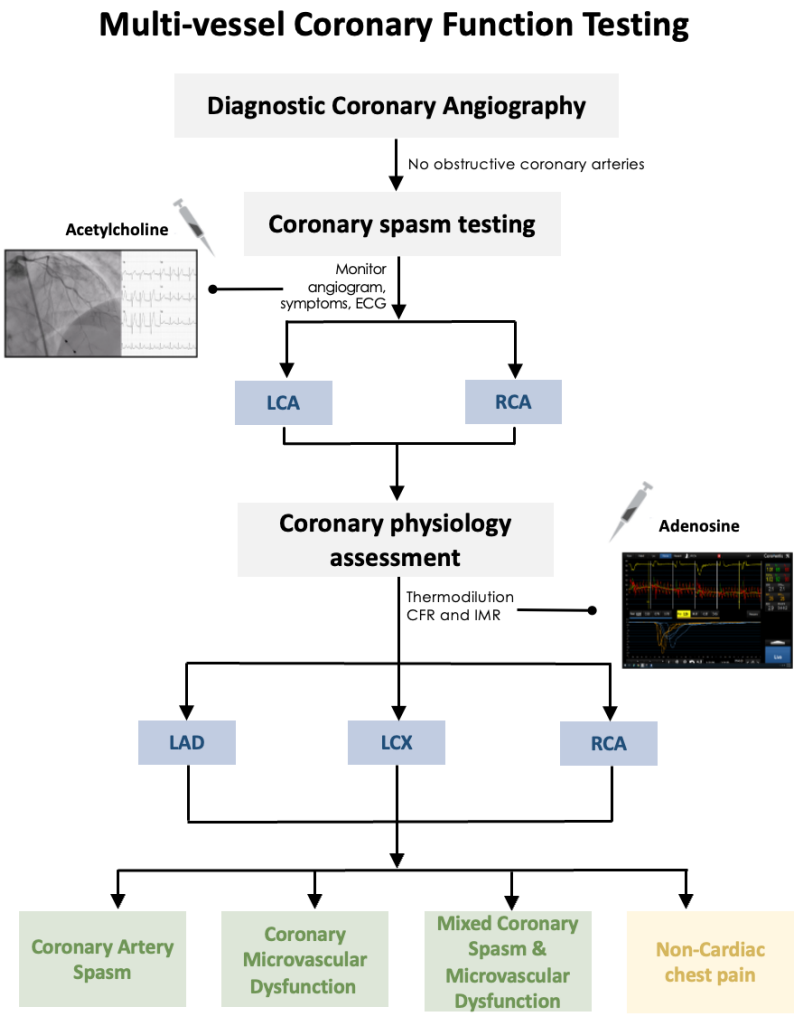
This multi-centre, prospective observational study assessed multi-vessel CFT in patients with ANOCA. All patients were referred by their treating cardiologist for suspected CVDys. Diagnostic ICA confirmed the absence of obstructive CAD, defined as >50% visual stenosis in combination with a measured RFR of ≤0.89 and/or FFR of ≤0.80 [124]. Patients (≥18 years of age) underwent ACh provocation followed by adenosine-mediated coronary physiology assessment in all three major epicardial arteries [Figure 7.1]. A minimum interval of 10 min was allowed between the two procedures. In the case of a small, non-dominant or severely tortuous coronary artery, CFT was avoided for safety reasons. Patients exhibiting resting or hyperaemic indices indicative of obstructive CAD were subsequently excluded from the study. In addition, those with a non-coronary indication for invasive angiography (e.g. valve disease), technical factors precluding safe passage of the pressure guidewire, an inability to receive adenosine, a recent (within 3 weeks before cardiac catheterisation) ST-segment elevation myocardial infarction and/or an inability to provide informed consent, were excluded.

Clinical data on patient characteristics, cardiac risk factors and symptom profiles were acquired before CFT. Information on conventional cardiovascular risk factors (e.g. diabetes, hypercholesterolaemia, hypertension and premature CAD in first-degree relatives) and non-conventional variables associated with vasomotor dysfunction (e.g. caffeine intake) [126,155] were

recorded. In addition, patients were asked to report angina characteristics and quality of life measures via the SAQ.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Human Research Ethics Review Board. Written informed consent was obtained from all participants. RR and ASCY had complete access to all study data and were responsible for their integrity and analysis.

Figure 7.1: Study protocol for multi-vessel coronary function testing



CFR = coronary flow reserve, ECG = electrocardiogram, IMR = index of micro-circulatory resistance, LAD = left anterior descending artery, LCA = left coronary artery, LCx = left circumflex**,** RCA = right coronary artery

7.2.1. Invasive coronary function testing

All CFT procedures were performed in the morning, and the patients were requested to withhold vasoactive medications (e.g. CCBs and long-acting nitrates) and methylxanthine-containing substances for >4 times the duration of the drug's half-life. A continuous 12-lead ECG was monitored throughout the procedure, and standard resuscitation equipment was available. ICA was performed as per standard institutional practice via the radial or femoral route. Administration of intra-arterial vasodilator drugs (e.g. NTG and CCB) was avoided before CFT in all patients. A standard diagnostic ICA was performed to confirm the absence of obstructive CAD. If obstructive CAD was ruled out, CFT was performed. The choice of the first vessel to be tested was left to the operator's discretion. Subsequently, CFT was performed on the remaining major epicardial coronary arteries.

Coronary vasoreactivity was determined using ACh provocation. A TTP was inserted via the femoral vein to compensate for potential bradycardia. Activation of the TTP was contingent upon a pause of >5 s. A 6-French angioplasty guiding catheter without side holes was inserted into either the LCA or the RCA according to the operator's clinical judgement. For LCA assessment, incremental doses of 20, 50, 100 and 200 μg of ACh were injected over 20 s, with a 2-min gap between the doses. After each injection, cine-images were obtained to evaluate alterations in coronary diameter via QCA, as previously described [125]. If CAS was induced, with reproducible symptoms and ST-segment changes (see definitions below), the provocation test was terminated and concluded to be positive. A similar protocol was adopted for the RCA, with incremental doses of 20, 50 and 80 μg of ACh. When CAS was induced and did not resolve spontaneously within 3 min after completing ACh testing or in instances of haemodynamic instability, NTG was administered.

Coronary physiology assessment was performed using a pressure–temperature sensor guidewire (PressureWire X; Abbott Corporation, Chicago, IL). The guidewire was advanced after equalisation to guide the catheter pressure, ensuring that the sensor was positioned in the distal third of the vessel and at least 80 mm from the guiding catheter. If not already done, intracoronary NTG was administered at a dose of 200 µg. The resting mean Pa and Pd were recorded. Three boluses of 3 mL room temperature saline were injected into the coronary artery via the guiding catheter. The transit time of the saline injections was determined using the thermodilution technique, and the average of the three resting transit times was recorded as the Tmn_{Rest}. Next, an intravenous adenosine infusion (140 µg/kg per min) was administered via a large-bore peripheral cannula or femoral venous sheath to induce a steady state of maximal hyperaemia. The hyperaemic mean proximal pressure and distal pressure were documented. Thermodilution curves were constructed in the same manner to determine the Tmn_{Hyp}. The calculations for the respective coronary haemodynamic indices included (a) Pd/Pa during hyperaemia for FFR, (b) Tmn_{Rest} /Tmn_{Hyp} for CFR and (c) Pd × Tmn_{Hyp} for IMR. All measurements were recorded using the Coroflow system (Coroventis Research AB, Uppsala, Sweden).

7.2.2. Definitions

CVDys was defined according to the underlying pathophysiological endotype as per previously published international consensus [110]. Epicardial CAS was defined as a focal or diffuse epicardial coronary diameter reduction of >90% in response to ACh compared with the relaxed state after intracoronary NTG, with a reproduction of recognisable symptoms and ischaemic ECG changes. Micro-vascular CAS was diagnosed when recognisable symptoms were reproduced with ischaemic ECG changes in the absence of >90% epicardial diameter reduction in response to ACh. Ischaemic ECG alterations were defined as transient ST-segment elevation or depression of >0.1 mV or

ischaemic T-wave changes in at least two contiguous leads. CMD was confirmed if coronary physiology assessment indicated a CFR of <2.5 and/or IMR of ≥25, as defined by current consensus documents [123]. A diagnosis of non-cardiac chest pain required non-obstructive epicardial CAD (FFR > 0.80) and normal CFT (CFR ≥ 2.5, IMR < 25 and negative ACh testing).

7.2.3. Statistical analysis

Continuous data were presented as mean ± SD or median and IQR, as appropriate. Categorical data were expressed as count (%). The chi-squared or Fisher's exact test was used, as appropriate, for categorical data analysis. McNemar's test was utilised to evaluate the alteration in diagnostic yield of multi-vessel compared with single-vessel CFT. A P-value of <0.05 was considered statistically significant. Assuming a 50% diagnostic rate using conventional single-vessel CFT in patients with ANOCA, a sample size of 78 patients was calculated to achieve 80% statistical power for detecting a 10% difference in diagnosis. The significance level was set at 5%. All analyses were performed using R version 4.2.2. (Vienna, Austria) [127].

7.3. Results

This study was conducted across two tertiary referral institutions and included 228 vessels from 80 patients. The patient cohort had a mean age of 57.8 ± 11.8 years, with 60% of the participants being women. Clinical and medication data are listed in Table 7.1. Patients with CVDys were more likely to receive beta-blocker therapy (45.5% vs 14.3%, P = 0.03). In addition, this group tended to be overweight (66.6% vs 35.7%, P = 0.074). Rest angina was observed in 25%, exertional angina in 35% and mixed rest and exertional angina in the remaining 40%.

Statistically significant differences were noted in age (P = 0.043), presence of hypertension (P = 0.048) and results of functional cardiac assessment (P = 0.037) among patients with no CMD, single-vessel CMD and multi-vessel CMD [Table 7.2]. Moreover, within the no CAS, single-vessel CAS and multi-vessel CAS sub-groups, a statistically significant difference was seen in the presence of minor CAD (P = 0.003) [Table 7.3].

Table 7.1: Patient characteristics

	All patients (n = 80)
Age, years	57.8 ± 11.8
Women	48 (60%)
Body mass index, kg/m^2	27.2 ± 4.1
Ethnicity	
Caucasian	54 (67.5%)
Asian	12 (15%)
Southeast Asian	4 (5%)
Middle Eastern	9 (11.2%)
Tongan	1 (1.3%)
Coronary risk factors	
Hypertension	39 (48.8%)
Hypercholesterolaemia	51 (63.7%)
Diabetes	14 (17.5%)
Family history of CAD	22 (27.5%)
Current smoking	18 (22.5%)
Previous coronary intervention	10 (12.5%)
Other risk factors	
Cerebrovascular accident	6 (7.5%)
Obstructive sleep apnoea	9 (11.2%)
Excessive alcohol	9 (11.2%)
Excessive caffeine intake	9 (18%)
Medications	
Aspirin	55 (68.8%)

P2Y12 Inhibitor	11 (13.8%)
Statin	53 (66.2%)
ACE-I/ARB	27 (33.8%)
Beta-blocker	28 (35%)
Calcium channel blockers	24 (30%)
Nicorandil	5 (6.25%)
Nitrates	18 (22.5%)
Angina characteristics	
Predominately rest angina	20 (25%)
Predominately exertional angina	28 (35%)
Mixed rest and exertional angina	32 (40%)
Predominately daytime angina (7 am–7 pm)	24 (30%)
Predominately nocturnal angina (7 pm–7 am)	14 (17.5%)
No circadian pattern	42 (52.5%)
Functional cardiac assessment	
Normal	44 (55%)
Inconclusive	8 (10%)
Abnormal	28 (35%)
Coronary dominance	
Right-dominant	60 (75%)
Left-dominant	13 (16%)
Co-dominant	7 (9%)

ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease

Values are mean ± or n (%)

Table 7.2: Patient characteristics according to coronary microvascular dysfunction sub-groups

	No CMD (n = 30)	Single-vessel CMD (n = 27)	Multi-vessel CMD (n = 23)	P-value
Age, years	54.6 ± 10.1	59.3 ± 13.7	61.3 ± 10.7	0.043
Women	19 (63.3%)	13 (48.1%)	13 (56.5.7%)	0.270

Body mass index, kg/m^2	26.7 ± 4.4	27.1 ± 3.1	27.9 ± 4.8	0.571
Ethnicity				0.864
Caucasian	21 (70.0%)	20 (59.1%)	13 (56.5%)	
Asian	3 (3.8%)	4 (14.8%)	5 (21.7%)	
Southeast Asian	1 (1.3%)	1 (3.7%)	2 (8.7%)	
Middle Eastern	3 (3.8%)	4 (14.8%)	2 (8.7%)	
Tongan	1 (1.3%)	0 (0.00%)	0 (0.00%)	
Coronary risk factors				
Hypertension	10 (33.3%)	14 (51.9%)	15 (65.2%)	0.048
Hypercholesterolaemia	19 (63.3%)	18 (66.6%)	14 (60.9%)	0.963
Diabetes	4 (13.3%)	7 (8.6%)	3 (13.0%)	0.605
Family history of CAD	10 (33.3%)	8 (10%)	4 (17.4%)	0.434
Current smoking	9 (30.0%)	7 (8.6%)	2 (8.7%)	0.444
Previous coronary intervention	5 (16.6%)	3 (3.8%)	4 (17.4%)	0.791
Other risk factors				
Cerebrovascular accident	5 (16.6%)	1 (3.7%)	0 (0.00%)	0.055
Obstructive sleep apnoea	2 (6.6%)	3 (11.1%)	4 (17.4%)	0.544
Excessive alcohol	4 (13.3%)	3 (11.1%)	2 (8.7%)	0.914
Excessive caffeine intake	3 (10.0%)	2 (7.4%)	2 (8.7%)	0.901
Medications				
Aspirin	22 (73.3%)	18 (66.6%)	15 (65.2%)	0.525
P2Y12 Inhibitor	5 (16.6%)	2 (7.4%)	4 (17.4%)	0.527
Statin	19 (63.3%)	18 (66.6%)	16 (69.6%)	0.724
ACE-I/ARB	7 (23.3%)	10 (37.0%)	10 (43.5%)	0.279
Beta-blocker	9 (30.0%)	10 (37.0%)	9 (39.1%)	0.763
Calcium channel blockers	6 (20.0%)	11 (40.7%)	7 (30.4%)	0.356
Nicorandil	2 (6.6%)	2 (7.4%)	1 (4.3%)	0.998
Nitrates	7 (23.3%)	7 (25.9%)	4 (17.4%)	0.894
Angina characteristics				
Angina pattern				0.053
Predominately rest angina	11 (36.6%)	4 (14.8%)	4 (17.4%)	

