Characterising mechanisms to stratify therapy in patients with angina and nonobstructive coronary arteries

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2. Sinha A, Rahman H, Douiri A, Demir OM, De Silva K, Clapp B, Webb I, Gulati A, Pinho P, Dutta U, Ellis H, Shah AM, Chiribiri A, Marber M, Webb AJ, Perera D. ChaMP-CMD: A Phenotype-Blinded, Randomized Controlled, Cross-Over Trial. Circulation. 2024 Jan 2;149(1):36-47. doi: 10.1161/CIRCULATIONAHA.123.066680.
3. Sinha A, Dutta U, Demir OM, De Silva K, Ellis H, Belford S, Ogden M, Li Kam Wa M, Morgan HP, Shah AM, Chiribiri A, Webb AJ, Marber M, Rahman H, Perera D. Rethinking False Positive Exercise Electrocardiographic Stress Tests by Assessing Coronary Microvascular Function. J Am Coll Cardiol. 2024 Jan 16;83(2):291-299. doi: 10.1016/j.jacc.2023.10.034.
4. Sinha A, Rahman H, Rajani R, Demir OM, Li KamWa M, Morgan H, Ezad SM, Ellis H, Hogan D, Gulati A, Shah AM, Chiribiri A, Webb AJ, Marber M, Perera D. Characterizing Mechanisms of Ischemia in Patients With Myocardial Bridges. Circ Cardiovasc Interv. 2024 Jan;17(1):e013657. doi: 10.1161/CIRCINTERVENTIONS.123.013657.
5. Demir O, Rahman H, Sinha A, Scannell, C, Ryan M, O'Gallagher K, Ellis H, Li Kam Wa M, Saraf S, Alfakih K, Webb I, Melikian N, De Silva K, Chiribiri A, Plein S, Perera D. Should coronary physiology be indivdualised to each vessel? Insights from an in vivo study of the diseased and unobstructed left main coronary artery. JACC Cardiovascular Interventions – under review.

6. Sinha A, Rahman H, Perera D. Coronary microvascular dysfunction and heart failure with preserved ejection fraction: what are the mechanistic links? Curr Opin Cardiol. 2023 Nov 1;38(6):521-526. doi: 10.1097/HCO.0000000000001082.
7. Dutta U*, Sinha A*, Demir OM, Ellis H, Rahman H, Perera D. Coronary Slow Flow Is Not Diagnostic of Microvascular Dysfunction in Patients With Angina and Unobstructed Coronary Arteries. J Am Heart Assoc. 2023 Jan 3;12(1):e027664. doi: 10.1161/JAHA.122.027664 (*joint first authors)
8. Perera D, Berry C, Hoole SP, Sinha A, Rahman H, Morris PD, Kharbanda RK, Petraco R, Channon K; UK Coronary Microvascular Dysfunction Working Group. Invasive coronary physiology in patients with angina and non-obstructive coronary artery disease: a consensus document from the coronary microvascular dysfunction workstream of the British Heart Foundation/National Institute for Health Research Partnership. Heart. 2022 Dec 22;109(2):88-95. doi: 10.1136/heartjnl-2021-320718
9. Aziz W, Morgan H, Demir OM, Sinha A, Rua T, Rajani R, Chang AL, Woo E, Mak SM, Benedetti G, Villa A, Preston R, Navin R, O'Kane K, Hunter L, Ismail T, Carr-White G, Beckley-Hoelscher N, Peacock J, Marber M, Razavi R, Perera D. Prospective RandOmised Trial of Emergency Cardiac Computerised Tomography (PROTECCT). Heart. 2022 Nov 24;108(24):1972-1978. doi: 10.1136/heartjnl-2022-320990
10. Rahman H, Sinha A, Demir OM, Perera D. EDIT-CMD: Are We Comparing Apples With Oranges? JACC Cardiovasc Imaging. 2022 Sep;15(9):1674-1676. doi: 10.1016/j.jcmg.2022.04.027
11. Morgan H, Sinha A, Mcentegart M, Hardman SM, Perera D. Evaluation of the causes of sex disparity in heart failure trials. Heart. 2022 Sep 12;108(19):1547-1552. doi: 10.1136/heartjnl-2021-320696

12. Couch LS, Sinha A, Navin R, Hunter L, Perera D, Marber MS, Kaier TE. Rapid risk stratification of acute coronary syndrome: adoption of an adapted European Society of Cardiology 0/1-hour troponin algorithm in a real-world setting. Eur Heart J Open. 2022 Jul 29;2(4):oeac048. doi: 10.1093/ehjopen/oeac048
13. Sinha A, Rahman H, Perera D. Vasospastic Angina: A Contemporary Review of its Pathophysiology, Diagnosis and Management. Heart Int. 2022 Jul 26;16(2):99-104. doi: 10.17925/HI.2022.16.2.99
14. Sinha A, Demir OM, Ellis H, Perera D. Dizziness in an avid cyclist: an unusual presentation of a common problem. Eur Heart J Case Rep. 2021 Dec 6;5(12):ytab459. doi: 10.1093/ehjcr/ytab45
15. Sinha A, Rahman H, Webb A, Shah AM, Perera D. Untangling the pathophysiologic link between coronary microvascular dysfunction and heart failure with preserved ejection fraction. Eur Heart J. 2021 Nov 14;42(43):4431-4441. doi: 10.1093/eurheartj/ehab653
16. Sinha A, Rahman H, Perera D. Ischaemia without obstructive coronary artery disease: the pathophysiology of microvascular dysfunction. Curr Opin Cardiol. 2020 Nov;35(6):720-725. doi: 10.1097/HCO.0000000000000788
17. Sinha A, Rahman H, Perera D. Coronary microvascular disease: current concepts of pathophysiology, diagnosis and management. Cardiovasc Endocrinol Metab. 2020 Jul 16;10(1):22-30. doi: 10.1097/XCE.0000000000000223

Abstracts

1. Sinha A, Rahman H, Rajani R, Demir O, Morgan H, De Silva K, Ryan M, Likamwa M, Ezad S, Ellis H, Hogan D, Shah AM, Webb AJ, Marber M, Perera D. Comparison of adenosine, dobutamine and bicycle exercise on intracoronary physiological indices in patients with myocardial bridges. **Submitted for ESC Congress 2024.**
2. Sinha A, Rahman H, Douiri A, Demir O, Morgan H, Li KamWa M, Ryan M, Ezad S, Pinho P, Ellis H, Shah AM, Marber M, Webb AJ, Perera D. Comparing response of coronary microvascular disease endotypes to anti-ischemic therapies. A phenotype-blinded randomized crossover trial. **Accepted for presentation at the ACC Congress 2024** (Atlanta).
3. Sinha A, Rahman H, Demir O, Rajani R, Gulati A, Li KamWa M, Morgan H, Ezad S, Ellis H, Hogan D, Shah AM, Webb AJ, Marber M, Perera D. Characterising mechanisms of ischemia in patients with muscle bridges. **Presented at the American Heart Association at the Samuel A Levine Early Career Clinical Investigator Award** (Philadelphia, 2023)
4. Sinha A, Rahman H, Douiri A, Demir O, De Silva K, Clapp B, Webb I, Gulati A, Pinho P, Dutta U, Ellis H, Shah AM, Chiribiri A, Marber M, Webb AJ, Perera D. Coronary flow reserve predicts response to anti-ischaemic therapy in patients with angina and nonobstructive coronary arteries. **Presented at the European Society of Cardiology Congress 2023 at the Young Investigator Awards session** (Coronary pathophysiology and microcirculation)
5. Sinha A, Dutta U, Demir O, De Silva K, Ellis H, Belford S, Ogden M, Li Kam Wa M, Morgan H, Shah AM, Chiribiri A, Webb AJ, Marber M, Rahman H, Perera D. Rethinking the false positive exercise electrocardiogram stress test in the context of

coronary microvascular dysfunction. **Presented at the European Society of Cardiology Congress 2023 at the Coronary Artery Disease session**

6. Sinha A, Rahman H, Webb I, Scott P, Perera D. Ventricular tachyarrhythmias secondary to coronary artery spasm. **Presented at the European Society of Cardiology Congress 2023 at the Clinical Cases Session**
7. Demir O, Rahman H, Sinha A, Scannell, C, Ryan M, O'Gallagher K, Ellis H, Li Kam Wa M, Saraf S, Alfakih K, Webb I, Melikian N, De Silva K, Chiribiri A, Plein S, Perera D. Should coronary physiology be indivdualised to each vessel? Insights from an in vivo study of the diseased and unobstructed left main coronary artery. **Presented at the European Society of Cardiology Congress 2023 and EuroPCR 2023**

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Abstract

Background: Up to half of all patients presenting to the catheter laboratory with angina have nonobstructive coronary arteries (ANOCA). ANOCA is an umbrella term comprising several distinct pathophysiological entities, including coronary microvascular disease (CMD) and myocardial bridging (MB). CMD is defined as an inability of the coronary vasculature to adequately augment coronary blood flow (CBF) in response to adenosine, i.e., an impaired coronary flow reserve (CFR). An impaired CFR identifies a substrate for ischaemia and is a sensitive marker that identifies perturbations early in the ischaemic cascade. We have previously demonstrated that patients with an impaired CFR have abnormal exercise physiology and high prevalence of inducible ischaemia on noninvasive imaging. We have also reported that CMD may itself be a heterogenous condition comprising two distinct endotypes, structural and functional CMD, which are phenotypically similar but have distinct underlying pathobiology. However, whether physiology-stratification according to CFR and more granular endotyping leads to improved patient-centric outcomes, such as exercise time on a treadmill and quality of life on angina questionnaires, is not known. Finally, there is now increasing recognition that certain myocardial bridges can cause myocardial ischaemia. However, the mechanisms underpinning this are not well understood. Coronary wave intensity analysis (WIA) has previously been used to identify the mechanisms of ischaemia in different pathophysiological states and represents a powerful tool to study the mechanisms of ischaemia in patients with MB.

Methods: Patients with typical and limiting angina underwent coronary angiography with invasive physiology assessment using a Combowire to measure coronary flow and pressure simultaneously in response to adenosine and acetylcholine (clinical protocol) but also supine bicycle exercise and dobutamine (in-lab study protocol). CFR (measure of endothelium-

independent microvascular function) and acetylcholine flow reserve (AChFR; measure of endothelium-*dependent* microvascular function) were calculated as the ratio of CBF in response to the vasoactive agent and the resting CBF. Patients and researchers were blinded to the coronary physiology measurements. All eligible patients were enrolled into the exercise electrocardiogram treadmill test (ETT) study. The accuracy of ischaemic ECG changes during ETT in identifying an underlying ischaemic substrate was compared against the reference standard of coronary endothelium-*independent* and -*dependent* microvascular function. The apparent false positive rates with different reference standards were also compared.

Eligible patients were then randomised to a phenotype-blinded crossover therapy trial, which was designed to assess the utility of coronary physiology measurements in predicting response to anti-ischaemic therapy (amlodipine and ranolazine) in patients with ANOCA. The primary outcome was the difference in change in exercise time in response to anti-ischaemic therapy *between* those with an impaired CFR (coronary microvascular disease group; **CMD**) and those with a normal CFR (**reference**). The incremental value of measuring minimal microvascular resistance and acetylcholine flow reserve, in predicting response to anti-ischaemic therapy, was also assessed.

Finally, for our mechanistic in-lab study, coronary perfusion efficiency in response to supine bicycle exercise was calculated as the ratio of accelerating wave energies and the total energy flux. The change in perfusion efficiency during exercise was compared between patients with MB and no MB (the latter being further dichotomised into CMD and reference groups). We also explored the prevalence of endothelial dysfunction in the MB, CMD and reference groups.

Results: One hundred and two patients (65% females; 60±8 years old) were enrolled into the ETT study. Thirty-two patients developed ischaemic ECG changes during their ETT (ischaemic group), whilst 70 patients did not (non-ischaemic group); both groups were phenotypically similar. Ischaemic ECG changes during ETT were 100% specific for underlying endothelium-*independent* and/or -*dependent* microvascular dysfunction. AChFR was the strongest predictor of ischaemic ECG changes during exercise. Using endothelium-*independent* and/or -*dependent* microvascular dysfunction as the reference standard, the false positive rate of ETTs dropped to 0%.

Eighty-seven patients (62% females, 61±8 years old) underwent randomisation as part of the phenotype-blinded crossover therapy trial (57 impaired CFR (**CMD group**) and 30 normal CFR (**reference group**)). Baseline exercise time and Seattle Angina Questionnaire (SAQ) summary scores were similar between the groups. Patients with CMD had a greater increment in exercise time compared to the reference group with both amlodipine (mean difference in change 82 seconds, 95% CI 37 to 126 seconds, $p<0.001$) and ranolazine (mean difference in change 68 seconds, 95% CI 21 to 115 seconds, $p=0.005$). The change in SAQ summary score in response to amlodipine was similar between the CMD and reference groups (mean difference in change 2, 95% CI -5 to 8, $p=0.549$). There was a greater increment in SAQ summary score with ranolazine in the CMD group compared to the reference group (mean difference in change 7, 95% CI 0 to 15, $p=0.048$). CFR was independently associated with change in exercise time and CFR$\leq$2.5 was the optimal threshold to predict response to therapy. Patients with functional CMD responded equally well to both anti-ischaemic agents, whereas those with structural CMD had a numerically greater response to amlodipine than ranolazine (mean difference in change 46s, 95% CI -2 to 93s, $p=0.056$). Patients with sole coronary endothelial dysfunction demonstrated a numerical increment in exercise time in response to anti-ischaemic therapy, whereas no such effect was seen in the reference group. These findings support the incremental

value of measuring minimal microvascular resistance and AChFR, in addition to CFR, in patients with typical limiting ANOCA.

Ninety-two patients were enrolled into the in-lab mechanistic study (30 MB, 33 CMD and 29 reference). FFR in these 3 groups was 0.86±0.05, 0.92±0.04 and 0.94±0.05; CFR was 2.5±0.5, 2.0±0.3 and 3.2±0.6. Perfusion efficiency improved numerically during exercise in the reference group (65±9% to 69±13%, p=0.063), but decreased in patients with CMD (68±10% to 50±10%, p<0.001) and MB (66±9% to 55±9%, p<0.001). The reduction in perfusion efficiency had distinct causes: in CMD, this was driven predominantly by microcirculation derived energy in early diastole, whereas in MB, this was driven by diminished accelerating energy arising from the upstream epicardial vessel in early systole. 54% of patients with MB, versus 29% reference and 38% CMD, had epicardial endothelial dysfunction. Overall, 93% of patients with a MB had an identifiable ischemic substrate.

Conclusions: Our findings have several important implications for both future research and clinical practice. First, in patients with ANOCA, ischaemic ECG changes on an ETT were *always* attributable to an underlying ischaemic substrate secondary to abnormalities in the coronary microcirculation. Therefore, a positive ETT (defined as ischaemic ECG changes during exercise) may be an excellent tool to *rule-in* CMD in patients with typical and limiting angina who have nonobstructive coronary arteries. Second, amongst a phenotypically similar group of patients with ANOCA, only those with an impaired CFR responded to anti-ischaemic therapy. Our data also suggests that measuring minimal microvascular resistance and acetylcholine flow reserve, in addition to CFR, *may* add incremental value in predicting response to therapy. Third, patients with MB and CMD demonstrated impaired coronary perfusion efficiency during exercise, whereas those with a normal CFR had a numerical

increase. The mechanisms driving the attenuated perfusion efficiency during exercise were disparate between patients with MB and CMD, with diminution of accelerating wave energies arising from the epicardial artery during early systole being the predominant mechanism in patients with MB and perturbation of the microcirculation derived wave energies being the predominant mechanism in patients with CMD. Patients with MB also had a high prevalence of epicardial and microvascular endothelial dysfunction. Both mechanisms may lead to ischaemia in patients with MB and represent therapeutic targets.

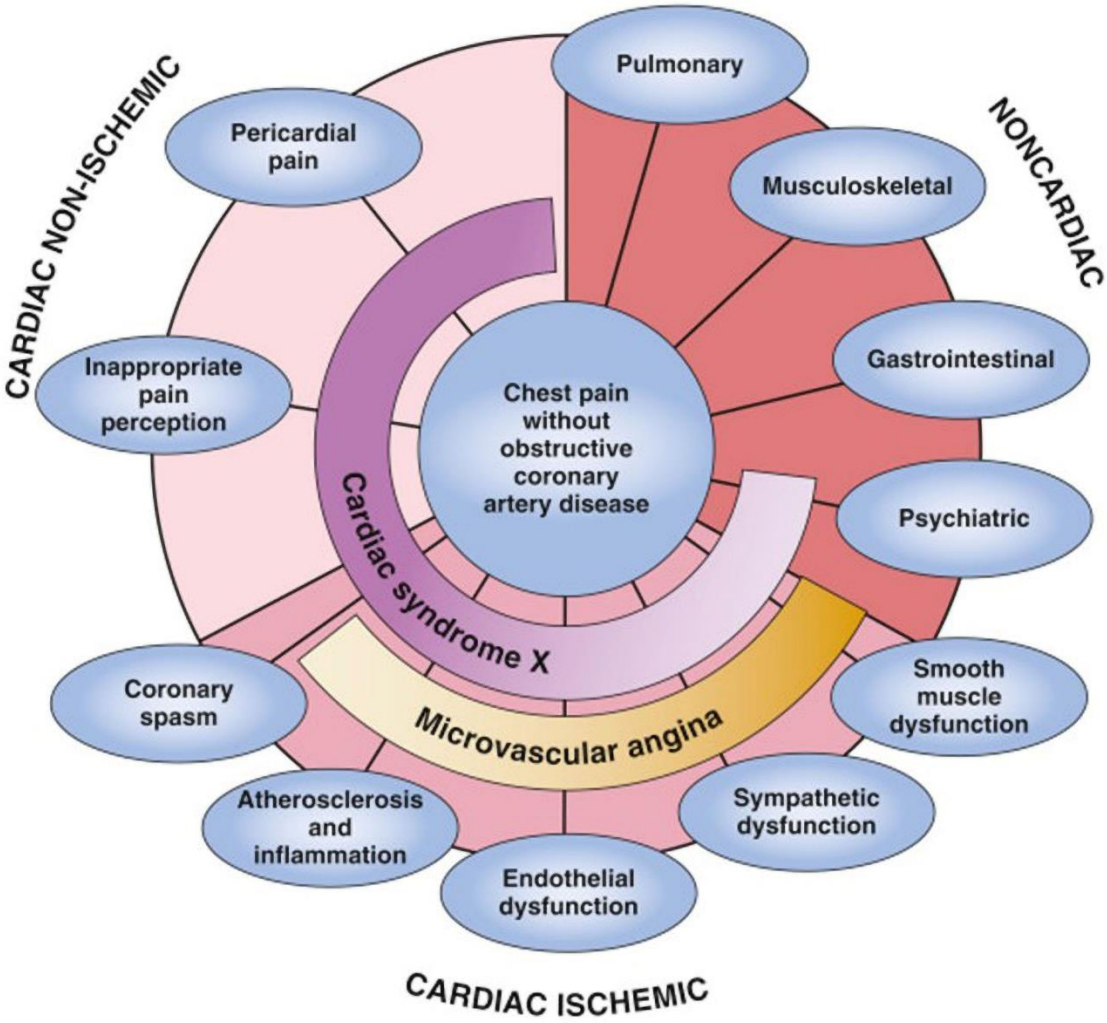
Chapter 1

Background

Published in Heart (2023), Current Opinions in Cardiology (2020) and Cardiovascular, Endocrinology and Metabolism (2020)

Up to half of all patients presenting to the catheter laboratory with angina have nonobstructive coronary arteries (ANOCA)[1]. ANOCA is an umbrella term that comprises several distinct pathophysiological processes[2] (Error! Reference source not found.), a mechanistic exploration o f which is the overriding aim of this thesis.

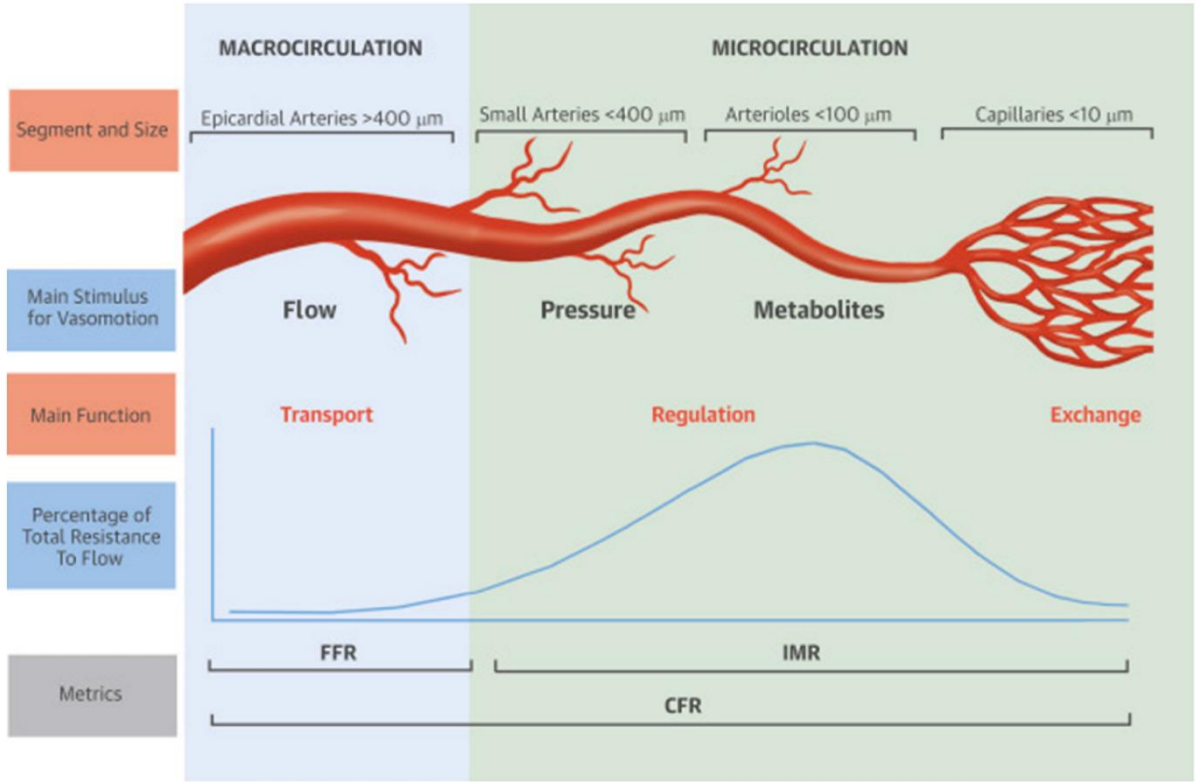
Figure 1. Angina with nonobstructive coronary arteries comprises several distinct pathophysiological processes (courtesy of Marinescu et al[2]).



1.1. Coronary vasculature and autoregulation

The coronary vasculature comprises epicardial arteries (>400 μm), pre-arterioles (100–400 μm), arterioles (<100 μm) and capillaries (<10 μm). The epicardial arteries function as capacitance vessels and respond to shear forces by endothelium-mediated dilatation. Epicardial arteries represent only 5–10% of the coronary vasculature. The pre-arterioles, arterioles and capillaries form the coronary microvasculature; pre-arterioles are characterised by a measurable pressure drop along their length, arterioles have a high resting tone and are responsible for most of the coronary vascular resistance, and capillaries deliver oxygen and substrates to the myocytes[3] (**Figure 2**).

Figure 2. Coronary artery compartmentalisation and function (courtesy of De Bruyne et al[3]).

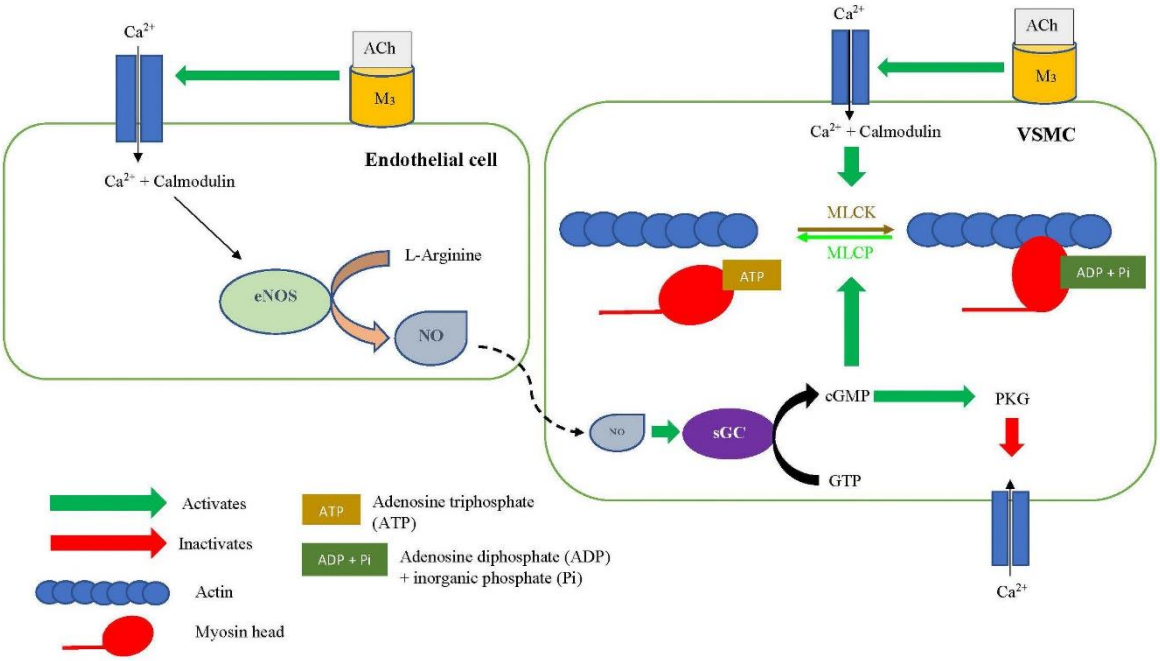


Large arterioles and endothelial control: these translate flow-related stimuli (shear stress) into vasomotor responses through endothelium-dependent mechanisms, most notably through the

generation of nitric oxide (NO). Medium-sized arterioles and myogenic control: these alter vascular smooth muscle tone to changes in intraluminal pressures detected via stretch receptors, with vasoconstriction following an increase in intraluminal pressure and vasodilatation following decrease in intraluminal pressure. Small arterioles and metabolic control: these are subject to regulation by local metabolic activity. Increased myocardial metabolism results in the release of metabolites like adenosine and carbon dioxide, which cross the interstitial space and interact directly with arteriolar smooth muscle cell. The small arterioles have a high resting tone and their dilatation reduces resistance in the overall network and pressure in the distal pre-arterioles. This induces dilatation of the myogenically sensitive vessels, with subsequent shear stress triggered flow-dependent dilatation in larger pre-arterioles and epicardial vessels. Each microvascular compartment is governed by distinct regulatory control enabling compensatory mechanisms when there is disruption at any one level; myocardial perfusion is dependent upon the interplay between these intimately linked compartments.

The coronary circulation matches myocardial oxygen demand with supply via a complex interplay between myogenic tone, metabolic signals and circulating hormones[4]. The endothelium plays an important role in the modulation of vascular tone by synthesising and releasing several vasodilator substances, such as nitric oxide (NO). Increased endothelial wall shear stress is the determinant of coronary blood flow (CBF) in health and leads to the biosynthesis of NO, which acts on the neighbouring smooth muscle cells to induce vasodilation via the NO pathway. Acetylcholine, when bound to the M_3 muscarinic receptors on the surface of endothelial cells, also promotes the biosynthesis of NO and is used to interrogate the endothelium-*dependent* microvascular function, in a dose-dependent manner, in clinical practice[5] (**Figure 3**).

Figure 3. The endothelial and vascular smooth muscle cellular pathways (courtesy of Sinha et al[5]).



Acetylcholine (ACh) has dual effects on coronary microvasculature. It binds to the muscarinic 3 (M_3) receptor on endothelial cells and leads to an influx of intracellular calcium (Ca^{2+}) via the L-type calcium channels. Intracellular Ca^{2+} binds to the protein calmodulin, and the calcium-calmodulin complex activates the endothelial nitric oxide synthase (eNOS) enzyme, which catalyses the conversion of L-Arginine into nitric oxide (NO). NO then diffuses into the neighbouring vascular smooth muscle cell (VSMC) and activates soluble Guanylate Cyclase (sGC) enzyme to catalyse the conversion of Guanosine Triphosphate (GTP) into cyclic Guanosine Monophosphate (cGMP). cGMP activates the protein kinase G (PKG), which, via a series of intracellular events, inactivates the calcium channels on the VSMC. This reduces the intracellular influx of Ca^{2+} into the VSMC, therefore leading to vasodilation. ACh also binds to the M_3 receptor on the surface of VSMCs and, in the presence of endothelial dysfunction, leads to unopposed vasoconstriction.

Calcium enters VSMCs via the L-type calcium channels and binds to the protein calmodulin. The calcium-calmodulin complex activates myosin light chain kinase (MLCK), which

phosphorylates myosin light chains (MLCs). MLCs are found on the myosin heads and MLC phosphorylation leads to cross-bridge formation between the myosin heads and the actin filaments, leading to VSM contraction. MLC phosphatase (MLCP) dephosphorylates MLC and promotes unbinding of the myosin-actin filaments, therefore leading to vasodilatation. cGMP promotes MLCP activity. The myosin head detaches from the actin binding site after adenosine triphosphate (ATP) attaches to the myosin head. This ATP is then hydrolysed to adenosine disphosphate (ADP) and inorganic phosphate (Pi) by the myosin head; the ADP and Pi is then released by the myosin head after the power stroke. At this point, the myosin head is ready for the next ATP to allow detachment from the myosin head.

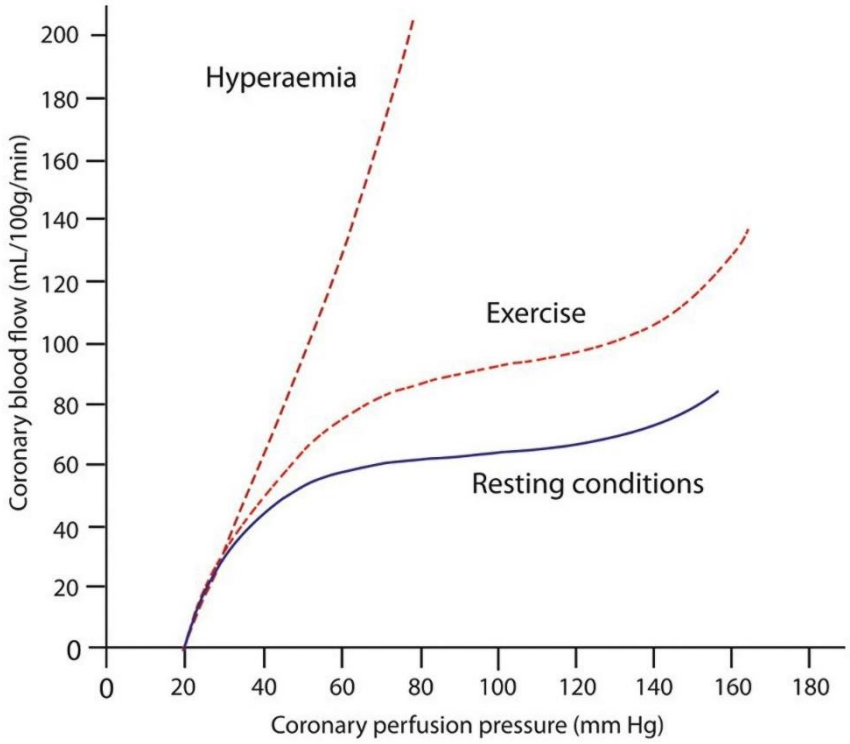
The use of acetylcholine has become standard practice for the assessment of endothelium-dependent function in clinical practice, owing to the wealth of data correlating intracoronary response to acetylcholine to non-invasive assessment of ischaemia, as well as cardiovascular outcomes. However, other agents, such as substance P, can also interrogate the endothelium-dependent pathway. Substance P acts solely on the endothelial cells[6]; the lack of a direct effect on the vascular smooth muscle cells renders it incapable of causing vasoconstriction. However, despite this advantage, a threshold that distinguishes abnormal responses has not been established. Furthermore, it is unknown whether an abnormal response to intracoronary substance P predicts ischaemia on non-invasive assessment or adverse cardiovascular outcomes. In view of these factors, we elected to use acetylcholine as the agent of choice to assess endothelium-dependent function in our studies.

Energy production in the normally functioning heart is primarily dependent upon oxidative phosphorylation, with increased cardiac activity relying on parallel increases in oxygen availability. At rest, oxygen consumption of the heart is 20-fold higher than skeletal muscle

(when normalised per gram of tissue). As an adaptation to the high oxygen demands, the heart maintains a high level of oxygen extraction, with 70-80% of arterial oxygen extracted (compared to 30-40% in skeletal muscle)[4]. This high level of oxygen extraction is facilitated by a high capillary density of 3,000–4,000/mm^2 [7], which is substantially higher than the 500–2,000 capillaries/mm^2 found in skeletal muscle[8]. Oxygen consumption by the heart is principally required for contraction, with requirements for maintaining basal metabolism comprising only 10–20% of total oxygen consumption. The oxygen requirement for myocardial contraction is governed by the heart rate, ventricular contractility, and ventricular work, which all are increased during exercise; the increase in heart rate contributes to 50-70% of the heightened myocardial oxygen demand. Exercise leads to an increase in contractility, which is a result of β-adrenergic activation[9]. Left ventricular work increases during exercise in proportion to the increased systolic arterial pressure and secondary to a modest increase of left ventricular end-diastolic volume[10]. Stroke volume, and therefore external work, is augmented as the increased contractility during exercise causes the ventricle to eject to a smaller end-systolic volume[10] so that total ventricular work increases. The effects of pacing and inotropic agents on cardiovascular haemodynamic response have been studied and compared with physical exercise and important differences exist between these. Pacing, for example, decreases end-diastolic volume and stroke volume, thereby reducing ventricular work and resulting in a lower myocardial oxygen demand[11]. Because of the high level of oxygen extraction by the myocardium during resting conditions, increases in oxygen demand during physical exercise are met principally by augmenting coronary blood flow (CBF). The increase in CBF results from a combination of attenuated coronary vascular resistance and an increase in effective perfusion pressure[12].

The effective perfusion pressure is determined by the pressure drop across the coronary vascular bed, with the entrance pressure being aortic pressure. During exercise, the increase in aortic pressure only slightly exceeds the increase in effective back pressure (sum of right atrial pressure and compressive extravascular forces) so that the effective perfusion pressure increases by 20–30% only[13]. Consequently, the exercise-induced four- to sixfold increase in CBF is mediated principally by a decrease in coronary vascular resistance, whereupon maximal exercise is associated with decreases in calculated coronary vascular resistance to 20–30% of basal resting values[14]. Total coronary resistance is the sum of passive (structural) and active (smooth muscle tone) components. In the completely vasodilated bed, flow to the different regions of the heart is determined by the cross-sectional area of the vessels, the length of the vasculature, and the number of parallel vessels that supply a defined perfusion territory. The total length of the vessels supplying the subendocardium is longer than those supplying the subepicardium. In addition, cardiac contraction compresses the intramural vasculature during systole, impeding blood flow especially to the subendocardium. To facilitate augmented flow during diastole to compensate for systolic underperfusion, the subendocardium has a 10% higher arteriolar and capillary density[15] so that during maximal pharmacological vasodilation, flow to the subendocardium is similar to flow to the subepicardium[16]. These structural and functional adaptations aid in maintaining blood flow to the subendocardial layers. When coronary driving pressure remains between 45 and 120 mmHg, coronary autoregulation ensures that capillary driving pressure and blood volume remain unaltered[17] (**Figure 4**).

Figure 4. Coronary-pressure flow relationship at rest, during exercise and with hyperaemia (courtesy of van de Hoef et al[17]).



At a constant myocardial oxygen consumption level, coronary flow is autoregulated: coronary blood flow is constant within a physiological range of perfusion pressures (resting conditions). An increase in myocardial oxygen demand results in an increase in the autoregulatory plateau, termed metabolic adaptation (exercise). During hyperaemia, the relationship between coronary pressure and flow tends towards a linear relationship, which allows a change in pressure to become an adequate surrogate of change in flow. This latter concept is harnessed during coronary physiology assessment using adenosine.

1.2. Angina with nonobstructive coronary arteries: pathophysiology

ANOCA is an umbrella term comprising several distinct underlying pathophysiological entities; these include endothelium-*independent* microvascular dysfunction, endothelium-*dependent* microvascular dysfunction, coronary artery spasm and myocardial bridging (MB); the former two are usually combined into coronary microvascular disease (CMD).