Predominately exertional angina	4 (13.3%)	9 (33.3%)	10 (43.5%)	
Mixed rest and exertional angina	14 (46.6%)	16 (53.3%)	8 (34.8%)	
Circadian rhythm				0.317
Predominately daytime angina (7 am–7 pm)	6 (20.0%)	10 (37.0%)	7 (30.4%)	
Predominately nocturnal angina (7 pm–7 am)	4 (13.3%)	3 (11.1%)	6 (26.1%)	
No circadian pattern	19 (63.3%)	16 (59.3%)	9 (39.1%)	
Functional cardiac assessment				
Exercise stress echocardiogram				0.037
Normal	21 (70.0%)	16 (55.2%)	8 (36.4%)	
Inconclusive	6 (20.0%)	9 (31.0%)	13 (59.1%)	
Abnormal	2 (6.6%)	4 (13.8%)	1 (4.55%)	
Coronary dominance				
Right-dominant	23 (76.6%)	21 (72.4%)	16 (72.7%)	0.798
Left-dominant	3 (9.9%)	6 (20.7%)	4 (18.2%)	0.645
Co-dominant	1 (3.3%)	2 (6.90%)	2 (9.09%)	0.850
Angiographic features				
Minor CAD	15 (50.0%)	13 (48.1%)	13 (56.5%)	0.600
Seattle Angina Questionnaire				
Summary	56.9 (17.4)	55.6 (12.5)	54.7 (11.7)	0.887
Limitation	63.7 (21.8)	67.0 (17.3)	66.7 (17.8)	0.786
Stability	44.0 (18.5)	40.7 (16.8)	37.1 (17.2)	0.425
Frequency	57.6 (27.1)	53.8 (15.2)	52.1 (18.9)	0.835
Satisfaction	59.5 (32.5)	64.9 (16.4)	56.5 (18.3)	0.362
Angina QoL	53.2 (16.0)	50.8 (13.1)	40.4 (10.3)	0.255

ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease, CMD = coronary microvascular dysfunction, QoL = Quality of life

Values are mean ± or n (%)

Table 7.3: Patient characteristics according to coronary artery spasm sub-groups

	No CAS (n = 32)	Single-vessel CAS (n = 33)	Multi-vessel CAS (n = 15)	P-value
Age, years	59.2 (13.3)	56.8 (11.7)	56.9 (8.62)	0.693
Women	22 (68.8%)	13 (39.4%)	10 (66.7%)	0.059
Body mass index, kg/m^2	26.5 (4.07)	27.9 (4.32)	26.9 (3.16)	0.364
Ethnicity				0.890
Caucasian	22 (68.8%)	20 (60.6%)	12 (80.0%)	
Asian	6 (18.8%)	5 (15.2%)	1 (6.67%)	
Southeast Asian	1 (3.12%)	2 (6.06%)	1 (6.67%)	
Middle Eastern	3 (9.38%)	5 (15.2%)	1 (6.67%)	
Tongan	0 (0.00%)	1 (3.03%)	0 (0.00%)	
Coronary risk factors				
Hypertension	19 (59.4%)	16 (48.5%)	4 (26.7%)	0.112
Hypercholesterolaemia	21 (65.6%)	19 (57.6%)	11 (73.3%)	0.552
Diabetes	3 (9.38%)	8 (24.2%)	3 (20.0%)	0.314
Family history of CAD	8 (25.0%)	9 (27.3%)	5 (33.3%)	0.897
Current smoking	6 (18.8%)	8 (24.2%)	4 (26.7%)	0.704
Previous coronary intervention	1 (3.12%)	1 (3.03%)	0 (0.00%)	0.870
Other risk factors				
Cerebrovascular accident	2 (6.25%)	0 (0.00%)	4 (26.7%)	0.065
Obstructive sleep apnoea	4 (12.5%)	4 (12.1%)	1 (6.67%)	0.817
Excessive alcohol	2 (6.25%)	4 (12.1%)	3 (20.0%)	0.408
Excessive caffeine intake	1 (3.12%)	6 (18.2%)	2 (13.3%)	0.182
Medications				
Aspirin	16 (50.0%)	27 (81.8%)	12 (80.0%)	0.216
P2Y12 Inhibitor	2 (6.25%)	3 (9.09%)	6 (40.0%)	0.105
Statin	21 (65.6%)	22 (66.7%)	10 (66.7%)	0.995
ACE-I/ARB	13 (40.6%)	10 (30.3%)	4 (26.7%)	0.552
Beta-blocker	9 (28.1%)	16 (48.5%)	3 (20.0%)	0.091
Calcium channel blockers	5 (15.6%)	4 (12.1%)	4 (26.7%)	0.487

Nicorandil	2 (6.25%)	3 (9.09%)	0 (0.00%)	0.641
Nitrates	4 (12.5%)	10 (30.3%)	4 (26.7%)	0.246
Angina characteristics				
Angina pattern				0.087
Predominately rest angina	3 (9.38%)	10 (30.3%)	6 (40.0%)	
Predominately exertional angina	13 (40.6%)	8 (24.2%)	2 (13.3%)	
Mixed rest and exertional angina	16 (50.0%)	15 (45.5%)	7 (46.7%)	
Circadian rhythm				0.118
Predominately daytime angina (7 am–7 pm)	15 (46.9%)	8 (24.2%)	0 (0.00%)	
Predominately nocturnal angina (7 pm–7 am)	3 (9.38%)	6 (18.2%)	4 (26.7%)	
No circadian pattern	14 (43.8%)	19 (57.6%)	11 (73.3%)	
Functional cardiac assessment				
Exercise stress echocardiogram				0.850
Normal	3 (10.7%)	2 (6.45%)	0 (0.00%)	
Inconclusive	15 (53.6%)	17 (54.8%)	8 (61.5%)	
Abnormal	10 (35.7%)	12 (38.7%)	5 (38.5%)	
Coronary dominance				
Right-dominant	23 (71.9%)	29 (87.9%)	8 (57.1%)	0.083
Left-dominant	1 (3.1%)	7 (21.2%)	5 (35.7%)	0.058
Co-dominant	2 (6.2%)	3 (9.1%)	0 (0.00%)	0.850
Angiographic features				
Minor CAD	6 (18.8%)	23 (69.7%)	12 (80.0%)	0.003
Seattle Angina Questionnaire				
Summary	59.0 (13.2)	54.3 (13.2)	47.9 (9.21)	0.035
Limitation	70.9 (18.2)	64.3 (14.6)	59.9 (13.4)	0.176
Stability	42.3 (19.7)	40.5 (14.2)	37.5 (13.0)	0.772
Frequency	60.4 (26.0)	52.4 (14.8)	44.4 (12.0)	0.220
Satisfaction	72.4 (24.2)	61.5 (22.7)	58.9 (21.6)	0.214
Angina QoL	45.0 (13.6)	42.1 (15.8)	38.3 (12.9)	0.381

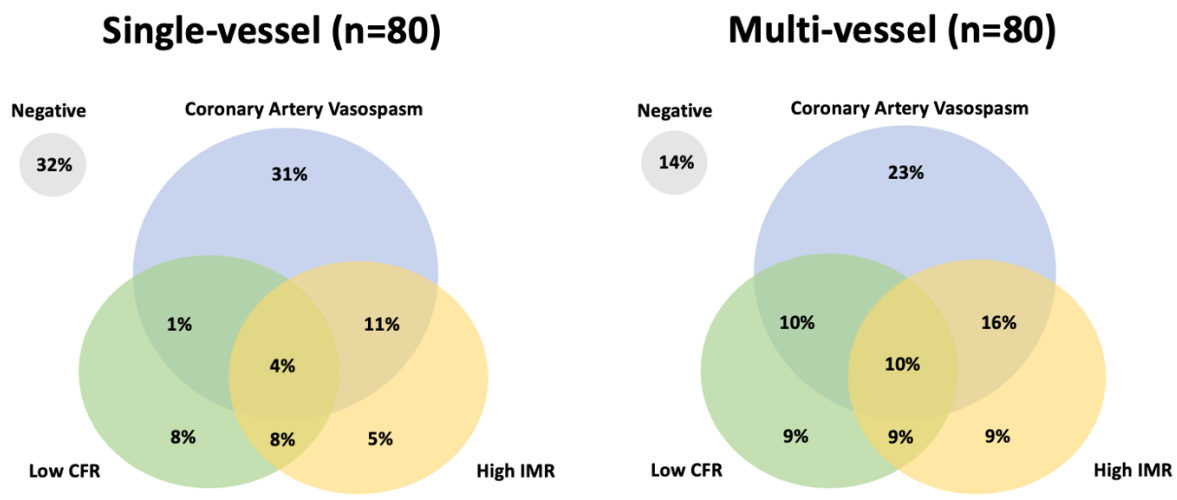
ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease, CAS = coronary artery spasm, QoL = Quality of life

Values are mean ± or n (%)

7.3.1. Diagnostic value of multi-vessel CFT

Among the cohort of 80 patients, the multi-vessel protocol demonstrated evidence of CVDys in 86.3% (69/80) cases compared with 68.8% (55/80) in the single-vessel protocol [Figure 7.2]. Therefore, 17.5% of the patients remained unidentified using single-vessel testing. Multi-vessel CFT modified the diagnosis in 33.8% (27/80) of the patient population [Figure 7.3]. Compared with single-vessel CFT, multi-vessel testing yielded a significantly greater prevalence of CVDys (86.3% vs 68.8%, P = 0.0005), CAS (60.0% vs 47.5%, P = 0.004) and CMD (62.5% vs 37.5%, P < 0.001) [Figure 7.4].

Figure 7.2: Distribution and co-existence of coronary functional abnormalities

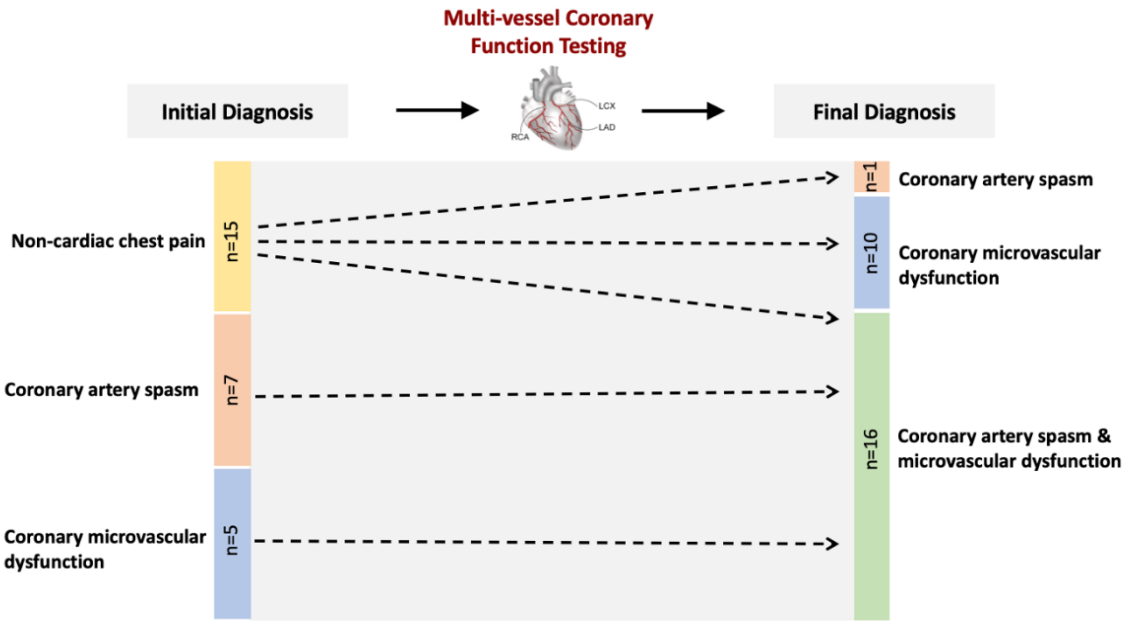


(A) Venn diagram illustrating the prevalence of coronary functional abnormalities in single-vessel coronary function testing

(B) Venn diagram illustrating the prevalence of coronary functional abnormalities in multi-vessel coronary function testing