1.2.1. Endothelium-*independent* microvascular dysfunction

The ability of the coronary vasculature to augment CBF in response to a stressor is known as flow reserve; when in response to adenosine this is referred to as the coronary flow reserve (CFR), which is a measure of endothelium-*independent* microvascular function. Coronary flow reserve represents the maximal theoretical flow that is possible through the interrogated vessel and, therefore, informs about the presence/absence of an ischaemic substrate rather than myocardial ischaemia itself. Therefore, this metric has a high sensitivity, but low specificity, to identify patients who are likely to develop myocardial ischaemia during exercise. Myocardial ischaemia requires a combination of an ischaemic substrate, i.e., an impaired CFR, and heightened myocardial oxygen demand that exceeds the supply; the latter is not accounted for by measurements made in the catheter laboratory. In the context of ANOCA, a diminished CFR is associated with inducible ischaemia, impaired quality of life, and increased risk of adverse cardiovascular outcomes[18–21]. The diagnostic threshold of CFR has historically varied between <2.0 and <2.5[22], with a so-called "grey zone" existing in the CFR range of 2.0-2.5. However, our group has previously demonstrated that patients with CFR 2.0-2.5 exhibit similar degrees of maladaptive exercise physiology and inducible ischaemia as patients with CFR<2.0, therefore dispelling the concept of a CFR grey-zone. The CFR<2.5 threshold provides a higher sensitivity, whereas CFR<2.0 provides a higher specificity, in the diagnosis of CMD; with the CFR<2.5 providing a greater overall diagnostic accuracy. For these reasons, I have used

CFR<2.5 as diagnostic of endothelium-independent microvascular dysfunction in my PhD studies.

Traditionally, endothelium-*independent* microvascular dysfunction has been attributed to microvascular architectural changes (such as microvascular obstruction and rarefaction) impairing the ability of the microvasculature to reduce microvascular resistance, and consequently augment CBF, in response to stress[23]. However, recent animal models and clinical physiology evaluations suggest that endothelium-*independent* microvascular dysfunction may be a heterogeneous condition comprising distinct entities that form part of a disease spectrum. Based on physiology assessment in the catheter laboratory, we have described the presence of two distinct endotypes, termed 'structural coronary microvascular disease (structural CMD)' and 'functional coronary microvascular disease (functional CMD)'[24,25]. Both endotypes display impaired augmentation of CBF in response to intravenous adenosine (CFR < 2.5). However, whilst patients with structural CMD have an elevated minimal microvascular resistance (MR) (which translates to reduced maximal CBF), patients with functional CMD have a normal minimal MR but an attenuated vasodilatory reserve as they have reduced tone at rest. The endotypes have a similar core phenotype, with both groups demonstrating high prevalence of inducible ischaemia and inefficient cardiac–coronary coupling during physical exercise, but their pathogenesis differs at the microvascular level[24,25].

Patients with functional CMD have heightened resting CBF, reflecting a near-maximal vasodilatory state at rest leading to an attenuated vasodilatory capacity in response to physiological stress[24]. Neuronal nitric oxide synthase (nNOS) regulates resting tone and CBF[6,26]. The elevated resting CBF in patients with functional CMD is likely due to up-

regulation of nNOS either as an appropriate response to an increased myocardial oxygen demand at rest or due to disordered autoregulation. Conversely, patients with structural CMD have normal resting CBF but an impaired ability to augment CBF in response to physiological stress and diminished peripheral endothelium-dependent dilatation, precipitating exercise-induced hypertension[24,25]. The attenuated reduction in afterload with exercise interrupts the usual synergistic response of the coronary and peripheral circulations and predisposes to ischaemia in patients with structural CMD[25]. However, it remains unclear whether patients with structural CMD have an impaired ability to augment their CBF as a result of irreversible architectural changes, such as microvascular hypertrophy and fibrosis, limiting their ability to vasodilate, or whether this reflects a reversible disequilibrium of the pathways that mediate vasomotor tone during stress, such as endothelial nitric oxide synthase (eNOS) dysfunction.

Similar pathobiological endotypes have been described by other groups. A bimodal distribution of impaired CFR has been reported in patients with type 2 diabetes mellitus (T2DM) depending on the duration of diabetes[27]. In the early stages of diabetes (<10-year duration), CFR was diminished due to elevated resting CBF whereas in the latter stages of the disease (>10-year duration), this was mainly due to a reduction in maximal CBF (secondary to heightened minimal MR). The elevated resting flow in the early stages of T2DM may represent impaired coronary microvascular autoregulation or an appropriate adaptive response to altered myocardial energy metabolism. Furthermore, it is conceivable that the increased resting CBF in the early stages of T2DM may lead to shear stress-induced architectural changes in the coronary microvasculature, contributing to heightened minimal MR, leading to an attenuated maximal CBF in the later stages of the disease.

Finally, whilst the NO pathway is central to the development of microvascular dysfunction, perturbations of the endothelin-1 (ET-1) pathway have also been implicated[28,29]. ET-1 is a highly potent coronary arteriolar vasoconstrictor; this effect is mediated by activation of the G-protein coupled endothelin A receptors on vascular smooth muscle (VSM) cells. A specific genetic allele, which is associated with higher serum ET-1 levels, impaired myocardial perfusion on cardiac magnetic resonance imaging and reduced exercise tolerance, has been identified in patients with angina and microvascular dysfunction[30]. This supports the role of ET-1 dysregulation in the pathogenesis of microvascular dysfunction.

1.2.2. Endothelium-*dependent* microvascular dysfunction

In the presence of functional endothelium, the balance of shear stress-induced vasodilation and vasoconstriction tips towards the former; however, in the presence of endothelial dysfunction, the balance tips towards the latter[31]. Under normal physiological circumstances, shear stress, by activating mechanoreceptors on endothelial cells, triggers eNOS in the presence of its cofactor tetrahydrobiopterin to convert L-arginine into NO[31]. This NO then diffuses into the neighbouring VSM cell and promotes vasodilatation via the cyclic guanosine monophosphate-protein kinase G pathway (**Figure 3**). However, certain conditions, such as a systemic inflammatory state, impair the ability of eNOS to produce NO; this is known as 'eNOS uncoupling'[31]. This is characteristic of coronary endothelial dysfunction (CED) and is characterised by an inability to augment CBF by ≥50% in response to acetylcholine infusion in clinical practice (i.e., acetylcholine flow reserve ≤1.5). Acetylcholine flow reserve (AChFR)≤1.5 is associated with myocardial ischaemia on noninvasive imaging[32], high symptom burden[33] and adverse cardiovascular outcomes[34,35].

1.2.3. Myocardial bridge

Coronary arteries may dip into the myocardium for varying lengths, and then reappear on the surface. The muscle overlying the intramyocardial segment of the epicardial coronary artery is termed a myocardial bridge (MB), and the arterial segment running within the myocardium is referred to as the tunnelled segment. Most MBs are located in the left anterior descending artery[36]. The rates of MB detection vary according to the imaging modality used, varying from 2-6% during coronary angiography to 20% when using intravascular ultrasound imaging and 30% when using computed tomography coronary angiography (CTCA)[37].

Myocardial bridges were previously considered a benign phenomenon, as 85% of myocardial perfusion occurs during diastole and compression of the tunnelled segment was thought to occur only in systole ("systolic milking"). However, seminal studies from the 1990s reported delayed decompression of the tunnelled segment during early diastole, resulting in persistent luminal narrowing, during times of a high sympathetic tone[38]. This delay impedes rapid early diastolic hyperaemia most significantly in the subendocardium, which is more prone to ischaemia[39]. Furthermore, the heightened sympathetic tone during exercise leads to increased heart rate and, consequently, reduced diastolic perfusion time; the combination of delayed vessel decompression and reduced diastolic perfusion time is thought to lead to ischaemia during physical exercise in certain MBs. However, the effects of physical exercise on coronary flow, pressure and perfusion efficiency have never been examined before and, therefore, our understanding of the mechanisms leading to ischaemia in patients with MBs remains incomplete.

1.2.4. Coronary vasospasm

Vasospastic angina (VSA) refers to a dysfunctional state of coronary vasomotion where there is sudden coronary flow attenuation as a result of either epicardial or microvascular spasm, leading to downstream myocardial ischaemia and angina. VSA has been associated with poor quality of life and increased risk of adverse cardiovascular outcomes[40]. However, its underlying pathophysiology remains incompletely defined, and the factors contributing to the development of VSA are poorly understood. It is likely that VSA occurs secondary to a combination of coronary endothelial dysfunction and/or vascular smooth muscle (VSM) hyperreactivity. In the setting of coronary artery spasm, several clinical studies have demonstrated reduced NO[41] and heightened endothelin-1 (ET-1)[42] activity. Ford et al reported an attenuated vasorelaxation in response to ACh and augmented vasoconstrictive response to ET-1 in gluteal biopsy samples of patients with VSA compared with control subjects, indicating a state of systemic endothelial dysfunction in these patients[43]. On the other hand, whilst the mechanisms leading to VSM hyperreactivity are not fully understood, it is thought to be a manifestation of an alteration of the signal transduction pathway somewhere between, but not including, the cellular receptors and the contractile proteins in the VSM cell. Porcine models of coronary spasm have demonstrated that the calcium handling mechanism of contractile proteins remains unaltered, as does the expression of cellular receptors involved in promoting vasoconstriction[44]. Animal studies have also implicated the protein kinase C-mediated pathway in the pathogenesis of coronary artery spasm[45]. These results suggest that calcium (Ca^{2+}) entry through L-type Ca^{2+} channels into VSM cells is the initial trigger for coronary artery spasm and that Ca^{2+} entry might be augmented via protein kinase C-dependent mechanisms. Indeed, it has been demonstrated that L-type Ca^{2+} channels are functionally upregulated at the spastic site in a porcine model of coronary artery spasm[46]. Animal studies have also reported that rho kinase is upregulated at the spastic site and plays a key role in

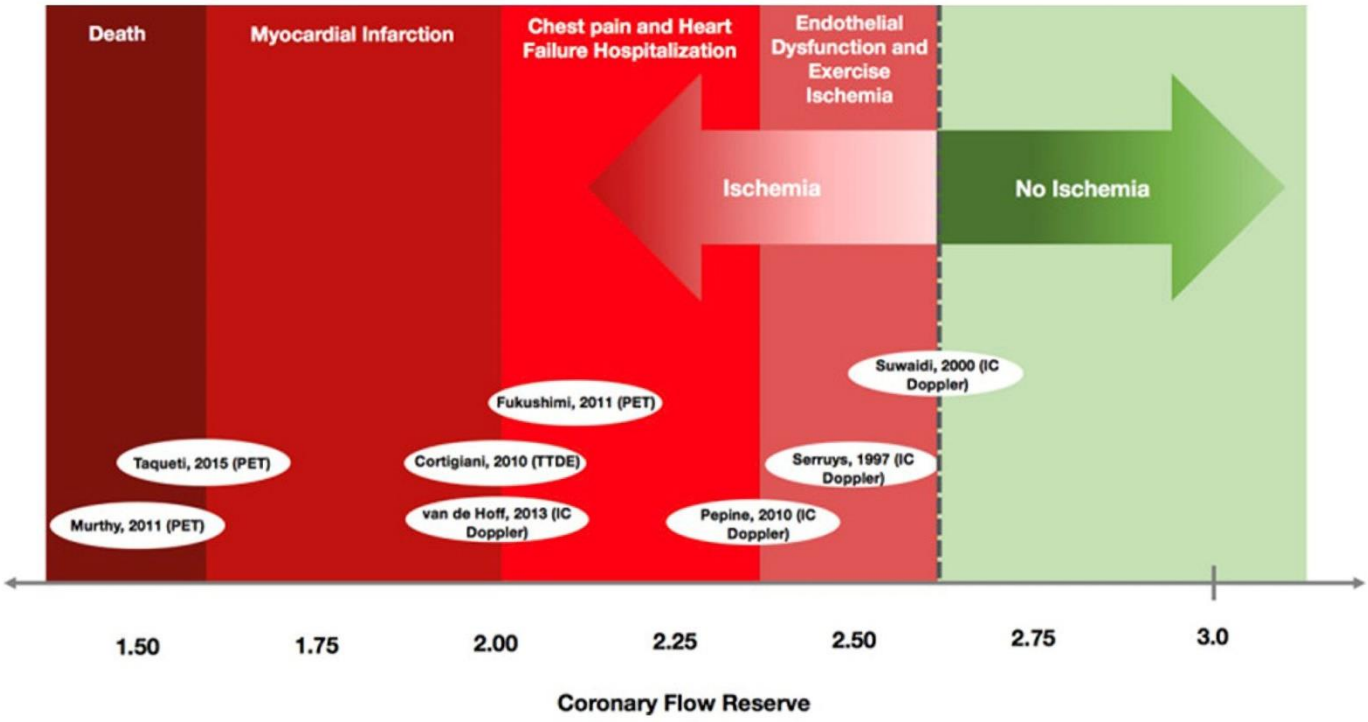
inducing VSM hypercontraction by inhibiting myosin light chain phosphatase[47]. It has been hypothesised that coronary endothelial dysfunction plays a greater role in the development of diffuse multi-vessel spasm, whereas VSM hyperreactivity plays a greater role in focal spasm.

1.3. Angina with nonobstructive coronary arteries: clinical implications

1.3.1. Endothelium-*independent* and endothelium-*dependent* microvascular dysfunction (coronary microvascular disease; CMD)

Recent studies have consistently reported adverse outcomes in patients with CMD. Of note, AlBadri et al have reported that CFR < 2.3 independently predicted a higher risk of composite endpoint of death, myocardial infarction (MI), stroke and hospitalisation for heart failure in women with ANOCA at a median follow-up of 9.7 years[21]. They also reported a trend towards higher rates of the composite endpoint in women with endothelium-*dependent* microvascular dysfunction[21]. Pepine et al, similarly, showed an increase in the composite outcome of death, nonfatal MI, nonfatal stroke, or hospitalisation for heart failure in women with ANOCA and CFR < 2.32[48]. Murthy et al reported an increased incidence of major adverse cardiac events (MACE), defined as cardiac death, nonfatal MI, late revascularisation and hospitalisation for heart failure, in patients with ANOCA and CFR < 2.0 on positron emission tomography (PET) imaging after a median follow-up of 1.3 years[49]. Suwaidi et al assessed endothelium-*dependent* microvascular function in 157 patients with ANOCA[34]. Over a 28-month follow-up, none of their patients with normal coronary endothelial function suffered from adverse events. However, 14% of patients with endothelium-*dependent* microvascular dysfunction suffered from MACE, which included MI, revascularisation, or cardiac death[34]. These findings are summarised in **Figure 5**[50]**.**

Figure 5. The spectrum of coronary microvascular disease (courtesy of Rahman et al[50]).



The figure summarises the relationship between coronary flow reserve thresholds and the prognostic spectrum of cardiovascular outcomes, based on event rates from previously published studies. 3T CMR: 3-Tesla perfusion cardiac magnetic resonance imaging; IC: intracoronary; PET: position emission tomography; TTDE: transthoracic dipyridamole echocardiography.

More recently, it has been reported that patients with structural and functional CMD have a similar prognosis (5-year incidence of MACE; composite of revascularisation, myocardial infarction, death and target vessel failure)[51]. Abnormal CFR was associated with an increased risk for 5-year MACE and target vessel failure in this patient cohort. In contrast, microvascular resistance parameters alone were not associated with outcomes[51].

1.3.2. Myocardial bridges

The majority of MBs are superficial and incidental findings on CTCA or invasive coronary angiography; however, certain MBs, such as those with longer and deeper tunnelled segments, are purported to be linked with an ischaemic substrate. These characteristics are thought to lead to a greater delay in decompression of the tunnelled segment during diastole, whereas atherosclerotic disease is thought to occur due to perturbed wall shear stress 10-20mm proximal to the mouth of the MB and increases the risk of plaque rupture in patients with otherwise no traditional vascular risk factors[52]. Acute coronary syndrome presentations in patients with MB can also be due to prolonged coronary spasm or coronary dissection within the tunnelled segment.

1.3.3. Coronary vasospasm

Vasospastic angina should be suspected in patients with anginal symptoms occurring predominantly at rest, especially if the resting symptoms follow a diurnal pattern (being worse at night and in the early morning). Although Prinzmetal's reports had linked episodes of coronary vasospasm predominantly with ST-segment elevation, there is now a greater appreciation that an episode of coronary artery spasm can present with disparate ischaemic ECG changes commensurate with the degree of coronary flow attenuation. Prolonged and more occlusive episodes of coronary artery spasm have a greater propensity to lead to ventricular arrhythmias; this is thought to be due to an increased inhomogeneity of ventricular depolarisation and repolarisation due to acute, severe and transient myocardial ischaemia[53]. These factors increase ventricular vulnerability and heighten the risk of sudden cardiac death[54]. The major adverse cardiac events rate, a composite of death, non-fatal myocardial infarction (MI), unstable angina and heart failure, has been reported to be around 5–6% over a median follow-up period of 3–4 years in patients with VSA[55,56]. Two endotypes of coronary

artery spasm are recognised: epicardial spasm (defined as ≥90% epicardial artery vasoconstriction in response to ACh stimulation, ischaemic ECG changes and characteristic chest pain) and microvascular spasm (a diagnosis of exclusion, namely <90% epicardial artery vasoconstriction in response to ACh stimulation, ischaemic ECG changes and characteristic chest pain)[57]. A recent study reported a 7.5% incidence of all-cause mortality, 1.4% MI and 2.2% stroke over a median 7-year follow-up in patients with invasively characterised coronary artery spasm[40]. Recurrent symptoms were reported in 64% of patients and 12% of patients underwent a repeat coronary angiography. Multivariate analysis revealed epicardial spasm as a predictor of non-fatal MI and repeat angiography, whereas patients with microvascular spasm more often had recurrent angina at follow-up[40]. Whilst the overall prognosis of patients with ANOCA and coronary artery spasm is generally favourable, patients with obstructive coronary artery disease (CAD) who are predisposed to spasm have a worse outlook[58]. Furthermore, patients with obstructive CAD who develop spasm within the stenotic segment are more likely to suffer from adverse cardiovascular outcomes compared with those who develop spasm in non-stenotic coronary segments or those who do not develop spasm at all[59]. The mechanisms underlying this are unclear; however, animal studies have demonstrated that intimal injury is prevalent in stenotic segments that develop spasm with pharmacological stimulation[60]. Therefore, it is conceivable that spasm within a stenotic segment can cause plaque disruption and, therefore, predispose to acute coronary syndrome. Finally, patients with myocardial infarction with non-obstructed coronary arteries secondary to coronary vasospasm have a heightened risk of all-cause mortality, cardiac death and readmission with acute coronary syndrome[61].

1.4. Angina with nonobstructive coronary arteries: diagnostic testing

1.4.1. Invasive assessment

1.4.1.1. Endothelium-independent microvascular function

In the absence of physiologically flow-limiting epicardial disease (fractional flow reserve >0.80), microvascular function testing elucidates an ischaemic substrate in patients with ANOCA. Endothelium-*independent* microvascular function is determined through calculating CFR following administration of the vasodilator adenosine, which acts on the $A2_A$ receptors on vascular smooth muscle cells and leads to vasodilatation (hyperaemia). CFR is defined as the ratio of maximal blood flow during hyperaemia to resting blood flow and is the clinical reference standard for quantitative assessment of microvascular vasodilatory reserve; CFR<2.5 is associated with exercise perfusion inefficiency and inducible ischaemia on noninvasive imaging[25]. CBF is difficult to assess in vivo; therefore, surrogates are measured in clinical practice. Doppler based techniques measure coronary flow velocity and CFR is calculated as the ratio of hyperaemic flow velocity to resting flow velocity. Doppler based techniques are considered the gold standard invasive diagnostic modality and have excellent concordance with PET-based CFR measurements[62]. Thermodilution techniques can also be used and as flow is inversely proportional to the transit time of a cold bolus of saline, CFR can be defined as the ratio of mean transit time at baseline and hyperaemia. Along with CFR, microvascular resistance (MR) can be measured as the ratio between myocardial perfusion pressure (which approximates to distal coronary pressure (Pd) and flow; when flow is estimated by Doppler, the resulting index is called hyperaemic microvascular resistance (hMR= Pd/APV, where APV is Average Peak Velocity) and when flow is estimated by thermodilution, the resulting index is called the Index of Microvascular Resistance (IMR= Pd x hyperaemic Tmn, where Tmn is the mean transit time). In patients with an impaired CFR, minimal microvascular resistance provides further insight into the endotype of microvascular disease; those with elevated

minimal microvascular resistance (≥2.5mmHg.cm^{-1}.s^{-1}) termed structural CMD and those with normal minimal microvascular resistance (<2.5mmHg.cm^{-1}.s^{-1}) termed functional CMD[24,25].

1.4.1.2. Endothelium-dependent microvascular function

Coronary endothelium-*dependent* microvascular dysfunction is characterised by an inability of the endothelial cells to produce adequate nitric oxide in response to physiological stress. This ultimately culminates in impaired augmentation of CBF due to a dampened cyclic guanosine monophosphate mediated pathway. Acetylcholine is the most frequently used endothelium-dependent vasodilating agent in clinical practice and its effects on cellular function is summarised in **Figure 3**. In coronary arteries with normal endothelium, a graded infusion of intracoronary acetylcholine should augment the CBF by ≥50% of the resting CBF[32]; this ratio is referred to as the acetylcholine flow reserve (AChFR) and AChFR≤1.5 is diagnostic of endothelium-dependent microvascular dysfunction[50]. The estimation of volumetric CBF from Doppler flow velocity incorporates vessel diameter. Given that acetylcholine can cause either epicardial vasodilatation or vasoconstriction, volumetric CBF is calculated using quantitative coronary angiography (QCA) to estimate epicardial diameter. It is calculated as:

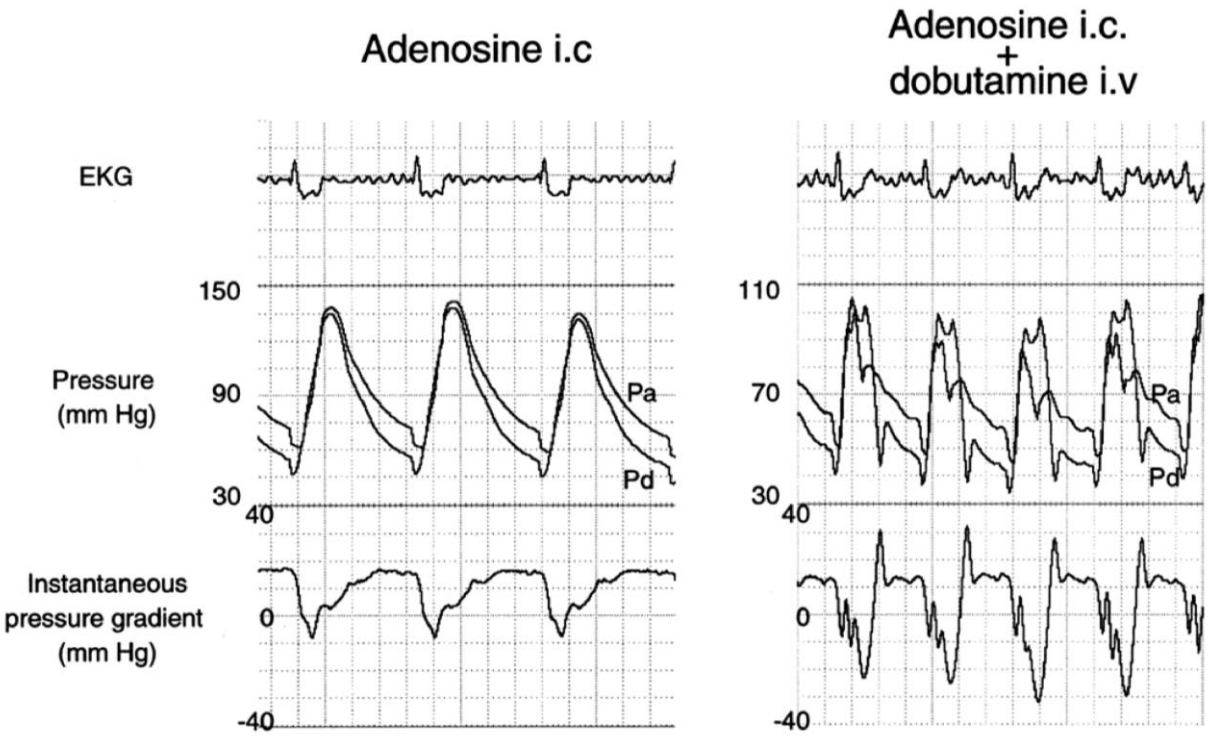
$$0.5 \times APV \times \pi \left(\frac{vessel\ diameter}{2}\right)^2$$

1.4.1.3. Myocardial bridges

Coronary physiology assessment is purported to identify MBs that may be capable of causing myocardial ischaemia. However, the cyclic changes in luminal dimensions reduce the sensitivity and specificity of pancylic physiological indices; furthermore, because the dynamic vessel calibre reduction is dependent on the degree of extravascular compression and intramyocardial tension, an accurate assessment of these vessels requires an agent that

increases both chronotropy and inotropy (i.e., one that emulates physical exertion). Accordingly, although the use of adenosine-mediated vasodilatation, and the resultant fractional flow reserve (FFR), is the gold standard for assessment of fixed coronary stenoses, these are thought to be inappropriate for the assessment of haemodynamic significance of MBs[63]. MBs can cause significant diastolic pressure gradients and negative systolic pressure gradients (where the distal pressure is greater than proximal pressure during systole as a result of systolic pressure overshooting)[63]. This phenomenon may produce an artificial elevation in the mean pressure used by conventional FFR, resulting in an underestimation of haemodynamic significance of the MB[63]. Furthermore, adenosine does not lead to inotropic changes, which is precisely when the physiological haemodynamics underpinning MB are borne out. Based on these assumptions, Escaned et al have demonstrated that measuring the diastolic Pd/Pa during dobutamine infusion (termed *dobutamine dFFR*) identified a significant proportion of *potentially* haemodynamically relevant MBs that conventional FFR did not[64]. **Figure 6** demonstrates an example of the physiological changes observed during the assessment of MBs with dobutamine and adenosine[64]. Other groups have since reported similar findings with dobutamine-induced wave-free pressure ratio (termed hyperaemic wave-free pressure ratio; HWPR)[65]. Finally, Aleksandric et al have recently identified *dobutamine dFFR*$\leq$0.76 as being the optimal threshold that predicts inducible ischaemia on exercise stress echocardiography in patients with MBs[66].

Figure 6. Physiological changes observed during the assessment of myocardial bridges with intravenous dobutamine (courtesy of Escaned et al[64]).



Intracoronary hyperaemic pressure measurements at baseline and during dobutamine infusion. The graph shows the recorded electrocardiogram (EKG), aortic pressure (Pa), intracoronary pressure distal to the myocardial bridge (Pd), as well as the instantaneous pressure gradient resulting from the difference between the two pressures. The overshooting of Pd over Pa noted during dobutamine infusion contributes to the characteristic negative systolic pressure gradient.

1.4.1.4. Coronary artery spasm

Coronary vascular assessment with ACh stimulation can be performed readily and safely in patients with suspected VSA[67]. There is variation in the doses and delivery rates of ACh used during spasm assessment, although the underlying scientific rationale remains the same. The

consensus is to deliver a 100mcg bolus of ACh down the left anterior descending artery over 20 seconds; this dose needs to be halved (i.e., 50mcg over 20 seconds) if being delivered into the right coronary artery due to the higher risk of bradyarrhythmias[68]. A diagnosis of epicardial artery spasm is made when ACh bolus leads to ≥90% coronary vasoconstriction, ischaemic ECG changes and chest pain; this protocol and diagnostic threshold is associated with a high degree of sensitivity and specificity for the detection of coronary spasm in patients with symptoms of VSA[69]. The diagnosis of microvascular spasm is made when ACh bolus leads to ischaemic ECG changes and chest pain in the absence of ≥90% coronary vasoconstriction; in the absence of significant epicardial spasm, an AChFR <1.0 with ACh bolus is also suggestive of microvascular spasm as it demonstrates flow attenuation[68].

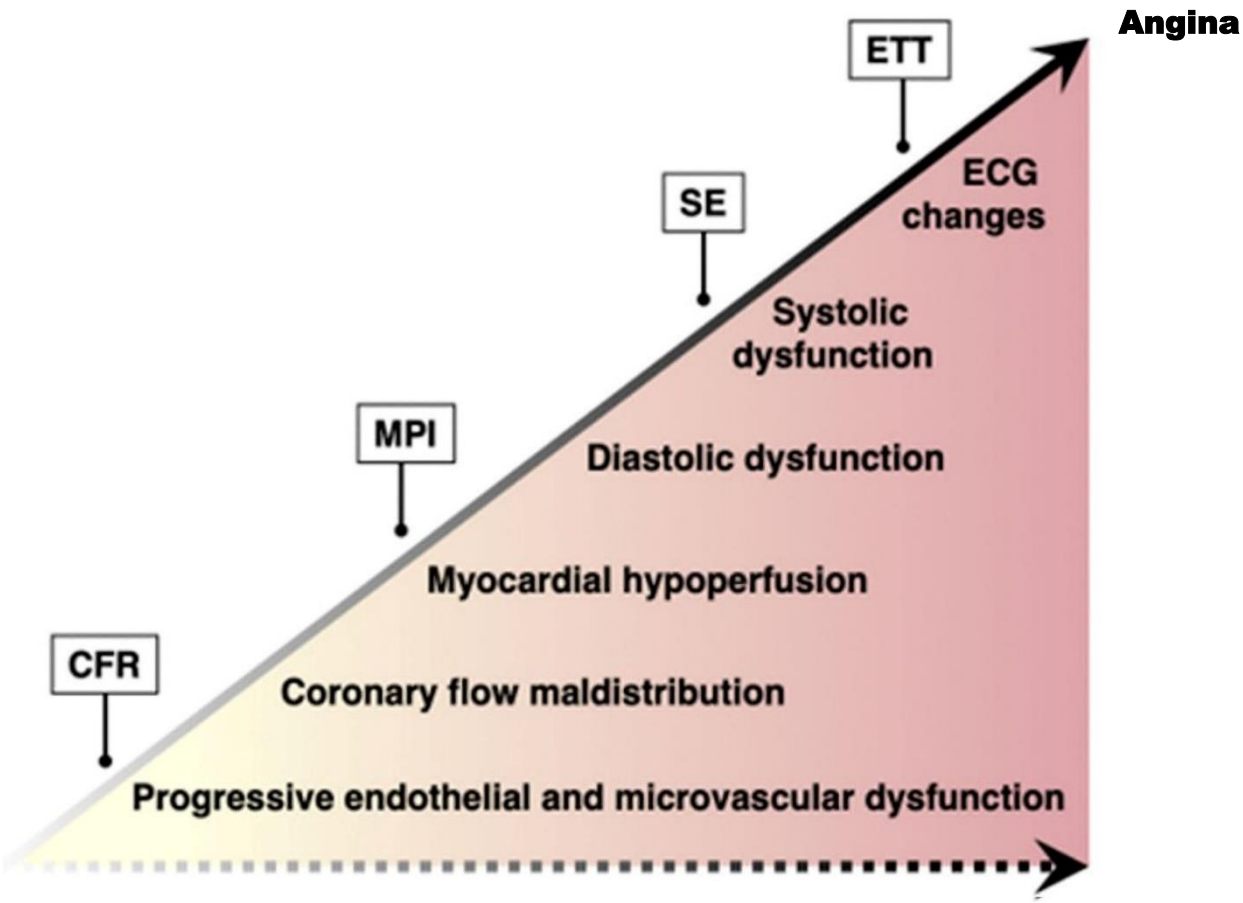
Whilst the aforementioned ACh protocol is the most commonly used one, other derivations exist and are associated with varying degrees of sensitivity and specificity. These include the incremental infusion of ACh at 0.86, 8.63, 86.3, 863 mcg/mL over 3 minutes or incremental boluses of ACh at 100–200mcg over 20 seconds[70,71]. These derivations have important clinical implications; for example, a 200mcg bolus is more likely to lead to multivessel spasm than a 100mcg bolus, and a 20-second bolus is more likely to lead to vasospasm than a 3-minute infusion of the same dose[72,73]. Furthermore, it is not known what dose and infusion rate of ACh correlates with physiological degrees of spasm; the caveat being that beyond a certain threshold of dose and infusion rate ACh may provoke spasm in any individual[74]. This phenomenon was observed in a study investigating the effects of varying concentrations of ACh in patients with normal epicardial arteries[75]. The authors reported an increase in epicardial vessel diameter and CBF with ACh concentrations of up to 10^{-4} mol/L; however, significant vasoconstriction, accompanied by chest pain, was observed with concentrations of 10^{-3} mol/L. This led the authors to conclude that the local ACh concentration and the coronary vascular segment under question may play a significant role in the observed response to ACh.

Finally, the diagnostic threshold for the degree of epicardial vasoconstriction in response to ACh can also vary between centres. The majority of centres use the 90% threshold; however, some centres use different arbitrarily chosen thresholds, such as 75% vasoconstriction[67,68]. Using different diagnostic thresholds will, of course, alter the diagnostic sensitivity and specificity.

1.4.2. Noninvasive assessment (exercise electrocardiogram treadmill testing)

CFR measures the theoretical maximal ability of the vessel to augment CBF. An abnormal CFR, therefore, identifies a substrate for ischaemia that may or may not manifest itself during physical exertion. On the other hand, electrocardiographic changes, such as ST segment depression, identify the presence of actual ischaemia (although, technically, the true gold standard marker of ischaemia would be delta coronary sinus lactate levels). Therefore, CFR interrogates the early part of the ischaemic cascade and is a sensitive diagnostic test, whereas an exercise ECG treadmill test interrogates the latter part of the ischaemic cascade and is a specific diagnostic test[76] (**Figure 7**).

Figure 7. The ischaemic cascade and diagnostic tools used to interrogate it (courtesy of Reynolds et al[76]).



CFR: coronary flow reserve; MPI: myocardial perfusion imaging; SE: stress echocardiography; ETT: exercise ECG treadmill test

Exercise electrocardiogram treadmill testing (ETT) is a low-cost and widely available non-invasive stress test, with long-standing use in clinical cardiology to study the cardiovascular response to physical stress. Historically, ETT has received a class I indication for initial evaluation of suspected or known coronary artery disease (CAD)[77,78]; however, in current practice it is primarily indicated for assessment of exercise tolerance, symptoms, arrhythmias and blood pressure response in select patients[79]. The use of ETT to assess for ischaemia in patients with new onset angina has been downgraded to a IIb C indication[79]; this is due to its

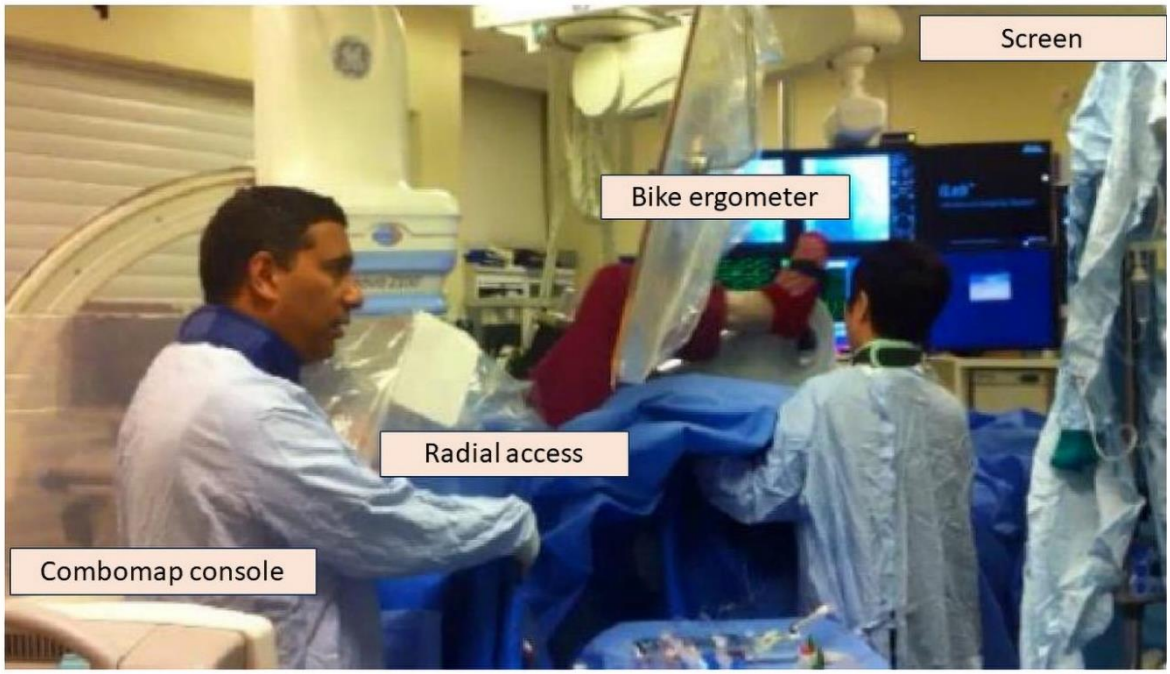
perceived high false positive rate. However, historically ETTs were validated against the reference standard of the presence of obstructive coronary artery disease (CAD) on coronary angiography. We now know that myocardial ischaemia can, and indeed in nearly half of all cases does, occur secondary to coronary microvascular disease in the absence of obstructive CAD. Therefore, it is conceivable that the historical high false positive rates of ETTs may have been due to the fallacy of using obstructive CAD as the reference standard of ischaemia. Reappraising the diagnostic accuracy of ETTs against a contemporary gold standard of comprehensive coronary physiology assessment is an important unmet clinical need.

1.5. Exercise versus pharmacological stress

Pharmacologically induced hyperaemia is aimed at creating conditions of minimal microvascular resistance, where the flow and pressure relationship become linear and, therefore, pressure becomes a surrogate for flow (**Figure 4**)[17]. However, there are several physiological differences between exercise and hyperaemia, with the former being accompanied by heightened inotropy, chronotropy and myocardial work, none of which occur in response to adenosine. Furthermore, the mechanism leading to augmentation of CBF is disparate between exercise and adenosine. Whilst the latter acts on A_{2A} receptors to mediate vasodilatation, the former leads to shear stress induced NO production via endothelial cells. Therefore, a functioning endothelial and vascular smooth muscle compartment is imperative for the flow responses to exercise, whereas adenosine acts directly on the vascular smooth muscle cells and, therefore, does not interrogate the endothelial layer. Finally, as CBF is determined by both perfusion pressure and microvascular resistance, and both change throughout the cardiac cycle due to phasic effects of the beating heart on the microvasculature, the effects of exercise on CBF are different to those of hyperaemia. Our unit has developed a clinical model that allows invasive coronary physiological evaluation during exercise in the

cardiac catheter laboratory[25,80]. This technique involves supine bicycle ergometer exercise during cardiac catheterisation (**Figure 8**).

Figure 8. Setup of coronary physiology assessment during in-lab supine bicycle exercise.



1.6. Phasic versus Pan-Cyclic Assessment: Wave Intensity Analysis

Myocardial perfusion depends on aortic pressure, epicardial artery patency and microvascular resistance (broadly characterised by CFR) but it is also dependent on the dynamic interaction between myocardium and microvasculature. As a result of phasic compression and decompression of intramyocardial vessels by surrounding myocytes, coronary flow is intimately linked to myocardial relaxation and contraction; this process is called cardiac–coronary coupling[81]. The energy fluxes accelerating and impeding myocardial perfusion can be quantified by wave intensity analysis (WIA). WIA was introduced over 20 years ago for the study of cardiovascular dynamics; wave intensity can be calculated as the product of the rate of change of pressure and rate of change of flow. It also separates waveforms into their forward

and backward components, which is particularly valuable in the coronary circulation as this differs from systemic circulatory beds in that pressure is generated from both the proximal and distal ends of the coronary artery. In a coronary artery, forward-traveling waves arise from the epicardial artery or aorta and are associated with congruent changes in flow and pressure; when pressure rises, flow increases and vice versa. Backward-traveling waves originate from the intramyocardial microvasculature and are associated with opposite changes in flow and pressure; when pressure falls, flow increases and vice versa. Each wave is a product of changes in pressure and flow velocity at any individual time point in the cardiac cycle (equations 1-3). It is, therefore, imperative that high quality pressure and flow velocity envelopes are obtained when measurements are taken to ensure the subsequent derivation of wave-intensity is an accurate reflection of the underlying phasic coronary haemodynamics. The main assumption in the derivation of wave intensity itself lies with the use of the single point measure of local wave-speed[82].

Equation 1 (forward travelling wave energy):

$$WI_{+} = \frac{1}{4\,\rho\,c} \times \left(\frac{dP}{dt} + \rho c \frac{dU}{dt}\right)^2$$

Equation 2 (backward travelling wave energy):

$$WI_{-} = -\frac{1}{4\,\rho\,c} \times \left(\frac{dP}{dt} + \rho c \frac{dU}{dt}\right)^2$$

Equation 3 (net wave energy):

$$WI_{net} = WI_{+} + WI_{-} = \left(\frac{dP}{dt}\right)\left(\frac{dU}{dt}\right)$$

Locally induced changes in pressure and flow are not transmitted instantaneously through the arterial wall, but propagate as waves at a certain speed, inversely proportional to vessel wall dispensability (D) and known as the wavespeed (c):

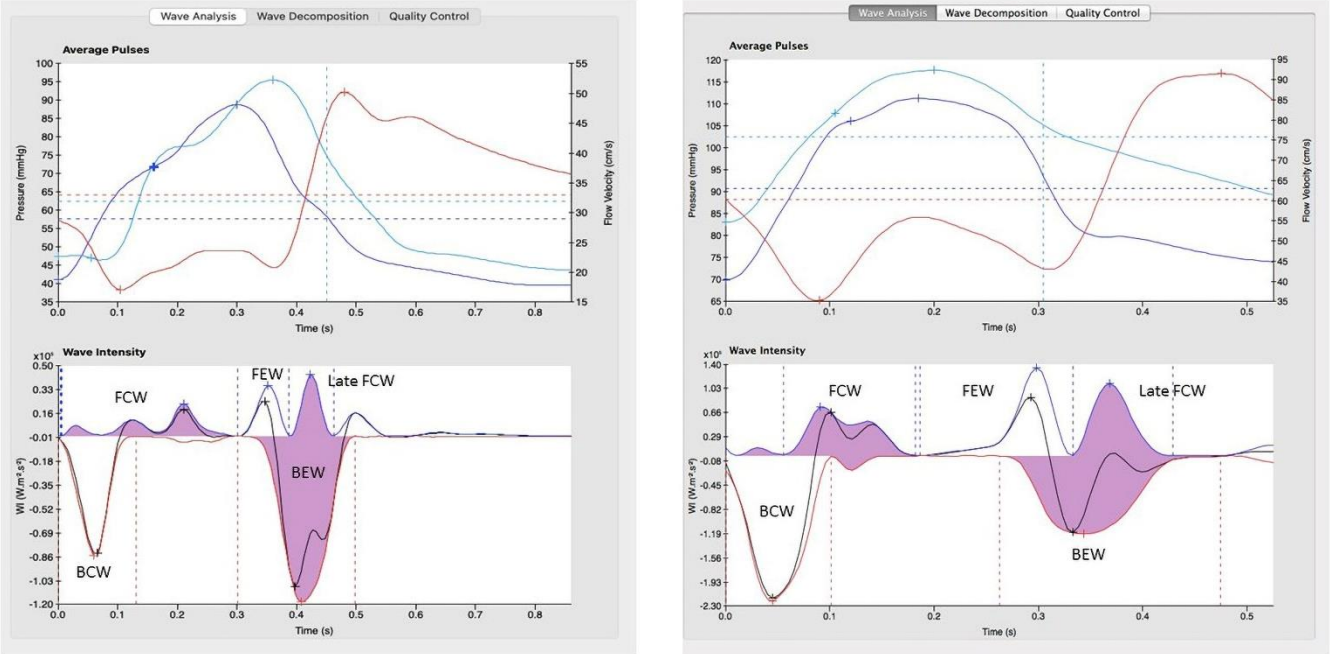
$$c = \frac{1}{\sqrt{\rho D}}$$

ρ is the density of blood, c wave-speed, dU change in flow velocity and dP change in pressure.

These waves can be further classified according to their effect on coronary flow (accelerating or decelerating) and pressure (compression or expansion). The backward compression wave (BCW) is a distally originating wave that arises during isovolumetric contraction (at the onset of systole) and decelerates coronary flow. The forward compression wave (FCW) is a proximally originating accelerating wave that reflects transmission of rising aortic pressure in early systole. The forward expansion wave (FEW) arises proximally, decelerates flow, and reflects the slight fall of aortic pressure in late systole. The backward expansion wave (BEW), the main driver of flow and myocardial perfusion in the normal coronary circulation, originates distally due to decompression of the microvasculature in early diastole. The late FCW originates from the epicardial artery, coinciding with closure of the aortic valve when aortic pressure is briefly augmented. This proximal originating wave accelerates flow, augmenting the actions of the BEW[81,83]. The relative balance of accelerating and decelerating waves has been termed coronary perfusion efficiency. Perfusion efficiency increases with exercise and pharmacologically induced microvascular dilatation in the healthy heart[80]. Coronary wave intensity analysis has been used by our group and others to study several different human disease processes including coronary microvascular disease[25] (**Figure 9**), aortic stenosis[80],

warm-up angina[84], response to intra-aortic counterpulsation balloon pump[85] and cardiac resynchronisation therapy[86].

Figure 9. Comparison of wave intensity analyses between patients with normal CFR (left hand side panel) and impaired CFR (right hand side panel).



Ensemble averaged aortic pressure (top, light blue), coronary pressure (top, dark blue), flow velocity (top, red) and wave intensity analysis (bottom). BCW: backward compression wave; FCW: forward compression wave; FEW: forward expansion wave; BEW: backward expansion wave; late FCW: late forward compression wave.

1.7. Current management strategy in patients with coronary microvascular disease

Currently, there is a limited evidence base to guide therapeutic options in patients with CMD. For the most part, therapies used for angina due to obstructive CAD do not distinguish the underlying pathophysiological process. Management of CMD should encompass controlling

cardiovascular risk factors, improving endothelium-*dependent* and endothelium-*independent* function (disease-modifying therapy), and achieving symptomatic relief. Appropriate management of hypertension, diabetes mellitus and hyperlipidaemia, as well as smoking cessation advice, is imperative as these contribute towards impaired microvascular function[87,88]. The current European Society of Cardiology (ESC) guidelines recommend treatment with aspirin and statins (class I indication), and consideration of angiotensin-converting enzyme (ACE) inhibitors for patients with ANOCA[79]. Aspirin is recommended by extrapolation of obstructive CAD data. Statin therapy improves vascular inflammation and endothelial function. Angiotensin II is a potent vasoconstrictor and may modulate coronary microvascular tone directly, therefore blocking it using an ACE inhibitor may prove beneficial in patients with CMD. These two agents may lead to improved coronary microvascular function over time but may have no immediate effect on symptom relief. A handful of studies have investigated the role of anti-ischaemic therapy in symptom control in patients with ANOCA.

Bairey-Merz et al carried out a double-blinded, placebo-controlled, therapeutic study with 2 weeks of ranolazine and 2 weeks of placebo in 128 patients with ANOCA[89]. The diagnosis of CMD was made either invasively (CFR<2.5 or <0% coronary vasodilatation in response to intracoronary acetylcholine) or non-invasively (myocardial perfusion reserve index (MPRI)<2.0 on stress perfusion cardiac magnetic resonance imaging). Their patient cohort included 35 patients with CFR<2.5, 36 patients met the acetylcholine criteria for CMD, and 67 patients had MPRI<2.0. Ranolazine did not improve symptoms, MPRI or diastolic filling in the overall patient cohort; however, the inclusion criteria inevitably led to a highly heterogenous cohort of patients, many of whom possibly did not have CMD. Interestingly, MPRI improved in the subgroup of patients with lower baseline CFR. This suggests that patients with invasively diagnosed CMD, using the robust dichotomous threshold of CFR<2.5,

were most likely to benefit from the anti-ischaemic effects of ranolazine; however, these were *posthoc* analyses, and the study was not powered for this treatment effect.

Koh et al have carried out a pilot therapeutic study in patients with ANOCA[90]. In their randomised, double-blinded, placebo-controlled trial, 22 patients with angina, an abnormal stress test and nonobstructive coronary arteries were randomised to ranolazine or placebo for 12 weeks. The primary end point was change in Seattle Angina Questionnaire (SAQ) angina frequency score. SAQ angina frequency score, Duke Activity Status Index score, FFR, CFR, hMR, and cardiopulmonary exercise testing were performed at baseline and at 12 weeks' follow up. There were no significant differences in SAQ angina frequency scores between ranolazine and placebo groups at 12 weeks. The change in coronary physiology indices at 12 weeks was not significantly different between groups. Thirteen of the 22 patients had CFR<2.0 at baseline; these patients had a greater increase in CFR, in response to ranolazine, when compared with patients with CFR≥ 2.0 at baseline. This increase in CFR was not observed in patients treated with placebo. There were no differences in response to ranolazine in delta SAQ angina frequency, Duke Activity Status Index and CPET parameters between patients with CFR<2.0 and CFR≥2.0.

Jansen et al have reported that 6 weeks of diltiazem did not lead to improvement in CFR or angina-specific quality of life in patients with ANOCA[91]. However, only 25% of their patient cohort had an impaired CFR at baseline; the rest having a mixture of epicardial spasm, microvascular spasm, and elevated index of microvascular resistance.

Corban et al have recently investigated the effects of autologous intracoronary CD34+ infusion in patients with ANOCA[92]. CD34+ cells are involved in normal vascular repair with microcirculatory regenerative potential and paracrine anti-inflammatory effects; these have been reported to be attenuated in patients with coronary endothelium-*dependent* microvascular dysfunction. Invasive coronary physiology assessment and ETT were repeated 6 months after cell infusion. Intracoronary CD34+ cell infusion improved endothelium-*dependent* microvascular function, decreased Canadian Cardiovascular Society angina class and improved SAQ scores with no significant change in exercise time at 6 months of follow-up. The same group of investigators also reported an improvement in CFR, in response to a single dose of intracoronary CD34+ cells, after 6 months[93].

Finally, perhaps the most influential therapeutic study in patients with ANOCA till date has been the Coronary Microvascular Angina (CorMicA) study, which demonstrated the superiority of physiology-guided management above empirical therapy in patients with ANOCA in relation to SAQ scores at 6- and 12-months in 151 patients randomised to either physiology-stratified therapy or empirical therapy[94,95]. This study also demonstrated an improvement in resource utilisation in the physiology-stratified group[96]. However, the definition of vasomotor abnormalities was broad in CorMicA (diminished CFR and/or elevated minimal microvascular resistance and/or epicardial/microvascular spasm with bolus acetylcholine provocation) and hence it was not possible to link the specific pathophysiological diagnosis with outcome. Furthermore, in CorMicA, the recommended therapies included a range of pharmacological and non-pharmacological measures (including referral to cardiac rehabilitation)[96], and, therefore, the mechanistic link between physiological findings, therapies instituted and improvement in outcomes remain incompletely understood.

Therefore, whilst there has been some advancement in our understanding of the therapeutics in patients with ANOCA, many questions remain unanswered. First, and foremost, is there a mechanistic link between impaired CFR and response to therapy using patient-centric outcome measures? Second, do measurements of minimal microvascular resistance and acetylcholine flow reserve, in addition to CFR, add incremental value in predicting response to therapy?

1.8. Aims and objectives

The overarching aims of my PhD studies were to determine the relationship between invasive coronary physiological metrics in patients with undifferentiated ANOCA and their a) clinical phenotype and b) response to physiology-stratified therapy. Three parallel clinical studies were performed in patients with ANOCA, classified according to endothelium-independent and -dependent vascular function and the dynamic response to exercise to address the following key aims:

1. To determine the specificity of a positive exercise ECG treadmill test in identifying an ischaemic substrate in patients with angina and nonobstructive coronary arteries, using coronary endothelium-*independent* and endothelium-*dependent* microvascular function as the reference standard. We will also compare the apparent false positive rates of the different reference standards. The findings of this study are presented in Chapter 3.

2. To determine whether coronary flow reserve predicts change in exercise time and quality of life in response to anti-ischaemic therapy in patients with ANOCA. We will carry out a randomised, controlled, phenotype-blinded, crossover trial using amlodipine and ranolazine as exemplar anti-ischaemic therapies. The differences in change in

exercise time and SAQ summary score from baseline will be compared between the two groups (impaired CFR (**CMD**) versus normal CFR (**reference**)). The findings of this study are presented in Chapter 4.

3. To determine whether measuring minimal microvascular resistance and acetylcholine flow reserve, in addition to CFR, adds incremental value in predicting response to anti-ischaemic therapy. Functional CMD is a recently identified endotype of CMD; we will assess the relative prevalence of this endotype and whether their response to therapy differs from that of the more traditionally recognised structural CMD. The findings of this study are reported in Chapter 5.

4. To determine the perfusion substrates for ischaemia in patients with myocardial bridges using supine bicycle exercise to determine coronary perfusion efficiency. We will also assess the prevalence of coronary endothelial dysfunction in these patients. Finally, we will compare these metrics against the CMD and reference groups.

Chapter 2 describes the derivation of methods used to address these hypotheses and carry out the studies.

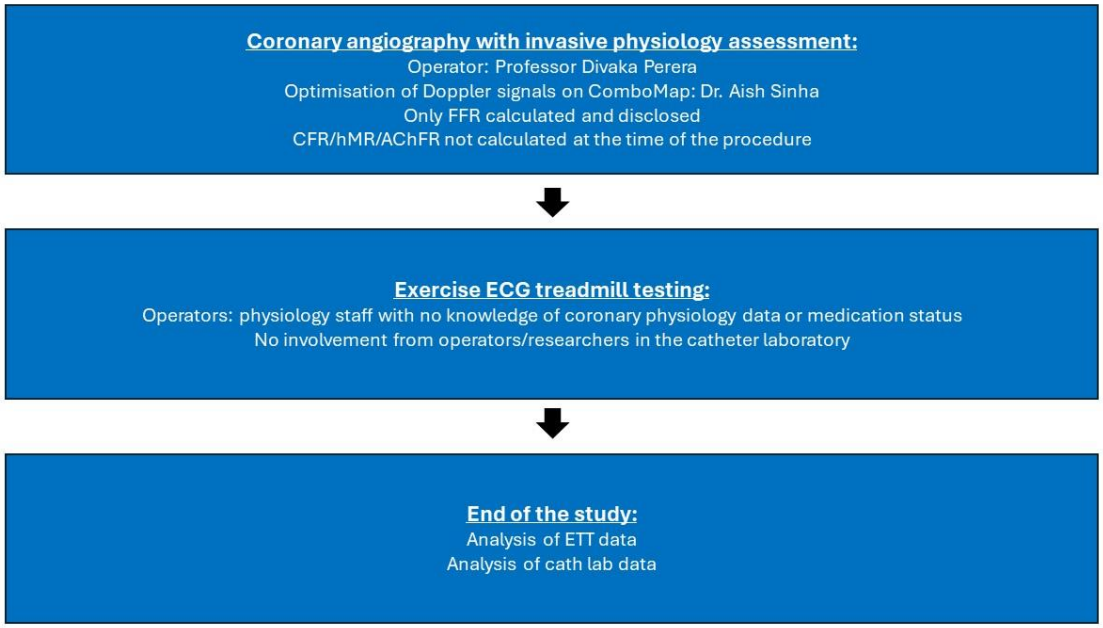
Chapter 2

Study design and methods

2.1. Designing the study protocol

The seminal findings from my predecessor, Dr. Haseeb Rahman, laid down the foundations for my PhD study. Dr. Rahman demonstrated that in a heterogenous group of patients with angina and nonobstructive coronary arteries (ANOCA), those with an impaired coronary flow reserve (CFR) in response to adenosine have an impaired exercise perfusion efficiency during exercise and high prevalence of inducible ischaemia on noninvasive stress imaging[25]. Other groups have demonstrated that, in patients with ANOCA, an impaired CFR results in poor quality of life on the account of a high angina burden[35]. However, whether measuring CFR can predict change in exercise capacity and quality of life has never been reported before. We felt that this was the key gap in our knowledge that would complete the link between the mechanisms leading to ischaemia and adverse outcomes in patients with ANOCA. This was the premise for our randomised, controlled, phenotype-blinded, crossover trial titled '**Cha**racterising **M**echanisms in **P**atients with **C**oronary **M**icrovascular **D**isease to stratify therapy (ChaMP-CMD)'. Importantly, unlike previous therapeutic studies in patients with ANOCA, all our patients had typical limiting angina and were invasively characterised in the catheter laboratory with the gold standard Doppler technique providing simultaneous intracoronary flow and pressure measurements. Meticulous blinded acquisition and, at the end of the study, offline processing of the coronary physiology data was fundamental to my PhD, with the former being the entry point into all my separate studies (**Figure 10**).

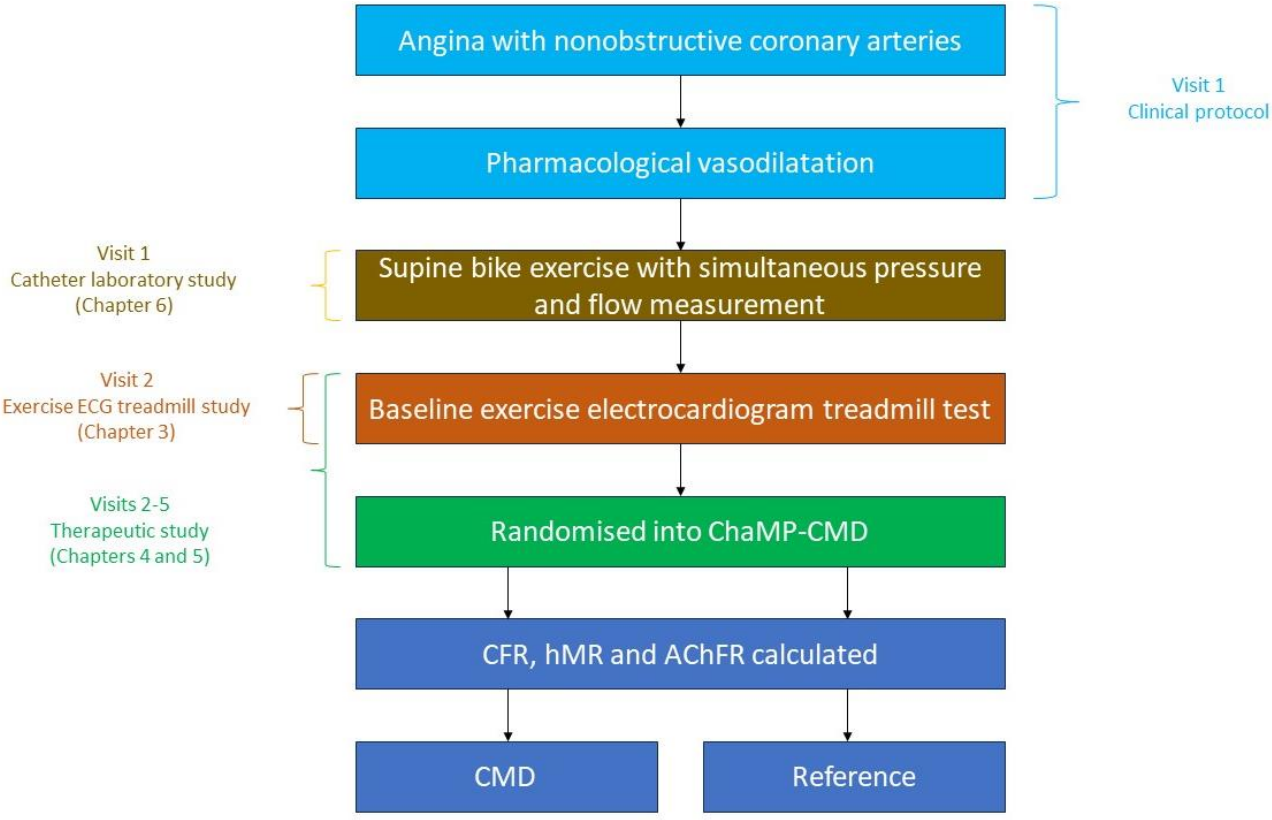
Figure 10. How patient and researcher blinding was maintained.



With guidance from my supervisors Professor Divaka Perera and Dr. Andrew Webb, and my colleague Dr. Haseeb Rahman, I designed the study protocol and secured funding via the Medical Research Council Clinical Research Training Fellowship (MR/T029390/1). I then obtained ethical approval to commence the study after review by the Bromley Research Ethics Committee (20/LO/1294). I was the main point of call for referrals pertaining to further assessment in patients with ANOCA. These referrals came from the rapid access chest pain clinic, other consultants at our institute, primary healthcare clinicians and tertiary referrals from other hospitals. Only those patients with typical limiting angina and a high pretest probability of coronary vasomotor dysfunction were offered coronary angiography with comprehensive physiology assessment. I also screened and consented suitable patients from elective angiography lists who were being investigated for exertional chest pain. At the end of the clinical protocol, we optimised patients' medications and I followed up every patient in clinic thereafter to evaluate their symptom control and clinical progress.

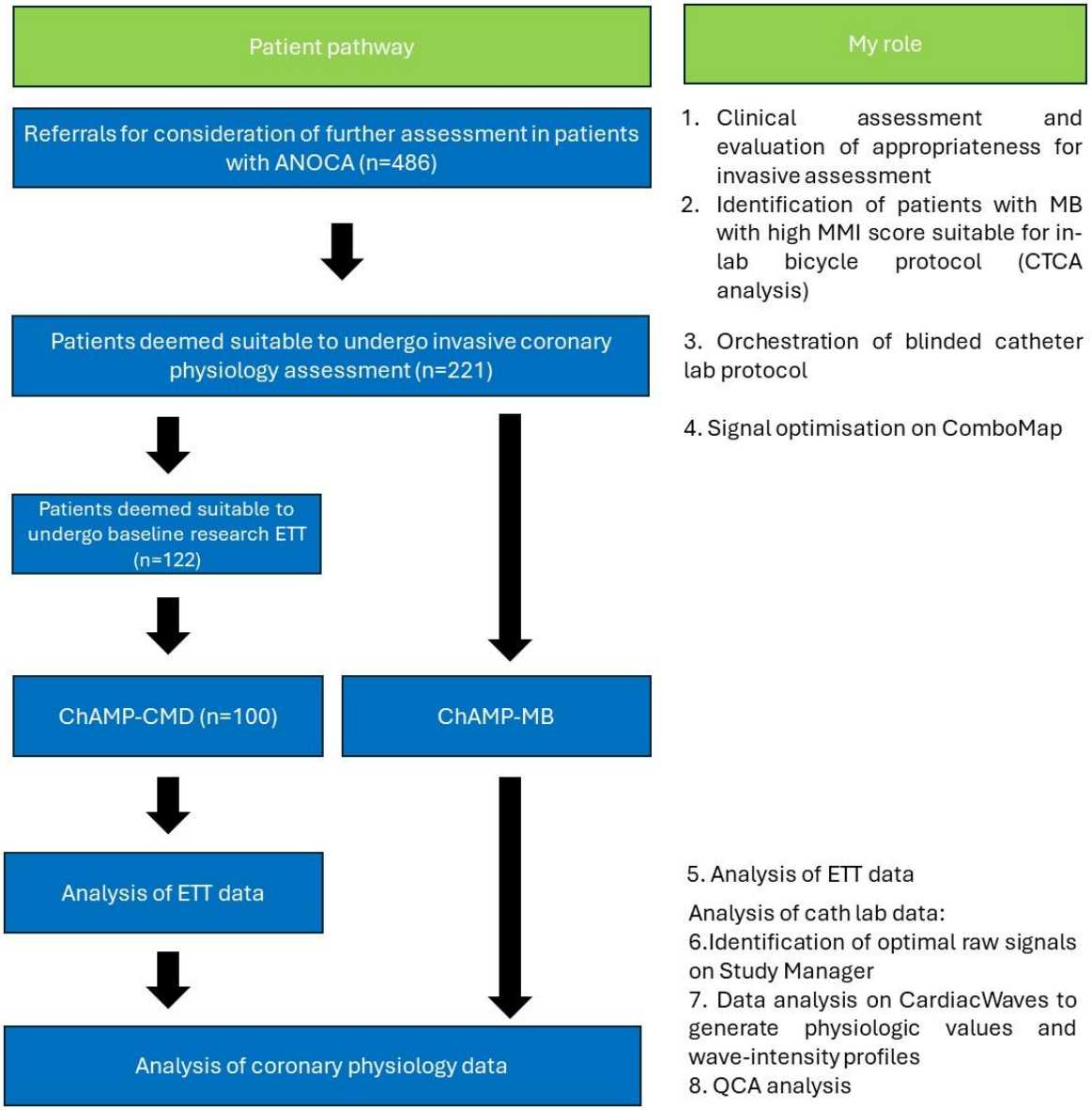
Inclusion criteria for the clinical protocol, and entry into any of the studies pertaining to my PhD, were typical limiting angina, preserved left ventricular systolic function (ejection fraction >50%) and nonobstructive coronary arteries (FFR > 0.80). Exclusion criteria were intolerance to adenosine, chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/m^2), concomitant valve disease (greater than moderate on echocardiography), previous acute coronary syndrome or revascularisation (including percutaneous coronary intervention), and cardiomyopathy (including left ventricular hypertrophy). The three study components (catheter laboratory study, exercise electrocardiogram treadmill study and randomised controlled trial) are described in the following section and **Figures 11 and 12** depict the overall study flowchart and my role respectively.

Figure 11. Study flow chart.



*ChaMP-CMD: **Cha**racteristing **M**echanisms in **P**atients with **C**oronary **M**icrovascular **D**isease; CFR: coronary flow reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve; CMD: coronary microvascular disease*

Figure 12. Patient pathway and my role.

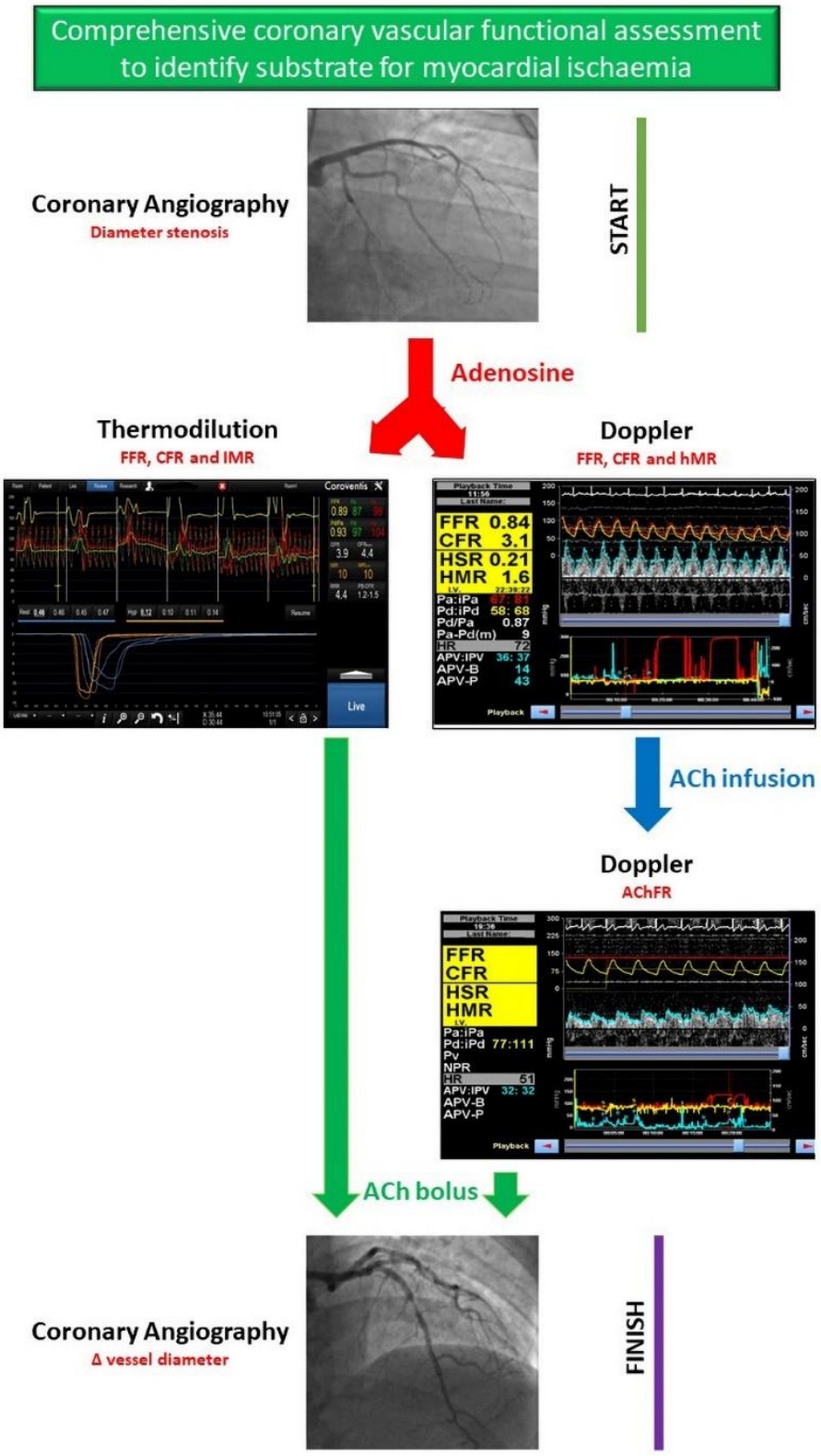


ANOCA: angina with nonobstructive coronary arteries; MMI: myocardial bridge muscle mass index; CTCA: computed tomography coronary angiography; ETT: exercise treadmill test; QCA: quantitative coronary angiography

2.2. Clinical protocol and catheter laboratory study

Our clinical catheter laboratory protocol is a systematic and standardised approach to coronary physiology assessment in patients with angina and nonobstructive coronary arteries (ANOCA). Our protocol has been published as part of the Coronary Microvascular Dysfunction Working Group in BMJ Heart[68] and has been adopted by centres across the country (**Figure 13**).

Figure 13. Standardised clinical protocol in patients with angina and nonobstructive coronary arteries (courtesy of Perera et al[68]).



FFR: fractional flow reserve; CFR: coronary flow reserve; hMR: hyperaemic microvascular resistance; IMR: index of microvascular resistance; AChFR: acetylcholine flow reserve

All patients with ANOCA undergo comprehensive physiology assessment with simultaneous measurement of intracoronary pressure and flow in response to adenosine and acetylcholine. This is the entry point to all the studies in my PhD. Acquiring high-quality measurements of coronary pressure and flow velocity was key to performing wave intensity analysis and accurately dichotomising patients into study groups.

Professor Divaka Perera, one of the most experienced Combowire operators in the country, manipulated the Combowire to acquire this data and several seconds of recordings were made across each condition to ensure adequate traces were obtained for offline analysis. The Combomap receives analogue signals and produces digitalised traces, however the data are refined between these steps to ensure that reliable traces can be analysed offline. I ensured veracity of the data being collected by optimising settings on the Combomap pertaining to flow velocity, instantaneous peak velocity (IPV) threshold and display threshold. I was also involved with the preparation and delivery of acetylcholine infusion.

2.2.1 Cardiac catheter laboratory study protocol overview

Coronary angiography was performed via the right radial artery in all patients who performed supine bicycle exercise (**Figure 8 and Figure 14**).

Figure 14. Catheter laboratory setup during in-lab supine bicycle exercise protocol.