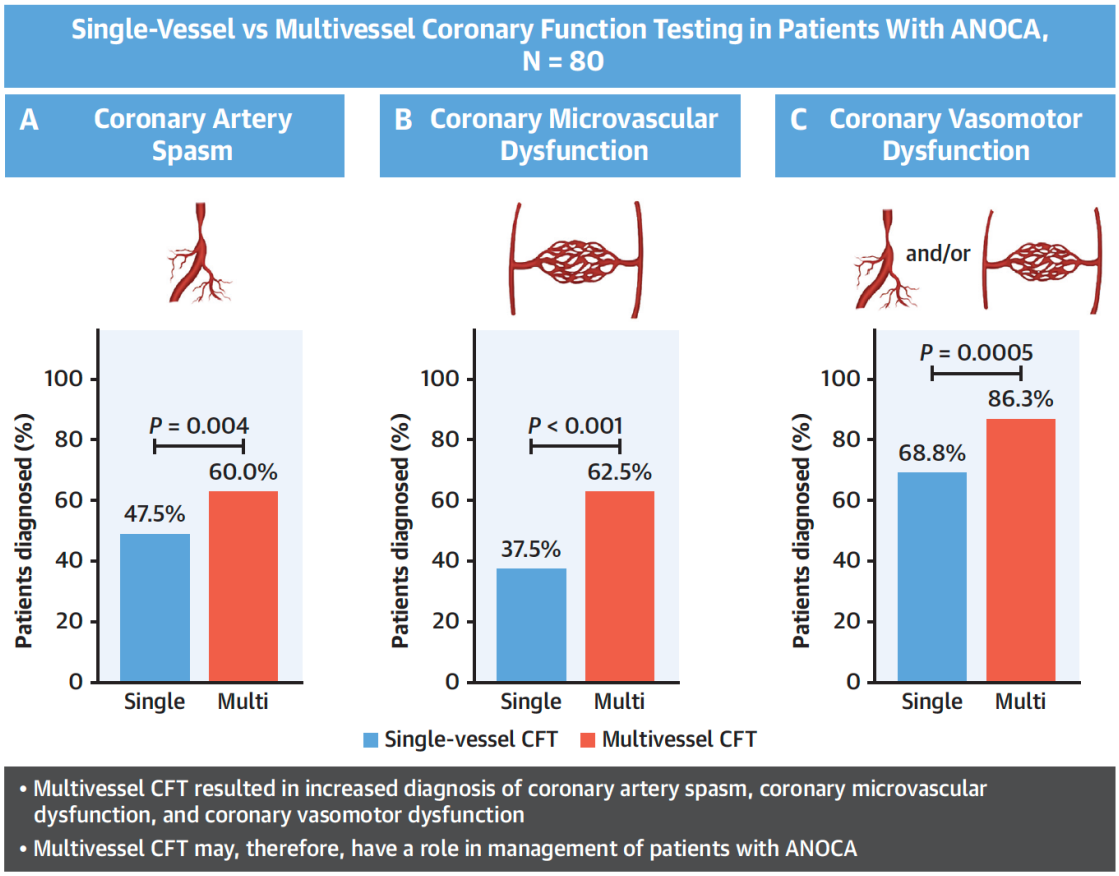
CFR = coronary flow reserve, IMR = index of microcirculatory resistance

Figure 7.3: Efficacy of multi-vessel coronary function testing in re-classifying ANOCA endotypes



The figure demonstrates that multi-vessel coronary function testing altered the final diagnosis in 33.8% (27/80) of patients with ANOCA.

Figure 7.4: Efficacy of multi-vessel coronary function testing in patients with angina and non-obstructive coronary arteries



ANOCA = angina with non-obstructive coronary arteries, LAD = left anterior descending artery, LCx = left circumflex, RCA = right coronary artery

7.3.2. Procedural characteristics

Most ICAs were performed using radial arterial access (57.5%); all study participants underwent ACh provocation testing, with a back-up TTP in situ. The LCA was tested first in 96.3% (77/80) of the patients. There was a predominance of right-dominant circulation (75%), followed by left-dominant (16%), with the remaining classified as co-dominant (9%). The median procedural time was 56 min (IQR: 48–62 min), and the median total contrast volume was 155 mL (IQR: 135–180 mL). Although no fatal adverse events were reported, one serious adverse event was observed, which involved a guiding catheter-induced coronary dissection requiring percutaneous coronary intervention. During ACh provocation testing, TTP was required in 12 (15%) patients during the LCA assessment and 46

(57.5%) during the RCA evaluation. Among those requiring TTP, 8 (66%) received a dose of 200 µg during the LCA assessment and 12 (26.1%) received 80 µg during the RCA assessment. Transient atrial fibrillation was noted in 10 patients (12.5%), of which 2 required DC cardioversion and the remaining resolved spontaneously before discharge. The procedural characteristics are summarised in Table 7.4.

Table 7.4: Procedural characteristics

	All patients (n = 80)
Final access site	
Radial	46 (57.5%)
Femoral	34 (42.5%)
Intra-procedural details	
Total procedure time (min)	56 (48–62)
Total contrast (mL)	155 (135–180)
Angiographic features	
*Minor CAD	41 (51%)
Complications	
Atrial fibrillation	10 (12.5%)
Coronary dissection	2 (2.5%)
Activation of TTP	
LCA	12 (15%)
RCA	46 (57.5%)

*Minor CAD: visual irregularities on diagnostic angiography indicative of atherosclerotic plaque

CAD = coronary artery disease, LCA = left coronary artery, RCA = right coronary artery, TTP = temporary transvenous pacemaker

Values are median and IQR or n (%)

7.3.3. Acetylcholine provocation testing

Results of ACh provocation testing revealed CAS in 60% (48/80) of the patients, with 52.5% (42/80) exhibiting epicardial spasms and 7.5% (6/80) displaying microvascular spasm. Among the 42 patients with epicardial spasms, a diffuse pattern was observed in 24 (57.1%) patients, whereas 18 (42.9%) had focal spasm [Table 7.5]. CAS was predominantly evident in the LCA (38/48), although isolated RCA spasms were detected in 20.8% (10/48) of the patients [Figure 7.5]. In addition, multi-vessel spasms were seen in 16 patients (20.0%). When evaluating the dose–response relationship, most CAS in the LCA occurred with a 100 μg dose (50%) and a higher 200 μg dose induced CAS in an additional four patients (10.5%). An 80 μg dose for the RCA led to CAS in two more patients (13%). [Table 7.6]. Across vessels, 32 patients (40.0%) had one-vessel CAS, 13 (16.3%) had two-vessel CAS and 3 (3.8%) had CAS in all three vessels [Figure 7.6].

Table 7.5: Summary of invasive coronary function testing according to all coronary vessels

	LAD	**LCx**	**RCA**
Acetylcholine testing			
Epicardial spasm	30 (37.5%)	15 (18.8%)	15 (18.8%)
Diffuse spasm	21 (26.3%)	12 (15%)	5 (6.3%)
Focal spasm	9 (11.3%)	3 (3.8%)	10 (66.7%)
Microvascular spasm	5 (6.3%)	0 (0%)	1 (1.3%)
Coronary physiology testing			
Resting indices			
RFR	0.92 (0.90–0.95)	0.98 (0.96–0.99)	0.97 (0.93–0.99)
Mean resting transit time (s)	0.84 (0.58–1.15)	0.65 (0.43–0.94)	0.97 (0.67–1.27)
Hyperaemic indices			
FFR	0.91 (0.87–0.93)	0.98 (0.96–0.99)	0.97 (0.93–0.99)
Mean hyperaemic transit time (s)	0.24 (0.13–0.34)	0.21 (0.12–0.28)	0.21 (0.16–0.39)
CFR	3.35 (2.70–5.82)	3.30 (2.40–4.50)	4.50 (2.55–5.30)
IMR	16.0 (11–24)	15 (11.0–26)	17.0 (12.0–27.0)

LAD = left anterior descending, LCx = left circumflex, RCA = right coronary artery, RFR = resting full-cycle ratio, FFR = fractional flow reserve, CFR = coronary flow reserve, IMR = index of microcirculatory resistance

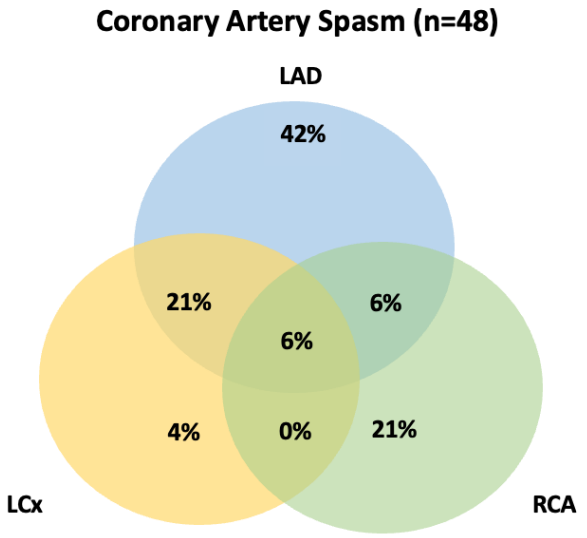
Table 7.6: Summary of acetylcholine dose response during coronary provocation testing

Left coronary artery (n = 38)		Right coronary artery (n = 16)	
Dosage (µg)	No. of CAS	Dosage (µg)	No. of CAS
20	4 (10.5%)	20	5 (31.3%)
50	11 (29.0%)	50	9 (56.3%)
100	19 (50%)	80	2 (13%)
200	4 (10.5%)		

CAS = coronary artery spasm

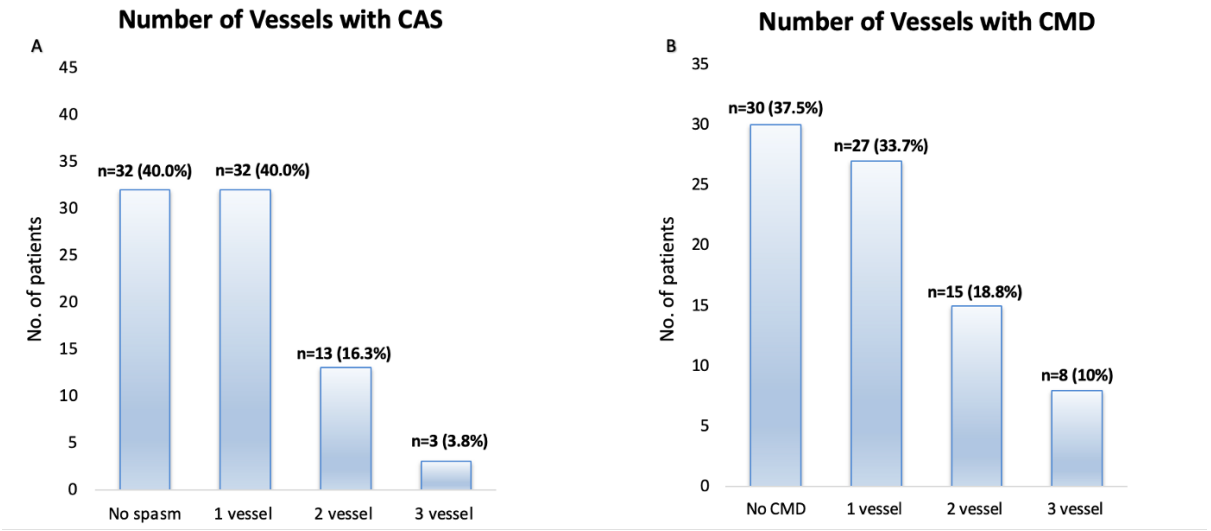
Values n (%)

Figure 7.5: Distribution of epicardial coronary artery spasm across all coronary vessels



LAD = left anterior descending artery, LCx = left circumflex**,** RCA = right coronary artery

Figure 7.6: Prevalence of coronary artery spasm and coronary microvascular dysfunction



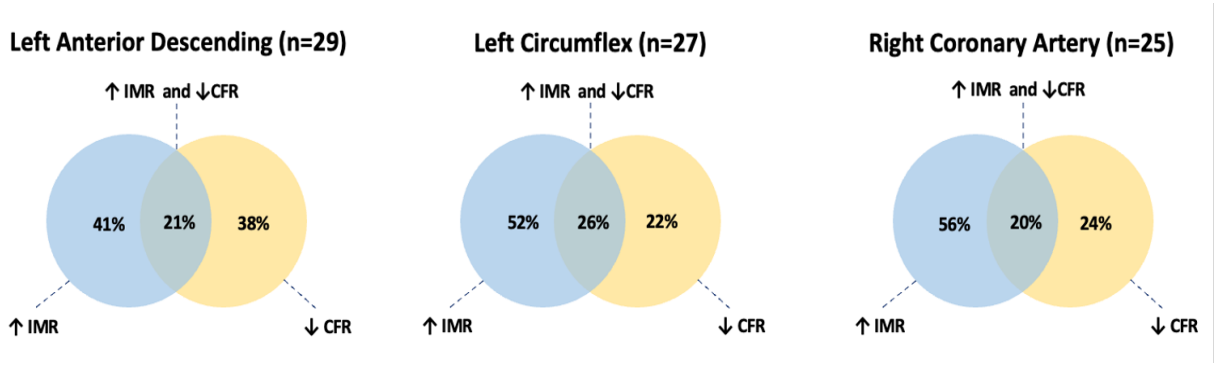
(A) Bar graph illustrating the prevalence of coronary artery spasm across all three coronary vessels; CAS (coronary artery spasm)

(B) Bar graph illustrating the prevalence of coronary microvascular dysfunction across all three coronary vessels; CMD (coronary micro-vascular dysfunction)

7.3.4. Coronary physiology testing

Functional assessment of the coronary microcirculation demonstrated an abnormality in 62.5% (50/80) of the study cohort and 35.5% (81/228) of all vessels. Among the 50 patients who exhibited an abnormal response, 40% (20/50) had an elevated IMR, 30% (15/50) had a reduced CFR and the remaining 30% (15/50) had both. CMD was observed at a similar rate in territories supplied by all three major coronary vessels (LAD = 36.3%, LCx = 33.8%, RCA = 31.3%; P = 0.486). Among the cohort with CMD, 27 patients (33.8%) had one-vessel CMD, 15 patients (18.8%) had two-vessel CMD and 8 patients (10%) had three-vessel CMD [Figure 7.6]. Vessel-specific analysis revealed a higher prevalence of abnormal IMR in the LCx (77.8%) and RCA (76.0%) than in the LAD (62.1%), although not statistically significant (P = 0.361). The presence of abnormal CFR did not differ significantly across the vessels (P = 0.706). The distribution of CMD endotypes across vessels is presented in Figure 7.7.

Figure 7.7: Distribution of coronary microvascular dysfunction across all coronary vessels



(A) Venn diagram illustrating the distribution of coronary microvascular dysfunction in the left anterior descending artery

(B) Venn diagram illustrating the distribution of coronary microvascular dysfunction in the left circumflex artery

(C) Venn diagram illustrating the distribution of coronary microvascular dysfunction in the right coronary artery

CFR = coronary flow reserve, IMR = index of micro-circulatory resistance, LAD = left anterior descending artery, LCx = left circumflex**,** RCA = right coronary artery

7.3.5. Angina and quality of life measures

Compared with non-cardiac chest pain, a statistically significant difference in the SAQSS (P = 0.040) was noted among the ANOCA endotypes. When comparing isolated LAD with non-LAD micro-vascular dysfunction, no significant differences were observed in the SAQSS (P = 0.585) and its individual domains, including angina limitation (P = 0.813), angina stability (P = 0.763), angina frequency (P = 0.974), treatment satisfaction (P = 0.630) and quality of life (P = 0.236). Similar results were observed when comparing isolated left and right CAS. The multi-vessel CMD group displayed a significantly lower angina quality of life score (mean 40 vs 51 units; −11 [95% CI −16 to −4], P = 0.045) than the single-vessel CMD group. When examining single vs multi-vessel CAS, a trend towards greater angina frequency was observed in the multi-vessel CAS group (mean 44 vs 52 units;

−8 [95% CI −14 to −2], P = 0.092), although not statistically significant. The SAQSS did not differ significantly between the CMD endotypes (P = 0.433). A comparison of angina and quality of life measures is summarised in Table 7.7.

Table 7.7: Comparison of angina and quality of life measures as per Seattle Angina Questionnaire

Table 7.7.1: Seattle Angina Questionnaire as per ANOCA endotype vs non-cardiac chest pain

	ANOCA endotype (n = 69)	Non-cardiac chest pain (n = 11)	P-value
Seattle Angina Questionnaire			
Summary	52.9 (11.5)	59.9 (14.1)	0.040
Limitation	64.7 (18.4)	71.6 (20.3)	0.134
Stability	39.1(19.6)	47.7 (19.8)	0.092
Frequency	52.3 (23.0)	60.9 (30.6)	0.142
Satisfaction	64.3 (27.8)	65.1 (27.5)	0.461
Angina QoL	41.3 (14.3)	45.8 (11.6)	0.167

ANOCA = angina with non-obstructive coronary arteries, QoL = Quality of life

Values are mean ± or n (%)

Table 7.7.2: Seattle Angina Questionnaire as per LAD vs non-LAD coronary microvascular dysfunction (CMD)

	LAD CMD (n = 14)	Non-LAD CMD (n = 21)	P-value
Seattle Angina Questionnaire			
Summary	56.5 (6.58)	55.1 (8.62)	0.585
Limitation	68.6 (12.4)	67.3 (18.2)	0.813
Stability	47.7 (13.5)	48.1 (15.3)	0.763
Frequency	51.8 (21.4)	52.1 (22.5)	0.974

Satisfaction	59.1 (24.6)	63.5 (25.9)	0.630
Angina QoL	49.2 (6.93)	45.8 (9.19)	0.236

CMD = coronary micro-vascular dysfunction, QoL = quality of life

Values are mean ± or n (%)

Table 7.7.3: Seattle Angina Questionnaire as per LCA vs RCA coronary artery spasm (CAS)

	LCA CAS (n = 32)	RCA CAS (n = 10)	P-value
Seattle Angina Questionnaire			
Summary	51.9 (13.3)	44.6 (12.2)	0.199
Limitation	65.2 (20.0)	58.9 (17.6)	0.432
Stability	39.7 (17.1)	39.3 (13.4)	0.952
Frequency	49.3 (25.9)	42.9 (13.8)	0.375
Satisfaction	67.0 (28.9)	65.5 (38.6)	0.927
Angina QoL	46.5 (10.1)	41.5 (14.6)	0.382

CAS = coronary artery spasm, QoL = quality of Life

Values are mean ± or n (%)

Table 7.7.4: Seattle Angina Questionnaire as per single- vs multi-vessel coronary microvascular dysfunction (CMD)

	Single-vessel CMD (n = 27)	Multi-vessel CMD (n = 23)	P-value
Seattle Angina Questionnaire			
Summary	55.6 (12.5)	54.7 (11.7)	0.774
Limitation	67.0 (17.3)	66.7 (17.8)	0.954
Stability	40.7 (16.8)	37.1 (17.2)	0.562
Frequency	53.8 (15.2)	52.1 (18.9)	0.741
Satisfaction	64.9 (16.4)	56.5 (18.3)	0.615
Angina QoL	50.8 (13.1)	40.4 (10.3)	0.045

CMD = coronary micro-vascular dysfunction, QoL = quality of life

Values are mean ± or n (%)

Table 7.7.5: Seattle Angina Questionnaire as per single- vs multi-vessel coronary artery spasm

	Single-vessel CAS (n = 33)	Multi-vessel CAS (n = 15)	P-value
Seattle Angina Questionnaire			
Summary	54.3 (13.2)	47.9 (9.21)	0.312
Limitation	64.3 (14.6)	59.9 (13.4)	0.647
Stability	40.5 (14.2)	37.5 (13.0)	0.644
Frequency	52.4 (14.8)	44.4 (12.0)	0.092
Satisfaction	61.5 (22.7)	58.9 (21.6)	0.701
Angina QoL	42.1 (15.8)	38.3 (12.9)	0.308

CAS = coronary artery spasm, QoL = quality of life

Values are mean ± or n (%)

Table 7.7.6: Seattle Angina Questionnaire as per coronary microvascular dysfunction endotypes

	Group 1: abnormal CFR and IMR (n = 15)	Group 2: isolated abnormal CFR (n = 15)	Group 3: isolated abnormal IMR (n = 20)	P-value
Seattle Angina Questionnaire				
Summary	57.9 (10.1)	53.6 (8.64)	54.3 (10.7)	0.433
Limitation	71.3 (16.1)	59.8 (14.6)	68.5 (19.2)	0.157
Stability	39.1 (25.8)	34.7 (20.0)	41.2 (16.8)	0.652
Frequency	54.4 (21.9)	61.4 (24.0)	49.0 (21.3)	0.272
Satisfaction	60.9 (26.5)	72.8 (24.5)	68.7 (24.3)	0.411
Angina QoL	47.9 (11.2)	36.7 (14.7)	45.4 (13.6)	0.055

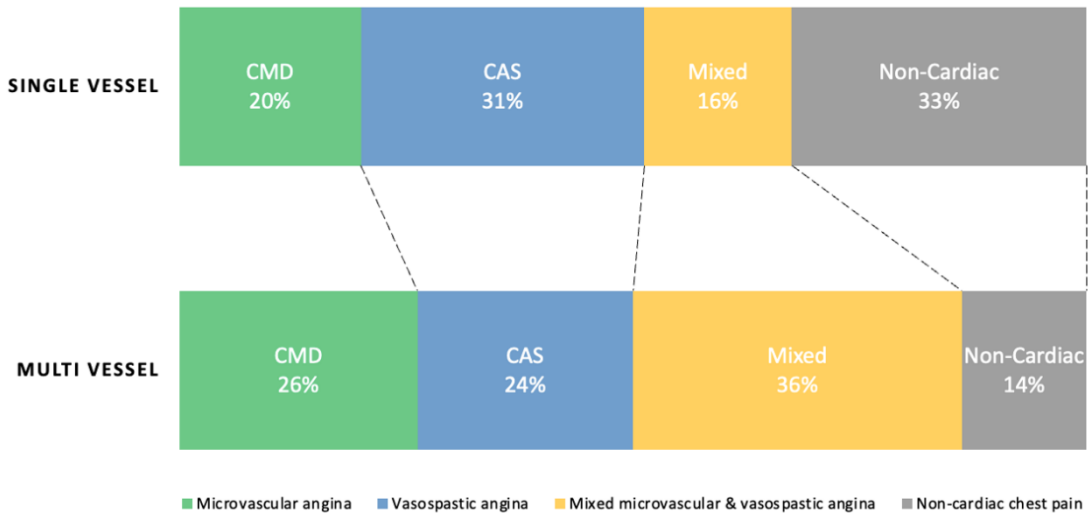
CFR = coronary flow reserve, IMR = index of micro-circulatory resistance

Values are mean ± or n (%)

7.4. Discussion

This study is the first to report the diagnostic value of multi-vessel CFT, encompassing ACh provocation and adenosine-medicating physiology assessment, in patients with ANOCA. Existing research has focused on single-vessel CFT, primarily the left coronary circulation. In contrast, this investigation has highlighted the ability of multi-vessel CFT to identify more patients across different ANOCA endotypes [Figure 7.8]. The findings suggest that regional variations in coronary vasomotion and microvascular function may be present, and single-vessel CFT could result in an under-diagnosis, ultimately affecting clinical management and patient outcomes.

Figure 7.8: Prevalence of ANOCA endotypes, as determined using single- and multi-vessel coronary function testing



CMD = coronary microvascular dysfunction, CAS = coronary artery spasm

7.4.1. Diagnostic impact of multi-vessel CFT in coronary artery spasm

Among the patient cohort, the multi-vessel protocol revealed CAS in 60% (48/80), whereas the single-vessel protocol showed the condition in only 47.5% (38/80). These findings are aligned with those of Di Fiore et al., who reported inducible spasm in 62% of the patients undergoing multi-vessel testing in a similar population cohort [105]. Interestingly, other studies have presented a diverse prevalence of CAS, ranging from 59% to 71% [121,156]. This variability could be attributed to the heterogeneity in current diagnostic protocols, including differences in administration time, dosage regimen and the specific vessels assessed. A previous study by Sueda and Kohno explored the distribution of CAS within specific coronary arteries in a retrospective analysis of 1,392 patients [84]. The results demonstrated that spasms in the LCx (28.3%) were significantly less frequent ($P < 0.001$) than those in the RCA (73.3%) and LAD (72%) [84]. Other studies subsequently validated these observations, implying that the LCx may be less responsive to ACh provocation owing to its smaller myocardial territory [85,86]. These results mirror those of the present study, in which spasm in the LCx was observed in only 31.3% (15/48) of the patients.

Certain studies have avoided provocative testing in the RCA to reduce procedural time and contrast administration [77,83,86,119]. However, such an approach may compromise diagnostic yield and overlook the presence of multi-vessel spasm, a well-known poor prognostic indicator [157]. In the current study, 10 patients (20.8%) exhibited isolated RCA spasms, emphasising the significance of comprehensive multi-vessel testing. As stated by Feenstra et al. [132], the omission of RCA testing could result in overlooked diagnoses, significantly impacting these patients' clinical management and prognosis.

7.4.2. Diagnostic impact of multi-vessel CFT in coronary microvascular dysfunction

Multi-vessel CFT provides greater insights into coronary microcirculation abnormalities than single-vessel CFT. This study revealed that almost two-thirds (62.5%) of the participants who underwent

multi-vessel coronary physiology testing demonstrated an abnormal response. In contrast, only 36.3% who underwent single-vessel testing exhibited abnormalities. These results agree with earlier research that reported prevalence rates ranging from 29% to 44% in single-vessel testing of the coronary micro-vasculature [119,154,158]. Kobayashi et al. conducted a prospective study utilising multi-vessel coronary physiology testing to identify clinical and angiographic predictors of an abnormal IMR [159]. The study observed that only 68% of the patients with CMD were diagnosed in the LAD territory, highlighting that a considerable proportion (32%) were missed in single-vessel testing [159]. Their findings are consistent with those from the present study, which noted a comparable prevalence of CMD in territories supplied by all three major coronary vessels (LAD = 36.3%, LCx = 33.8%, RCA = 31.3%; P = 0.486). The incidence of abnormal CFR, a well-established prognostic marker in this population, did not differ significantly among the three vessels.

Unlike our study, Kobayashi et al. omitted comprehensive CFT, specifically the inclusion of ACh testing. Nevertheless, our collective findings allude that abnormalities of the coronary microcirculation may be regional and conventional CMD testing in a single coronary territory may be limited in its ability to detect abnormalities. This raises important questions about the validity of current methods for assessing CMD and suggests that multi-vessel testing may be more effective in identifying abnormalities in coronary microcirculation.