All patients received 1mg intravenous midazolam before local anaesthetic was administered and arterial puncture took place. This was both to minimise the risk of radial artery spasm and to ensure that patients were calm ahead of any physiological measurements. Patients received 1mg of isosorbide dinitrate into the radial artery before advancement of the diagnostic catheters. Intracoronary acetylcholine measurements were made at least 15 minutes after administration of nitrates. This was to mitigate for the latter impacting on the pharmacological effects of the former. Prior to the acquisition of any research measurements, diagnostic views were taken and patients' FFR, CFR and hMR were measured. After delivery of intracoronary nitrates,140mcg/kg/min adenosine was administered through a venous cannula until hyperaemia was achieved; the latter was confirmed by maximal augmentation and maintenance of steady state coronary blood flow and ventricularisation of distal coronary pressure. Patients

with FFR≤0.80 were not recruited into any of the studies and were treated as per the standard guidelines. We carried out acetylcholine assessment at the end of the protocol (after the research measurements) because of concerns regarding occurrence of vasospasm resulting in ischaemia and affecting subsequent measurements. All recordings were made via guide catheters in the left anterior descending (LAD) artery. This is the standard practice in both clinical and research settings, with the majority of existing data on corresponding non-invasive ischaemia assessment and cardiovascular outcomes being derived from invasive measurements made in the LAD artery. However, there is now a growing understanding that coronary microvascular disease may not necessarily be a panmyocardial phenomenon but may rather be associated with patchy areas of regional microvascular dysfunction. Marroquin et al have demonstrated that the mean CFR, measured during positron emission tomography (PET) scans, was disparate in the three epicardial vessels in 34 women with ANOCA (2.85±1.35 in the LAD, 2.58±0.94 in the LCx and 3.24±1.42 in the RCA territories)[97]. Using CFR<2.5 as diagnostic of CMD, there was only moderate concordance between the three epicardial arteries (with strong Spearman's and Intraclass correlation between LAD and RCA but weak correlation between LAD and LCx and LCx and RCA territories). The disparate CFR values between LAD and LCx were due to a lower hyperaemic flow in the LCx territory. These findings suggest that either CMD is a regional condition with local variations in CFR or that CFR is not agnostic of the vessel being interrogated (i.e., different vessels have disparate CFR thresholds). It has been hypothesised that as the LCx artery subtends a smaller myocardial mass compared to the LAD artery, a lower hyperaemic flow may, in fact, be adequate for the myocardial oxygen demand[97]. As such, we currently do not have the diagnostic thresholds for RCA and LCx arteries to accurately diagnose patients as having an impaired CFR in these two vessels. In view of these data, applying the diagnostic threshold of <2.5 may not be appropriate for non-LAD arteries. Therefore, until a vessel-specific diagnostic threshold is defined, a standardised approach in

the LAD artery is likely to remain the standard practice as this allows an inter- and intra-patient comparison. In the majority of cases 5F guide catheters were used, due to the preponderance of radial artery spasm within the study population. Once the minimum clinical dataset was acquired, the guide catheter was disengaged from the left coronary ostium to minimise catheter trauma to the vessel. Extra backup guide catheters were used in all cases to minimise accidental suction of the tip of the catheter into the vessel ostium during exercise, which can theoretically happen with the Judkins left guide catheters. Patients who could not exercise were excluded from this protocol. A ramped exercise protocol starting at 30W with 20W increments every 2 minutes whilst maintaining a cadence of 60 revolutions per minute was employed. Where resistance limited further exercise, the workload was maintained and, therefore, tailored to the individual patient but continued for sufficient time for the patient to develop exhaustion. Aortic pressure, distal coronary pressure, coronary flow velocity and ECG were monitored continuously throughout. Once the exercise protocol was completed and all measurements returned to baseline levels, patients were infused with intravenous dobutamine at incremental doses of 10-, 20- and 30mcg/kg/min until 85% of the target heart rate was achieved and/or the patient developed significant chest tightness and/or ischaemic ECG changes. Patients were requested to withhold any negative inotropic and chronotropic agents 48 hours prior to the catheter laboratory study protocol.

Once all the parameters returned to baseline, patients were infused with intracoronary acetylcholine (18 mcg/ml) via the guide catheter. We started the infusion at 1ml/min and assessed for response (change in Pd and/or APV). After this, we acquired a cine and, if no evidence of spasm, increased the rate of delivery to 2ml/min for up to 3 minutes; this is equivalent to 10^{-4} mol/L. The Combowire allows continuous measurement of distal coronary blood flow (CBF) (in the form of APV) and distal coronary pressure (Pd) even during

intracoronary infusions. This permits measurement of CBF in response to acetylcholine infusions as a measure of coronary endothelium-*dependent* microvascular function. At the end of the acetylcholine infusion, the guide catheter was aspirated, and a cine image taken to check for evidence of vessel calibre reduction. If >90% vessel calibre reduction was observed with the acetylcholine infusion (which is diagnostic of vasospastic angina), then no further acetylcholine boluses were infused. In the absence of >90% vessel calibre reduction with the infusion, 100mcg intracoronary acetylcholine (5.5.ml of the 18mcg/ml solution) was delivered as a bolus over 20 seconds as per the standard guidelines for vasospasm assessment[68]. A diagnosis of vasospastic angina was confirmed by the reproduction of patients' characteristic chest tightness, ischaemic ECG changes and >90% reduction in vessel calibre. Using the Doppler wire offers an advantage over non-Doppler wires when assessing for coronary artery spasm. One can infer the presence of spasm, in response to acetylcholine infusion or bolus, by observing the change in APV and Pd and their relationship. If the Pd and APV both fall, then there is likely to be spasm proximal to the flow sensor; if the Pd falls but the APV rises then there is likely to be spasm at the site of the pressure and flow sensor; if the Pd remains static but the APV falls then there is likely to be spasm distal to the pressure and flow sensor. These changes precede the appearance of visual coronary artery diameter reduction on coronary angiography and are, therefore, a very sensitive marker of spasm. At the end of the protocol, intracoronary nitrates were infused to ameliorate any acetylcholine-induced spasm and final cine images taken. This concluded the clinical and catheter laboratory protocols. On average, a clinical catheter laboratory protocol (comprising adenosine and acetylcholine assessment) took 45-60 minutes, whereas the combined clinical and research catheter laboratory protocol (comprising the clinical protocol, in-lab supine bicycle exercise and dobutamine infusion) took 120 minutes. There were no complications during our clinical and catheter laboratory protocols. A minority of patients developed transient atrioventricular block that improved

immediately after the cessation of acetylcholine (as the latter has a half-life of a few seconds). None of the patients required atropine or pacing. A very small number of patients developed paroxysmal atrial fibrillation with acetylcholine infusion/bolus; only one patient required flecainide and no patients required direct current cardioversion. All patients who developed rhythm abnormalities during acetylcholine infusion/bolus were followed up with a 7-day Holter tape and no further rhythm abnormalities were uncovered during this monitoring period. The prevalence of paroxysmal atrial fibrillation during acetylcholine infusion is quoted to be 5% in the literature and is associated with left ventricular diastolic dysfunction and a history of known paroxysmal atrial fibrillation[98]. No ventricular arrhythmias were observed during acetylcholine infusion/bolus.

The concentration of ACh infusion used for the interrogation of endothelium-dependent function is in line with the current international guidelines[68]. Newman et al, in the 1990s, demonstrated that a normal coronary artery should dilate and increase coronary blood flow in response to ACh infusion at doses of up to 10^{-4} mol/L and most healthy volunteers would vasoconstrict at concentrations of 10^{-3} mol/L or higher[75]. Subsequently, Hasdai et al demonstrated that patients with an inability to augment coronary blood flow by ≥50% in response to intracoronary acetylcholine infusion at 10^{-4} mol/L had a higher prevalence of myocardial ischaemia on myocardial perfusion imaging[32]. This data was complemented by the evidence of worse cardiovascular outcomes in patients with an AChFR<1.5[34]. My predecessor, Dr. Haseeb Rahman, had carried out ACh infusion assessments at 10^{-6} – 10^{-4} mol/L as part of his PhD studies and had demonstrated an excellent safety profile of these ACh concentrations. We elected to use the 10^{-4} mol/L concentration and vary the rate of delivery from 1ml/min to 2ml/min after 2 minutes. For our coronary artery spasm provocation assessment, we elected to

use 100mcg ACh bolus, delivered over 20s, in the LAD artery; this is in keeping with the international guidelines and the rationale has been discussed in detail earlier.

2.2.2. Calibration and Optimisation of Pressure and Flow Velocity Signals

Following intubation of the left coronary artery ostium, the Combowire (fitted with Doppler sensor at the distal tip and pressure sensor 15mm proximal to the Doppler sensor) was advanced until the pressure sensor was between the guide catheter and the ostium of the coronary artery. The introducer needle was withdrawn, the catheter flushed with saline and the ComboWire pressure equalized to the catheter signal. The ComboWire was manipulated into the mid-to-distal LAD artery (at least 5cm from the ostium) and fine rotational movements applied to obtain optimal and stable Doppler traces using the density of the signal on the visual display of the console as well as the phasic auditory signal. Optimal readings occur when the Doppler probe is aligned co-axially with the vessel. When using a wire with offset sensors, it is desirable to manipulate the wire so that the tip is in a retroflex (or looped) orientation, which allows a more stable signal and ensures that the pressure and Doppler sensors are in the same location within the artery (**Figure 15**). Once an optimal Doppler signal was obtained, I optimised the signal to noise ratio by varying the IPV threshold, display threshold and the velocity scale (**Figure 16**).

Figure 15. Looped Combowire in the distal left anterior descending artery.

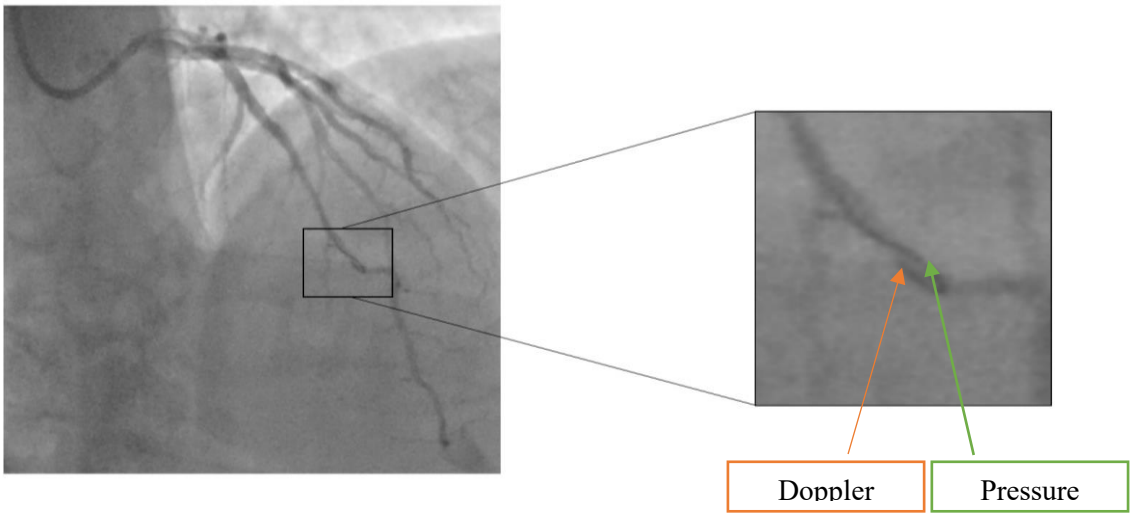
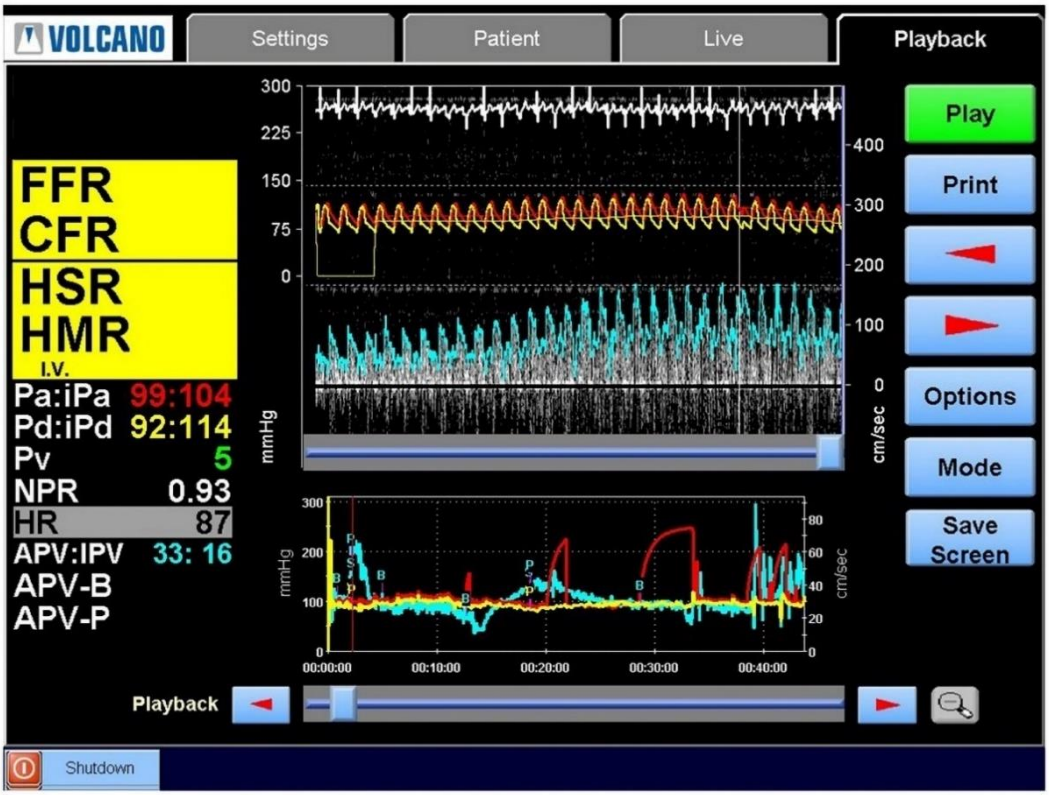


Figure 16. Example of Combomap screen. Signal to noise ratio can be optimised by altering IPV threshold, display threshold and velocity scale in 'Settings'.

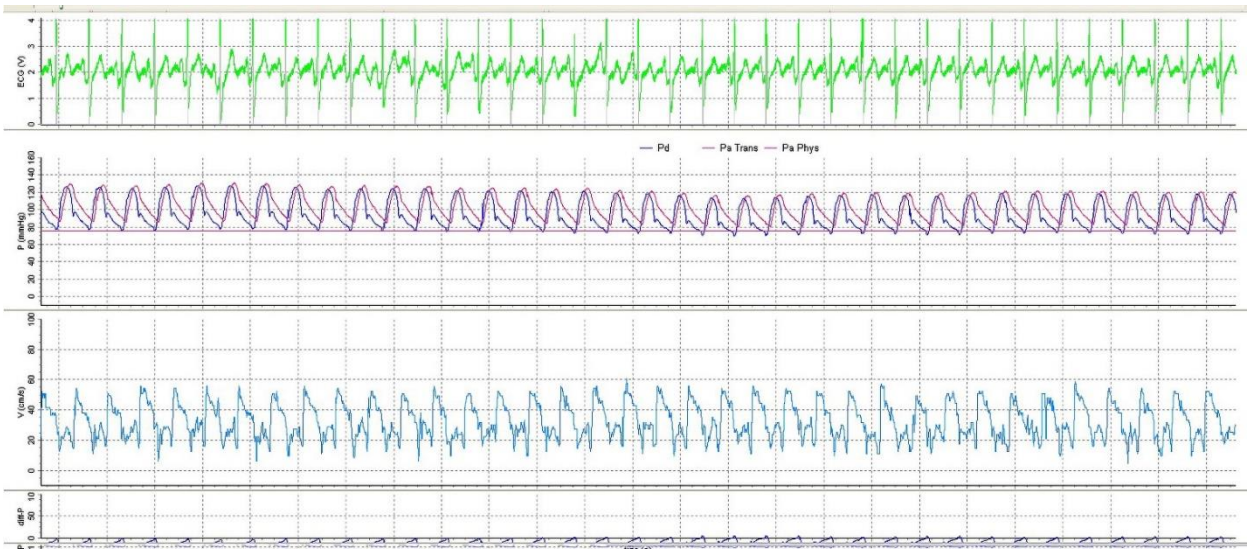


ECG (top panel), proximal aortic (red) and distal coronary (yellow) pressures (middle panel, Doppler flow velocity (bottom panel).

2.2.3 Off-line Data Processing

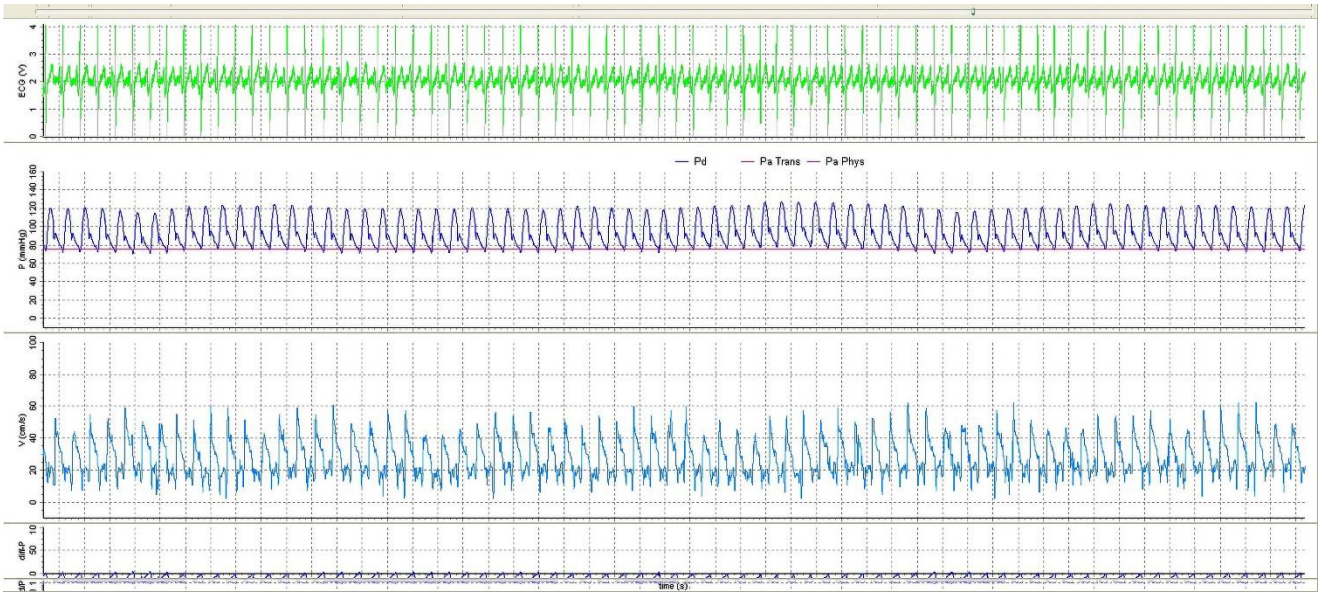
I performed all the post-processing and coronary physiology analysis using dedicated software packages at the end of the study. Raw physiology data were exported in the form of .SDY files and analysed using Study Manager, which is a custom-made software. The user can view all the collected physiological variables, select the cardiac cycles of interest (in our case, this included rest #1, hyperaemia, rest #2, supine exercise, rest #3, dobutamine infusion, rest #4, acetylcholine infusion and bolus) and convert the .SDY file in text file format for further analysis. Setting markers during the coronary physiology measurements in the catheter laboratory aids in recognition of specific cycles in Study Manager. A minimum of 10, but typically 25-50 cardiac cycles, were selected for further analysis during adenosine, exercise and dobutamine stress, whereas 10 cardiac cycles were most appropriate during peak acetylcholine response given its short half-life (**Figure 17** and **Figure 18**).

Figure 17. Selection of appropriate cardiac cycle on Study Manager.



ECG traces (top panel), proximal aortic (red) and distal coronary (dark blue) pressures (middle panel), coronary flow velocity (bottom panel)

Figure 18. Selection of appropriate cardiac cycle on Study Manager during acetylcholine infusion.

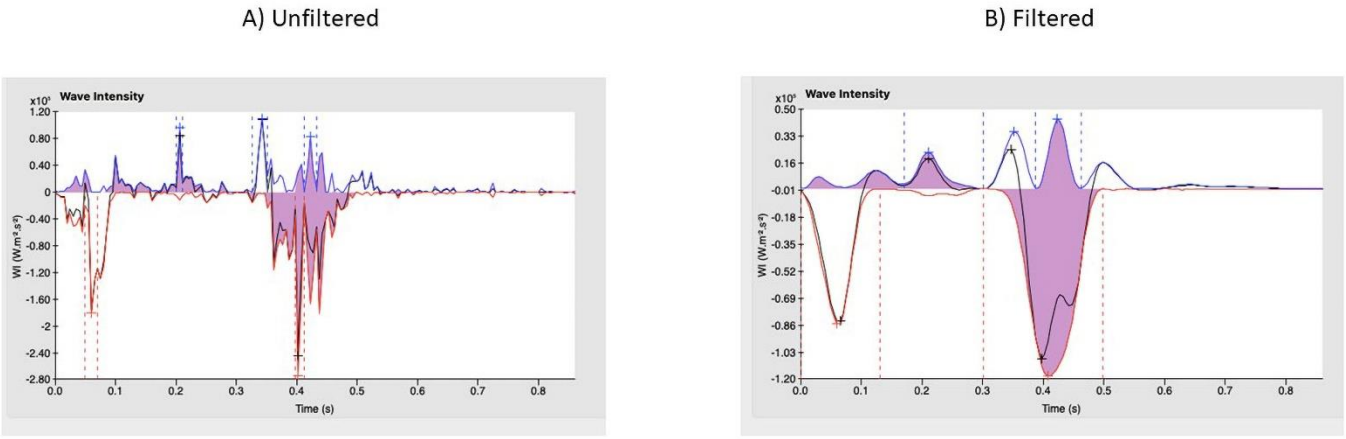


ECG traces (top panel), distal coronary pressure (middle panel), coronary flow velocity (bottom panel). Note: the proximal aortic pressure is absent as the acetylcholine is directly being infused into the coronary arteries through the manifold. Distal coronary pressure, coronary flow velocity and ECG traces are being measured continuously.

The corresponding text files were analysed on a custom-made software called Cardiac Waves, which has been developed between King's College London and Academic Medical Centre, Amsterdam. The software allows the user to select which cardiac cycles to include, and which to exclude, for the final analysis. Premature ventricular contractions and poor flow signal were the main reasons for exclusion of certain cardiac cycles. The flow and pressure signals were passed through Savitzky-Golay smoothing filters, which work by fitting a polynomial of a chosen order to a chosen number of points about the centre point using least squares. This has the advantage of preserving peaks in the data during smoothing, whilst the level of filtering can

be directly controlled by changing the order of the polynomial and the frame width constants. All datasets included in the results were subjected to the same level of Savitzky-Golay filtering. The effectiveness of these filters is demonstrated in **Figure 19**. The post-processing of data was carried out by two independent researchers at least twice to test the veracity of the analyses and to ensure there was no significant inter- and intra-observer variability.

Figure 19. Coronary wave intensity analysis of identical haemodynamic data, without (A) and with (B) Savitzky-Golay filtering.



2.2.4 Pan-cardiac Cycle Indices

Average peak velocity (APV) was used as a surrogate of coronary blood flow (CBF) during adenosine-mediated vasodilatation, as there is negligible variation in epicardial vessel diameter in response to adenosine[99]. The following measurements were made during hyperaemia: Fractional flow reserve (FFR), Coronary flow reserve (CFR) and hyperaemic microvascular resistance (hMR)

As exercise, dobutamine stress and acetylcholine infusion can all lead to changes in vessel calibre, volumetric CBF was calculated incorporating the vessel calibre using quantitative

coronary angiography (as described previously). The following measurements were made during each of these stressors:

Exercise Pd/Pa = Pd/Pa during peak exercise

Exercise flow reserve = CBF during peak exercise/resting CBF

Dobutamine Pd/Pa = Pd/Pa during peak dobutamine infusion

Dobutamine flow reserve = CBF during peak dobutamine infusion/resting CBF

AChFR = CBF at peak acetylcholine infusion/resting CBF

2.2.5 Wave Intensity Analysis

Net wave intensity normalised for the sampling interval represents the energy flux carried per cross sectional area of a vessel, determined by the product of change in distal coronary pressure (Pd) and velocity (U) at a single location:

$$\text{Wave intensity} = \frac{dP}{dt} \cdot \frac{dU}{dt}$$

When dP and dU change in the same direction, wave intensity (WI) is positive, and the wave is classified as forward travelling; when the dP and dU change in the opposite direction then the WI is negative, and the wave is classified as backward travelling. Positive (forward travelling) waves are propagated from the proximal end of the circulation (aortic or epicardial), whereas negative (backward travelling) waves arise from the distal microvasculature. This characteristic, of flow arising from both proximal and distal ends, is unique of the coronary circulation[100] (**Figure 20**). Net wave intensity is made up of the contribution of forward travelling (WI^+) and backward travelling (WI^-) waves arriving at the same measurement site:

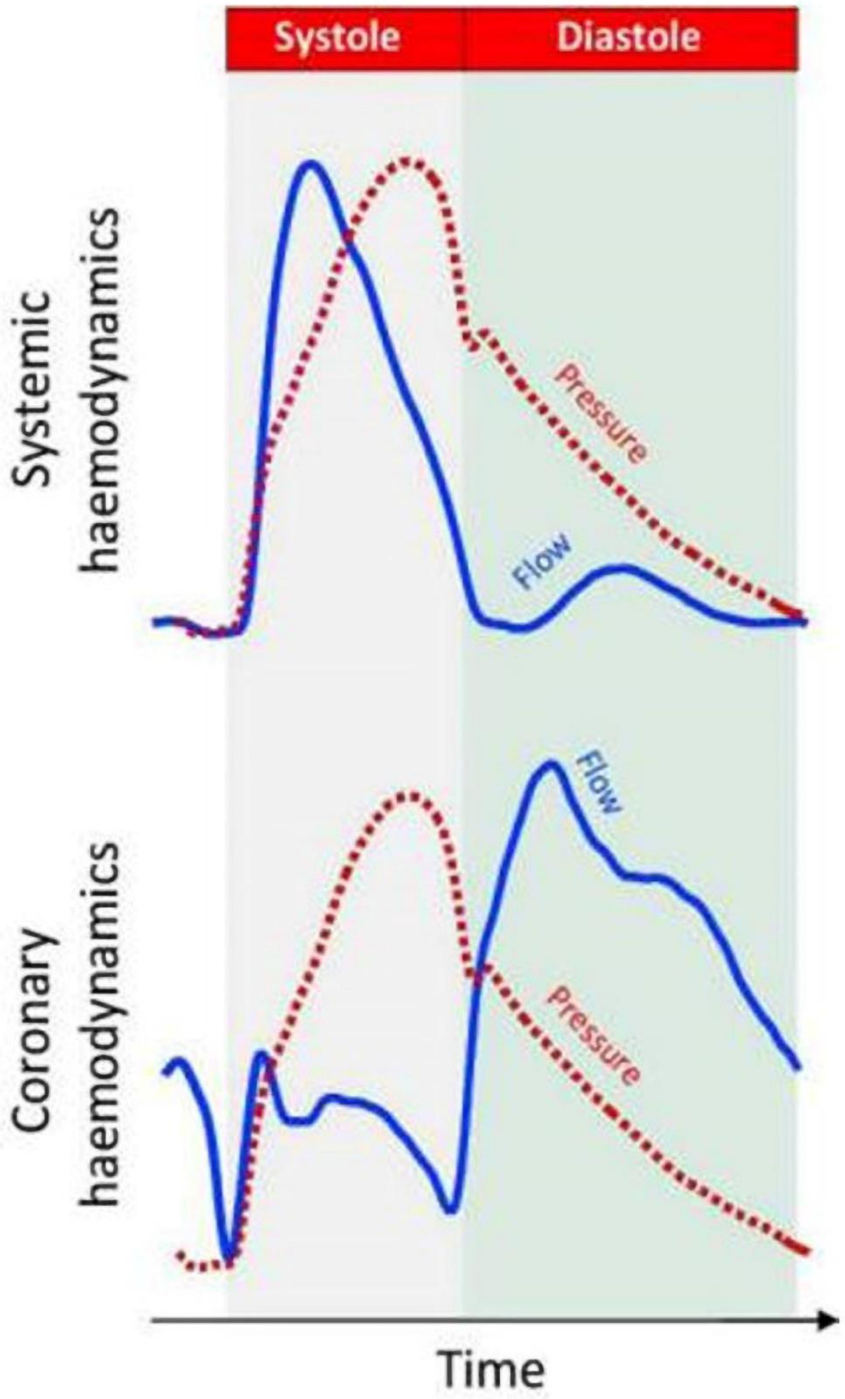
$WI = WI^+ + WI^-$

Using CardiacWaves, the integrals for WI (areas under the curve) were calculated for the five main waves described, providing a surrogate of wave energy rather than energy flux. The proportion of wave energies during each physiological condition was calculated, with coronary perfusion efficiency (PE) being the proportion of accelerating wave energy divided by total wave energy:

$$\mathrm{PE} = \frac{BEW_{AUC} + FCW_{AUC} + late\ FCW_{AUC}}{BEW_{AUC} + FCW_{AUC} + BCW_{AUC} + FEW_{AUC} + late\ FCW_{AUC}}$$

Coronary perfusion efficiency provides a simple metric into the efficiency of cardiac-coronary coupling and the energy requirement to achieve the degree of flow augmentation measured using conventional pan-cyclic indices of coronary blood flow.

Figure 20. Differences between coronary and systemic pressure-flow relationship (courtesy of Sen et al)[100].



In the systemic circulation, there is only one source of forward traveling blood flow (aortic pressure); however, in the coronary circulation there are two sources (aortic pressure and distal coronary microvasculature).

2.3. Exercise electrocardiogram treadmill test protocol

Exercise electrocardiogram treadmill testing (ETT) was performed with a Marquette Case 8000 system (GE Medical Systems, Milwaukee, WI, USA) according to American College of Cardiology and American Heart Association practice guidelines using the standard Bruce protocol[77,78]. The stages of the standard Bruce protocol are summarised in **Table 1**.

Table 1. Standard Bruce exercise electrocardiogram treadmill test protocol.

Stage	Time (min)	Speed (km/h)	Slope
1	0	2.74	10%
2	3	4.02	12%
3	6	5.47	14%
4	9	6.76	16%

12-lead electrocardiogram (ECG), heart rate (HR) and blood pressure (BP) were recorded at rest, at 3-minute intervals during exercise, as well as 2 minutes and 5 minutes after start of the cool-down period. The test was supervised by an exercise treadmill test physiologist who was blinded to the patients' coronary physiology data as well as any medications that they were taking. The test was terminated upon patients' request, with minimal prompting given to patients in order to minimise bias. At the end of the protocol, the 12-lead ECG traces were interpreted by three independent researchers who were blinded to the coronary physiology data; ischaemic changes were adjudicated as per the majority interpretation. Ischaemic ECG changes were defined as the appearance of ≥0.1mV horizontal or downsloping ST-segment depression

80 milliseconds from the J-point during exercise. Patients were not requested to halt any medications prior to the exercise ECG treadmill test, which is representative of real-world practice. Other recorded outcome measures were exercise time (ET), which was defined as the time from the start of the exercise protocol to the cessation of exercise. Patients' reason for stopping the test was recorded and, where applicable, time to angina and time to ≥0.1mV ST depression were recorded as the time from the start of exercise protocol to the time of onset of angina and to the time of onset of ≥0.1mV horizontal or downsloping ST-segment depression respectively. The rate pressure product, which is a surrogate of myocardial work, was calculated as the product of heart rate and systolic blood pressure. **Figure 21** illustrates the setup of our ETT protocol.

Figure 21. Exercise electrocardiogram treadmill test setup.

2.4. Phenotype-blinded randomised crossover trial protocol

To assess if physiology-stratified therapy predicts response to anti-ischaemic therapy in patients with angina and nonobstructive coronary arteries, we carried out a phenotype-blinded, randomised, crossover trial. The primary outcome was change in exercise time on treadmill test and the secondary outcome was change in quality of life as measured on the Seattle Angina Questionnaire (SAQ) summary score. Importantly, the patients, researchers and ETT physiologists were all blinded to the coronary physiology data. Data analysis was carried out once the database was locked at the end of the study.

2.4.1. Outcome measures

Change in exercise time (in seconds) compared with baseline on ETT is a meaningful outcome to patients and has been the gold standard measure for therapy trials in patients with stable angina. A within-subject improvement in exercise time of 30 seconds on the standard Bruce protocol is considered to be clinically relevant[101]. Our study was powered to identify an improvement in exercise time of 60 seconds or longer in response to anti-ischaemic therapy, which is in line with seminal anti-ischaemic therapies in patients with obstructive coronary artery disease[102–105].

The Seattle Angina Questionnaire (SAQ) is a validated, disease-specific questionnaire that quantifies limitations caused by angina, the frequency of angina, treatment satisfaction, and subjective perception of quality of life[106]. Each component score is converted and collated to give a total score out of 100, where a higher score indicates better function. SAQ scores are independently associated with mortality, hospitalisation, and resource use[107]. The SAQ is a

sensitive instrument in patients with coronary microvascular disease[94,95], and a change in 8 points has previously been demonstrated to represent a minimal clinically important difference.

2.4.2. Randomisation

Patients were randomised in a 1:1 manner to amlodipine or ranolazine after the first ETT. An automated randomisation tool was used for this purpose and was carried out by a member of the research team that was blinded to the coronary physiology assessment. The crossover nature of the trial means that each patient served as their own control and, therefore, a more complex means of randomisation (such as block or stratified randomisation) was not necessary.

2.4.3. Blinding

This trial was phenotype-blinded, i.e., the patients, researchers and ETT physiologists were blinded to patients' coronary physiology data. Patients were informed that their epicardial arteries do not have significant narrowing but were not informed of the results of the microvascular function assessment until the end of the study. Researchers who were involved with the coronary physiology assessment had no further involvement with the therapeutic protocol. Outcome assessment (end point adjudication) was undertaken in a blinded fashion.

2.4.4. Trial medication

Adherence with trial medication was assessed at visits 3 and 4 by tablet count and patient-reported adherence with therapy. If patients reported < 80% adherence to a medication, then they were not included in the per protocol analysis pertaining to that medication. Ranolazine

was started at 375mg twice a day regimen, and uptitrated within 1-2 weeks after a repeat ECG (to ensure absence of QTc prolongation). Amlodipine was started at 5mg and then uptitrated to the maximal tolerated dose within 1-2 weeks of commencing the drug. As ChaMP-CMD was a phenotype-blinded strategy trial assessing the utility of physiology-stratified therapy, rather than a trial assessing the efficacy of a certain medication, placebo-blinding was not employed. Neither the patients nor the researchers were aware of the patients' phenotype and, therefore, were not able to predict the response to anti-ischaemic medications.

The novel phenotype-blinded cross-over design of ChaMP-CMD allows for a cost-effective way to simultaneously compare response to therapies in different endotypes through one study design. The crossover design means that each patient acts as their own control; therefore, increasing the study power. Our study design is different to conventional double-blind placebo-controlled trials, such as CARISA[108], which were designed to assess the response to certain pharmacotherapy, such as ranolazine, where patients and physicians were blinded to the medication and not the anatomy or physiology.

No serious adverse events were reported during our trial.

2.5. Development of methods

This section describes the iterative changes I have made to the study protocols, including methodological insights gained that have facilitated my growth as a researcher. I have implemented some of these into grant applications for upcoming studies.

2.5.1. Changes to the catheter laboratory protocol during my PhD

My predecessor, Dr. Haseeb Rahman, had previously demonstrated that resting coronary blood flow was elevated in patients with functional CMD, which was likely secondary to elevated nitric oxide synthase levels in this cohort[24]. Previous studies have demonstrated that resting coronary blood flow is mediated by neuronal nitric oxide synthase (nNOS)[6,26]; therefore, we wanted to test the hypothesis that nNOS activity was upregulated in patients with functional CMD. This could be an appropriate upregulation secondary to heightened resting myocardial oxygen demand or disordered autoregulation. The use of S-methyl-L-thiocitrulline (SMTC), an nNOS inhibitor, has allowed researchers to determine whether nNOS plays a role in coronary vascular tone regulation[6]. Therefore, SMTC is an ideal vasoactive agent to study whether the elevated resting coronary blood flow in patients with functional CMD is mediated by heightened nNOS activity.

We carried out the in-lab SMTC protocol between March 2021 and August 2021. SMTC was obtained from Merck Millipore (Massachusetts, USA) at a purity of >99% and formulated for human injection to good manufacturing practices by the Pharmacy Aseptics department at Guy's Hospital, London, UK. It was formulated as an aqueous solution with an appropriate pH for injectables. After bioburden testing, SMTC was processed as a sterile injection and tested at an external pharmaceutical testing laboratory. Each batch was tested for absence of endotoxin according to British Pharmacopoeia standards. SMTC was stored at -80 degrees Celsius and was thawed on the day of the procedure.

The planned study dose of intracoronary SMTC (0.625 μmol/min) was chosen based on previous coronary studies to achieve a local coronary artery concentration of 5 μmol/L and to be devoid of eNOS-inhibitory effects[6,26]. After patients had undergone adenosine and

acetylcholine infusion as part of the clinical protocol, and where there was no evidence of coronary vasospasm, SMTC was infused into the LAD artery for 7 minutes at 2ml/min with close monitoring of coronary physiology and systemic haemodynamics. The 7-minute timer for SMTC was only turned on after the dead space, within the catheter, had been overcome. SMTC infusion was tolerated safely in all patients and there was no evidence of ischaemic ECG changes or chest pain.