7.4.3. Clinical relevance of multi-vessel CFT in ANOCA

ANOCA is a complex clinical entity that often poses diagnostic challenges to clinicians. Patients with this condition experience a decreased quality of life, are subjected to repeated coronary angiograms and face an elevated risk of adverse events [1,160]. Identifying ANOCA endotypes precisely is crucial for determining the most appropriate pharmacological therapy for such patients. CCBs are the first-line

drug of choice for patients with epicardial or micro-vascular spasms [161]. Nitrates are preferred as add-on agents for partial or non-responders [162,163]. Beta-blockers, in combination with anti-atherosclerotic therapy, are recommended in CMD [164-168]. The latter involves ACE inhibitors, angiotensin receptor blockers, and statins, which may enhance endothelial function and improve hyperaemic myocardial blood flow [169].

Multi-vessel CFT can considerably increase the proportion of patients diagnosed with CVDys and even alter the initially diagnosed ANOCA endotype, necessitating re-evaluation of the treatment approach. This reassessment is crucial as distinct pathophysiological mechanisms may exist, and current diagnostic methods can misclassify patients. This technique has implications in treating current patients and poses challenges for future therapeutic clinical trials, where inaccurate identification of ANOCA endotypes can confound results. Moreover, multi-vessel CFT can uncover coexisting coronary functional abnormalities, such as enhanced coronary vasoreactivity and elevated micro-vascular resistance, which are associated with a poor prognosis [154]. Interestingly, when evaluating symptoms using the SAQ, no difference in angina burden was observed in patients with CVDys restricted to a non-LAD vessel. The presence of multi-vessel CMD was linked to a lower angina-related quality of life. This finding highlights the significance of multi-vessel CFT in refining the diagnostic process and potentially improving patient outcomes via a more sophisticated therapeutic approach.

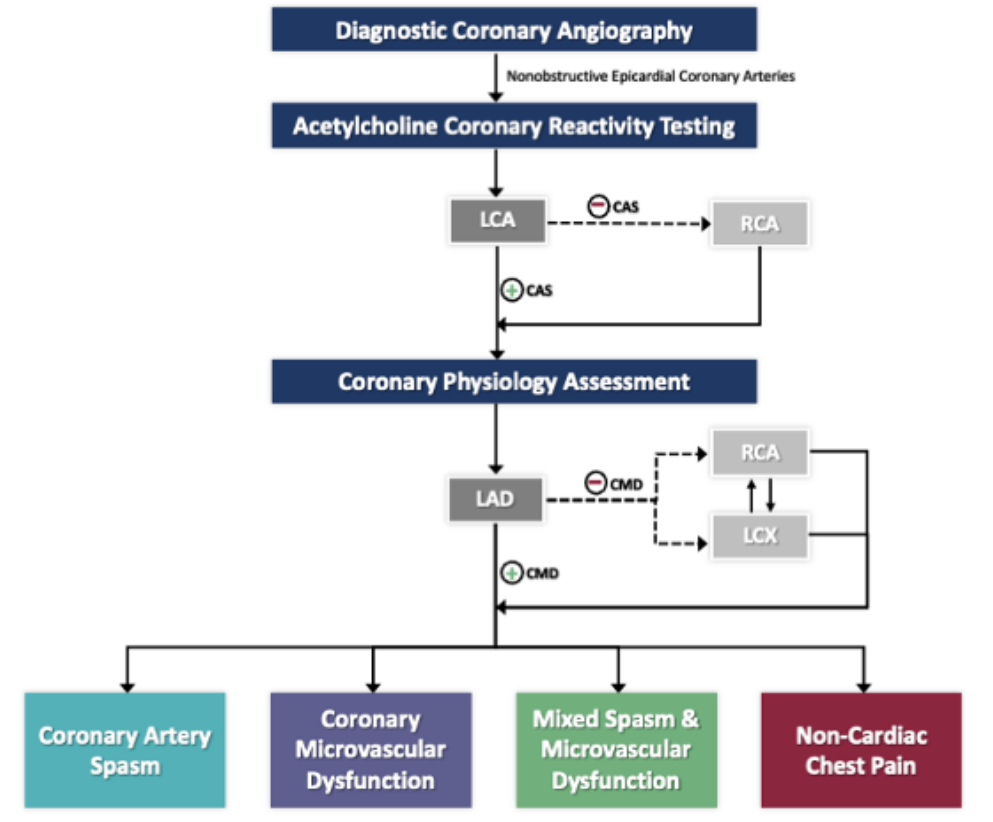
7.4.4. Clinical recommendation

Drawing from our experience and critical evaluation of prior studies, we advocate for multi-vessel CFT in patients with ANOCA (Figure 7.9). We recommend initiating testing in the LAD unless there is a strong clinical suspicion for regional CMD or spasm. Should uncertainty persist after ACh provocation and/or coronary physiology assessment, we suggest testing the contralateral epicardial artery (LCA and/or RCA). This pragmatic approach facilitates precise identification of the underlying

ANOCA endotype, influencing clinical management. During CFT, ACh testing can be performed either before or after coronary physiology assessment as the optimal sequence remains debatable.

Although multi-vessel CFT offers comprehensive diagnostic insights, the potential downsides associated with this approach should be considered. Compared with single-vessel, multi-vessel testing required a longer median procedural time (56 min, [IQR]: 48–62 min vs 40.5 min, [IQR]: 35.5–46 min) and increased contrast administration (155 mL, [IQR]: 135–180 ml vs 110 mL, [IQR]: 90–120 mL). Additionally, one serious adverse event occurred. Hence, careful patient selection and operator expertise are essential for minimising the potential risks of multi-vessel CFT.

Figure 7.9: Invasive diagnostic algorithm for multi-vessel coronary function testing



CAS = coronary artery spasm, CMD = coronary microvascular dysfunction, LAD = left anterior descending artery, LCA = left coronary artery, LCx = left circumflex**,** RCA = right coronary artery

7.4.5. Limitations

The limitations of this study are inherent to the internationally recognised protocols and diagnostic criteria for chronic coronary syndromes. The study population comprised a selectively chosen group of patients referred to expert ANOCA testing centres by their treating physicians, which might have influenced the high rate of abnormalities observed. Nevertheless, the prevalence of abnormalities noted in this study was consistent with that reported in previous literature. Specific testing for endothelial dysfunction using a low-dose, slow ACh infusion was not performed, which could have potentially affected the diagnostic yield of CFT. Given the ease of use, availability and clinician expertise, the thermodilution method was used in coronary physiology assessment. Using Doppler flow velocity or absolute flow measurements might have provided a more accurate assessment [65,170]. ACh rechallenge was not integrated into our protocol, potentially overlooking the presence of coexisting epicardial and microvascular spasm [171]. Owing to the recency of the data, information on the effect of diagnosis and medical therapy on quality of life and angina burden was not available. This unavailability limited the ability to draw definitive conclusions regarding the clinical impact of multi-vessel CFT in the current patient cohort.

7.5. Conclusions

This study suggests that multi-vessel CFT enhances the diagnostic utility in patients with ANOCA compared with single-vessel CFT. Multi-vessel testing identifies more patients with CVDys and facilitates a more precise classification of ANOCA endotypes. This strategy may improve clinicians' ability to determine the appropriate therapeutic strategy in this heterogeneous patient population. Further studies are required to elucidate the prognostic implications of this approach.

Chapter 8: Multi-vessel Compared with Single-vessel Coronary Function Testing in Diagnosing Patients with Non-obstructive Coronary Artery Disease: The MAD-NOCA Trial

8.1. Introduction

ANOCA, including MVA and VSA, affects nearly half of the patients undergoing ICA for suspected ischaemic heart disease [1]. The underlying pathophysiology is coronary vasomotor disorders, resulting in a supply–demand mismatch of myocardial perfusion [10,172,173]. Patients with ANOCA often face a high burden of symptoms, leading to impaired quality of life and recurrent hospitalisations [1,2,4].

Advancements in scientific understanding and diagnostic tools now allow the routine evaluation of ANOCA in the cardiac catheterisation laboratory via invasive CFT. Both the American College of Cardiology/American Heart Association and the ESC guidelines recommend CFT to categorise patients with ANOCA into distinct endotypes [144,174]. This approach facilitates tailored therapy to address the underlying pathology, alleviating the angina burden and improving the quality of life [80].

CFT typically targets a single coronary vessel, often the LAD, as it subtends the largest myocardial territory [63]. Preliminary research by our group demonstrated that single-vessel CFT may lead to an under-diagnosis, potentially overlooking regional variations in coronary vasomotion and micro-vascular function [134]. Therefore, this study aimed to assess the clinical efficacy of multi-vessel CFT in patients diagnosed with ANOCA. We hypothesized that multi-vessel CFT to guide management would improve angina severity and overall quality of life for these patients.

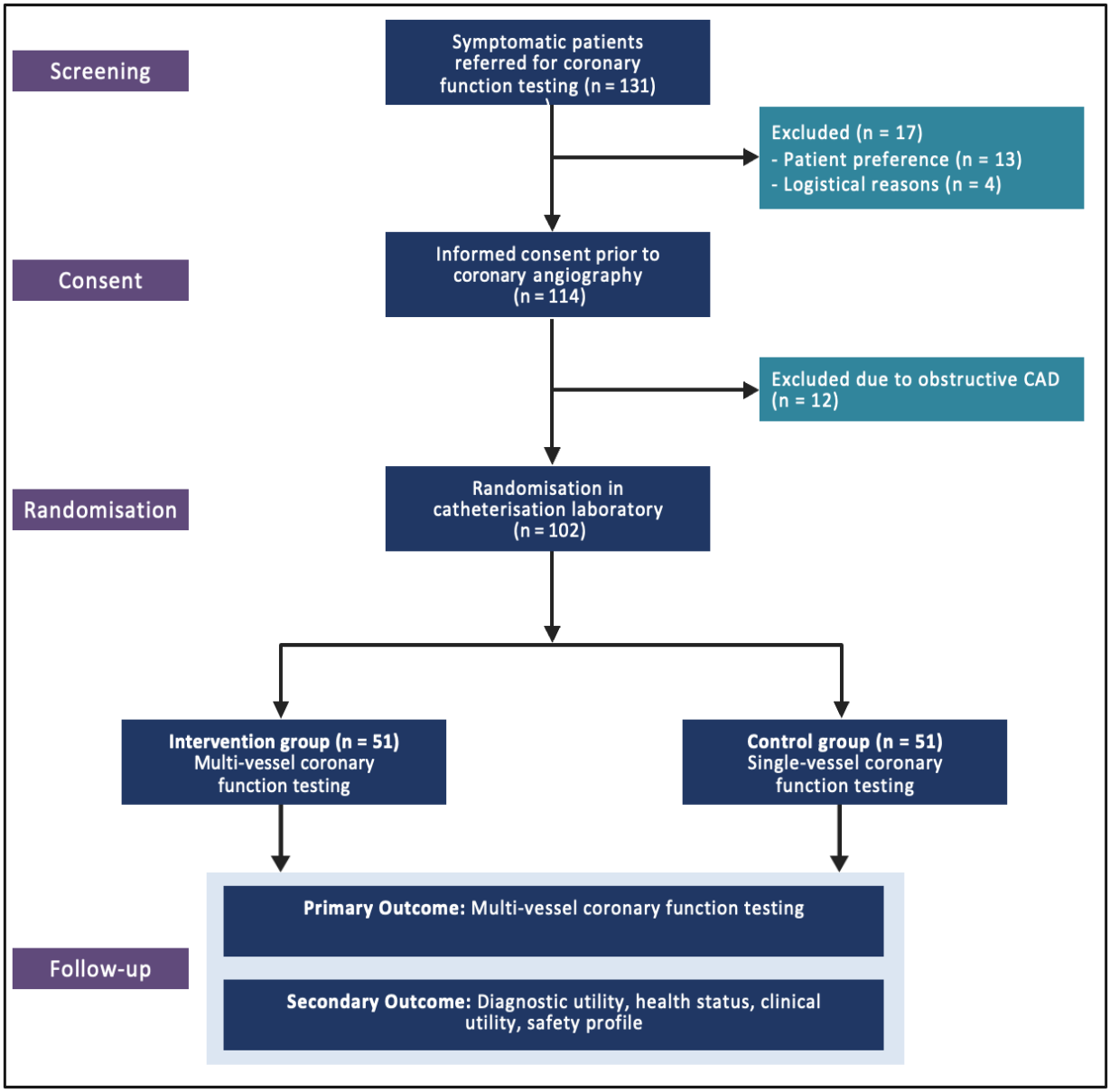
8.2. Methods

This prospective, multi-centre, randomised controlled trial assessed single- vs multi-vessel CFT in patients with ANOCA [Figure 8.1]. All outpatients referred by their treating cardiologists for suspected ANOCA were screened. A non-coronary indication for invasive angiography (e.g. valve

disease), technical factors precluding safe passage of the pressure guidewire, an inability to receive adenosine, a recent (within 3 weeks before cardiac catheterisation) myocardial infarction and/or an inability to provide informed consent were the exclusion criteria. Patients with an unexpected finding of obstructive CAD (>50% diameter stenosis and/or FFR ≤ 0.80) during ICA after obtaining informed consent were also excluded.

Patients were enrolled from the cardiac day unit, and clinical data, including patient demographics, cardiac risk factors, and symptom profiles, were obtained before CFT. Furthermore, the patients were requested to provide details on angina characteristics and quality of life measures using validated questionnaires [175-179]. After enrolment, they were randomly assigned in a 1:1 ratio to either the intervention (multi-vessel CFT) or the control group (single-vessel CFT). Trained staff in the catheterisation laboratory utilised a web-based randomisation tool to immediately randomise the patients after initial coronary angiography revealed non-obstructive CAD. The randomisation sequence employed block lengths randomised in blocks of 4, with every 20 allocations comprising 2 blocks of length 4 and 2 blocks of length 6, arranged in a random order. The patients were considered randomised once the allocation was assigned on the web-based portal. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Human Research Ethics Review Board. Written informed consent was acquired from all participants. RR and ASCY had complete access to the study data and were responsible for their integrity and analysis.

Figure 8.1: Trial profile



CAD = coronary artery disease

8.2.1. Invasive coronary function testing

All CFT procedures were performed in the morning, and the patients were requested to withhold vasoactive medications (e.g. CCBs and long-acting nitrates) and methylxanthine-containing substances for >4 times the duration of the drug's half-life. A continuous 12-lead ECG was monitored throughout the procedure, and standard resuscitation equipment was available. ICA was performed as per standard institutional practice via the radial or femoral route. Administration of intra-arterial vasodilator drugs (e.g. NTG and CCB) was avoided before CFT in all patients. A standard diagnostic ICA was performed to confirm the absence of obstructive CAD. If obstructive CAD was not present, CFT was performed. The first vessel to be tested was left to the operator's discretion. In the intervention group, CFT was performed on the remaining major epicardial vessels. In the case of a small, non-dominant or severely tortuous coronary artery, CFT was avoided for safety reasons.

Coronary vasoreactivity was evaluated via ACh provocation. A 6-French angioplasty guiding catheter without side holes was inserted into either the LCA or the RCA. For the LCA assessment, incremental doses of 20, 50, 100 and 200 μg of ACh were injected over 20 s, with at least a 2-min gap between the doses. After each injection, cine-images were obtained to assess the change in coronary diameter via QCA, as previously described [125]. If CAS was induced, with reproducible symptoms and ST-segment alterations (see definitions below), the provocation test was terminated and concluded to be positive. A similar protocol was adopted for the RCA, with incremental doses of 20, 50 and 80 μg of ACh.

Coronary physiology assessment was conducted using a pressure–temperature sensor guidewire (PressureWire X; Abbott Corporation, Chicago, IL). Following equalisation to guide the catheter pressure, the guidewire was carefully advanced to ensure that the sensor was positioned in the distal third of the vessel and at least 80 mm from the guiding catheter. The assessment included CFR (abnormal <2.5), IMR (abnormal ≥25) and FFR (abnormal ≤0.80) under steady-state conditions of

maximal hyperaemia induced by the intravenous infusion of adenosine (140 µg/kg per min), and a thermodilution bolus technique was applied. Upon completion, the results were documented and disclosed to the patient and the treating cardiologist, who had discretion over final treatment decisions in both study groups.

8.2.2. Definitions of endotypes

CFT was used to categorise patients into ANOCA endotypes, including MVA, VSA or both, according to the diagnostic criteria outlined in the guidelines [5,110,180]. A diagnosis of vasospastic angina required the occurrence of three conditions during ACh: (1) clinically significant epicardial vasoconstriction (≥90%); (2) reproduction of usual chest pain symptoms; and (3) ischaemic changes on ECG [110]. MVA was defined based on standardised COVADIS diagnostic criteria: symptoms of myocardial ischaemia, unobstructed coronary arteries and proven CMD (any of abnormal IMR, CFR or micro-vascular spasm to ACh) [180]. Microvascular spasm was diagnosed when recognisable symptoms were reproduced with ischaemic ECG changes without >90% epicardial diameter reduction in response to ACh. Non-cardiac chest pain was diagnosed in the case of non-obstructive epicardial CAD (FFR > 0.80) and normal CFT (CFR ≥ 2.5, IMR < 25 and negative ACh testing).

8.2.3. Questionnaire and follow-up

The SAQ is a self-administered tool designed to gauge angina severity and is known for its validity, reproducibility and sensitivity to changes [175]. This questionnaire evaluates various aspects such as physical limitations owing to angina, symptom frequency and recent variations, treatment satisfaction and the impact of the disease on overall quality of life. Scores on each scale range from 0 to 100, with higher scores indicating better functioning, such as reduced physical limitations, fewer

angina episodes and enhanced quality of life. The SAQSS amalgamates domains, including angina limitation, frequency and quality of life, to comprehensively measure angina severity.

The health status was assessed using the EQ-5D-5L, a validated self-administered questionnaire widely recognised as a standard tool for evaluating generic quality of life [176]. The EQ-5D-5L scores range from 0.59 to 1.00, with higher scores indicating better quality of life. Furthermore, the Brief Illness Perception Questionnaire (B-IPQ) was used to ascertain patients' perceptions of their illness [177], the PHQ-4 for depression and anxiety screening [178] and the Treatment Satisfaction Questionnaire (TSQM-9) [179]. At 6-month follow-up, patients' angina symptoms and quality of life were re-assessed using the same questionnaires for consistency and comparability.

8.2.4. Outcomes

The primary outcome was angina severity at 6-month follow-up, according to the SAQSS. The following were the pre-specified secondary outcomes:

1. Diagnostic Utility: between-group difference in the diagnosis of ANOCA endotypes
2. Health Status: change from baseline using validated questionnaires, including the EQ-5D-5L, TSQM-9, B-IPQ and PHQ-4, at 6-month follow-up
3. Clinical Utility: impact on medical therapy and downstream investigations
4. Safety profile of multi-vessel CFT

Research staff conducted follow-up assessments for outcomes and adverse events, including in-person visits, telephonic follow-ups and electronic health record reviews. Downstream treatment decisions were made the discretion of the treating physician. Major adverse cardiac and cerebrovascular events, defined as cardiovascular death, non-fatal myocardial infarction,

hospitalisation for heart failure, non-fatal stroke or transient ischaemic attack and resuscitated cardiac arrest, were monitored. Potentially relevant clinical events were assessed by the Clinical Safety Committee, which operated independently of the investigators. This committee, blinded to baseline data and randomised groups, ensured an unbiased evaluation of safety outcomes.

8.2.5. Statistical analysis

The study population included all participants who provided informed consent, and there were no exclusions. Interim analyses were not performed, and trial enrolment was considered complete upon reaching the predetermined recruitment target. Continuous data were represented as mean ± SD or median and IQR, as appropriate. Categorical data were expressed as count (%). Continuous outcome measures recorded at baseline and 6 months were compared between randomised groups using a mixed-effects linear regression model, including random effects for patients and fixed effects for time points (baseline or follow-up), randomised groups and their interaction. The baseline-adjusted intervention effect was estimated as the interaction term from this model. The chi-squared or Fisher's exact test was used, as appropriate, for categorical data analysis. A two-tailed analysis was conducted, and a P-value of <0.05 was considered statistically significant. All analyses were performed using R version 4.2.2 (Vienna, Austria) [127].

To detect a mean group difference of change in SAQSS of 11 units, a sample size of 47 patients per group was calculated to provide 80% power to determine a between-group difference in SAQSS. This calculation assumed a two-tailed 5% significance level and an SD of 19 units, consistent with previous studies such as the CorMICA trial, which observed a change in SAQSS score of 11.7 units (P = 0.001) with CFT-linked medical therapy compared with placebo in patients with ANOCA [80].

8.3. Results

From 15 November 2021 to 12 August 2023, 131 elective patients referred for invasive CFT because of suspected ANOCA were screened. Thirteen patients (9.9%) were excluded owing to patient preference, and four (3%) were excluded for logistical reasons. In total, 114 (87%) patients provided informed consent before undergoing ICA. Among them, obstructive CAD was detected in 12 (9.2%) patients, and the remaining 102 were randomised (n = 51 intervention group; n = 51 control group).

The patient cohort had a mean age of 58.5 ± 0.9 years, with 57.8% of the participants being women. Clinical, laboratory and medication data are listed in Table 8.1. Both groups exhibited a high prevalence of cardiovascular risk factors and the use of preventative and anti-anginal medications. Almost half (48%) had previously undergone ICA. At baseline, the mean SAQ angina frequency score was 60.4 ± 23.5, indicating a frequency of weekly to monthly angina episodes (SAQ frequency score 31–60 denotes weekly angina, whereas 61–99 denotes monthly angina). The mean SAQ angina limitation score was 59.9 ± 20.9, reflecting mild to moderate angina limitation. Over one-third displayed evidence of cardiac ischaemia on non-invasive stress testing (stress echocardiography n = 27 [32.5%], myocardial perfusion scan n = 6 [40%]).

Table 8.1: Patient characteristics

Characteristics	**Randomised**		
	All patients (n = 102)	**Control (n = 51)**	**Intervention (n = 51)**
Age, years	58.5 ± 10.9	58.1 ± 11.7	58.8 ± 10.0
Female	59 (57.8%)	31 (60.8%)	28 (54.9%)
Body mass index, kg/m^2	26.6 ± 3.3	26.2 ± 2.8	27.1 ± 3.7

Ethnicity			
Caucasian	75 (73.5%)	40 (78.4%)	35 (68.6%)
Asian	9 (8.8%)	4 (7.8%)	5 (9.8%)
Southeast Asian	6 (5.9%)	1 (1.96%)	5 (9.8%)
Middle Eastern	11 (10.8%)	6 (11.8%)	5 (9.8%)
Others	1 (1.0%)	0 (0%)	1 (2.0%)
Smoking status, n (%)			
Never	63 (61.8%)	30 (58.8%)	33 (64.7%)
Former	13 (12.7%)	9 (17.6%)	4 (7.%)
Current	26 (25.5%)	12 (23.5%)	14 (27.5%)
Hypertension	50 (49.0%)	23 (45.1%)	27 (52.9%)
Hypercholesterolaemia	58 (56.9%)	27 (52.9%)	31 (60.8%)
Diabetes	16 (15.7%)	6 (11.8%)	10 (19.6%)
Overweight	61 (59.8%)	31 (60.8%)	30 (58.8%)
Family history of CAD	37 (36.3%)	22 (43.1%)	15 (29.4%)
Peripheral vascular disease	6 (5.9%)	3 (5.9%)	3 (5.9%)
CAD	10 (9.8%)	6 (11.8%)	4 (7.8%)
Previous invasive coronary angiogram	49 (48.0%)	25 (49.0%)	24 (47.1%)
Cerebrovascular accident	2 (2.0%)	0 (0%)	2 (3.9%)
Obstructive sleep apnoea	13 (12.7%)	4 (7.8%)	9 (17.6%)
Excessive alcohol	8 (7.8%)	2 (3.9%)	6 (11.8%)
Excessive caffeine intake	12 (11.8%)	4 (7.8%)	8 (15.7%)
Migraine	27 (26.5%)	13 (25.5%)	14 (27.5%)
Chronic pain syndrome	24 (23.5%)	11 (21.6%)	13 (25.5%)
Preventative therapy, n (%)			
Aspirin	49 (48.0%)	25 (49.0%)	24 (47.1%)
ACE-I/ARB	30 (29.4%)	15 (29.4%)	15 (29.4%)
Statin	66 (45.1%)	22 (43.1%)	24 (47.1%)
Angina therapy, n (%)			
Beta-blocker	38 (37.3%)	16 (31.4%)	22 (43.1%)
Calcium channel blockers	24 (23.5%)	13 (25.5%)	11 (21.6%)
Nitrates	19 (18.6%)	8 (15.7%)	11 (21.6%)

Nicorandil	10 (9.8%)	6 (11.8%)	4 (7.8%)
Laboratory			
Total cholesterol	3.96 ± 0.73	3.93 ± 0.63	3.97 ± 0.80
LDL	1.89 ± 0.69	1.82 ± 0.73	1.93 ± 0.67
HDL	1.35 ± 0.37	1.40 ± 0.31	1.31 ± 0.41
Triglycerides	1.42 ± 0.55	1.33 ± 0.55	1.49 ± 0.55
Hba1c	5.43 ± 0.58	5.19 ± 0.69	5.61 ± 0.42
CCS angina class:			
1	32 (31.4%)	18 (35.3%)	14 (27.5%)
2	37 (36.3%)	16 (31.4%)	21 (41.2%)
3	27 (26.5%)	12 (23.5%)	15 (29.4%)
4	4 (3.9%)	3 (5.9%)	1 (1.96%)
Angina pattern:			
Predominately rest	32 (31.4%)	17 (33.3%)	15 (29.4%)
Predominately exertion	27 (26.5%)	16 (31.4%)	11 (21.6%)
Mixed	43 (42.2%)	18 (35.3%)	25 (49.0%)
Circadian pattern:			
Predominately daytime (7 am–7 pm)	36 (35.3%)	19 (37.3%)	17 (33.3%)
Predominately nocturnal (7 pm–7 am)	19 (18.6%)	9 (17.6%)	10 (19.6%)
Throughout the day	47 (46.1%)	23 (45.1%)	24 (47.1%)
Seattle Angina Questionnaire			
Summary	55.9 ± 15.8	56.4 ± 15.1	55.3 ± 16.7
Limitation	59.9 ± 20.9	58.8 ± 21.9	61.1 ± 20.1
Stability	48.4 ± 23.1	51.3 ± 23.3	45.6 ± 22.8
Frequency	60.4 ± 23.5	61.7 ± 22.7	59.2 ± 24.5
Satisfaction	67.0 ± 24.1	65.7 ± 21.4	68.4 ± 26.6
Angina QoL	41.1 ± 19.2	40.3 ± 18.8	42.0 ± 19.6
Quality of life (EQ-5D-5L)			
Index score	0.58 ± 0.22	0.59 ± 0.22	0.57 ± 0.2
VAS score	62.0 ± 14.2	62.8 ± 15.6	61.2 ± 12.7
Treatment satisfaction (TSQM-9)			
Effectiveness	55.0 ± 15.6	55.4 ± 15.9	54.5 ± 15.4