However, due to unforeseen circumstances, our ability to recruit patients for the in-lab SMTC study was abruptly impacted in August 2021. Firstly, from September 2021-March 2022, there was a halt in the production of this substance. On further introspection, it became obvious that the logistics around the SMTC protocol were proving too difficult to make it a tenable study. SMTC has a short window period from production to expiry date (two weeks), which led to high levels of wastage due to the inability to list large number of research cases on catheter laboratory lists; this was further compounded by the COVID pandemic. If our patients had demonstrated coronary vasospasm with acetylcholine infusion and/or bolus, requiring nitrates to ameliorate the spasm, then we are unable to use SMTC, which led to a sizeable drop-out rate. SMTC needs to be infused for at least 7 minutes to observe an effect. It has previously been demonstrated that, with time, there is a natural drop off in APV values even in the absence of vasoactive substances. Therefore, unless we allowed for a 7-minute period of monitoring followed by a 7-minute period of SMTC infusion, it would have been difficult to distinguish whether attenuation of APV values, in response to SMTC, was due to the nNOS inhibitory effects of SMTC or whether it represented the natural drop off in Doppler signal that is expected over time. This limitation was further compounded by the fact that basal coronary blood flow attenuation in response to SMTC was modest at best during our interim analysis; this made distinguishing between the real nNOS inhibitory effects and the natural drop off in APV even

more difficult. As a result of these limiting factors, we discussed the logistics of the SMTC study at my upgrade viva presentation and we decided to disinvest in this study. I had had an interest in assessing the ischaemic mechanisms of patients with myocardial bridges from an early stage in my PhD and this was the perfect opportunity to replace the in-lab SMTC study with an in-lab bicycle exercise study. I was able to get the required ethics amendment and started recruiting for the in-lab bicycle exercise study in due time. This was an excellent learning process for me and taught me how to handle a study/experiment that has had unforeseen logistic complications. I feel that we acted decisively and avoided loss of resources and momentum; these are skills that will no doubt be very useful in my future academic ventures.

2.5.2. Intracoronary nitrate administration and pre-medication

With regards to pre-medication, this was a cohort with a high proportion of female patients with smaller radial artery diameters. The need to perform bicycle exercise via the right radial route meant that preventing spasm was vital to data acquisition. All patients received 1mg of intravenous midazolam and 1mg of isosorbide dinitrate through the radial artery as standard. We started a timer from the onset of nitrate delivery and ensured that any intracoronary acetylcholine assessment was carried out at least 15 minutes after the nitrate was delivered. This was to prevent any interaction between nitrates and acetylcholine.

Excessive sedation could not be achieved due to the need to undergo bicycle exercise. Our group has described the phenomenon of coronary flow variability prior to the start of each condition by simple catheter laboratory commands[109]; therefore, we carried out repeat rest measurements at the end of each stage to facilitate paired comparisons between stressors and their corresponding resting measurements.

2.5.3. Order of pharmacological and exertional stressors

The order of pharmacological agents and exercise was carefully chosen. We elected to use adenosine as the first pharmacological agent given that all our catheter laboratory studies had a clinical indication (to identify an ischaemic substrate in patients with limiting angina and nonobstructive coronary arteries) and answering this was of utmost importance. Only after robust FFR and CFR measurements were made did we move on to our research protocol. Once the physiology had returned to the baseline, we commenced the supine bicycle exercise protocol. Patients were requested to trial the mounted bicycle before laying supine on the catheter laboratory table; this was to ensure the bicycle was placed in an optimal position. The extra backup guide catheter was backed out of the left main coronary artery ostium under x-ray guidance. Intracoronary flow and pressure were simultaneously monitored throughout the bicycle exercise. In case of deterioration of the Doppler signal, the looped Combowire was gently manipulated until optimal signals were regained. A researcher was constantly communicating with the patient to ensure patient comfort and to inform them of upcoming increases in resistance, as well as ensuring optimal cadence. The supine bicycle exercise protocol was completed after acquisition of peak exercise data and/or after patient prompting (one patient reported angina during the in-lab bicycle protocol).

The concentrations of intravenous dobutamine infusion (10-, 20-, and 30mcg/kg/min) were decided upon after consulting with our imaging specialists, as well as contemporary literature. Most patients demonstrate peak response with 20-30mcg/kg/min, with no incremental gain from 40mcg/kg/min; furthermore, many patients, especially if hypovolaemic, develop presyncope/syncope with the 40mcg/kg/min dose due to a hypercontractile left ventricle and reduced preload. The intracoronary physiology assessment was complete once the patient

reached 85% of their target heart rate or requested to stop the assessment due to symptoms. Intracoronary acetylcholine infusion was reserved to the end due to the possibility of spasm, and therefore ischaemia and resultant catecholamine response to pain, which may have affected the responses to exercise and dobutamine. Furthermore, reserving the acetylcholine assessment to the end ensured that at least 15 minutes had transpired between the intracoronary nitrate and acetylcholine testing.

2.6. Statistical analyses

Normality of data was assessed using the Kolmogorov-Smirnov test. Normally-distributed continuous data are presented as mean ± standard deviation (SD) and compared using the independent sample's Student *t*-test. Continuous data without normal distribution are presented as median (interquartile range) and compared using the Mann-Whitney test (unpaired analyses) or the Wilcoxon matched-pairs signed rank test (paired analyses). Categorical variables are presented as n (%) and compared using the chi-squared test. Binary logistic regression was performed using univariate and multivariate analysis and reported as standard coefficients (95% CI). Biologically plausible variables were assessed in the univariate model, and those that correlated were included in the multivariate model. Specific statistical analyses are described in detail in each chapter. All analyses were performed using SPSS Statistics 27 (IBM, NY, USA) and GraphPad Prism software version 9.0 for Windows (GraphPad software, San Diego, CA, USA). We deemed a p value less than 0.05 to be significant.

Chapter 3

Rethinking the false positive exercise electrocardiogram treadmill test in the context of coronary microvascular disease

Abstract presented at European Society of Cardiology 2023 congress

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3.1. Abstract

Background

Exercise electrocardiogram treadmill testing (ETT) has historically been validated against the demonstration of obstructive coronary artery disease (CAD). However, myocardial ischaemia can occur due to coronary microvascular disease (CMD) in the absence of obstructive CAD. This study aimed to assess the specificity of ETT to detect an ischaemic substrate against the reference standard of coronary endothelium-*independent* and endothelium-*dependent* microvascular function in patients with angina and nonobstructive coronary arteries (ANOCA).

Methods

Patients with ANOCA underwent invasive coronary physiology assessment using adenosine and acetylcholine. CMD was defined as impaired endothelium-*independent* and/or endothelium-*dependent* function. ETT was performed using a standard Bruce treadmill protocol, with ischaemia defined as the appearance of ≥0.1mV ST-segment depression 80 milliseconds from the J-point on electrocardiography. The study was powered to detect a specificity of ≥91%.

Results

One hundred and two patients were enrolled (65% females, 60±8 years old). Thirty-two patients developed ischaemic ECG changes (ischaemic group) during their ETT, whilst 70 patients did not (non-ischaemic group); both groups were phenotypically similar. Ischaemic ECG changes during ETT were 100% specific for underlying coronary microvascular disease. Acetylcholine flow reserve was the strongest predictor of ischaemic ECG changes during exercise. Using endothelium-*independent* and endothelium-*dependent* microvascular dysfunction as the reference standard, the false positive rate of ETTs dropped to 0%.

Conclusion

In patients with ANOCA, ischaemic ECG changes on ETT were *always* attributable to an underlying ischaemic substrate. These findings challenge the traditional belief that ETT has a high false positive rate.

3.2. Introduction

Exercise electrocardiogram treadmill testing (ETT) represents a ubiquitous, non-invasive, and low-cost functional test for the evaluation of patients with new onset angina. However, its use has declined over the past decade due to the higher sensitivity of other non-invasive stress imaging modalities and the perceived high false positive rate of ETT. In view of this, ETT has been downgraded to a class IIb recommendation in the latest European Society of Cardiology (ESC) guidelines[79]. It is important to remember that the accuracy of ETT has historically been assessed and validated against its ability to detect the presence of obstructive coronary artery disease (CAD) with the reference standard being visual diameter stenosis on coronary angiography. However, we now know that myocardial ischaemia can occur in the absence of obstructive CAD due to coronary microvascular disease (CMD)[110]. Therefore, it is conceivable that the historical false positive ETTs were not due to the poor specificity of ETT as a diagnostic test, but rather due to the limitations of obstructive CAD as a reference standard for myocardial ischaemia. The aim of this study was to examine the specificity of ETT in detecting an ischaemic substrate when compared against the robust reference standard of coronary endothelium-*independent* and endothelium-*dependent* microvascular function in patients with angina and nonobstructive coronary arteries (ANOCA).

3.3. Methods

3.3.1. Study population

We prospectively enrolled consecutive patients presenting with angina who were referred for further assessment. Inclusion criteria were angina with nonobstructive coronary arteries (ANOCA; fractional flow reserve>0.80) and preserved left ventricular ejection fraction (>50%). Exclusion criteria were inability to undergo adenosine and acetylcholine assessment, chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/m^2), significant

valvular disease, history of acute coronary syndrome, previous revascularisation, cardiomyopathy (including left ventricular hypertrophy), limitation by non-anginal symptoms, existing bundle branch block, poor ECG traces during exercise or paced rhythm hindering ECG interpretation. All patients provided written informed consent in accordance with the protocol approved by the UK National Research Ethics Service (20/LO/1294).

3.3.2. Intracoronary physiology assessment

Our protocol for systematic evaluation of ANOCA has been described in full in Chapter 2. Briefly, all coronary physiology measurements were made in the left anterior descending artery. A 0.014-inch dual sensor-tipped intracoronary guidewire was used for measurement of distal coronary pressure (P_d) and average peak velocity (APV). Aortic pressure (P_a) was measured via the fluid-filled guide catheter. Patients were asked to withhold any vasodilating agents for at least 24 hours prior to the invasive coronary physiology assessment. All patients received 1 mg intravenous midazolam, 200 mcg intracoronary glyceryl trinitrate and 70 U/kg unfractionated heparin prior to angiography and physiology assessment. We first assessed endothelium-*independent* microvascular function using intravenous adenosine (140 mcg/kg/min), followed by endothelium-*dependent* microvascular function using graded intracoronary infusions of acetylcholine (18mcg/ml of acetylcholine solution delivered at 1ml/min followed by 2ml/min) via the guide catheter. All intracoronary acetylcholine measurements were made at least 15 minutes after the intracoronary nitrate injection. Patients, researchers, and ETT physiologists were blinded to the results of the coronary physiology assessment.

3.3.3. Physiology data analysis

Signals were sampled at 200 Hz, with data exported into a custom-made study manager program (Academic Medical Centre, University of Amsterdam, Netherlands) and analysed on custom-made software: Cardiac Waves (Kings College London, UK). Coronary flow reserve (CFR) was derived as hyperaemic APV/basal APV; endothelium-*independent* microvascular dysfunction was defined as CFR<2.5[25,68]. Hyperaemic microvascular resistance (hMR) was calculated as P_d/APV during hyperaemia. Elevated hMR was defined as hMR≥2.5mmHg.cm^{-1}.s^{-1}. Acetylcholine flow reserve (AChFR) was calculated as the ratio of coronary blood flow (CBF) in response to acetylcholine infusion compared to basal CBF; endothelium-*dependent* microvascular dysfunction was defined as AChFR≤1.5[50,68]. The estimation of volumetric flow from Doppler flow velocity also incorporates vessel diameter. Given that acetylcholine can cause either epicardial vasodilation or vasoconstriction, volumetric CBF was calculated as quantitative coronary angiography (QCA)-derived cross-sectional area × APV × 0.5, with QCA performed 5mm distal to the tip of the guidewire. Coronary microvascular disease (CMD) was defined as endothelium-*independent* and/or endothelium-*dependent* microvascular dysfunction (i.e., CFR<2.5 and/or AChFR≤1.5)[68].

3.3.4. Exercise electrocardiogram treadmill test protocol

Exercise electrocardiogram treadmill testing (ETT) was performed after coronary angiography with physiology assessment. ETT was performed with a Marquette Case 8000 system (GE Medical Systems, Milwaukee, WI, USA) according to the American College of Cardiology and American Heart Association practice guidelines using a standard Bruce protocol[77,78]. 12-lead electrocardiogram (ECG), heart rate and blood pressure were recorded at regular intervals before, during and after the ETT. All ETTs were supervised by cardiac physiologists who were

blinded to the coronary physiology measurements. The only criterion for termination of the test was patient request.

Exercise time was defined as the time from the start of the exercise protocol to exercise cessation. Exercise-induced angina was documented where the patient reported chest tightness during exercise. Ischaemic ECG changes were defined as the appearance of ≥0.1mV horizontal or downsloping ST-segment depression 80 milliseconds from the J-point during exercise. All ECG tracings were reviewed by three independent observers blinded to the coronary physiology data; ischaemic changes were adjudicated as per the majority interpretation. Patients who developed ischaemic ECG changes were classified as the 'ischaemic' group and those who did not as the 'non-ischaemic' group. Patients were not requested to halt any medications prior to the ETT, which is representative of real-world practice.

3.3.5. Statistical analyses

Normality of data was assessed using the Kolmogorov-Smirnov test. Normally-distributed continuous data are presented as mean ± standard deviation (SD) and compared using the independent sample's Student *t*-test. Continuous data without normal distribution are presented as median (interquartile range) and compared using the Mann-Whitney test. Categorical variables are presented as n (%) and compared using the chi-squared test. A positive ETT was defined as development of ischaemic ECG changes during the ETT. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for the development of ischaemic ECG changes and exercise-induced angina against different reference standards of ANOCA with i) endothelium-*independent* microvascular dysfunction (CFR<2.5), ii) endothelium-*dependent* microvascular dysfunction (AChFR≤1.5) and iii) CMD (CFR<2.5 and/or AChFR≤1.5). Values are presented as percentages. An apparent false positive

rate was calculated as apparent false positives divided by the sum of apparent false positives and true negatives for the above reference standards. Apparent false positive rates, with the different reference standards, were compared using the McNemar's test. Binary logistic regression was performed using univariate analysis and all statistically significant variables were entered into a multivariate model; data are presented as standardised coefficient (95% CI). Inter-observer reliability in interpreting the exercise ECGs for presence or absence of ischaemic changes was assessed using the intraclass correlation coefficient. All analyses were performed using SPSS Statistics 27 (IBM, NY, USA) and GraphPad Prism software version 9.0 for Windows (GraphPad software, San Diego, CA, USA).

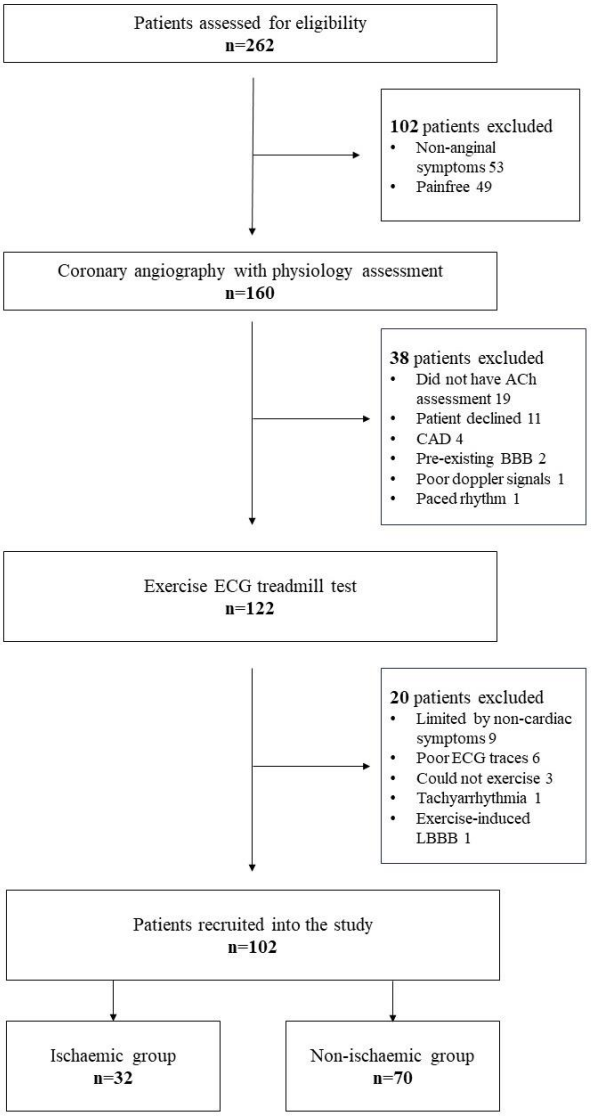
3.3.6. Sample size calculation

In a previous study, ischaemic ECG changes on ETT had 80% specificity in detecting coronary vasomotor dysfunction[111]. Assuming a 30% rate of ischaemic ECG changes on ETT, we calculated that a sample size of 100 patients would give an absolute precision of 0.1 (95% CI) for a specificity of 91%[112].

3.4. Results

Between March 2021 and July 2023, 262 patients with stable angina were assessed for eligibility. Out of these, 160 underwent coronary angiography with physiology assessment. One hundred and twenty-two patients with ANOCA underwent both adenosine and acetylcholine assessment in the catheter laboratory and were deemed suitable to enrol into the study, of which 102 patients were included in the final analysis (**Figure 22**).

Figure 22. Consort diagram demonstrating the ETT study flow.



ACh: acetylcholine; CAD: coronary artery disease; BBB: bundle branch block

Twelve patients (12%) did not have any prior coronary-based investigations, 50 (52%) had prior computed tomography coronary angiography scan, 8 (8%) had previous stress imaging (stress echocardiography or cardiac magnetic resonance imaging) and 27 (28%) had previous coronary angiography. Exercise ECG treadmill testing took place 29 (20, 139) days after the coronary angiography with physiology assessment. Thirty-two patients developed ischaemic ECG changes during their ETT (**ischaemic group**), whilst 70 did not (**non-ischaemic group**).

There were no differences in gender, age, body mass index, cardiovascular risk factors, Canadian Cardiovascular Society angina grade and New York Heart Association class between the two groups (**Table 2**). Patients in the ischaemic group had a higher percentage of typical angina (91% vs 73%, p=0.043) and lower haemoglobin levels (130±12 vs 137±14 g/L, p=0.008) than those in the non-ischaemic group (**Table 2**).

Table 2. Baseline characteristics of patients in the ETT study.

	Ischaemic group (n=32)	**Non-ischaemic group (n=70)**	**P value**
Patient demographics			
Female sex, n (%)	18 (56)	48 (69)	0.227
Age, years	62±6	59±9	0.131
BMI, kg/m^2	30 (25, 32)	29 (25, 34)	0.600
Hypertension, n (%)	20 (63)	31 (44)	0.088
Diabetes mellitus, n (%)	8 (25)	15 (21)	0.689
Hyperlipidaemia, n (%)	18 (56)	41 (59)	0.826
Smoking history, n (%)	7 (22)	12 (17)	0.569
Symptomology			
Typicality score			0.043
Non-anginal, n (%)	0	0	
Atypical, n (%)	3 (9)	19 (27)	
Typical, n (%)	29 (91)	51 (73)	
CCS grade			0.266
I, n (%)	3 (9)	6 (9)	
II, n (%)	8 (25)	31 (44)	
III, n (%)	20 (63)	30 (43)	
IV, n (%)	1 (3)	3 (4)	

NYHA class I, n (%) II, n (%) III, n (%) IV, n (%)	 17 (53) 14 (44) 1 (3) 0	 34 (48) 30 (43) 6 (9) 0	0.593
SAQ Summary score	50±19	53±19	0.619
Lab results			
Haemoglobin (g/L)	130±12	137±14	0.008
eGFR (ml/min/1.72 m^2)	79±23	82±18	0.555
NT-proBNP (pg/mL)	74 (50, 119)	65 (50, 108)	0.540
HbA1c (mmol/mol)	38 (36, 43)	40 (38, 42)	0.230
Total cholesterol (mmol/L)	4.3±1.2	4.2±1.0	0.724
Medications			
Antiplatelet agents	18 (56)	33 (47)	0.393
Statins	23 (72)	48 (69)	0.736
ACE inhibitors or ARBs	14 (44)	21 (30)	0.175
Beta blockers	4 (13)	7 (10)	0.960
CCBs	1 (3)	6 (9)	0.231

BMI: Body mass index; CCS: Canadian Cardiovascular score; NYHA: New York Heart Association; eGFR: estimated glomerular filtration rate, NT-proBNP: N-terminal pro-brain natriuretic peptide; HbA1c: glycated haemoglobin; ACE inhibitor: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker

Typicality score: 1: non-anginal chest pain; 2: atypical angina; 3: typical angina

There were no differences in the epicardial coronary physiology metrics (mean FFR > 0.90 in both groups) or exercise time (348±164 vs 342±164 seconds, p=0.860) during ETT between the ischaemic and non-ischaemic groups (**Table 3**). There was a trend towards greater

prevalence of exercise-induced angina in the ischaemic group (78% vs 60%, p=0.074). The time to ≥0.1mV ST depression in patients in the ischaemic group was 268±135 seconds, whilst the time to resolution of ischaemic ECG changes was 156±112 seconds.

Table 3. Coronary physiology and exercise ECG treadmill test parameters.

	Ischaemic group (n=32)	**Non-ischaemic group (n=70)**	**P value**
Coronary physiology			
Pd/Pa	0.95±0.03	0.95±0.03	0.807
FFR	0.90±0.06	0.90±0.04	0.971
CFR	2.3 (2.0, 2.9)	2.6 (2.0, 2.9)	0.507
hMR, $mmHg.cm^{-1}.s^{-1}$	2.0±0.8	2.1±0.7	0.372
AChFR	1.2±0.3	1.5±0.6	<0.001
hMR≥$2.5mmHg.cm^{-1}.s^{-1}$	9 (28)	15 (21)	0.459
Endothelium-*independent* microvascular dysfunction (CFR<2.5), n (%)	20 (63)	30 (43)	0.066
Endothelium-dependent microvascular dysfunction (AChFR≤1.5), n (%)	31 (97)	39 (56)	<0.001
CMD, n (%)	32 (100)	46 (66)	<0.001
Exercise ECG treadmill testing			
Exercise time, seconds	348±164	342±164	0.860

Presence of angina during ETT, n (%)	25 (78)	42 (60)	0.074
Time to angina, seconds	204±118	185±119	0.550
Resting heart rate, bpm	84±19	87±15	0.398
Resting systolic blood pressure, mmHg	146±25	134±19	0.006
Resting rate pressure product, mmHg.bpm	12101±3098	11651±3019	0.508
Peak heart rate, bpm	145±15	136±23	0.035
Peak systolic blood pressure, mmHg	191±26	180±34	0.137
Peak rate pressure product, mmHg.bpm	27662±4846	24678±7130	0.039
Achieved ≥85% target heart rate, n (%)	27 (84)	58 (83)	0.849
Reasons for stopping exercise, n (%)			0.967
Angina	13 (40)	26 (37)	
Dyspnoea	7 (22)	15 (21)	
Fatigue	8 (25)	22 (31)	
Dizziness	2 (6)	3 (4)	
Leg pain	2 (6)	4 (6)	

FFR: Fractional Flow Reserve; CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve; CMD: coronary microvascular disease

All patients in the ischaemic group had coronary microvascular disease, compared to 66% of patients in the non-ischaemic group (p<0.001). There were no differences in the coronary flow reserve or hyperaemic (minimal) microvascular resistance between the two groups; however, patients in the ischaemic group had lower acetylcholine flow reserve (1.2±0.3 vs 1.5±0.6, p<0.001), as well as higher peak heart rate (145±15 vs 136±23 bpm, p=0.015) and rate pressure product (27662±4846 vs 24678±7130 mmHg.bpm, p=0.039) during exercise (**Table 3**).

Using linear regression analysis, acetylcholine flow reserve, peak heart rate and haemoglobin levels were associated with ischaemic ECG changes during exercise (**Table 4**). Coronary endothelium-*dependent* microvascular dysfunction, but not coronary endothelium-*independent* microvascular dysfunction (both CFR<2.5 and CFR<2.0 thresholds), was associated with ischaemic ECG changes during exercise (**Table 5)**. Elevated hyperaemic (minimal) microvascular resistance alone was not associated with ischaemic ECG changes during exercise (**Table 5).**

Table 4. Linear regression analysis to test the association between biologically plausible variables and ischaemic ECG changes on ETT (continuous variables).

	Odds ratio (95% CI)	**P value**
Univariate		
Age	1.045 (0.991, 1.101)	0.103
Gender	0.589 (0.249, 1.395)	0.229
Hypertension	2.097 (0.890, 4.941)	0.090
Haemoglobin	**0.956 (0.923, 0.990)**	**0.012**

Peak heart rate	**1.025 (1.001, 1.049)**	**0.039**
Peak systolic blood pressure	1.010 (0.997, 1.024)	0.138
CFR	0.979 (0.920, 1.042)	0.506
AChFR	**0.854 (0.776, 0.939)**	**0.001**
hMR	0.980 (0.924, 1.039)	0.498
Multivariate (R^2 0.228)		
AChFR	**0.817 (0.720, 0.928)**	**0.002**
Haemoglobin	**0.935 (0.894, 0.979)**	**0.004**
Peak heart rate	**1.035 (1.006, 1.064)**	**0.018**

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; hMR: hyperaemic microvascular resistance

Table 5. Linear regression analysis to test the association between biologically plausible variables and ischaemic ECG changes on ETT (binary variables).

	Odds ratio (95% CI)	**P value**
Univariate		
CFR<2.5	2.222 (0.942, 5.241)	0.068
CFR<2.0	0.963 (0.368, 2.523)	0.939
AChFR≤1.5	**24.641 (3.184, 190.723)**	**0.002**
hMR≥2.5	1.357 (0.519, 3.547)	0.534
Multivariate (R^2=0.184)		
CFR<2.5	1.305 (0.507, 3.358)	0.581
AChFR≤1.5	**22.570 (2.854, 178.463)**	**0.003**

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; hMR: hyperaemic microvascular resistance

Ischaemic ECG changes during ETT had poor sensitivity and moderate specificity to detect endothelium-*independent* microvascular dysfunction, and poor sensitivity but excellent specificity to detect endothelium-*dependent* microvascular dysfunction (**Table 6**).

Table 6. Diagnostic accuracy of ischaemic ECG changes on ETT to detect an abnormality in coronary microvascular function.

	Endothelium-*independent* microvascular dysfunction (CFR < 2.5)	**Endothelium-*dependent* microvascular dysfunction** (AChFR ≤ 1.5)	**CMD** (CFR<2.5 and/or AChFR≤1.5)
Sensitivity	40%	44%	41%
Specificity	77%	97%	100%
PPV	63%	97%	100%
NPV	57%	44%	34%

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; PPV: positive predictive value; NPV: negative predictive value

If obstructive coronary artery disease were to be used as the reference standard, then the assumed false positive rate of ETT would be 31% (as all 102 patients had nonobstructive coronary arteries but 32 patients had a positive ETT). With the addition of endothelium-*independent* microvascular dysfunction (i.e., CFR<2.5) to the reference standard, the apparent false positive rate remained high at 23%. With the further addition of endothelium-*dependent* microvascular dysfunction (i.e., AChFR≤1.5) to the reference standard, the apparent false

positive rate came down to 0% (p=0.002 for comparison of apparent false positive rate with endothelium-*independent* vs endothelium-*dependent* microvascular dysfunction as the reference standard).

Ischaemic ECG changes during ETT had poor sensitivity and moderate specificity to detect CFR<2.0 or hMR≥2.5mmHg.cm^{-1}.s^{-1}. (**Table 7**). Exercise-induced angina during ETT had an excellent positive predictive value but poor negative predictive value to detect an abnormality in the coronary microvascular function (**Table 8**). The composite of ischaemic ECG changes and/or exercise-induced angina during ETT had an excellent sensitivity and positive predictive value, but poor specificity and negative predictive value to detect an abnormality in the coronary microvascular function (**Table 9**).

Table 7. Diagnostic accuracy of ischaemic ECG changes on ETT to detect coronary flow reserve <2.0 or hyperaemic microvascular resistance≥2.5mmHg.cm^{-1}.s^{-1}.

	CFR<2.0	**hMR≥2.5mmHg.cm^{-1}.s^{-1}**
Sensitivity	31%	38%
Specificity	68%	69%
PPV	25%	28%
NPV	74%	78%

CFR: coronary flow reserve; hMR: hyperaemic microvascular resistance; PPV: positive predictive value; NPV: negative predictive value

Table 8. Diagnostic accuracy of exercise-induced angina during ETT to detect an abnormality in the coronary microvascular function.

	Endothelium-*independent* microvascular dysfunction (CFR < 2.5)	**Endothelium-*dependent* microvascular dysfunction** (AChFR ≤ 1.5)	**CMD** (CFR<2.5 and/or AChFR≤1.5)
Sensitivity	74%	76%	73%
Specificity	42%	58%	58%
PPV	55%	81%	85%
NPV	63%	51%	40%

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; PPV: positive predictive value; NPV: negative predictive value

Table 9. Diagnostic accuracy of a composite of ischaemic ECG changes and/or exercise-induced angina on ETT to detect an abnormality in the coronary microvascular function.

	Endothelium-*independent* microvascular dysfunction (CFR < 2.5)	**Endothelium-*dependent* microvascular dysfunction** (AChFR ≤ 1.5)	**CMD** (CFR<2.5 and/or AChFR≤1.5)
Sensitivity	84%	86%	82%
Specificity	38%	58%	58%
PPV	57%	82%	86%
NPV	71%	64%	50%

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; PPV: positive predictive value; NPV: negative predictive value

There was a strong degree of inter-observer reliability when interpreting exercise ECGs for the presence or absence of ischaemic changes (intraclass correlation coefficient=0.843, 95% CI 0.778 to 0.891).

3.5. Discussion

The main findings of our study are: i) ischaemic ECG changes during ETT had 100% specificity in detecting an abnormality in the coronary microvascular function, ii) patients who developed ischaemic ECG changes during exercise had a lower acetylcholine flow reserve and iii) acetylcholine flow reserve (and endothelium-*dependent* microvascular dysfunction) was the strongest predictor of ischaemic ECG changes during exercise.

In recent years, there has been a paradigm shift in our understanding of ischaemic heart disease, with the emphasis moving away from detecting obstructive coronary artery disease (CAD) to confirming a physiological substrate for myocardial ischaemia in the setting of chronic coronary syndrome. However, the diagnostic accuracy of traditional non-invasive tests has not been systematically re-evaluated against contemporary standards of assessing ischaemia. Our study found that the specificity and positive predictive value of ETT is much higher when assessed against comprehensive physiological evaluation of the coronary circulation, in contrast to validation against the frequency of obstructive epicardial CAD. Intriguingly, these concepts were first introduced more than three decades ago and described as cardiac syndrome X at the time[113].

3.5.1. Specificity of exercise ECG treadmill testing to detect an abnormality in coronary microvascular function

Previous studies that have examined the diagnostic accuracy of ETT to detect abnormalities in coronary microvascular function in patients with ANOCA (largely defined by an impaired CFR) have reported sensitivity values between 38-54% (**Table 10**)[111,114–117]. Our study also

found that ETT has poor sensitivity, but in contrast to previous studies[114–117], we found it to have excellent specificity to detect abnormalities in coronary microvascular function (i.e., an ischaemic substrate). The reasons for this are likely two-fold. First, previous studies have included angina (in the absence of ischaemic ECG changes) as a criterion for a positive ETT. Our study demonstrates that angina revealed during ETT is in fact poorly specific to detect abnormalities in coronary microvascular function. Therefore, the use of angina as a criterion for a positive ETT may have led to under-reporting of specificity in previous studies. Second, several studies have employed reference standards that do not interrogate the endothelium-*dependent* compartment of microvascular function, such as elevated index of microvascular resistance (IMR>25)[114,117], CFR<2.0 on positron emission tomography[116], and angiographic vasoconstriction in response to acetylcholine[115]. Cassar et al used similar reference standards to our study and reported a specificity of 80% of ETT to detect abnormalities in coronary microvascular function[111]. However, it is noteworthy that the symptomology of their patient cohort was unknown, along with delays of up to 6 months between ETT and invasive physiology assessment.

Table 10. Summary of previous studies investigating the accuracy of exercise ECG treadmill testing to detect an abnormality in coronary microvascular function in patients with ANOCA.

Definition of CMD	**Sensitivity (%)**	**Specificity (%)**	**Reference**
CFR < 2.5 (IC Doppler)	16	75	(Cassar et al., 2009)[111]
AChFR ≤ 1.5 (IC Doppler)	18	78	(Cassar et al., 2009)[111]
IMR ≥ 25	38	63	(Pargaonkar et al., 2019)[115]

(IC Thermo)			
Reduction in diameter > 20% after IC ACh (QCA)	43	73	(Pargaonkar et al., 2019)[115]
CFR < 2 / IMR > 25 / microvascular spasm (IC Thermo)	54	76	(Ford et al., 2019)[117]
CFR < 2 (PET)	35	65	(Lopez et al., 2022)[84]

CFR: coronary flow reserve; AChFR: acetylcholine flow reserve; IC: intracoronary; IMR: index of microvascular resistance; QCA: quantitative coronary angiography; PET: positron emission tomography

3.5.2. Physiological relevance of acetylcholine

Only AChFR, haemoglobin levels and peak heart rate were associated with ischaemic ECG changes during exercise, with AChFR and haemoglobin being lower and peak heart rate being higher in the ischaemic group. This is suggestive that a combination of attenuated coronary blood flow and heightened myocardial oxygen demand was the underlying pathophysiology leading to ischaemic ECG changes during exercise. There were no baseline demographic differences between the ischaemic and non-ischaemic groups to account for the difference in peak heart rate between the two groups; it is noteworthy that although haemoglobin levels were associated with ischaemic ECG changes during exercise, the mean haemoglobin levels were well within the normal limits in both ischaemic and non-ischaemic groups. Finally, whilst peak heart rate and haemoglobin levels can be expected to be associated with ischaemic ECG changes during exercise, we identified AChFR (and endothelium-*dependent* microvascular

dysfunction) as the strongest predictor of ischaemic ECG changes during exercise. This reiterates the physiological relevance of acetylcholine testing in the evaluation of patients with ANOCA. Whilst adenosine acts on the A_{2A} receptors on vascular smooth muscle cells to promote cyclic adenosine monophosphate-mediated vasodilatation, acetylcholine acts on endothelial muscarinic receptors leading to cyclic guanosine monophosphate (cGMP)-mediated vasodilatation (**Figure 3**). Therefore, by assessing the response to adenosine, CFR reflects the theoretical (supra)maximal vasodilatory capacity of the vessel and may not be as physiologically relevant as AChFR. The latter is likely to be a better surrogate of the physiological flow-mediated vasodilatation that occurs during exercise as it assesses the functionality of both the endothelial and vascular smooth muscle pathways (nitric oxide-cGMP-protein kinase G pathway). It is, therefore, unsurprising that AChFR, rather than CFR, was associated with ischaemic ECG changes during ETT. We have previously demonstrated a close link between coronary and peripheral endothelial function as assessed using venous occlusion plethysmography[25]. Therefore, it would be enticing to assess whether measures of peripheral endothelial function can be used as a surrogate for coronary endothelial function testing in the future, obviating the need for instrumenting the coronary arteries for this purpose.

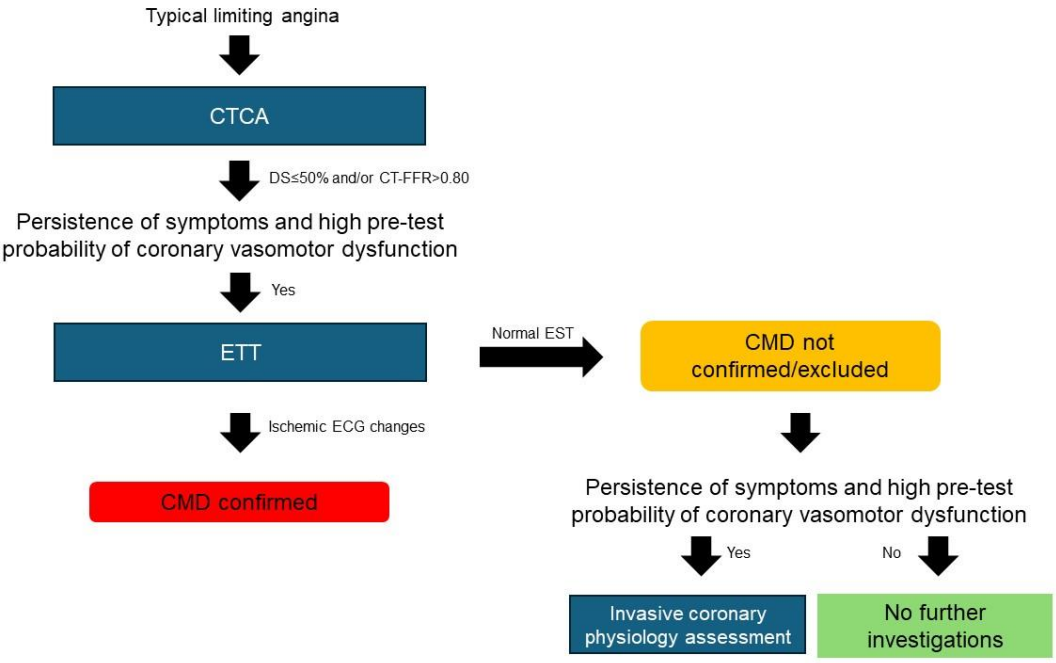
Previous studies have demonstrated the safety and low complication rate of acetylcholine testing in the catheter laboratory[118,119], whilst others have demonstrated the prognostic significance of endothelium-*dependent* microvascular dysfunction[34,35]. These findings may not only strengthen recommendations for intracoronary acetylcholine testing in future guidelines, but also have implications from a therapeutic viewpoint, given the pleiotropic effects of statins and angiotensin converting enzyme inhibitors on endothelium-*dependent* function and their prognostic benefit in patients with CMD[120].