Convenience	61.9 ± 15.0	61.8 ± 15.3	62.0 ± 14.8
Global satisfaction	54.6 ± 13.0	55.5 ± 14.5	53.8 ± 1.4
Psychological distress (PHQ-4)			
Total score	4.11 ± 3.11	4.06 ± 2.79	4.16 ± 3.44
Illness perception (B-IPQ)			
Total score	47.2 ± 9.10	48.1 ± 8.72	46.2 ± 9.50
Stress echocardiography			
Normal	50 (60.2%)	23 (56.1%)	27 (64.3%)
Inconclusive	6 (7.2%)	4 (9.8%)	2 (4.8%)
Abnormal	27 (32.5%)	14 (34.1%)	13 (31.0%)
Radionuclide myocardial perfusion			
Negative or inconclusive	9 (60.0%)	6 (66.7%)	3 (50.0%)
Abnormal	6 (40.0%)	3 (33.3%)	3 (50.0%)

ACE-I = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, CAD = coronary artery disease,

Values are mean ± or n (%)

Most ICAs were performed using radial arterial access (75.5%). In the intervention group, the median procedural time was 53 min (IQR: 50–61), compared with 36 min (IQR: 32–41.5) in the control group. Similarly, the median total volume of contrast used was higher at 140 min in the intervention group (IQR: 120–160) compared with 100 min (IQR: 90–110) in the control group. The LAD was the target vessel in 94% (48/51) of the patients in the control group and 96% (49/51) in the intervention group. A total of 145 (94.8%) vessels were interrogated in the intervention group. Across all randomised patients, the median FFR in the LAD was 0.90 (IQR: 0.87–0.93), indicative of non-obstructive CAD. Of the 102 patients, 35 (34.3%) had isolated MVA, 22 (21.6%) had isolated VSA and 27 (26.5%) had mixed (both) disease. Coronary vasomotor disorder was absent in 18 (17.6%) patients. The procedural characteristics are summarised in Table 8.2.

Table 8.2: Procedural characteristics

	All patients (n = 102)	Control (n = 51)	Intervention (n = 51)
Final access site			
Radial	77 (75.5%)	38 (74.5%)	39 (76.5%)
Femoral	25 (24.5%)	13 (25.5%)	12 (23.5%)
Intra-procedural details			
Total procedure time (min)	45.0 (35.2, 53.8)	36.0 (32, 41.5)	53.0 (50, 61)
Fluro time (s)	493 (381, 687)	395 (297, 456)	673 (546, 801)
Total contrast (mL)	118 (100, 150)	100 (90, 110)	140 (120, 160)
Angiographic features			
Angiographically normal	40 (39.2%)	19 (37.3%)	21 (41.2%)
Acetylcholine testing			
Epicardial spasm	49 (48%)	20 (39.2%)	29 (56.9%)
Diffuse spasm	32 (31.4%)	13 (25.4%)	19 (37.3%)
Focal spasm	17 (16.7%)	7 (13.7%)	10 (19.6%)
Microvascular spasm	8 (7.8%)	2 (3.9%)	5 (9.8%)
Invasive physiology*			
LAD target artery	95 (93.1%)	48 (94.1%)	47 (92.2%)
Resting transit time, s	0.82 (0.62, 1.15)	0.83 (0.62, 1.11)	0.81 (0.63, 1.19)
Hyperaemic transit time, s	0.25 (0.13, 0.36)	0.23 (0.11, 0.40)	0.25 (0.16, 0.34)
RFR	0.93 (0.91, 0.95)	0.93 (0.91, 0.95)	0.92 (0.90, 0.95)
FFR	0.90 (0.87, 0.93)	0.90 (0.88, 0.94)	0.91 (0.86, 0.93)
Index of micro-circulatory resistance	18.0 (12.0, 29.0)	18.0 (12.2, 31.0)	18.0 (12.0, 24.0)
Coronary flow reserve	3.25 (2.38, 4.73)	3.35 (2.30, 4.77)	3.25 (2.52, 4.65)
Endotypes			
MVA	35 (34.3%)	17 (33.3%)	18 (35.3%)
VSA	22 (21.6%)	11 (21.6%)	11 (21.6%)
Mixed	27 (26.5%)	9 (17.6%)	18 (35.3%)
Non-cardiac	18 (17.6%)	14 (27.5%)	4 (7.8%)
Complications			
Atrial fibrillation	10 (9.8%)	3 (5.9%)	5 (9.8%)
Coronary dissection	2 (2.5%)	1 (2%)	1 (2%)

*First vessel tested

FFR = fractional flow reserve, MVA = microvascular angina, RFR = resting flow reserve, VSA = vasospastic angina

Values are median and IQR or n (%)

8.3.1. Primary outcome

All enrolled patients (n = 102) completed the primary outcome assessment (SAQ). At 6-month follow-up, the SAQSS was not significantly different between the randomised groups. At 6 months, the SAQSS in the intervention and control groups were 66.3 ± 14.6 (change of 10.9 ± 13.9 from baseline) vs 64.3 ± 11.5 (change of 7.9 ± 13.5 from baseline), with no significant difference (P = 0.260) [Table 8.3]. This finding was consistent across all SAQ domains. Stratification by ANOCA endotype revealed no difference in the SAQSS outcome between the randomised groups.

8.3.2. Secondary outcome

8.3.2.1. Diagnostic utility

The intervention yielded a significantly greater prevalence of coronary vasomotor disorders in the patient cohort than in the control group (92.2% vs 72.5%, P = 0.019) [Table 8.4]. Across ANOCA endotypes, the intervention demonstrated a higher prevalence of mixed disease (35.3% vs 17.6%, P = 0.043). However, no significant differences were observed in the incidences of isolated MVA (35.3% vs 33.3%, P = 1.00) and VSA (21.6% vs 21.6%, P = 1.00). Multi-vessel CFT modified the diagnosis in 35.3% (18/51) of the patients in the intervention group.

8.3.2.2. Health status

Health-related quality of life (as assessed using the EQ-5D-5L questionnaire) at 6 months was higher in the intervention arm (utility index score 0.095; 95% CI: 0.01–0.18; P = 0.036, visual analogue score 7.6; 95% CI: 1.1–14.0; P = 0.023). Treatment satisfaction for the convenience domain of TSQM-9 demonstrated a between-group difference of 8.8 points (95% CI: 2.0–15.5; P = 0.012). For the global satisfaction domain, the between-group difference was 7.4 points (95% CI: 0.7–14.1; P = 0.032). Significant differences were not observed in the illness perception (B-IPQ, P = 0.789) or psychological distress levels (PHQ-4, P = 0.708).

8.3.2.3. Clinical utility

Physicians were more inclined to include anti-anginal therapy in the intervention arm than in the control arm (84.3% vs 58.8%, P = 0.008). There was no difference in the use of preventative therapy between the groups at the 6-month mark (66.7% vs 60.8%, P = 0.680). In the control group, physicians were more likely to refer for non-cardiovascular investigations following CFT (23.5% vs 9.8%, P = 0.031). Physician inclination towards downstream cardiovascular-specific investigations did not differ (P = 0.603).

8.3.2.4. Safety profile

Adverse events related to coronary function testing

Although no fatal adverse events were reported during CFT, two serious adverse events were observed, one in each group. Both incidents entailed guiding catheter-induced coronary dissections that necessitated percutaneous coronary intervention. One of the incidents resulted from multi-vessel testing, and the other occurred from single-vessel testing. ACh provocation-induced atrial fibrillation happened in 5.9% (3/51) of the patients in the control group and 9.8% (5/51) in the intervention group. In the latter group, two patients required DC cardioversion and were discharged

without prolonged hospital stay. The remaining cases of atrial fibrillation resolved spontaneously within 30 min, with no need for treatment or consequent thromboembolic events.

Adverse events following coronary function testing

All participants' vital status at 6 months was obtained via face-to-face consultations, teleconsultations and verification of electronic health records. MACE (5.9% in the control group vs 3.9% in the intervention group; P = 1.00) or unplanned hospitalisations owing to angina (13.7% in the control group vs 7.8% in the intervention group; P = 0.52) did not differ significantly throughout the 6-month follow-up period. Furthermore, no mortality events occurred during this duration.

Table 8.3: Primary outcome and changes in health status at 6 months

	Control (n = 51)		**Intervention (n = 51)**		**Intervention effect***		
	6 months	**Change from baseline**	**6 months**	**Change from baseline**	**Estimate**	**95% CI**	**P-Value**
Primary efficacy endpoint – Seattle Angina Questionnaire							
Angina summary score	64.3 ± 11.5	7.87 ± 13.5	66.3 ± 14.6	10.9 ± 13.9	3.067	–8.369 to 2.236	0.26
Angina limitation	67.0 ± 14.8	8.20 ± 21.5	69.8 ± 15.5	8.66 ± 24.2	0.462	–9.342 to 8.417	0.919
Angina stability	55.9 ± 20.4	4.61 ± 24.5	58.4 ± 23.4	12.8 ± 28.0	8.235	–18.426 to 1.956	0.117
Angina frequency	69.2 ± 18.9	7.55 ± 23.6	68.3 ± 21.4	9.12 ± 23.6	1.569	–10.717 to 7.58	0.738
Angina treatment satisfaction	71.2 ± 20.1	5.56 ± 29.1	74.1 ± 20.6	5.76 ± 28.6	0.204	–11.392 to 10.983	0.972
Angina QoL	53.0 ± 15.4	12.8 ± 18.7	52.7 ± 21.7	10.7 ± 23.0	-2.087	–6.033 to 10.207	0.616
Secondary efficacy endpoint – Health status							

Index score	0.60 ± 0.09	0.02 ± 0.25	0.68 ± 0.10	0.11 ± 0.19	0.095	0.008 to 0.183	0.036
VAS score	65.5 ± 12.1	2.65 ± 18.2	71.4 ± 11.5	10.2 ± 14.9	7.588	1.136 to 14.04	0.023
Illness perception (B-IPQ)	45.5 ± 8.02	–2.72 ± 8.40	43.1 ± 9.55	–3.27 ± 12.3	-0.559	–3.529 to 4.646	0.789
Psychological distress (PHQ-4)	3.63 ± 2.05	–0.43 ± 3.32	3.53 ± 2.29	–0.71 ± 4.03	-0.275	–1.158 to 1.707	0.708
Effectiveness	57.6 ± 10.7	2.17 ± 16.5	63.0 ± 16.1	8.51 ± 20.8	6.336	0.952 to 13.623	0.092
Convenience	62.4 ± 10.1	0.65 ± 16.2	71.5 ±15.0	9.43 ± 18.5	8.779	2.038 to 15.52	0.012
Global satisfaction	57.3 ± 9.74	1.82 ± 15.2	63.0 ± 16.3	9.24 ± 19.0	7.423	0.747 to 14.098	0.032

Values mean ± or n (%) unless otherwise indicated.
*Adjusted mean difference at 6 months (intervention–control). Seattle Angina Questionnaire (SAQ): lower scores represent worse angina symptoms. Illness perception: a lower score reflects a less threatening view of the illness. Patient Health Questionnaire-4 (PHQ4) is a four-item brief screening tool for anxiety and depression; higher scores indicate more psychological distress. VAS is a visual analogue score of EQ-5D validated quality of life tool, and higher scores indicate a better quality of life. B-IPQ = Brief Illness Perception Questionnaire

Table 8.4: Secondary outcomes

	All patients (n = 102)	**Control (n = 51)**	**Intervention (n = 51)**	**P-value**
Clinical events				
Major cardiovascular events	5 (4.9%)	3 (5.9%)	2 (3.9%)	1.000
All-cause death	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Cerebrovascular event	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Heart failure hospitalisation	1 (1%)	1 (2.0%)	0 (0.0%)	1.000
Non-fatal myocardial Infarction:	4 (3.9%)	2 (3.9%)	2 (3.9%)	1.000
Hospitalisation for angina	11 (10.8%)	7 (13.7%)	4 (7.8%)	0.523
Clinical utility				
Preventative therapy	63 (61.8%)	31 (60.8%)	32 (62.7%)	1.000

Anti-anginal therapy	73 (71.6%)	30 (58.8%)	43 (84.3%)	0.008
Change in medical therapy	53 (52.0%)	33 (64.7%)	20 (39.2%)	0.017
Additional cardiovascular tests	4 (3.9%)	3 (5.9%)	1 (2%)	0.603
Additional non-cardiovascular tests	17 (16.7%)	12 (23.5%)	5 (9.8%)	0.031
Diagnostic utility				
Coronary vasomotor disorder	84 (82.4%)	37 (72.5%)	47 (92.2%)	0.019
MVA	35 (34.3%)	17 (33.3%)	18 (35.3%)	1.000
VSA	22 (21.6%)	11 (21.6%)	11 (21.6%)	1.000
Mixed	27 (26.5%)	9 (17.6%)	18 (35.3%)	0.043
Non-cardiac	18 (17.6%)	14 (27.5%)	4 (7.84%)	0.019

MVA = microvascular angina, VSA = vasospastic angina

Values are mean ± or n (%)

8.4. Discussion

In this randomised controlled trial, compared with single-vessel, multi-vessel CFT did not significantly improve the angina burden despite its superior diagnostic ability to identify the cause of angina. However, the intervention was associated with improved health-related quality of life and treatment satisfaction. Patients in the control group were more often referred for downstream investigations, likely due to a diagnosis of non-cardiac chest pain.