3.5.3. Clinical implications

Coronary physiology assessment detects the *substrate* for myocardial ischaemia (CFR<2.5 and/or AChFR≤1.5), which are sensitive markers as they detect perturbations early in the ischaemic cascade. ETT, on the other hand, detects electrocardiographic manifestations of *actual* myocardial ischaemia and is a specific marker. It is, therefore, unsurprising that all patients who developed ischaemic ECG changes during the ETT had an identifiable ischaemic substrate in the catheter laboratory, but not vice versa. ETT has the advantage of detecting ischaemia with very high specificity and has the potential of being used as a readily available and cost-effective diagnostic test for patients with ANOCA to identify underlying CMD. Our results show that a positive result on ETT is highly specific for (and suggestive of) the presence of an ischaemic substrate, but as this does not distinguish the relative contributions of the epicardial and microvascular compartments, ETT will always have to be combined with a test that specifically evaluates the epicardial coronary arteries, namely, invasive or noninvasive coronary angiography. The pathway that is being increasingly adopted worldwide is to use computed tomographic coronary angiography as the first line investigation for patients presenting with chest pain that might be consistent with inducible ischaemia. In this setting, patients who are found to have unobstructed epicardial arteries are managed in 1 of 3 ways: discharge without further investigation (a common strategy), consideration for a noninvasive functional test (adopted by networks in which there is good awareness of microvascular dysfunction), or referral for invasive testing (usually reserved for patients with a high burden of symptoms despite several antianginal medications). In this context, ETT may have a role as a second-line test with good rule-in utility. This is likely to expedite the diagnosis of CMD in a large proportion of patients and streamline the use of (less widely available and more costly) tests, such as invasive physiology and/or stress perfusion cardiac magnetic resonance imaging. The efficacy of this proposed strategy (**Figure 23**) would need to be tested in a future diagnostic

trial. The more historical pathway (and one that is decreasingly recommended in international guidelines because of the perceived false positive rate) is to use ETT as a first-line investigation. In this scenario, patients with positive results on ETT would often go on to undergo invasive angiography to exclude epicardial disease. The findings of our study suggest that invasive microvascular physiological assessment may not be required if no obstructive coronary disease is found in these cases, as the positive ETT result makes a diagnosis of CMD highly likely. Finally, longitudinal ETT assessment may play an important role in monitoring response to therapy in patients who have been diagnosed with CMD based on a positive ETT.

Figure 23. Proposed novel diagnostic pathway in patients with new onset angina.



This figure denotes our suggested novel diagnostic pathway in patients with new onset angina. Patients should first undergo a computed tomography coronary angiography (CTCA) as per international guidelines. Those with obstructive coronary artery disease should be managed as per standard of care. Those with nonobstructive coronary arteries (diameter stenosis <50% and/or CT-FFR>0.80) with typical and limiting symptoms should undergo exercise ECG stress

testing (EST). If patients develop ischemic ECG changes on their ETT, then a diagnosis of CMD is confirmed and they can be managed as per current guidelines. If the ETT is normal, then patients should again be re-evaluated for symptom burden. Only those with persistent limiting symptoms and a high pretest probability of coronary vasomotor dysfunction should undergo invasive coronary physiology assessment to confirm/exclude a substrate for ischemia. This novel pathway needs to be tested in future clinical trials for its efficacy and cost-effectiveness.

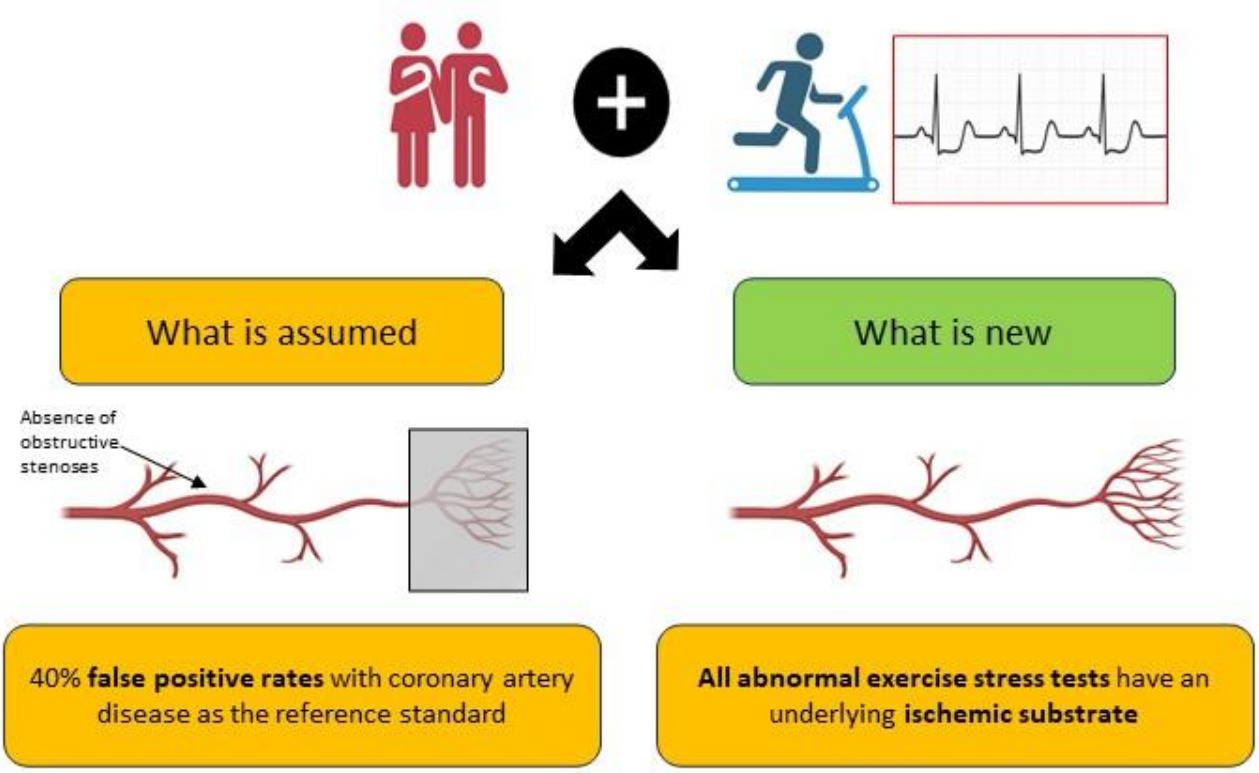
3.5.4. Study limitations

Our study has some limitations that should be considered when interpreting the findings. Firstly, this was a single centre study with its inherent limitations of generalisability. Secondly, all patients in this study had angina as their main symptom that necessitated further investigations. Therefore, these results may not necessarily apply to patients without angina (such as those with breathlessness as their predominant symptom). Thirdly, long-term outcome data are currently unavailable for these patients, precluding us from identifying features on ETT that enable risk stratification of patients; however, this will form the basis for future studies.

3.6. Conclusions

Using comprehensive coronary physiology assessment, we have demonstrated that ischaemic ECG changes during ETT in patients with ANOCA are always indicative of abnormalities in coronary microvascular function. This is an important finding that highlights the limitations of using obstructive coronary artery disease as a reference standard to assess the accuracy of non-invasive imaging modalities. **Figure 24** summarises the findings of our study.

Figure 24. Summary of findings from the ETT study.



Chapter 4

Characterising mechanisms in patients with coronary microvascular disease to stratify therapy (ChaMP-CMD) – a phenotype-blinded, randomised controlled, crossover trial.

Finalist at the European Society of Cardiology Young Investigator Award 2023 (Amsterdam)

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4.1 Abstract

Background: Angina with nonobstructive coronary arteries (ANOCA) is a common condition for which no effective treatment has been established. We hypothesised that measurement of coronary flow reserve (CFR) allows identification of patients with ANOCA who would benefit from anti-ischaemic therapy.

Methods: Patients with ANOCA underwent blinded invasive CFR measurement and were randomly assigned to receive four weeks of amlodipine or ranolazine. After a one-week washout, they crossed over to the other drug for four weeks; final assessment was after cessation of study medication for another four weeks. The primary outcome was change in treadmill exercise time, and the secondary outcome was change in Seattle Angina Questionnaire (SAQ) summary score, in response to anti-ischaemic therapy. Analysis was on a per protocol basis according to the following classification: coronary microvascular disease (**CMD group**) if CFR<2.5 and **reference group** if CFR≥2.5. The study protocol was registered before the first patient was enrolled (International Standard Randomised Controlled Trial Number: ISRCTN94728379).

Results: Eighty-seven patients (61±8 years, 62% females) underwent randomisation (57 **CMD group** and 30 **reference group**). Baseline exercise time and SAQ summary scores were similar between groups. The CMD group had a greater change in exercise time compared to the reference group, in response to both amlodipine (difference in change 82 seconds, 95% CI 37 to 126 seconds, p<0.001) and ranolazine (difference in change 68 seconds, 95% CI 21 to 115 seconds, p=0.005). The CMD group reported a greater change in SAQ summary score compared to the reference group in response to ranolazine (difference in change 7, 95% CI 0 to 15, p=0.048), but not to amlodipine (difference in change 2, 95% CI -5 to 8, p=0.549).

Conclusions: Amongst phenotypically similar patients with ANOCA, only those with an impaired CFR derive benefit from anti-ischaemic therapy. These findings support routine measurement of CFR to diagnose and guide management of this otherwise heterogenous patient group.

4.2. Introduction

Angina with non-obstructive coronary arteries (ANOCA) is a common clinical condition, although this umbrella term comprises several distinct pathophysiological entities[2], which include endothelium-*independent* microvascular dysfunction. For the purposes of this chapter, we will be using the term 'endothelium-*independent* microvascular dysfunction' interchangeably with the term 'coronary microvascular disease (CMD)'. CMD is defined as an inability of the coronary vasculature to augment coronary blood flow in response to heightened myocardial oxygen demand despite the absence of epicardial coronary artery disease[25]. CMD leads to impaired quality of life[121] and a heightened risk of adverse cardiovascular outcomes[35]. A diagnosis of CMD is traditionally made by invasive assessment in the cardiac catheter laboratory to delineate the hallmark of CMD, a diminished coronary flow reserve (CFR) in response to adenosine, representing the ratio of maximal achievable flow to resting flow[68]. CFR<2.5 is associated with impaired coronary perfusion efficiency during exercise and myocardial ischaemia on non-invasive assessment[25].

Whilst the Coronary Microvascular Angina (CorMicA) study has previously demonstrated the value of coronary physiology-stratified therapy in enhancing angina-specific quality of life in patients with ANOCA (including patients with CMD, vasospastic angina and noncardiac chest pain)[94,95], uptake by physicians has been low; this is partly because of scepticism about the mechanistic link between coronary physiology parameters and response to therapy. Furthermore, it is not known if physiology-stratified therapy leads to an improvement in exercise capacity, which is the reference standard for efficacy of anti-ischaemic therapies.

The **Cha**racterising **M**echanisms in **P**atients with **C**oronary **M**icrovascular **D**isease to stratify therapy (ChaMP-CMD) trial aims to assess whether a diminished CFR relates to the effects of anti-ischaemic therapy on exercise time in patients with ANOCA.

4.3. Methods

4.3.1. Study design and participants

ChaMP-CMD is a phenotype-blinded randomised crossover trial carried out at a tertiary referral cardiac centre in London, UK. This study was approved by the National Health Service Research Ethics Committee (reference 20/LO/1294), funded by the UK Medical Research Council and overseen by a trial steering committee. Written informed consent was obtained from all patients prior to their enrolment. The study protocol was registered before the first patient was enrolled (International Standard Randomised Controlled Trial Number: ISRCTN94728379).

We recruited patients with typical angina, preserved left ventricular ejection fraction (>50%) and nonobstructive coronary arteries (fractional flow reserve (FFR)>0.80). Exclusion criteria were intolerance to adenosine, advanced chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/m^2), significant valvular heart disease, history of acute coronary syndrome, previous revascularisation, cardiomyopathy, atypical/non-cardiac chest pain, low symptom burden, confirmed vasospastic angina (necessitating commencement of calcium channel antagonists), exercise incapacity due to non-cardiac causes, known contraindications to ranolazine or amlodipine, patients who were unable to exercise on a treadmill or those who could exercise for >540seconds in the absence of any revealed cardiac symptoms on the baseline exercise test.

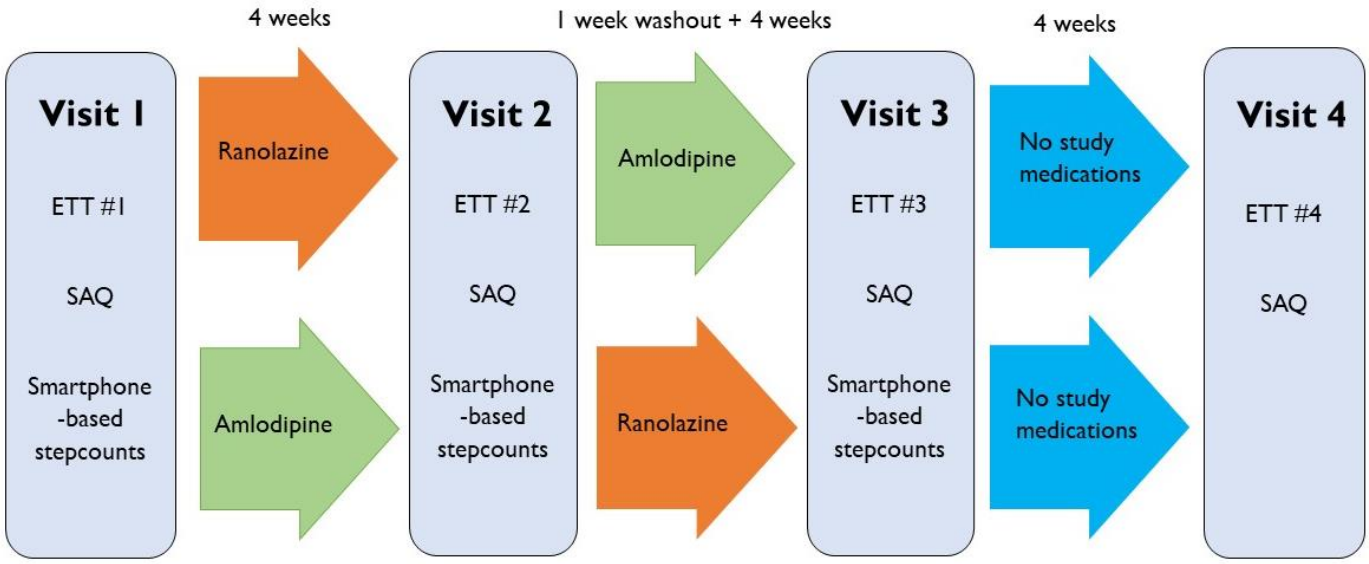
4.3.2. Randomisation, masking and cross-over

Patients, researchers, and clinical staff conducting exercise tests were blinded to the patients' invasive physiological characteristics (phenotype-blinding). All eligible study patients were randomised to receive either amlodipine or ranolazine (in a 1:1 allocation ratio) using an automated randomisation tool; no stratification or blocks were used. Patients who were already taking a calcium channel antagonist and/or ranolazine were requested to withhold them from four weeks prior to their baseline visit until the end of the study.

The initial doses were either amlodipine 5 mg once a day or ranolazine 375 mg twice a day, with an increase to amlodipine 10 mg once a day and ranolazine 500mg or 750 mg twice a day after one to two weeks. If patients did not tolerate amlodipine 5 mg, then the dose was reduced to 2.5 mg. Four weeks after randomisation, patients returned for the second ETT, following which a one-week drug washout period was applied (a duration longer than five half-lives of either medication, to minimise potential carryover effects[122]). They then crossed-over to the second drug arm in the sequence of amlodipine-ranolazine or ranolazine- amlodipine, and after 4 weeks of treatment, returned for the third ETT. Study medications were discontinued at this point and a subgroup returned for a fourth ETT, four weeks later, to assess for training effect. This was based on input provided at my interim thesis progression committee meeting. Patients were encouraged to continue their regular (non-study) medications throughout the study period. Treatment adherence was assessed using pill count at each visit and patients with <80% adherence to study medications, or those who were not able to attend their second or third visit within one week of the scheduled date, were excluded from that aspect of the per protocol analysis but included in the intention-to-treat analyses. Ambulant day to day activity was monitored by capturing 4-week step-counts from patients' smartphone application.

After the fourth ETT (or third for those who did not undertake the fourth ETT), study participation was complete, and patients and physicians were unblinded to the patients' phenotype. **Figure 25** illustrates the study flow.

Figure 25. Study flow of ChaMP-CMD.



ETT: Exercise Treadmill Test; SAQ: Seattle Angina Questionnaire

4.3.3. Procedures

4.3.3.1. Coronary angiography and physiology data analysis

Coronary angiography with invasive physiology assessment was carried out via the radial artery and is described in detail in Chapter 2 and Chapter 3. CFR was calculated as hyperaemic APV/resting APV, both measured at steady state. Patients were classified off-line into **reference** (CFR≥2.5) and **CMD** (CFR<2.5) groups.

4.3.3.2. Exercise ECG treadmill test

The exercise ECG treadmill test (ETT) was performed with a Marquette Case 8000 system (GE Medical Systems, Milwaukee, WI, USA) according to the American College of Cardiology and American Heart Association practice guidelines using a standard Bruce protocol[78], terminated at the patient's request. 12-lead electrocardiogram (ECG), heart rate and blood pressure were recorded at regular intervals before, during and after the ETT. All ETTs were supervised by physiologists who were blinded to the patients' coronary physiology data. Exercise time (ET) was defined as the time from the start of the exercise protocol to exercise cessation.

4.3.3.3. Seattle Angina Questionnaire (SAQ)

At each visit, patients completed the Seattle Angina Questionnaire (SAQ), which is a validated angina-specific quality of life assessment[106]. The SAQ comprises five components, namely physical limitation, angina stability, angina frequency, treatment satisfaction and quality of life; these are then incorporated into the summary score, which is sensitive to changes in response to therapy[106].

4.3.4. Outcomes

The primary outcome was change in exercise time in response to anti-ischaemic therapy. The secondary outcome was change in SAQ summary score in response to anti-ischaemic therapy.

4.3.5. Hypotheses and statistical considerations

Our primary hypothesis was that patients with CMD (impaired CFR) will have a greater improvement in their exercise capacity in response to anti-ischaemic therapy compared to

patients in the reference group (normal CFR). Assuming a 2:1 distribution (as reported in the literature for patients with high pre-test probability of coronary vascular dysfunction[94]), 49 patients with CMD and 25 in the reference group will provide 80% power (α=0.05) to detect a 60 second difference in exercise time between the groups (assumed standard deviation (SD) 85 seconds). A statistical analysis plan was finalised before data lock and analysis of data.

Normality of data was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous data are presented as mean$\pm$SD, unless specified otherwise, and compared using the independent sample's Student *t*-test. Non-normally distributed data are presented as median (interquartile range) and compared using the Mann-Whitney test (unpaired analyses) or the Wilcoxon matched-pairs signed rank test (paired analyses). Categorical variables are presented as n (%) and compared using the chi-squared test. Continuous endpoints were compared with the two-sample t test of the difference between groups (**CMD** versus **reference** groups); the findings are reported as the difference in mean change between study groups with 95% confidence intervals (CIs) and p values. Binary logistic regression was performed using univariate and multivariate analysis and reported as standard coefficients (95% CI). Biologically plausible variables were assessed in the univariate model, and those that correlated were included in the multivariate model. Gain-or-loss of function was assessed using the repeated measures ANOVA analysis (mixed model, assuming missing values are missing at random). Sphericity was not assumed, and the Geisser-Greenhouse correction was applied to the analyses. The Youden's index in receiver operating characteristic curves was used to identify the optimal CFR threshold that predicted ≥60 seconds increment in exercise time in response to anti-ischaemic therapy. All randomised patients were included in these analyses. The accuracy of CFR thresholds in predicting ≥60 seconds increment in exercise time in response to anti-ischaemic therapy was calculated as [(true positives + true negatives) ÷ (true

positives + true negatives + false positives + false negatives)] x 100; the accuracy of different CFR thresholds was compared using the McNemar's test.

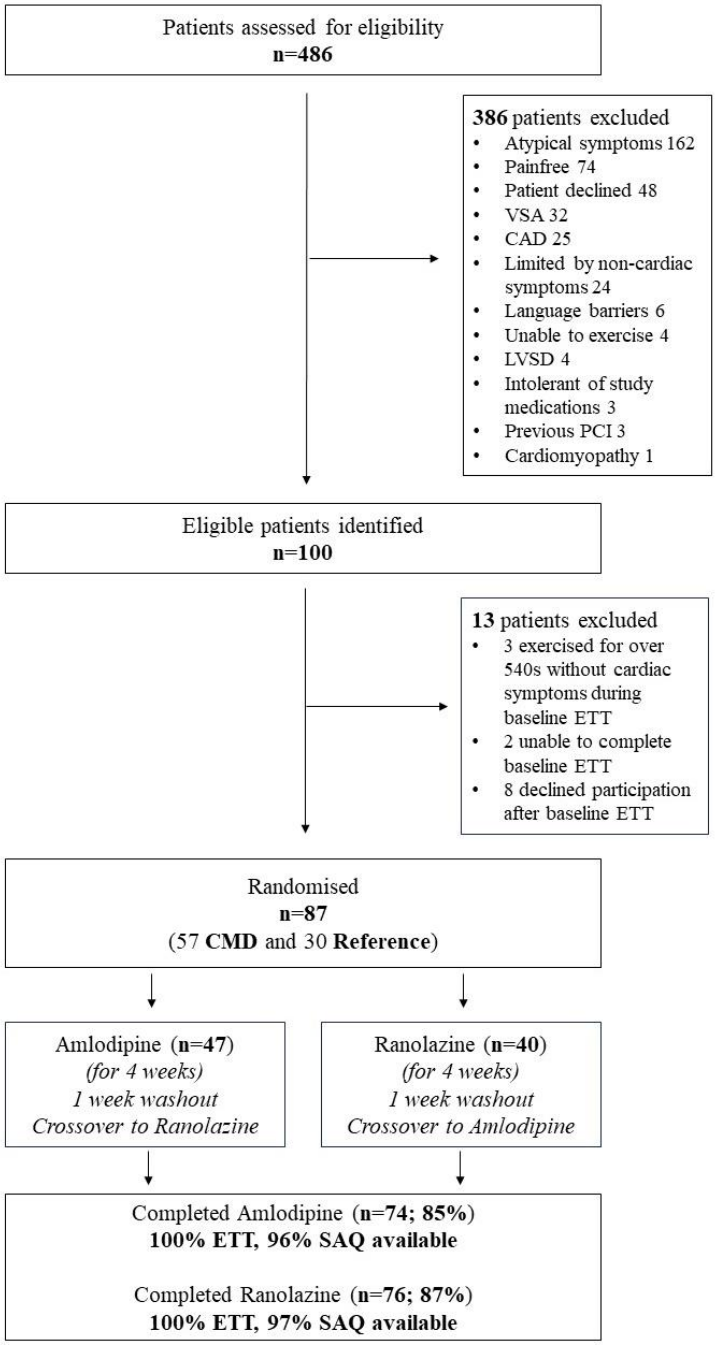
Data were analysed on a per protocol basis as stipulated in the Statistical Analysis Plan. Per protocol analysis was favoured over intention-to-treat analysis as this study addressed the efficacy of therapy as opposed to a treatment strategy. Intention-to-treat analyses are also reported in the **Appendix** section. All analyses were performed using SPSS Statistics 27 (IBM, NY, USA) and GraphPad Prism software version 9.0 (GraphPad software, San Diego, CA, USA). We deemed a p value less than 0.05 to be significant.

The study was registered with the National Institute for Health Research UK Clinical Research Network portfolio database (Central Portfolio Management System identifier: 47795) and International Standard Randomized Controlled Trial Number (identifier: ISRCTN94728379).

4.4. Results

Between December 2020 and August 2023, 486 patients with angina and nonobstructive coronary arteries were screened and 100 eligible patients identified, of which 87 were randomised (**Figure 26)**.

Figure 26. Patient screening and recruitment for ChaMP-CMD.



VSA: vasospastic angina; CAD: coronary artery disease; LVSD: left ventricular systolic dysfunction; PCI: percutaneous coronary intervention; ETT: exercise ECG treadmill test; SAQ: Seattle Angina Questionnaire

By blinded classification, 57 patients had impaired CFR (**CMD group**) and 30 patients had normal CFR (**reference group**). The exercise ECG treadmill test took place 25 (14, 41) days after the coronary physiology assessment. Patients in the CMD and reference groups were well matched for baseline demographics; the CMD group had a higher prevalence of hypertension (**Table 11**).

Table 11. Baseline demographics of patients recruited into ChaMP-CMD.

	CMD (n=57)	**Reference (n=30)**	**P value**
Patient demographics			
Age, years	62±8	60±7	0.385
Female, n(%)	36 (63)	18 (60)	0.773
BMI, kg/m^2	28 (25, 32)	31 (26, 34)	0.169
Hypertension, n(%)	33 (58)	9 (30)	0.013
Diabetes, n(%)	15 (26)	3 (10)	0.074
Hyperlipidaemia, n(%)	36 (63)	16 (53)	0.374
Smoking history, n(%)	9 (16)	7 (23)	0.388
Symptomology			
CCS, n(%)			0.913
I	3 (5)	2 (7)	
II	21 (37)	13 (43)	
III	28 (49)	13 (43)	
IV	5 (9)	2 (7)	
NYHA, n(%)			0.523
I	27 (47)	16 (53)	

II	27 (47)	11 (37)	
III	3 (5)	3 (10)	
IV	0	0	
Serum biomarkers			
Haemoglobin, g/L	134±14	135±12	0.726
eGFR, ml/min/1.73m^2	79±21	83±17	0.295
NTproBNP, pg/mL	75 (38, 126)	31 (25, 78)	0.009
HbA1c, mmol/mol	39 (37, 42)	42 (39, 45)	0.142
HbA1c in DM, mmol/mol	53 (50, 96)	62 (54, 92)	0.497
Total cholesterol, mmol/L	4.1±1.1	4.3±1.2	0.373
Medications			
Antiplatelet agent	33 (58)	14 (47)	0.318
Statins	45 (79)	21 (70)	0.354
ACE inhibitors/ARB	27 (47)	11 (37)	0.339
Beta blockers	13 (23)	7 (23)	0.956
Nitrates	10 (18)	6 (20)	0.779

BMI: Body mass index; CCS: Canadian cardiovascular society; NYHA: New York heart association, eGFR: estimated glomerular filtration rate; NTproBNP: N-terminal pro-brain natriuretic peptide; HbA1c: glycated haemoglobin; DM: diabetes mellitus; ACE inhibitor: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker

There were no differences in the epicardial coronary physiology metrics (mean FFR > 0.90 in both groups), baseline exercise time (315±143 vs 317±128 seconds, p=0.954) or SAQ summary score (49±19 vs 51±19, p=0.651) between the CMD and reference groups (**Table 12**).

Table 12. Coronary physiology, exercise test and SAQ measurements at baseline in patients recruited into ChaMP-CMD.

	CMD (n=57)	Reference (n=30)	P value
Coronary physiology measurements			
Pd/Pa	0.95±0.03	0.95±0.04	0.909
FFR	0.92±0.04	0.91±0.04	0.368
CFR	2.0±0.3	3.0±0.4	<0.001
hMR, $mmHg.cm^{-1}.s^{-1}$	2.3 (1.7, 2.7)	1.9 (1.7, 2.2)	0.133
Exercise treadmill test			
Exercise time, seconds	315±143	317±128	0.954
Presence of angina during ETT, n (%)	43 (75)	22 (73)	0.722
Time to angina, seconds	191±112	180±115	0.716
Ischaemia during ETT, n (%)	18 (32)	4 (13)	0.041
Time to ≥0.1mV ST depression, seconds	254±143	198 (79, 347)	0.759
Resting heart rate, bpm	85±15	85±14	0.981
Resting systolic blood pressure, mmHg	140±22	138±20	0.658
Resting rate pressure product, mmHg.bpm	11922±2789	11850±3102	0.912
Peak heart rate, bpm	185±36	190±37	0.531

Peak systolic blood pressure, mmHg	139±19	141±19	0.684
Peak rate pressure product, mmHg.bpm	25808±6253	26924±7852	0.473
Seattle Angina Questionnaire			
Summary score	49±19	51±19	0.651

FFR: Fractional Flow Reserve; CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance

Exercise time and SAQ summary score at baseline were moderately correlated (r=0.39, r^2=0.15, p=0.003). Four patients reported peripheral oedema and 2 postural hypotension with amlodipine, whilst 4 reported headaches, 1 dizziness, 1 fatigue and 2 gastrointestinal symptoms with ranolazine. Thirteen patients did not tolerate amlodipine and/or attend their ETT at the scheduled date whilst on amlodipine, whilst 11 patients did not tolerate ranolazine and/or attend their ETT at the scheduled date whilst on ranolazine. Patients were taking the following medication at the time of the second/third ETT: amlodipine 2.5 mg once a day (4%), amlodipine 5 mg once a day (61%) or amlodipine 10 mg once a day (35%); ranolazine 375 mg twice a day (47%), ranolazine 500 mg twice a day (11%) or ranolazine 750 mg twice a day (42%).

Patients with CMD had a greater increment in exercise time compared to the reference group with both amlodipine (mean difference in change 82 seconds, 95% CI 37 to 126 seconds, p<0.001) and ranolazine (mean difference in change 68 seconds, 95% CI 21 to 115 seconds, p=0.005) (**Table 13 and Appendix Table 1**). In patients with a normal CFR, there was no

change in exercise time (compared to baseline) in response to either anti-ischaemic agent (amlodipine: mean change 9 seconds, 95% CI -20 to 38 seconds, p=0.526; ranolazine: mean change 11 seconds, 95% CI -20 to 42 seconds, p=0.469).

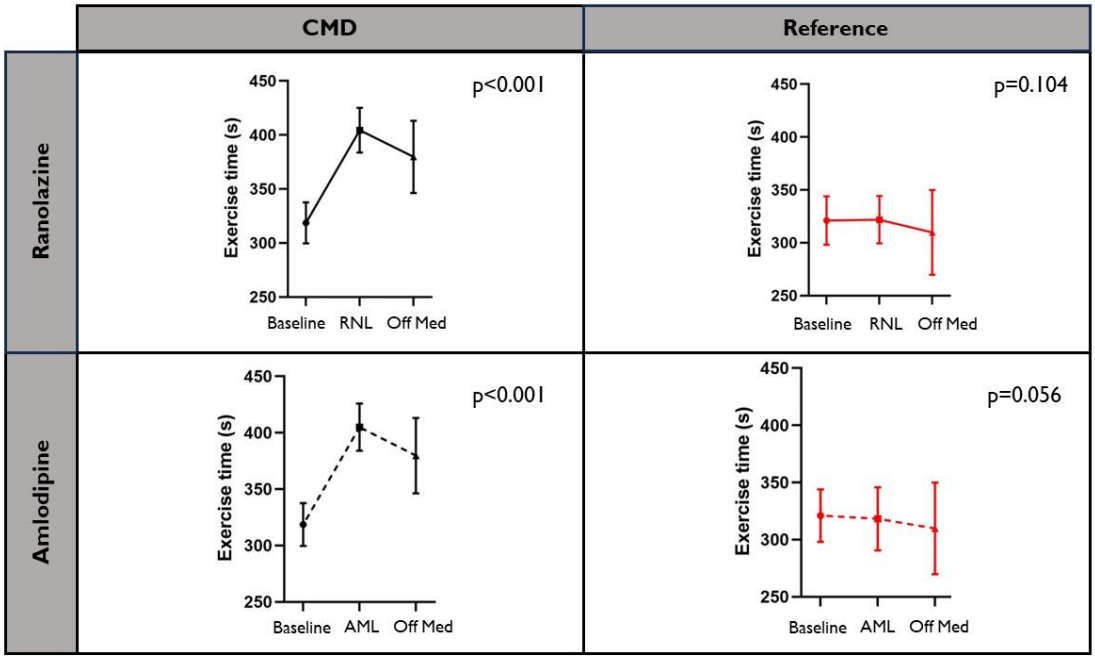
Table 13. Primary and secondary outcomes in ChaMP-CMD.

	Amlodipine (n=74)		**Ranolazine (n=76)**	
	CMD	**Reference**	**CMD**	**Reference**
Exercise time (seconds)				
Increment	91 (95% CI 62 to 120)	9 (95% CI -20 to 38)	79 (95% CI 49 to 109)	11 (95% CI -20 to 42)
Difference in change between groups	82 (95% CI 37 to 126)		68 (95% CI 21 to 115)	
P value	<0.001		0.005	
SAQ summary score				
Increment	7 (95% CI 3 to 12)	5 (95% CI 1 to 9)	13 (95% CI 8 to 17)	5 (95% CI 0 to 10)
Difference in change between groups	2 (95% CI -5 to 8)		7 (95% CI 0 to 15)	
P value	0.549		0.048	

The gain in exercise time observed in the CMD group was lost following cessation of each anti-ischaemic medication, as assessed on the 4th ETT (off study medication) (p<0.001 for both amlodipine and ranolazine by repeated measures ANOVA). In the reference group, exercise time remained unchanged, on or off study medication (**Figure 27**). Overall, patients exercised for 22 seconds longer during their fourth visit compared to the first visit (p=0.017); when

dichotomised according to CFR, those with CMD exercised for longer during their fourth visit compared to their first visit (p=0.004), whereas no such difference was observed in the reference group (p=0.995). Additional ETT-related parameters are reported in **Appendix Table 2.**

Figure 27. Gain-or-loss of function in exercise time with/without study medication.



CMD: coronary microvascular disease; RNL: ranolazine; AML: amlodipine; off med: without study medications

Using linear regression analysis on all randomised patients, CFR (as a continuous variable) was the only independent variable that was associated with an increment in exercise time ≥60 seconds in response to anti-ischaemic therapy; however, the R^2 for this model was only 11.9% (**Table 14**).

Table 14. Linear regression analysis to test the association between biologically plausible variables and ≥60 seconds increment in exercise time in response to anti-ischaemic therapy (ranolazine and/or amlodipine).

	Odds ratio (95% CI)	**P value**
Univariable		
Age	1.020 (0.966, 1.077)	0.475
Gender	2.101 (0.854, 5.174)	0.106
Hypertension	**2.651 (1.098, 6.403)**	**0.030**
CFR	**0.889 (0.816, 0.968)**	**0.007**
hMR	1.000 (0.952, 1.051)	0.993
Multivariable (R^2 0.119)		
CFR	**0.905 (0.830, 0.987)**	**0.024**
Hypertension	2.079 (0.824, 5.246)	0.121

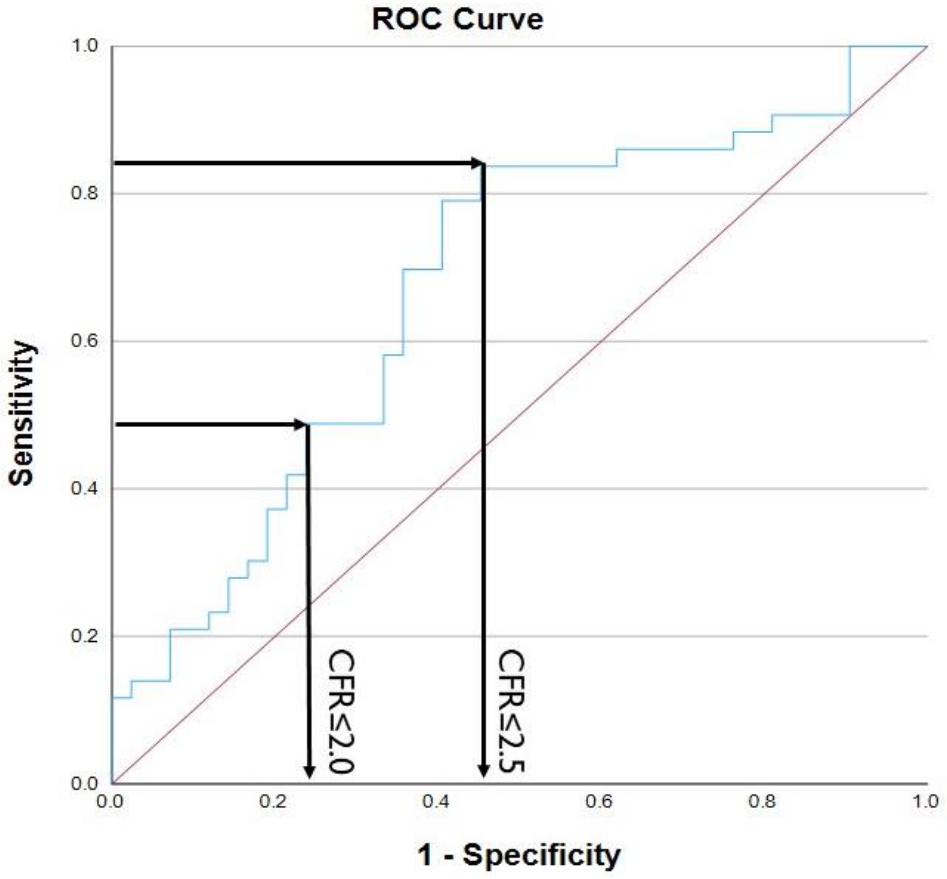
CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance

The increment in independent variables:

Age: 1 year increase; CFR: 0.1 unit increase; hMR: 0.1 mmHg.cm^{-1}.s^{-1} increase.

The optimal CFR to predict an increment in exercise time of ≥60 seconds in response to anti-ischaemic therapy (ranolazine and/or amlodipine) was ≤2.5 (sensitivity 84% and specificity 55%) (**Figure 28**). The CFR≤2.5 threshold was 69% accurate at predicting an increment in exercise time of ≥60 seconds in response to anti-ischaemic therapy, compared with the CFR≤2.0 threshold, which was 61% accurate (sensitivity 49% and specificity 74%) (p<0.001).

Figure 28. Receiver operating characteristic curves comparing the diagnostic accuracy of two CFR thresholds.

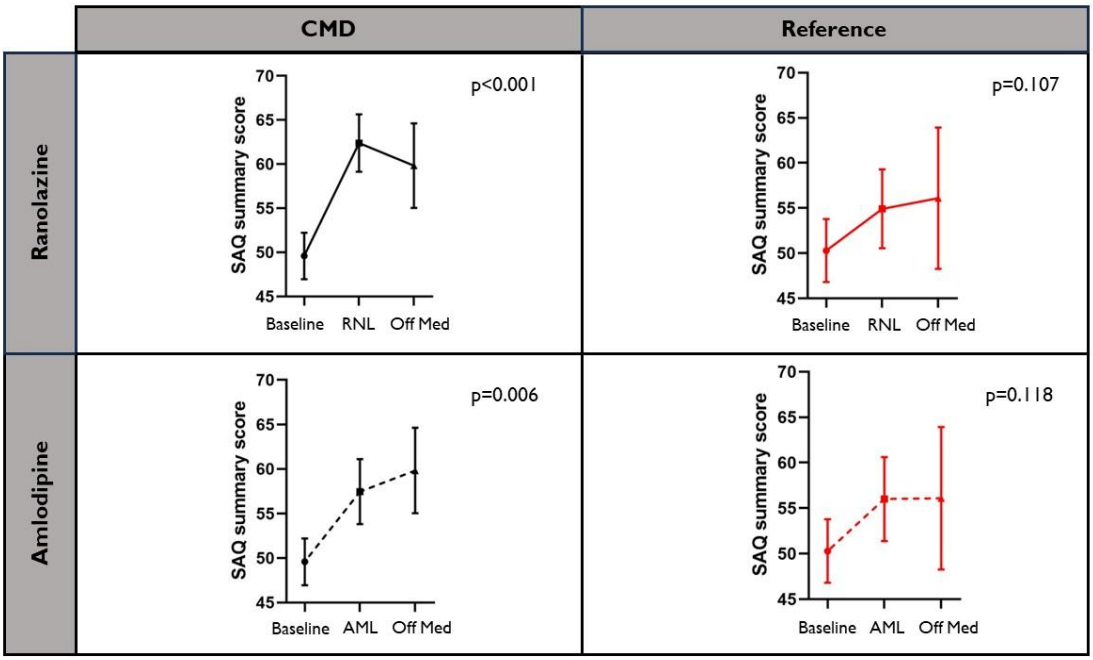


CFR: coronary flow reserve

The change in SAQ summary score in response to amlodipine was similar between the CMD and reference groups (mean difference in change 2, 95% CI -5 to 8, p=0.549). There was a greater increment in SAQ summary score with ranolazine in the CMD group compared to the reference group (mean difference in change 7, 95% CI 0 to 15, p=0.048) (**Table 13 and Appendix Table 1**). The gain in SAQ was not lost on cessation of study medications in the reference group; however, in the CMD group, gain-or-loss of function of SAQ summary score was observed with/without ranolazine (**Figure 29**). Overall, patients scored 5 units higher on their SAQ summary score compared to the first visit (p=0.035); when dichotomised according

to CFR, those with CMD had a numerically higher SAQ summary score during their fourth visit compared to their first visit (p=0.054), whereas no such difference was observed in the reference group (p=0.432).

Figure 29. Gain-or-loss of function in SAQ summary score with/without study medication.



CMD: coronary microvascular disease; RNL: ranolazine; AML: amlodipine; off med: without study medications.

There was no evidence of an interaction between allocated treatment sequence and the change in exercise time. Patients allocated to the ranolazine-amlodipine sequence (n=40) had a similar change in exercise time to those allocated to the amlodipine-ranolazine sequence (n=47), in response to amlodipine (mean difference in change 29 seconds, 95% CI -19 to 76 seconds, p=0.232) and ranolazine (mean difference in change 4 seconds, 95% CI -44 to 51 seconds, p=0.877).

4.5. Discussion

In ChaMP-CMD, patients with ANOCA and an impaired CFR had an improvement in exercise time in response to anti-ischaemic therapy, whereas those with a normal CFR did not. The gain in function with therapy was lost on cessation of therapy in patients with CMD, which suggests a causal link between the pathophysiological classification and response to therapy. We believe this to be the first robust demonstration of the association between CFR and an improvement in exercise capacity in response to anti-ischaemic therapy. This, in turn, demonstrates the practical utility of measuring CFR in patients with ANOCA as a means of personalising therapies.

We had previously demonstrated that an impaired CFR reliably identifies those with an ischaemic substrate and maladaptive physiology during exercise[25], but whether this classification also identifies patients who derive objective benefit in their exercise capacity from anti-ischaemic therapy was not known. Three contemporary trials designed to assess response to therapy in ANOCA patients have yielded equivocal results[89,123,124] and have been limited by inclusion of patients with atypical symptoms, low prevalence of impaired CFR at baseline and the use of subjective quality of life questionnaires as the primary outcome. Post-hoc analyses of two of these studies have suggested that those with an impaired CFR at baseline may derive an improvement in their quality of life in response to anti-ischaemic therapy[89,124]. The CorMicA study also demonstrated superiority of physiology-guided management above empirical therapy in relation to SAQ scores at 6- and 12-months[94,95], as well as improvement in resource consumption[96]. However, the definition of vasomotor abnormalities was broad in CorMicA (diminished CFR and/or elevated minimal microvascular resistance and/or epicardial/microvascular spasm with bolus acetylcholine provocation) and hence it was not possible to link the specific pathophysiological diagnosis with outcome. Furthermore, in

CorMicA, the recommended therapies included a range of pharmacological and non-pharmacological measures (including referral to cardiac rehabilitation)[96], and, therefore, the mechanistic link between physiological findings, therapies instituted and improvement in outcome remained incompletely understood. CFR was the only biologically plausible variable that was associated with an increment in exercise time ≥60 seconds in response to anti-ischaemic therapy in our study. However, the multivariate model that included CFR, gender and hypertension only accounted for 11.9% of the change in exercise time; this is suggestive that there must be other patient-related factors that affect the response to therapy that were not characterised in our study.

When objectively assessing efficacy of therapies on exercise capacity, the minimum clinically relevant increment in exercise time is often regarded to be 30 seconds. It is notable that patients with CMD had an increment in exercise time following anti-ischaemic therapy that was, on average, greater than twice this minimum difference; this treatment effect is in keeping with those reported in seminal anti-ischaemic trials of patients with obstructive coronary disease[102–105] and greater than the effect reported in many trials of heart failure patients[125,126] (**Table 15**).

Table 15. Comparison of previous exemplar cardiovascular trials using exercise time as an outcome measure.

	Study design	**Baseline ET**	**Increment in ET**
Obstructive CAD			
Fox KM et al[102] TIBET	Design: Double-blind parallel-group study Patient numbers: 319	417s (atenolol arm) 423s (nifedipine arm) 410 (combination arm)	91s (atenolol arm) 91s (nifedipine arm) 98s (combination arm)

	Follow-up: Six weeks		
Frischman WH et al[103]	Design: Double-blind, placebo controlled, RCT Patient numbers: 551 Follow-up: Four weeks	348s (verapamil) 360s (amlodipine) 348s (amlodipine and atenolol) 354s (placebo)	66s (verapamil) 60s (amlodipine) 72s (amlodipine and atenolol) 24s (placebo)
Chaitman BR et al[104] MARISA	Design: Double blinded placebo controlled RCT; Patient numbers: 191 Follow-up: One week of therapy at 3 sequentially doses	Baseline exercise times not reported	116s (Ranolazine 750mg) 70s (placebo)
Noman A et al[105]	Design: Double-blind, placebo-controlled, crossover RCT Patient numbers: 65 Follow-up: Six weeks	301s	6s (placebo) 93s (allopurinol)
Al-Lamee R et al[101] ORBITA	Design: Double-blinded sham procedure trial; Patient numbers: 230 Follow-up: Six weeks	528s (PCI group) 490s (sham group)	28s (PCI group) 12s (sham group)
Reynolds HR et al[127] ISCHEMIA-CIAO	Design: observational substudy Patient numbers: 116 Follow-up: One year	364s	46s

Heart failure with reduced ejection fraction			
Rouleau JL et al[125] IMPRESS	Design: Double-blinded parallel RCT Patient numbers: 573 Follow-up: 12 weeks	511s (omapatrilat arm) 500s (lisinopril arm)	24s (omapatrilat arm) 31s (lisinopril arm)
Australia/New Zealand Heart Failure Research Collaborative Group[126]	Design: Double-blind, placebo-controlled RCT Patient numbers: 415 Follow-up: One year	630s	7s 24s improvement) and placebo (roughly 17s improvement)

CAD: coronary artery disease; ET: exercise time

This therapeutic effect, and our observation that the diagnostic (based on our previous study comparing CFR with noninvasive myocardial ischaemia[25]) and therapeutic (based on this study comparing CFR with ≥60 seconds improvement in exercise time) thresholds of CFR are identical, suggest a mechanistic link between the presence of an ischaemic substrate and exercise capacity**.** We have demonstrated that the CFR≤2.5 threshold can predict a clinically relevant therapeutic response with greater accuracy than the CFR≤2.0 threshold. This follows on from our previous study, which demonstrated that the CFR<2.5 threshold had a better accuracy at predicting myocardial ischaemia and coronary perfusion efficiency during exercise than the CFR≤2.0 threshold[25]. This reaffirms a strong mechanistic link between coronary perfusion inefficiency during exercise, myocardial ischaemia, and response to therapy.

The patients enrolled in ChaMP-CMD reported a degree of impairment in their quality of life, on account of angina, that was comparable to that reported by patients with obstructive CAD in ORBITA[101] and ANOCA in CorMicA[94], but worse than patients with obstructive CAD in ISCHEMIA[128] and ANOCA in ISCHEMIA-CIAO[127] **(Table 16).** The minimum clinically meaningful change in the SAQ summary score, following an investigational therapy, has previously been regarded to be a difference of 8 points. By this metric, patients with CMD experienced an improvement in their SAQ summary score compared to the reference group in response to ranolazine but not amlodipine, whilst no meaningful improvement in SAQ summary score was observed within the reference group.

Table 16. Comparison of SAQ scores in other trials of patients with ischaemic heart disease.

	SAQ scores at baseline	**SAQ scores with treatment**
CorMicA[94]	Summary score 51 Angina frequency score 59	Summary score 52 (control arm) Summary score 68 (intervention arm) Angina frequency score 56 (control arm) Angina frequency score 75 (intervention arm) Follow-up: 6 months
ORBITA[101]	Angina frequency score 63 (PCI group) Angina frequency score 60 (sham group)	Angina frequency score 74 (PCI group) Angina frequency score 68 (sham group) Follow-up: 6 weeks

Spertus JA et al[128] ISCHEMIA	Summary score 73 (invasive arm) Summary score 75 (conservative arm) Angina frequency score 81 (invasive arm) Angina frequency score 82 (conservative arm)	Summary score 89 (invasive arm) Summary score 83 (conservative arm) Follow-up: 3 years
ISCHEMIA-CIAO[127]	Summary score 83 Angina frequency score 90	Summary score 92 Angina frequency score 100 Follow-up: 6 months

The discordance between treatment effect, as assessed by exercise time versus SAQ summary score, may be because the subjective assessment of quality of life is multifactorial (the coefficient of determination, r^2, between SAQ and exercise time at baseline was only 15%) and more prone to treatment bias than objective measures like exercise time. The uncoupling of ischaemia and SAQ scores was also seen in the CIAO-ISCHEMIA study, where patients reported improvement in their SAQ scores after medical treatment despite having persistence of objective ischaemia[127]. Similarly, in a study of patients with obstructive coronary artery disease, percutaneous coronary intervention led to a reduction in the ischaemic burden, as identified on stress echocardiography, but this did not translate to better angina-specific quality of life[129].

There was some evidence of a training effect, with participants walking for 22 seconds longer, on average, during their fourth ETT compared to the first one. While this may have led to slight over-estimation of the treatment effects overall, there is no evidence that this has affected the comparison of treatment effect between groups or between drugs, given the randomised cross-

over design. Similarly, patients scored 5 points higher on their fourth SAQ compared to the first one; however, this did not reach the minimum clinically relevant threshold of 8 points. Interestingly, patients with CMD exercised for longer and reported better angina-specific quality of life during their fourth visit compared to their baseline visit, whereas no such difference was observed in patients in the reference group. This might be because patients with CMD had an improvement in their coronary flow reserve in response to the preceding anti-ischaemic therapy, which led to an improvement in their exercise time even when off study medications. However, we did not carry out repeat coronary physiology assessment to assess changes in coronary flow reserve before and after the study to test this hypothesis. Of note, Bairey-Merz et al have previously reported that a two-week course of ranolazine led to an improvement in myocardial perfusion reserve index in patients with an impaired CFR, whereas no such effect was seen in patients with a normal CFR[89].

There is currently marked heterogeneity in the diagnosis and management of patients with ANOCA due to a variety of reasons, some of which we have addressed with the findings of our study. Firstly, there is a widely held belief that empirical management of patients with ANOCA (without embarking on definitive diagnostic testing to confirm or refute the presence of CMD) is an acceptable alternative. We have demonstrated that patients with ANOCA, but no CMD, do not experience any improvement in objective exercise capacity or subjective quality of life with anti-ischaemic therapies. To treat these patients empirically would not only potentially expose them to ineffective therapies but also delay the pursuit of alternative causes for their symptoms. The cause of chest pain in patients with ANOCA and normal CFR is unclear, but our findings suggest that inducible ischaemia may not be an appropriate treatment target in this group.

The rationale for using amlodipine and ranolazine as our exemplar drugs is provided in **Chapter 5**.

Our study has some limitations that should be considered when interpreting the findings. First, this was a single centre study with a relatively small sample size with inherent limitations of generalisability. Second, we did not include a placebo therapy arm and hence this was not double blinded in the conventional sense. However, patients and researchers were blinded to the physiological classification (phenotype-blinding) and had no means of knowing whether a given drug was expected to affect their exercise time or quality of life. Third, objective methods of assessing the efficacy of blinding, such as blinding questionnaires, were not undertaken. However, patients were not informed about their coronary physiology metrics and had no way of finding this out given that researchers and physiologists were blinded to the physiology as well. Furthermore, none of the researchers/staff present in the catheter laboratory were present during the exercise ECG stress testing protocol. Fourth, we did not repeat coronary physiology measurements, which may have provided an important mechanistic link and demonstrated the effects of the medications on patients' underlying physiology. However, repeating these measurements three times within a course of 12 weeks would have been highly burdensome for our patients and beyond the study resources. Fifth, we have studied two specific anti-ischaemic agents with different mechanistic profiles and therefore cannot be certain that the differential effect we observed (in CMD versus reference patients) would be replicated with other classes of drug. Sixth, we only interrogated the LAD artery for coronary physiology assessment. Whilst this is the standard of practice, in both clinical and research settings, there is some emerging data to suggest that CMD may not necessarily be a panmyocardial process.

It is possible that patients with a normal CFR in the LAD may have had an impaired CFR in the non-LAD vessels. However, whether the same CFR diagnostic threshold applies to non-LAD vessels is not known. Furthermore, whether an impaired CFR in non-LAD vessels correlates with inducible ischaemia on non-invasive assessment is also not known. Finally, it is important to emphasise that this was a carefully selected patient cohort with a high symptom burden and pretest probability of coronary vascular dysfunction (**Figure 26**); therefore, these results may not apply to patients with ANOCA who present with atypical or minimal symptoms.

4.5. Conclusions

In summary, we have demonstrated that an invasive diagnosis of CMD, based on CFR, distinguishes patients with ANOCA who derive objective benefit from anti-ischaemic therapy. The CFR threshold that is commonly used to diagnose CMD was also the most accurate in identifying patients who respond to anti-ischaemic therapy. These findings support routine measurement of CFR in patients with ANOCA and typical limiting symptoms to not only establish a diagnosis and predict prognosis, but also to guide medical therapy. Our findings should also inform future trials of anti-ischaemic therapies for CMD, which should selectively enrol patients with diminished CFR or be designed to allow comparison of ANOCA patients with versus without evidence of CMD.

Chapter 5

Does deep endotyping in the catheter laboratory predict response to anti-ischaemic therapy in patients with angina and nonobstructive coronary arteries?

Abstract accepted as poster presentation at American College of Cardiology Congress 2024

Manuscript submitted to *Circulation: Cardiovascular Interventions*

5.1. Abstract

Background: Angina with nonobstructive coronary arteries (ANOCA) is a common condition and an impaired coronary flow reserve in response to adenosine (coronary endothelium-*independent* microvascular dysfunction; CMD) and acetylcholine (coronary endothelium-*dependent* microvascular dysfunction; CED) identifies those with an ischaemic substrate. Recently, we have shown that CMD itself may be a heterogenous condition comprising two endotypes that are distinguished by the minimal microvascular resistance (structural and functional CMD). Both endotypes have a high prevalence of inducible ischaemia and similarly impaired prognosis, but the underlying pathobiology is disparate. Whether these distinct endotypes respond differently to anti-ischaemic therapies is not known and is a key unmet clinical need.

Methods: Patients with ANOCA underwent blinded invasive coronary flow reserve (CFR), hyperaemic (minimal) microvascular resistance (hMR) and acetylcholine flow reserve (AChFR) measurement and were randomly assigned to receive four weeks of amlodipine or ranolazine. After a one-week washout, they crossed over to the other drug for four weeks. The primary outcome was change in treadmill exercise time, and the secondary outcome was change in Seattle Angina Questionnaire (SAQ) summary score, in response to anti-ischaemic therapy. Analysis was on a per protocol basis; we tested for *within* group differences, in response to anti-ischaemic therapy, in patients with **structural CMD** (**sCMD**; CFR<2.5 and hMR≥2.5 mmHg.cm^{-1}.s^{-1}) and **functional CMD** (**fCMD**; CFR<2.5 and hMR<2.5 mmHg.cm^{-1}.s^{-1}). We also assessed the incremental value of measuring AChFR in predicting response to anti-ischaemic therapy; sole **coronary endothelial dysfunction** (**CED**) was denoted by CFR≥2.5 and AChFR≤1.5 and the **reference** group by CFR≥2.5 and AChFR>1.5.

Results: Eighty-seven patients (61±8 years, 62% females) underwent randomisation (22 **sCMD**, 35 **fCMD**, 15 **CED** and 15 **reference** group). Baseline exercise time and SAQ summary scores were similar between the groups. CFR was independently associated with an increment in exercise time ≥60s in response to anti-ischaemic therapy. The optimal CFR threshold to predict response to therapy was ≤2.5. Patients with functional CMD had an increment in exercise time with both anti-ischaemic therapy (amlodipine: change from baseline 94s, 95% CI 58 to 130 s, p<0.001; ranolazine: change from baseline 100s, 95% CI 58 to 141s, p<0.001) with no *within* group differences between the agents (difference in change 3, 95% CI -27 to 32s, p=0.859). Patients with structural CMD had a numerically greater response to amlodipine than ranolazine (difference in change 46s, 95% CI -2 to 93s, p=0.056). Patients with sole CED had a numerical increment in exercise time (albeit statistically non-significant), compared to baseline, with both anti-ischaemic therapies, whereas no such effect was seen in the reference group.

Conclusions: Amongst phenotypically similar patients with ANOCA, those with an impaired CFR respond to anti-ischaemic therapy. Patients with functional CMD exhibited a clinically meaningful response in exercise time and quality of life with both anti-ischaemic therapies; this further confirms the veracity of this recently identified endotype. Patients with structural CMD exhibited a preferential response with amlodipine. This suggests that measurement of minimal microvascular resistance and acetylcholine flow reserve, in addition to CFR, provides incremental value in therapy stratification.

5.2. Introduction

We have demonstrated in **Chapter 4** that in a phenotypically similar group of patients with angina and nonobstructive coronary arteries (ANOCA), only those with an impaired CFR (CMD group) respond to anti-ischaemic therapy. We have previously demonstrated that CMD itself may be a heterogenous condition comprising two distinct endotypes termed functional and structural CMD[24,25], distinguished by elevated minimal microvascular resistance in the latter. While the two endotypes are phenotypically similar, the underlying pathobiology is distinct[24]; submaximal vasodilatation at rest appears to be the predominant mechanism of perturbed coronary physiology in patients with functional CMD, whereas an inability to adequately vasodilate in response to a stressor appears to be the predominant mechanism in patients with structural CMD[24]. It has recently been reported that both endotypes have similarly impaired long-term prognosis, when compared to patients with a normal CFR[51]. Furthermore, it is now being increasingly recognised that patients with a normal CFR but abnormal vasodilatory response to acetylcholine infusion (acetylcholine flow reserve; AChFR) have a substrate for ischaemia[32,50], a high angina burden[33] and adverse cardiovascular outcomes[34,35]. This substudy of ChaMP-CMD was designed to assess if deep endotyping in the catheter laboratory, using minimal microvascular resistance and acetylcholine flow reserve, provides incremental value in predicting response to anti-ischaemic therapy beyond that afforded by measuring CFR alone.

5.3. Methods

5.3.1. Study design and participants

This is discussed in detail in the relevant section of **Chapter 4 (4.3.1.).**

5.3.2. Randomisation, masking and cross-over

These protocols are described in detail in the relevant section of **Chapter 4 (4.3.2.).**

5.3.3. Procedures

5.3.3.1. Coronary angiography

Catheterisation was performed via the right radial artery; this protocol is described in more detail in the methods section (**Chapter 2**). Patients, researchers and ETT physiologists were all blinded to the patients' coronary physiology dataset.

5.3.3.2. Analysis of coronary physiology data

Signals were sampled at 200 Hz, with data exported into a custom-made study manager program (Academic Medical Centre, Amsterdam, Netherlands) and analysed on custom-made software, Cardiac Waves (King's College London, UK). CFR was calculated as hyperaemic APV/resting APV, both measured at steady state. Hyperaemic (minimal) microvascular resistance (hMR) was calculated as P_d/APV. Acetylcholine flow reserve (AChFR) was calculated as the ratio of coronary blood flow (CBF) in response to acetylcholine infusion compared to resting CBF. The estimation of volumetric CBF was calculated as quantitative coronary angiography (QCA)-derived cross-sectional area × APV × 0.5, with QCA performed 5mm distal to the tip of the guidewire[68]. Patients were classified off-line into **structural CMD** (CFR<2.5 and hMR≥2.5 $mmHg.cm^{-1}.s^{-1}$), **functional CMD** (CFR<2.5 and hMR<2.5 $mmHg.cm^{-1}.s^{-1}$), sole **coronary endothelial dysfunction (CED**; CFR ≥2.5 and AChFR≤1.5) and **reference** (CFR≥2.5 and AChFR>1.5) groups as previously described[50].

5.3.3.3. Exercise electrocardiogram treadmill test

The exercise ECG treadmill test (ETT) was performed with a Marquette Case 8000 system (GE Medical Systems, Milwaukee, WI, USA) according to the American College of Cardiology and American Heart Association practice guidelines using a standard Bruce protocol[78], terminated at the patient's request. 12-lead electrocardiogram (ECG), heart rate and blood pressure were recorded at regular intervals before, during and after the ETT. All ETTs were supervised by physiologists who were blinded to the patients' coronary physiology data. Exercise time (ET) was defined as the time from the start of the exercise protocol to exercise cessation.

5.3.3.4. Seattle Angina Questionnaire (SAQ)

At each visit, patients completed the Seattle Angina Questionnaire (SAQ), which is a validated angina-specific quality of life assessment[106]. The SAQ comprises five components, namely physical limitation, angina stability, angina frequency, treatment satisfaction and quality of life; these are then incorporated into the summary score, where a higher score indicates better function. SAQ scores are independently associated with mortality, hospitalisation, and resource use[130]. The SAQ is a sensitive instrument in patients with coronary microvascular disease[94,95] and a change in 8 points has been demonstrated to represent a minimal clinically important difference.

5.3.4. Outcomes

The primary outcome was change in exercise time in response to anti-ischaemic therapy. The secondary outcome was change in SAQ summary score in response to anti-ischaemic therapy.

5.3.5. Hypotheses and statistical considerations

Our primary hypothesis was that patients with functional CMD will have a greater improvement in their exercise capacity in response to ranolazine compared to amlodipine, whereas patients with structural CMD will have a greater improvement in their exercise capacity in response to amlodipine compared to ranolazine (justification for the selection of these agents is provided in the Discussion section). Assuming a 1:1 distribution, the sample size required to detect a *within-group* difference of 60 seconds (SD 85 seconds) in response to amlodipine and ranolazine, respectively, at 80% power and 5% significance was 18 patients of each endotype.

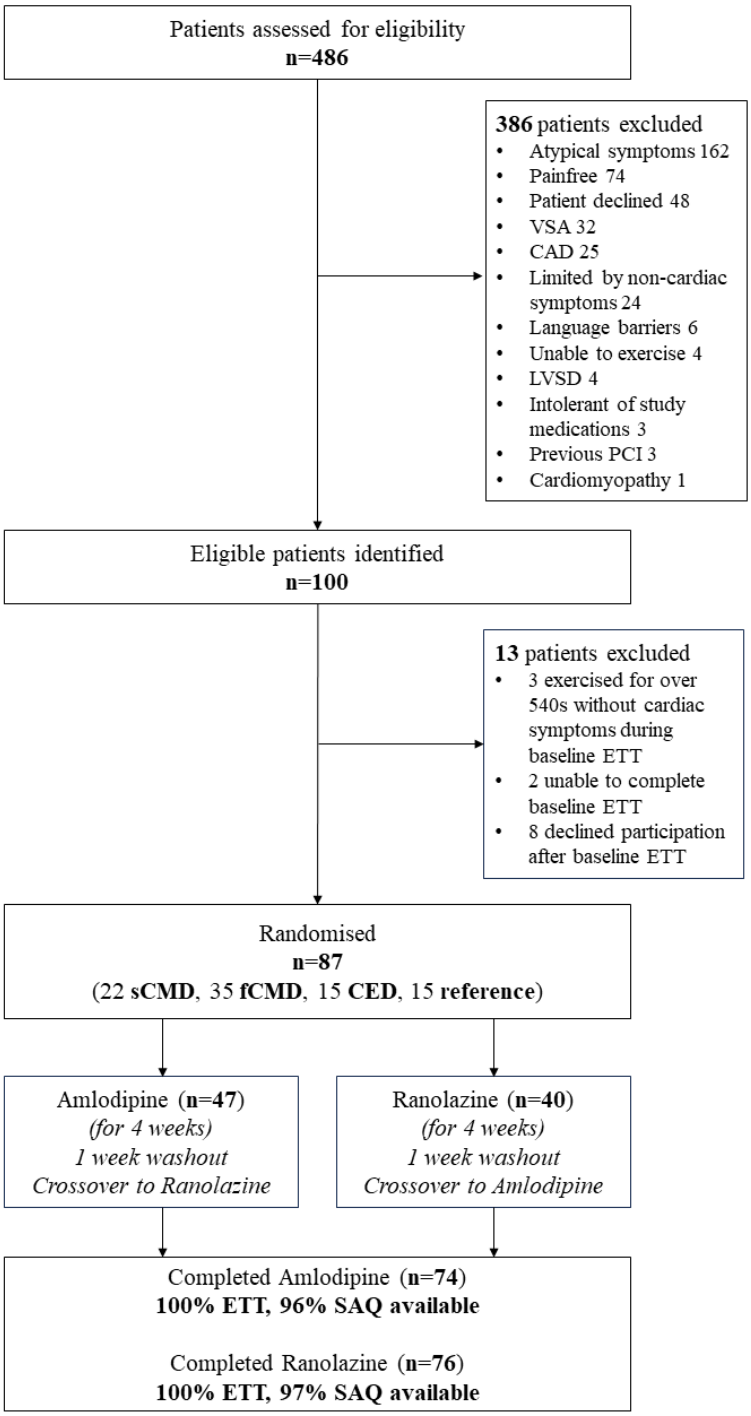
Normality of data was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous data are presented as mean±SD, unless specified otherwise, and compared using the independent sample's Student *t*-test. Non-normally distributed data are presented as median (interquartile range) and compared using the Mann-Whitney test (unpaired analyses) or the Wilcoxon matched-pairs signed rank test (paired analyses). Categorical variables are presented as n (%) and compared using the chi-squared test. Continuous endpoints were compared with the two-sample t test of the difference *within* groups (structural CMD and functional CMD); the findings are reported as the difference in mean change within study groups with 95% confidence intervals (CIs) and p values. Binary logistic regression was performed using univariate analysis and reported as standard coefficients (95% CI). Receiver operating characteristic curves were used to compare the area under the curves for CFR, hMR and AChFR to predict ≥60 seconds increment in exercise time in response to anti-ischaemic therapy. These area under the curves were compared using the Delong-Delong method. The optimal CFR threshold to predict ≥60 seconds increment in exercise time was derived using the Youden's index. All randomised patients were included in these analyses. Data were

analysed on a per protocol basis, which was favoured over intention-to-treat analysis as this study addressed the efficacy of therapy as opposed to a treatment strategy. All analyses were performed using SPSS Statistics 27 (IBM, NY, USA) and GraphPad Prism software version 9.0 (GraphPad software, San Diego, CA, USA). We deemed a p value less than 0.05 to be significant.

5.4. Results

Between December 2020 and August 2023, 486 patients with angina and nonobstructive coronary arteries were screened and 100 eligible patients identified. Eighty-seven of these patients underwent randomisation after the baseline visit (**Figure 30**). By blinded classification, 57 patients had **CMD** (22 patients had **structural CMD (sCMD)** and 35 **functional CMD (fCMD)**), 15 had sole **coronary endothelial dysfunction** (**CED)** and 15 were in the **reference** group.

Figure 30. Patient screening and recruitment for ChaMP-CMD substudy.



VSA: vasospastic angina; CAD: coronary artery disease; ACh: acetylcholine; LVSD: left ventricular systolic dysfunction; PCI: percutaneous coronary intervention; ETT: exercise ECG treadmill testing; sCMD: structural CMD; fCMD: functional CMD; CED: sole coronary endothelial dysfunction

Patients were well matched for age, gender, body mass index, cardiovascular risk factors, symptomology, routine blood tests and medications (**Tables 17 and 18**).

Table 17. Baseline demographics in patients with structural and functional CMD.

	Structural CMD (n=22)	**Functional CMD (n=35)**	**CFR>2.5 (n=30)**	**P value (sCMD vs fCMD)**
Patient demographics				
Age, years	61±9	62±8	60±7	0.446
Female, n(%)	14 (64)	22 (63)	18 (60)	0.953
BMI, kg/m^2	29 (27, 33)	27 (24, 32)	31 (26, 34)	0.332
Hypertension, n(%)	14 (64)	19 (54)	9 (30)	0.486
Diabetes, n(%)	7 (32)	8 (23)	3 (10)	0.454
Hyperlipidaemia, n(%)	12 (55)	24 (69)	16 (53)	0.285
Smoking history, n(%)	4 (18)	5 (14)	7 (23)	0.695
Symptomology				
CCS, n(%)				0.740
I	2 (9)	1 (3)	2 (7)	
II	7 (32)	14 (40)	13 (43)	
III	11 (50)	17 (49)	13 (43)	
IV	2 (9)	3 (9)	2 (7)	
NYHA, n(%)				0.691
I	9 (41)	18 (51)	16 (53)	
II	12 (55)	15 (43)	11 (37)	
III	1 (5)	2 (6)	3 (10)	

IV	0	0	0	
Serum biomarkers				
Haemoglobin, g/L	136±16	133±15	135±12	0.432
eGFR, ml/min/1.73m^2	79±22	78±21	83±17	0.848
HbA1c, mmol/mol	39 (38, 41)	40 (38, 46)	42 (39, 45)	0.384
Total cholesterol, mmol/L	3.9±1.1	4.2±1.1	4.3±1.2	0.420
Medications				
Antiplatelet agent	10 (46)	23 (66)	14 (47)	0.132
Statins	19 (86)	26 (74)	21 (70)	0.276
ACE inhibitors/ARB	10 (46)	17 (49)	11 (37)	0.819
Beta blockers	3 (14)	10 (29)	7 (23)	0.191
Nitrates	6 (27)	4 (11)	6 (20)	0.126

sCMD: structural CMD; fCMD: functional CMD; CFR: coronary flow reserve; BMI: Body mass index; CCS: Canadian cardiovascular society; NYHA: New York heart association, eGFR: estimated glomerular filtration rate; HbA1c: glycated haemoglobin; DM: diabetes mellitus; ACE inhibitor: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker.

Table 18. Baseline demographics in patients with normal coronary flow reserve.

	CED (n=15)	**Reference (n=15)**	**CMD (n=57)**	**P value (CED vs reference)**
Patient demographics				
Age, years	62±6	58±8	62±8	0.096
Female, n(%)	9 (60)	9 (60)	36 (63)	1.000
BMI, kg/m^2	32 (26, 34)	32 (28, 36)	28 (25, 32)	0.222
Hypertension, n(%)	6 (40)	3 (20)	33 (58)	0.232
Diabetes, n(%)	2 (13)	1 (7)	15 (26)	0.543
Hyperlipidaemia, n(%)	8 (53)	8 (53)	36 (63)	1.000
Smoking history, n(%)	2 (13)	5 (33)	9 (16)	0.195
Symptomology				
CCS, n(%)				0.709
I	1 (7)	1 (7)	3 (5)	
II	5 (33)	8 (53)	21 (37)	
III	8 (53)	5 (33)	28 (49)	
IV	1 (7)	1 (7)	5 (9)	
NYHA, n(%)				0.714
I	9 (60)	7 (47)	27 (47)	
II	5 (33)	6 (40)	27 (47)	
III	1 (7)	3 (!3)	3 (5)	
IV	0	0	0	

Serum biomarkers				
Haemoglobin, g/L	130±11	140±11	134±14	0.027
eGFR, ml/min/1.73m^2	82±14	85±20	79±21	0.593
HbA1c, mmol/mol	42 (39, 49)	41 (39, 43)	39 (37, 42)	0.270
Total cholesterol, mmol/L	4.3±1.1	4.3±1.3	4.1±1.1	0.916
Medications				
Antiplatelet agent	6 (40)	8 (53)	33 (58)	0.464
Statins	12 (80)	9 (60)	45 (79)	0.232
ACE inhibitors/ARB	7 (47)	4 (27)	27 (47)	0.256
Beta blockers	4 (27)	3 (20)	13 (23)	0.666
Nitrates	3 (20)	3 (20)	10 (18)	1.000

CED: isolated coronary endothelial dysfunction; CMD: coronary microvascular disease; BMI: Body mass index; CCS: Canadian cardiovascular society; NYHA: New York heart association, eGFR: estimated glomerular filtration rate; HbA1c: glycated haemoglobin; DM: diabetes mellitus; ACE inhibitor: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker.

Coronary flow reserve and acetylcholine flow reserve were similar between the structural CMD and functional CMD endotypes (CFR: 1.9±0.3 vs 2.0±0.3, p=0.073; AChFR: 1.2±0.3 vs 1.3±0.4, p=0.812). Baseline exercise time and SAQ summary scores were also similar (ET: 348±138 vs 295±144 s, p=0.171; SAQ summary score: 46±18 vs 52±19, p=0.290) (**Table 19**).