Participants were enrolled from four hospitals across Australia over a 20-month period. For the primary efficacy endpoint, the overall treatment effect of the intervention—a 6-month difference in angina burden—was not significant (3U, 95% CI: −8.37 to 2.24; P = 0.26). Several factors may account for the absence of a significant between-group improvement.

First, effective disease-modifying therapies are unavailable for patients with ANOCA. Current treatment recommendations lack robust evidence, with many supported by small-scale studies demonstrating varying degrees of efficacy [181]. Several of these studies did not differentiate between ANOCA sub-types, which is crucial for effectively managing these patients [182,183]. Despite the widespread use of anti-anginal agents such as beta-blockers and CCBs as first-line treatments, evidence is limited, particularly in patients with CMD [181]. Clinicians have limited options, as demonstrated in our cohort, where all patients diagnosed with an ANOCA endotype (n = 84) were on either a beta-blocker (n = 35) or a CCB (n = 49) at 6-month follow-up. Moreover, Sinha et al. showed that patients with an impaired CFR benefit the most from anti-ischaemic therapy [184]. The comparable prevalence of impaired CFR between the groups (45.1% vs 31.4%, P = 0.154) may explain the lack of improvement in angina burden across both cohorts.

Second, the treating cardiologist retained discretion over final treatment decisions. A recent survey highlighted the considerable heterogeneity among practising cardiologists regarding prescribed treatments, with one in three reporting a neutral sentiment or lack of confidence in managing patients with ANOCA [185]. Despite advancements, the optimal management of these patients remains challenging owing to the variability in underlying mechanisms and the potential for concurrent disorders. Many physicians face challenges in optimising the pharmacological therapy for these patients. Ideally, those with ANOCA should receive collaborative care from their referring physician and experts at specialised ANOCA centres, where the necessary expertise in managing this complex condition is available [181]. A personalised strategy involving sequential initiation of treatments, with careful titration every few weeks, is often necessary to evaluate therapeutic effectiveness and ensure patient tolerance and compliance. This task becomes difficult when patients have concurrent disorders that require navigating complex treatment regimens and considerations. The higher incidence of mixed disease in the intervention compared with the control group (35.3% vs 17.6%)

might have complicated matters for treating physicians, mainly if they were unfamiliar with managing concurrent ANOCA endotypes.

Third, because of potential limitations in experience, expertise and local availability, several second- and third-line pharmacological therapies for patients experiencing refractory symptoms were under-utilised. At 6-month follow-up, only two patients were prescribed cilostazol, and none received prescriptions for ranolazine, ivabradine or cyproheptadine. Although evidence supporting the efficacy of these therapies is limited, they have demonstrated some effectiveness in clinical practice [183,186-189].

Despite the lack of objective improvement in angina status, multi-vessel CFT significantly enhanced health-related quality of life and treatment satisfaction at 6-month follow-up. The effect of receiving a definitive diagnosis presumably alleviated the uncertainty and provided validation to a cohort that usually experiences repeated presentations to multiple medical providers. Furthermore, a diagnosis followed by physician-guided therapy likely served as a powerful motivator for patients to adopt lifestyle modifications and adhere more closely to medication regimens. Consistent with recent trials, obtaining a diagnosis was associated with reduced downstream investigations, namely non-cardiovascular related, alleviating patient burden and curbing healthcare costs linked to unnecessary repetitive testing.

8.4.1. Clinical relevance of multi-vessel CFT in ANOCA

Considering clinical implications, multi-vessel CFT increased the proportion of patients diagnosed with an ANOCA endotype. Moreover, it established the presence of coexisting coronary functional abnormalities, such as enhanced coronary vasoreactivity and elevated microvascular resistance,

which are indicative of a poor prognosis [120]. In addition, this intervention was associated with improvements in health-related quality of life and treatment satisfaction but not angina burden. An equal number of serious adverse procedural events occurred in both groups. The intervention group exhibited longer median procedural times and increased contrast administration. Therefore, clinicians should consider adjunctive CFT in contralateral epicardial arteries if uncertainty persists after single-vessel testing.

Notably, two guide catheter–induced dissections occurred (2%), exceeding contemporary rates for routine coronary angiography. Risk may be minimised by strict coaxial, shallow engagement, avoidance of pressure damping, and use of less aggressive guide shapes. Mechanistically, ANOCA patients may exhibit heightened vasomotor reactivity and vulnerable intimal surface, potentially increasing susceptibility to ostial injury though this hypothesis merits further evaluation. This reinforces that careful patient selection and the operator's expertise are vital to mitigate potential risks of such procedures.

8.4.2. Limitations

First, this study adhered to binary cut-offs for CFT, aligning with internationally recognised protocols and diagnostic criteria for chronic coronary syndromes. Given the intricate pathophysiology of these conditions, indeterminate (borderline) test results might have been misclassified. Second, while blinding patients to group allocation would have reduced the bias, our local ethics committee precluded this approach. Third, our reliance on patient-reported outcome measures, although valuable, introduces susceptibility to bias, given that patients and cardiologists were aware of group allocations. Incorporating objective measures of functional capacity or conducting repeated CFT assessments could enhance the study's robustness; however, the associated patient burden and increased risk from repeated invasive testing warrant caution. Fourth, endothelial dysfunction was not specifically tested using a low-dose, slow ACh infusion, which could have potentially affected the

diagnostic yield of CFT. Similarly, intra-vascular imaging to identify myocardial bridging was not performed due to the considerable demands on the study participants. Lastly, the study's duration might have limited the assessment of long-term outcomes, including the sustained impact of multi-vessel CFT on angina burden and clinical events.

8.5. Conclusions

In patients with ANOCA, multi-vessel CFT did not influence the angina burden despite its increased yield in diagnosing the cause of the condition. Nonetheless, multi-vessel CFT improved health-related quality of life and treatment satisfaction. This observation highlights the need for more effective therapy in patients with ANOCA.

Chapter Nine: Conclusions and Future Directions

ANOCA encompasses a heterogeneous group of pathologies that often result in recurrent angina, poor quality of life, frequent hospitalisations and adverse cardiovascular outcomes. Despite its clinical significance, the diagnosis and management of ANOCA remain challenging and require more nuanced approaches beyond conventional coronary assessments. This thesis investigated the diagnostic and prognostic value of invasive CFT in ANOCA via a series of multi-centre prospective studies. It explored the prevalence of coronary vasomotor disorders, evaluated the diagnostic accuracy of CFT protocols and assessed the impacts of these diagnostic procedures on patient health status. The findings aimed to refine diagnostic strategies, enhance patient care and improve long-term management for patients with ANOCA, offering valuable insights for clinical practice and future research.

Chapter three investigated the prevalence and predictive factors of CVDys in an Australian cohort of patients with ANOCA, utilising invasive CFT. Among 110 patients, MVA was identified in 31.8%, VSA in 25.5% and a mixed presentation of MVA and VSA in 24.5%. Patients with CVDys were older (59 ± 11 vs 51 ± 15 years, P = 0.024), more likely to be overweight (61.1% vs 15.0%, P < 0.001) and reported a lower quality of life (EQ-5D-5L; 0.61 vs 0.67, P = 0.043). MVA was significantly associated with being overweight (OR, 4.2; 95% CI, 1.9–9.3; P = 0.015) and ischaemia on stress testing (OR, 2.4; 95% CI, 1.1–4.3; P = 0.028), whereas VSA was strongly linked to smoking (OR, 9.1; 95% CI, 2.21–39.3; P = 0.007). These findings underscore the high prevalence of CVDys in patients with ANOCA and highlight the crucial role of CFT in their diagnostic evaluation.

Chapter four evaluated the diagnostic validity of current ACh provocation protocols, focusing on a maximum dose of 200 μg for the LCA and 80 μg for the RCA in diagnosing CAS. This multi-centre,

prospective observational study included 82 patients, encompassing those with suspected ANOCA and those referred for ICA for non-coronary indications. Among patients with inducible spasm on ACh provocation testing, 93.4% (61/65) had typical angina consistent with CAS. The conventional ACh dosage regimen (up to 100 μg in the LCA and 50 μg in the RCA) yielded a diagnostic rate of 67.1% (55/82). The higher dosage regimen 200 μg in the LCA and 80 μg in the RCA) led to an increased detection of symptomatic CAS (n=61) but was associated with a higher incidence of false positives (n = 4) compared to the conventional doses (n = 1). While higher ACh doses significantly improves sensitivity, there is a trend toward reduced specificity suggests a higher likelihood of false-positive results. These results suggest this approach should be reserved for patients with a high clinical suspicion of CAS, and results should be interpreted within the broader clinical context

Chapter five assessed the diagnostic effect of prior intra-vascular NTG administration on ACh provocation testing. This study, conducted across two tertiary referral centres, included 102 patients, 40 of whom were diagnosed with epicardial CAS and underwent an ACh rechallenge. To determine the re-inducibility of epicardial spasm, ACh was re-administered at 5-min intervals into the affected coronary artery in patients who initially tested positive. The results indicated that the sensitivity of ACh provocation testing decreased to 30% at 5 min and to 55% at 10 min post-NTG administration. Furthermore, a time-dependent reduction in MLD was observed, with a 47% (±0.30) decrease at 5-min post-NTG, which increased to 64% (±0.24) at 10 min. These findings suggest that clinicians should avoid NTG administration before ACh provocation testing or at least delay testing for greater than 10 min post-NTG to improve diagnostic accuracy.

Chapter six determined the incidence of back-up pacing during ACh provocation testing and identified potential predictive factors in patients with ANOCA suspected of CAS. A TTP was inserted

into the right ventricle via the femoral vein before ACh testing, with activation triggered by a prolonged sinus pause (>5 s) or profound bradycardia (<30 bpm for >30 s). During ACh provocation of the LCA, 25.5% of the patients required back-up pacing, with a higher incidence in those with left coronary dominance (42.3% vs 11.8%, P = 0.001) and at higher ACh doses. For the RCA, 61.7% of the patients required pacing, which was significantly more frequent in those with baseline sinus bradycardia than those without the condition (25.9% vs 5.2%, P = 0.036). These findings support the use of prophylactic TTP during ACh provocation testing of the RCA, especially in patients with baseline sinus bradycardia. For LCA testing, clinicians should consider TTP placement in patients with left coronary dominance or when administering high doses of ACh.

Chapter seven examined the diagnostic yield of multi-vessel vs single-vessel invasive CFT in patients with ANOCA. This multi-centre, prospective observational study included 228 vessels from 80 patients. Multi-vessel CFT resulted in more patients being diagnosed with CVDys (86.3% vs 68.8%, P = 0.0005), CAS (60.0% vs 47.5%, P = 0.004) and CMD (62.5% vs 36.3%, P < 0.001) compared with single-vessel testing. Among patients with CAS (n = 48), spasms predominantly affected the left coronary system (n = 38), with isolated right coronary spasms occurring in 20.8% of the patients. CMD was observed in 62.5% of the cohort, affecting one vessel in 33.8%, two in 18.8%, and three in 10%. These findings demonstrate that compared with single-vessel testing, multi-vessel CFT provides a higher diagnostic yield in patients with ANOCA. This observation suggests that multi-vessel CFT has a role in managing patients with ANOCA.

Chapter eight explored whether multi-vessel, compared with single-vessel, CFT would improve health outcomes in patients with ANOCA. In this multi-centre, randomised controlled trial, patients were randomised 1:1 to either the intervention (multi-vessel CFT) or the control group (single-vessel CFT). The primary outcome was the mean difference in angina severity at 6 months, assessed using

the SAQSS. Secondary outcomes included diagnostic utility, health-related quality of life and treatment satisfaction. A total of 102 participants were randomised (51 in each group). Multi-vessel CFT resulted in a higher diagnosis rate of CVDys than single-vessel testing (92.2% vs 72.5%, P = 0.019). Nonetheless, at 6 months, there was no significant difference in SAQSS between the groups (change from baseline: 10.9 ± 13.9 vs 7.9 ± 13.5; P = 0.260). Multi-vessel CFT was linked to significant improvements in health-related quality of life (EQ-5D-5L utility index score 0.095; 95% CI: 0.01–0.18; P = 0.036, visual analogue score 7.6; 95% CI: 1.1–14.0; P = 0.023) and global treatment satisfaction (an increase of 7.4 points; 95% CI: 0.7–14.1; P = 0.0325). Although multi-vessel CFT did not reduce the angina burden, it considerably improved health-related quality of life and patient satisfaction in those with ANOCA.

This thesis has offered critical insights into the diagnostic and prognostic value of invasive CFT in patients with ANOCA, shedding light on the prevalence of coronary vasomotor disorders and highlighting the diagnostic nuances of prevailing protocols. As diagnostic strategies continue to evolve, the findings presented in this thesis may guide development of a globally standardised CFT protocol to ensure consistent identification of ANOCA endotypes across centres. The next step lies in translating diagnostic insights into personalised therapies that target specific underlying mechanisms. Future research should prioritise randomised controlled trials evaluating endotype-specific treatments to improve both symptom control and long-term outcomes. Bridging diagnostic advances with therapeutic innovation will lead to improved patient care, enhanced quality of life, and more effective long-term management of ANOCA.

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