Table 19. Coronary physiology, exercise test and SAQ measurements at baseline in patients with structural and functional CMD.

	Structural CMD (n=22)	**Functional CMD (n=35)**	**CFR>2.5 (n=30)**	**P value (sCMD vs fCMD)**
Coronary physiology measurements				
Pd/Pa	0.95±0.02	0.95±0.03	0.95±0.04	0.368
FFR	0.92±0.03	0.92±0.05	0.91±0.04	0.678
CFR	1.9±0.3	2.0±0.3	3.0±0.4	0.073
hMR, $mmHg.cm^{-1}.s^{-1}$	2.8 (2.7, 3.8)	1.8 (1.5, 2.1)	1.9 (1.7, 2.2)	<0.001
AChFR	1.2±0.3	1.3±0.4	1.6±0.7	0.812
Exercise treadmill test				
ET, seconds	348±138	295±144	317±128	0.171
Resting HR, bpm	85±16	85±15		0.878
Resting systolic blood pressure, mmHg	142±24	139±21		0.617
Resting RPP, mmHg.bpm	12086±3043	11824±2396		0.737
Seattle Angina Questionnaire				
Summary score	46±18	52±19	51±19	0.290

CMD: structural coronary microvascular disease; fCMD: functional coronary microvascular disease; FFR: Fractional Flow Reserve; CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve; ET: exercise time

Coronary flow reserve and minimal microvascular resistance were similar between the isolated coronary endothelial dysfunction and reference endotypes (CFR: 3.1±0.5 vs 2.9±0.4, p=0.129; hMR: 1.8 (1.4, 2.1) vs 2.0 (1.8, 2.3) $mmHg.cm^{-1}.s^{-1}$, p=0.155). Baseline exercise time and SAQ summary scores were also similar (ET: 290±123 vs 344±132, p=0.253; SAQ summary score: 55±21 versus 48±16 p=0.338) (**Table 20**).

Table 20. Coronary physiology, exercise test and SAQ measurements at baseline in patients with normal coronary flow reserve.

	CED (n=15)	**Reference (n=15)**	**CMD (n=57)**	**P value (CED vs reference)**
Coronary physiology measurements				
Pd/Pa	0.95±0.03	0.96±0.04	0.95±0.03	0.592
FFR	0.90±0.04	0.91±0.05	0.92±0.04	0.765
CFR	3.1±0.5	2.9±0.4	2.0±0.3	0.129
hMR, $mmHg.cm^{-1}.s^{-1}$	1.8 (1.4, 2.1)	2.0 (1.8, 2.3)	2.3 (1.7, 2.7)	0.155
AChFR	1.1±0.2	2.1±0.4	1.3±0.4	<0.001
Exercise treadmill test				
ET, seconds	290±123	344±132	315±143	0.253

Resting HR, bpm	83±13	88±15		0.380
Resting systolic blood pressure, mmHg	144±19	132±20		0.102
Resting RPP, mmHg.bpm	12065±2825	11756±3540		0.796
Seattle Angina Questionnaire				
Summary score	55±21	48±16	49±19	0.338

CED: isolated coronary endothelial dysfunction; CMD: coronary microvascular disease; FFR: Fractional Flow Reserve; CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve; ET: exercise time

5.4.1. All randomised patients

Using linear regression analysis, CFR was the only biologically plausible variable that was independently associated with an increment in exercise time ≥60 seconds in response to anti-ischaemic therapy (**Table 21**).

Table 21. Linear regression analysis testing the association between biologically plausible variables and increment in exercise time ≥60 seconds in all randomised patients.

	Odds ratio (95% CI)	**P value**
Univariable		
Age	1.020 (0.966, 1.077)	0.475
Gender	2.101 (0.854, 5.174)	0.106
Hypertension	2.651 (1.098, 6.403)	0.030
CFR	0.889 (0.816, 0.968)	0.007
hMR	1.000 (0.952, 1.051)	0.993
AChFR	0.979 (0.897, 1.069)	0.637
Coronary perfusion efficiency	1.022 (0.975, 1.071)	0.366
Multivariable		
CFR	0.905 (0.830, 0.987)	0.024
Hypertension	2.079 (0.824, 5.246)	0.121

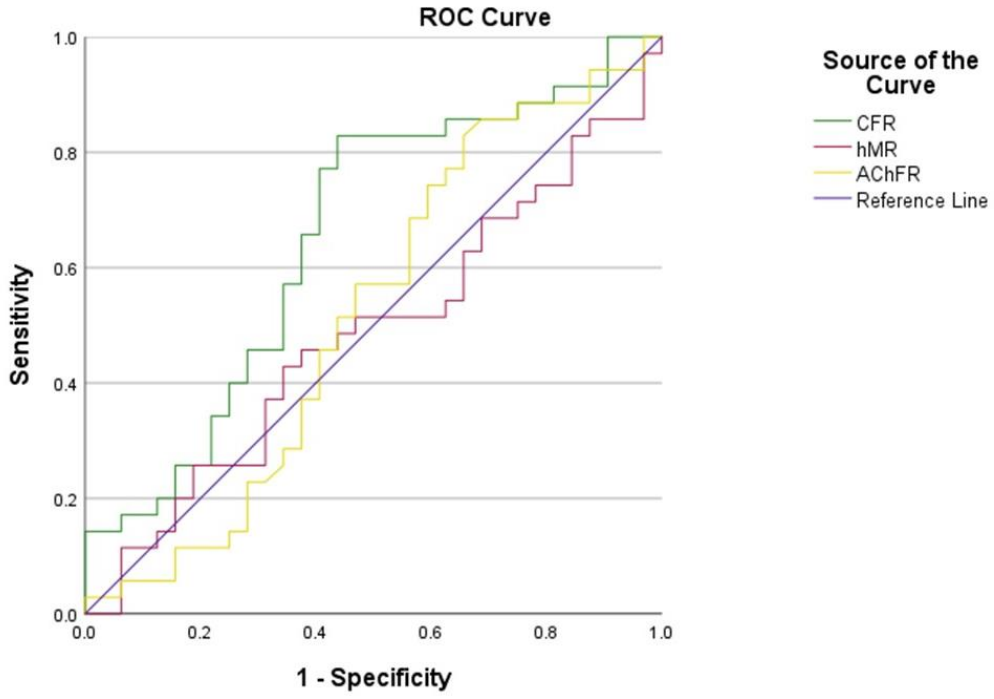
CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve

The increment in independent variables:

Age: 1 year increase; CFR: 0.1 unit increase; hMR: 0.1 mmHg.cm^{-1}.s^{-1} increase; AChFR: 0.1 unit increase

The area under the curves to predict an increment in exercise time of ≥60 seconds were 0.652, 0.474 and 0.508 for the CFR≤2.5, hMR≥2.5mmHg.cm^{-1}.s^{-1} and AChFR≤1.5 thresholds, respectively (p=0.128 for CFR vs hMR; p=0.120 for CFR vs AChFR; p=0.696 for hMR vs AChFR) (**Figure 31**). The optimal CFR threshold to predict an increment in exercise time of ≥60 seconds in response to anti-ischaemic therapy was ≤2.5 (sensitivity 82% and specificity 53%).

Figure 31. Receiver operating characteristics curve to compare the ability of CFR, hMR and AChFR to predict increment in exercise time ≥60 seconds in response to anti-ischaemic therapy in all randomised patients.



CFR: coronary flow reserve; hMR: hyperaemic microvascular resistance; AChFR: acetylcholine flow reserve

5.4.2. Structural and functional coronary microvascular disease

Patients with functional CMD had a significant change in exercise time with both anti-ischaemic agents (amlodipine: change from baseline 94s, 95% CI 58 to 130s, p<0.001; ranolazine: change from baseline 100s, 95% CI 58 to 141s, p<0.001) with no *within* group differences between the agents (mean difference in change -3s, 95% CI -32 to 27s, p=0.859). Patients with structural CMD had a numerically greater increment in exercise time in response to amlodipine than ranolazine (mean difference in change 46s, 95% CI -2 to 93s, p=0.056)

(**Table 22 and Appendix Table 3**). The peak rate pressure product standardized to exercise time was similar in response to amlodipine and ranolazine in patients with structural and functional CMD, respectively (structural CMD: 77±31 vs 76±30 mmHg.bpm.s^{-1}, p=0.840; functional CMD: 78±49 vs 70±35 mmHg.bpm.s^{-1}, p=0.440). The resting rate pressure product was not altered, compared to baseline, by either therapy in both structural and functional CMD endotypes (structural CMD: p=0.833 for amlodipine and p=0.601 for ranolazine; functional CMD: p=0.439 for amlodipine and p=0.610 for ranolazine).

Table 22. Primary and secondary outcomes (*within group* differences in patients with structural and functional CMD).

	Structural CMD		**Functional CMD**	
	AML	**RNL**	**AML**	**RNL**
Exercise time (seconds)				
Mean increment	84 (95% CI 29 to 138)	41 (95% CI 7 to 75)	94 (95% CI 58 to 130)	100 (95% CI 58 to 141)
Difference in delta within groups	46 (95% CI -2 to 93)		- 3 (95% CI -32 to 27)	
P value	0.056		0.859	
SAQ summary score				
Mean increment	4 (95% CI -4 to 13)	14 (95% CI 5 to 22)	9 (95% CI 4 to 14)	12 (95% CI 6 to 18)
Difference in delta within groups	-10 (95% CI -21 to 1)		-3 (95% CI -11 to 5)	
P value	0.081		0.435	

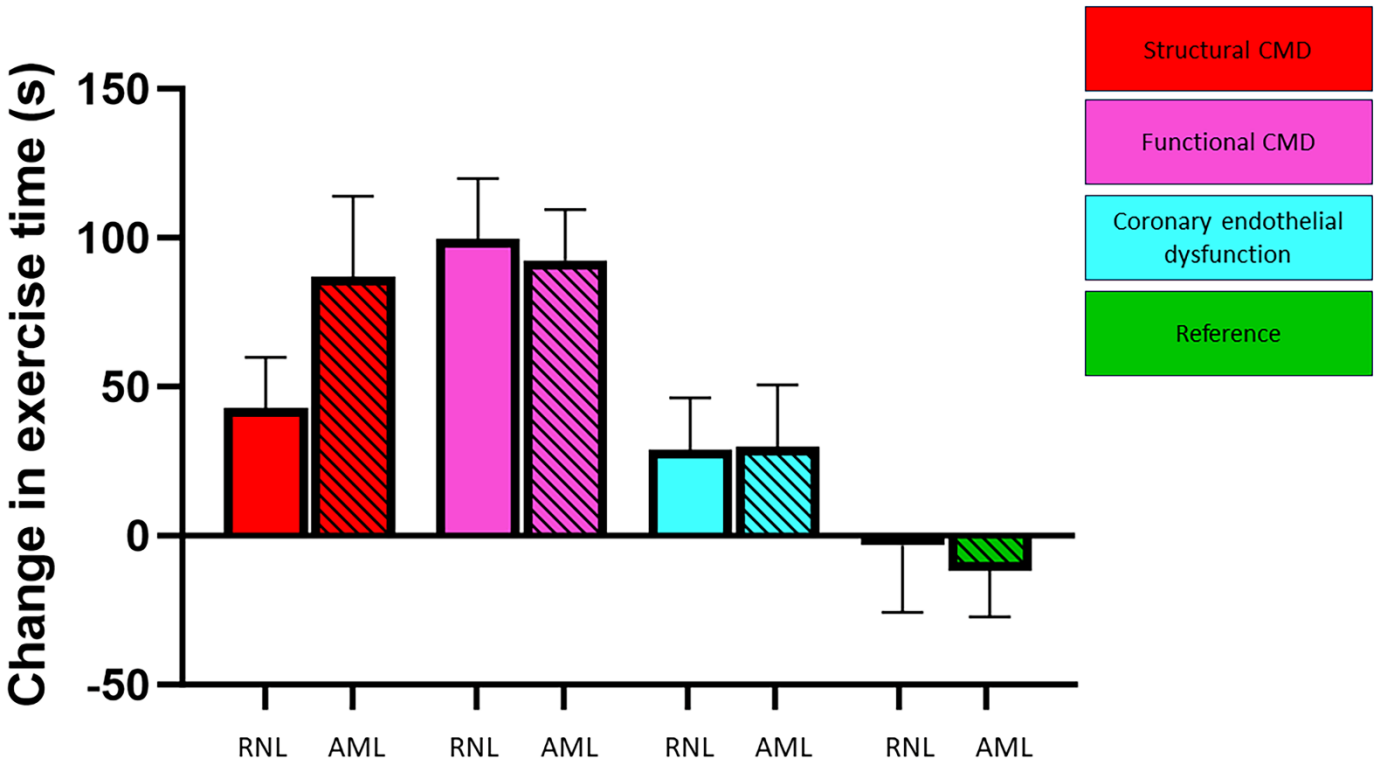
AML: amlodipine; RNL: ranolazine

Patients with structural CMD had a greater increment in SAQ score in response to ranolazine than amlodipine (mean difference in delta 10 units, p=0.081). Patients with functional CMD had a clinically meaningful increment in SAQ score in response to both anti-ischemic therapies (**Table 22**).

5.4.3. Coronary endothelial dysfunction and reference groups

Changes in exercise time and SAQ scores following treatment with either anti-ischaemic agent were modest in patients with a normal CFR (those with CED and the reference group) compared to those with CMD (structural or functional CMD) (**Figures 32 and 33**).

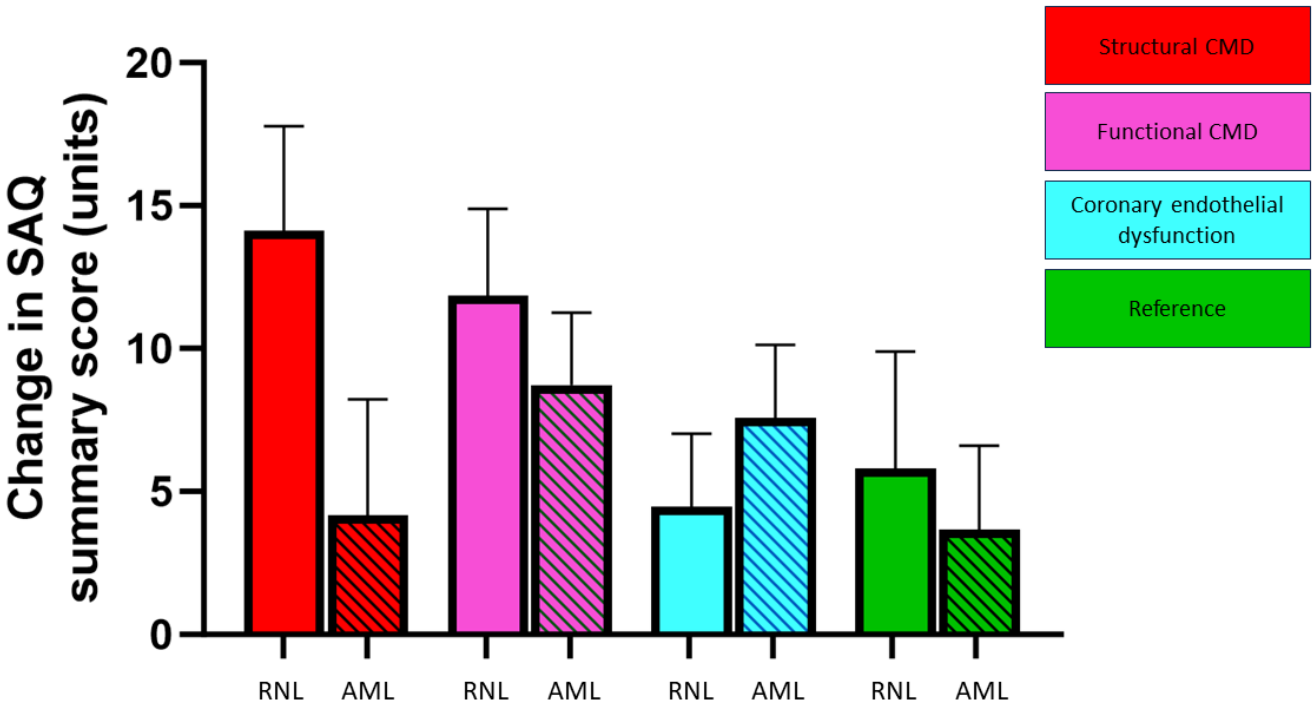
Figure 32. Comparison of change in exercise time in response to anti-ischaemic therapies across the different endotypes.



CMD: coronary microvascular disease; RNL: ranolazine; AML: amlodipine

Data are presented as mean±SEM.

Figure 33. Comparison of change in SAQ summary score in response to anti-ischaemic therapies across the different endotypes.



CMD: coronary microvascular disease; RNL: ranolazine; AML: amlodipine
Data are presented as mean±SEM.

On stratifying the former, patients with isolated coronary endothelial dysfunction had a greater increment in exercise time compared with the reference group with both amlodipine (mean difference in delta 42 s, 95% CI -13 to 96, p=0.129) and ranolazine (mean difference in delta 32 s, 95% CI -30 to 94 s, p=0.293), although these did not reach statistical significance (**Table 23**). Patients with isolated coronary endothelial dysfunction had a statistically significant increment in their SAQ summary score in response to amlodipine (change from baseline 7 units, 95% CI 2 to 13, p=0.011), whereas no change was reported in the reference group to either amlodipine or ranolazine (**Table 23**).

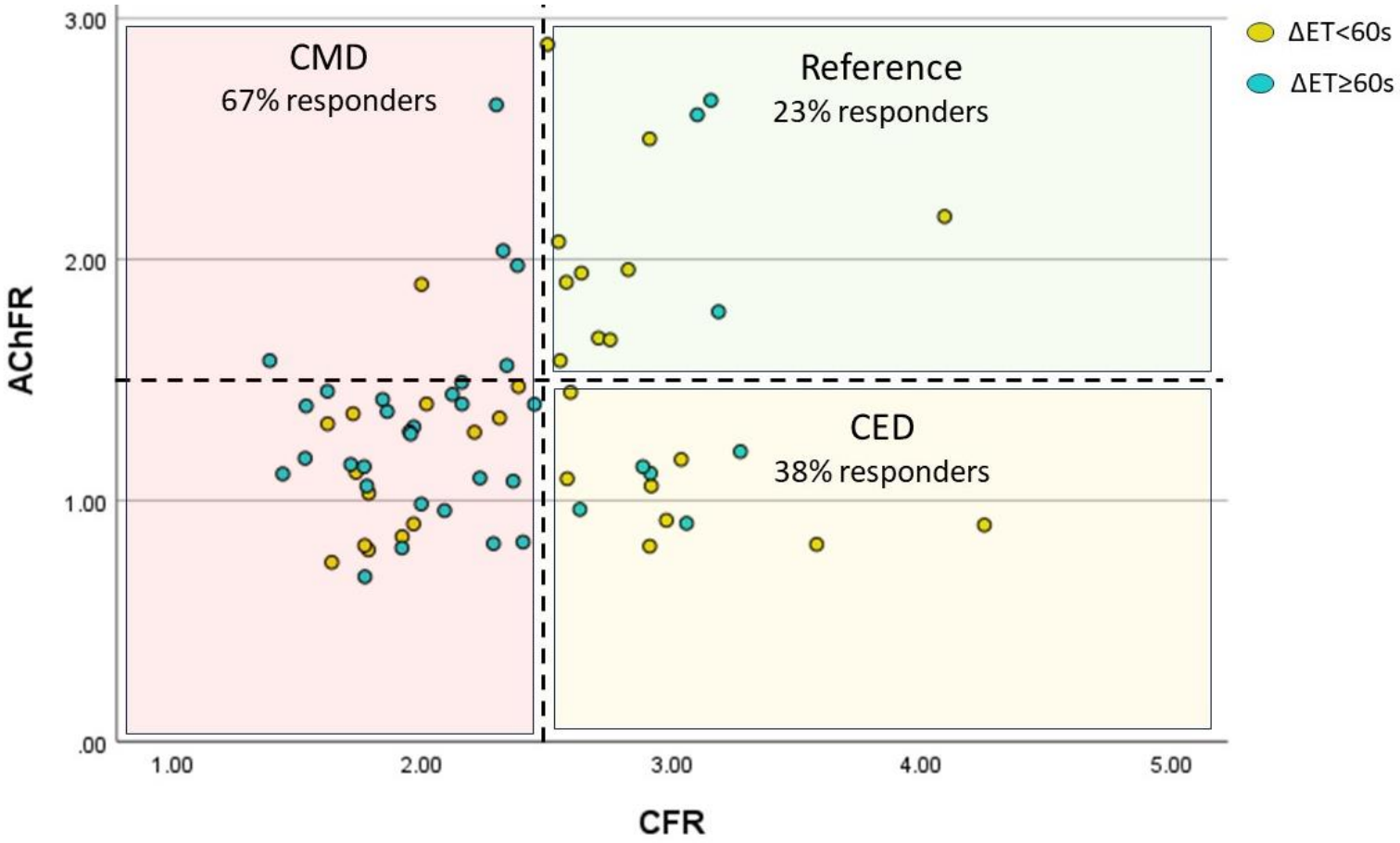
Table 23. Between group differences in exercise time and SAQ summary score in response to anti-ischaemic therapy in patients with isolated coronary endothelial dysfunction and patients in the reference group.

	Amlodipine		**Ranolazine**	
	CED	**Reference**	**CED**	**Reference**
Exercise time (seconds)				
Mean increment	30 (95% CI -15 to 74)	-12 (95% CI -46 to 22)	29 (95% CI -10 to 68)	-3 (95% CI -52 to 46)
Difference in delta between groups	42 (95% CI -13 to 96)		32 (95% CI -30 to 94)	
P value	0.129		0.293	
SAQ summary score				
Change from baseline	7 (95% CI 2 to 13)	4 (95% CI -3 to 10)	4 (95% CI -1 to 10)	6 (95% CI -3 to 15)
Difference in delta between groups	4 (95% CI -4 to 12)		-1 (95% CI -12 to 9)	
P value	0.323		0.799	

CED: isolated coronary endothelial dysfunction

67% of patients with an impaired CFR had ≥60 second increment in exercise time in response to anti-ischaemic therapy, versus 38% of patients with sole CED and 23% of patients in the reference group (**Figure 34**).

Figure 34. Comparison of coronary flow reserve, acetylcholine flow reserve and response to anti-ischaemic therapy.



CMD: coronary microvascular disease; CED: sole coronary endothelial dysfunction; ET: exercise time

5.5. Discussion

The main findings from this substudy of ChaMP-CMD are: i) CFR was independently associated with an increment in exercise time ≥60 seconds in all randomised patients, ii) functional CMD was the most prevalent endotype of CMD and these patients exhibited an increment in exercise time in response to both anti-ischaemic therapies, iii) measurement of hyperaemic (minimal) microvascular resistance and acetylcholine flow reserve, in addition to CFR, may offer incremental value in predicting response to anti-ischaemic therapy.

5.5.1. Pathophysiology of coronary microvascular disease: a changing paradigm

Traditionally, coronary microvascular disease has been attributed to a combination of microvascular architectural changes (such as microvascular obstruction and rarefaction), endothelial dysfunction, and/or vascular smooth muscle (VSM) dysfunction. However, recent animal models and clinical physiology evaluations suggest that coronary microvascular disease may be a heterogeneous condition comprising distinct entities that form part of a disease spectrum. Based on physiology assessment in the catheter laboratory, we have described the presence of two distinct CMD endotypes, termed 'structural CMD' and 'functional CMD'[24,25]. Both endotypes display impaired augmentation of CBF in response to intravenous adenosine (CFR<2.5). However, whilst patients with structural CMD have an elevated minimal microvascular resistance (MR) (which translates to reduced maximal CBF), those with functional CMD have a normal minimal MR but an attenuated vasodilatory reserve as they have reduced tone, i.e., increased flow, at rest[24,25]. The endotypes have a similar core phenotype, with both groups demonstrating high prevalence of inducible ischaemia and inefficient cardiac–coronary coupling during physical exercise, but their pathogenesis differs at the microvascular level[24,25]. Increased resting flow, in patients with functional CMD, appears to be mediated by heightened resting nitric oxide synthase activity but at present it is not clear whether this is an appropriate response to increased resting myocardial oxygen demand or whether this is a manifestation of disordered autoregulation[24]. The increased oxygen demand hypothesis is supported by the state of higher total resting wave energy in patients with functional CMD, as demonstrated by wave intensity analysis, suggesting a higher potential energy at rest in these patients[24]. Additionally, the supply:demand ratio, determined as the ratio of average peak velocity and rate-pressure product (a measure of myocardial work), is highest in patients with functional CMD, compared to patients with structural CMD and those with

normal coronary microvascular function[24]. A porcine model of CMD has provided further evidence supporting the increased oxygen consumption hypothesis. In this model, swine with CMD demonstrated an attenuated cardiac output at rest and during exercise[131]. This was accompanied by impaired systemic vasodilatation and increased circulating levels of lactate, suggestive of myocardial ischaemia. Oxygen consumption was elevated, suggestive of increased myocardial oxygen demand, at similar levels of cardiac work between swine with CMD and healthy swine. This increased oxygen demand was thought to be either due to a myocardial substrate shift towards fatty acid oxidation, leading to a reduced phosphate:oxygen ratio and an increased oxygen consumption for adenosine triphosphate production, or it was due to mitochondrial uncoupling leading to a reduction in phosphate:oxygen ratio, thereby increasing oxygen consumption at any given level of cardiac work[131]. Alternatively, disordered regulation of the neuronal nitric oxide synthase pathway may underlie an abnormality of autoregulation, which has been implicated in the regulation of resting CBF in both health and disease states[6,26]. On the other hand, patients with structural CMD have normal resting CBF but an impaired ability to augment flow in response to physiological stress, leading to ischaemia. In addition, patients in the structural CMD group demonstrate an impaired peripheral endothelium-dependent vasodilatation and exaggerated exercise-induced hypertension, leading to increased afterload and myocardial oxygen demand, exacerbating the supply deficit and predisposing to ischaemia. At present it remains unclear whether patients with structural CMD have an impaired ability to augment their CBF as a result of irreversible architectural changes, such as microvascular hypertrophy or fibrosis, limiting the microvasculature's ability to vasodilate, or whether it is due to a reversible dysregulation of the endothelial NOS pathway, which has been shown to regulate CBF in response to exertion[132].

Other groups have also demonstrated a high prevalence of normal minimal microvascular resistance in patients with CMD, which is characterised by a high resting flow[133]. Patients with functional CMD also have a similarly adverse prognosis compared to patients with structural CMD[51]. Our finding, that functional CMD is the most prevalent endotype of CMD and these patients derive as much benefit from anti-ischaemic therapy as those with structural CMD, provides further evidence for the veracity of this endotype. Further mechanistic studies are warranted to fully elucidate the underlying pathophysiology, which may lead to the development of novel therapeutic targets in these patients. Those with structural CMD demonstrated a numerically greater increment in exercise time with amlodipine. This is suggestive that dichotomising patients into functional and structural CMD *may* allow further stratification of therapy in patients with ANOCA. These are hypothesis-generating findings and need to be tested in future trials. It is important to remember that our power calculations were designed to identify ≥60 seconds increment in exercise time in response to therapy and we may not identify smaller, but clinically relevant, increments in exercise time. It is interesting that patients with structural CMD demonstrated a numerically greater increment in exercise time with amlodipine but a numerically greater increment in SAQ summary score with ranolazine. This discordance between exercise time and SAQ summary score has been addressed in detail in **Chapter 4.**

5.5.2. Rationale for using ranolazine and amlodipine as exemplar drugs

We chose to study ranolazine and amlodipine as the exemplar drugs in our study, as these were expected to address the pathobiological processes that are believed to underlie each CMD endotype. The hallmark of functional CMD is submaximal vasodilatation at rest, which could be due to heightened myocardial oxygen demand at rest. Ranolazine has been shown to

modulate myocyte metabolism (by switching fatty acid oxidation to glucose oxidation through an influence on pyruvate dehydrogenase activity[134]); therefore, leading to a more "oxygen-efficient" production of adenosine triphosphate (ATP). When fatty acid levels are elevated, the end products of beta-oxidation reduce the activity of pyruvate dehydrogenase, the enzyme that mediates the conversion of pyruvate to acetyl coenzyme A and permits its entry into the Krebs cycle. As a result, oxygen-wasting fatty acid oxidation predominates, pyruvate oxidation is inhibited, and lactate accumulates with deleterious consequences. Inhibiting fatty acid oxidation with ranolazine is purported to relieve the inhibition of pyruvate dehydrogenase, promoting oxidation of glucose and lactate, which phosphorylates a given amount of ATP using less oxygen than fatty acid oxidation. In addition, the coupling of pyruvate formation via glycolysis to pyruvate oxidation in the Krebs cycle is improved so that lactate accumulation is diminished, which could decrease myocardial oxygen demand at rest. For these reasons, we hypothesised that ranolazine would be an effective treatment strategy in patients with functional CMD. Along with the metabolic actions of ranolazine, it also selectively inhibits reactive oxygen species induced late sarcolemmal Na^+ channel current and reduces intracellular Ca^{2+} in cardiomyocytes, leading to improved myocardial relaxation, which should augment lusitropy and, consequently, myocardial perfusion[135]. On the other hand, structural CMD is characterised by failure of vasodilatation during exercise in both the myocardial and systemic vasculature[24], which makes amlodipine an attractive therapy. Amlodipine is a dihydropyridine calcium channel blocker and inhibits the transmembrane influx of Ca^{2+} into vascular smooth muscle cells. The precise mechanism by which amlodipine relieves angina has not been fully elucidated but a few mechanisms have been purported. Firstly, Amlodipine dilates peripheral arterioles and, therefore, reduces the systemic vascular resistance, i.e., left ventricular (LV) afterload. The attenuated LV afterload leads to reduced myocardial oxygen demand and re-establishes a synergistic relationship between the coronary and peripheral

circulation during exercise. Secondly, amlodipine possibly dilates coronary arteries and arterioles. The combination of these two effects would lead to a reduction in myocardial oxygen demand and improvement in coronary blood flow; therefore ameliorating myocardial ischaemia.

The exact mechanisms by which both ranolazine and amlodipine led to a significant increment in exercise time in patients with functional CMD remains unknown. Serial positron emission tomography scans before and after ranolazine may allude to the change in resting myocardial oxygen consumption in patients with functional CMD with/without ranolazine; whereas serial echocardiograms with LV diastolic measurements and coronary physiology with wave intensity analysis assessment may establish if ranolazine leads to enhanced lusitropy and, consequently, augments the accelerating backward expansion wave in patients with CMD. The mechanisms leading to the preferential beneficial effects of amlodipine in patients with structural CMD is not clear. There was no difference in the standardised rate pressure product (rate pressure product ÷ exercise time) in response to amlodipine or ranolazine in these patients; this is suggestive that reduction in myocardial work may not be the driving mechanism behind this beneficial effect of amlodipine in patients with structural CMD. Future studies can look at the effects of peripheral vasodilators on the Buckberg index, which is a diastolic to systolic pressure-time integral ratio and provides a surrogate measure of myocardial oxygen supply and demand. Serial coronary physiology assessment with wave intensity analysis may provide further insight into whether amlodipine leads to a greater positive change in perfusion efficiency and coronary flow reserve in patients with structural CMD.

5.5.3. The incremental value of acetylcholine flow reserve in predicting response to therapy

A functioning endothelial layer leads to the production of nitric oxide in response to shear stress that occurs during physical exertion, which, via a cascade of pathways, leads to vasodilatation (**Figure 3**). In practice, coronary endothelial function is assessed using intracoronary infusion of acetylcholine[68]. In healthy endothelium, acetylcholine infusion, at concentrations of up to 10^{-4} mol/L, should lead to vasodilatation and enhanced perfusion[75]. However, in pathological endothelium, acetylcholine infusion may not lead to appropriate augmentation in coronary blood flow, and a dichotomous threshold of <1.5 (i.e., <50% increase in coronary blood flow in response to acetylcholine infusion) has been established to diagnose coronary endothelial dysfunction[32]. Patients with endothelial dysfunction have evidence of myocardial ischaemia[50], coronary perfusion inefficiency during exercise[50] and adverse cardiovascular outcomes[34,35]. However, there is a paucity in data appraising the efficacy of anti-ischaemic therapy in patients with coronary endothelial dysfunction. Lerman et al performed a double-blinded study in 26 patients with ANOCA using L-Arginine and placebo[136]. They reported that 6 months of L-Arginine, compared to placebo, resulted in an improvement in AchFR and was also associated with an improvement in patient-specific quality of life score[136]. Reriani et al investigated the effects of an endothelin-receptor antagonist (Atrasentan) and placebo in patients with ANOCA. Atrasentan was associated with a greater improvement in AchFR than placebo at 6 months[137]; however, there were no patient-centric outcomes in this study. Within the limitations of a small sample size, we report a numerical increment in exercise time in response to both amlodipine and ranolazine in patients with sole coronary endothelial dysfunction, whereas no such increment was seen in the reference group. Furthermore, patients with sole coronary endothelial dysfunction reported a significant increment in their SAQ summary score in response to amlodipine; again, no such effect was observed in the reference group. These findings indicate that assessing endothelium-*dependent* microvascular function, in patients

with a normal endothelium-*independent* microvascular function, adds incremental value in predicting response to anti-ischaemic therapy in patients with limiting angina. This is complementary to our findings from Chapter 3, as well as our previous work that demonstrates the incremental value of measuring AChFR for the detection of an ischaemic substrate[50]. Further larger studies utilising therapies that target the NO-cGMP-PKG pathway, such as L-arginine, phosphodiesterase inhibitors and nitrites, are warranted in this patient cohort.

5.5.4. Study limitations

Our study has some limitations that should be considered when interpreting the findings. Dichotomising patients with CMD into structural and functional CMD with a hMR threshold of 2.5mmHg.cm^{-1}.s^{1} was based on historical natural history studies; the therapeutic threshold may be slightly disparate in reality. Furthermore, whilst we have used a binary threshold to split CMD into functional and structural endotypes, in reality microvascular resistance is a continuous variable and the higher or lower this value the closer the underlying pathophysiology will be to true structural and functional CMD, respectively. Finally, we did not interrogate non-LAD vessels and, therefore, it is possible that patients with a normal CFR and AChFR in the LAD (i.e., the reference group) may have had impaired CFR/AChFR in the non-LAD vessels. However, interrogating the LAD vessel is the norm in both clinical practice and research studies when assessing for coronary microvascular function; furthermore, the thresholds for impaired CFR/AChFR in non-LAD vessels have not been established.

5.6. Conclusions

In summary, we have demonstrated that functional CMD is the most common endotype of CMD and these patients derive clinically meaningful benefit from both amlodipine and ranolazine, whilst patients with structural CMD demonstrated a greater increment in exercise

time with amlodipine. Furthermore, patients with sole coronary endothelial dysfunction demonstrated a numerical increment in exercise time in response to anti-ischaemic therapy, whereas no such effect was seen in the reference group. These findings suggest that measuring minimal microvascular resistance and acetylcholine flow reserve, in addition to CFR, may provide incremental value in predicting response to anti-ischaemic therapy in patients with ANOCA.

Chapter 6

Characterising mechanisms of ischaemia in patients with myocardial bridges

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6.1. Abstract

Background: Myocardial bridges (MB) have been associated with ischaemic syndromes. Abnormal cardiac coronary coupling is implicated but has not been systematically evaluated. We sought to determine the substrates for ischaemia in patients with angina, nonobstructive coronary arteries (ANOCA) and a MB in the left anterior descending artery.

Methods: Patients with ANOCA underwent acquisition of intracoronary pressure and flow during rest, supine bicycle exercise and adenosine infusion. Coronary wave intensity analysis was performed, and perfusion efficiency defined as accelerating wave energy/total wave energy (%). Epicardial endothelial dysfunction was defined as a reduction in epicardial vessel diameter ≥20% and microvascular endothelial dysfunction as an inability to augment coronary blood flow by >50% in response to intracoronary acetylcholine infusion. Patients with ANOCA and MB were compared to those with microvascular angina (CMD: coronary flow reserve, CFR, <2.5) or ANOCA with normal CFR (reference: CFR≥2.5).

Results: Ninety-two patients were enrolled (30 MB, 33 CMD and 29 reference). FFR in these 3 groups was 0.86±0.05, 0.92±0.04 and 0.94±0.05; CFR was 2.5±0.5, 2.0±0.3 and 3.2±0.6. Perfusion efficiency improved numerically during exercise in the reference group (65±9% to 69±13%, p=0.063), but decreased in patients with CMD (68±10% to 50±10%, p<0.001) and MB (66±9% to 55±9%, p<0.001). However, the reduction in perfusion efficiency had distinct causes: in CMD, this was driven predominantly by microcirculation derived energy in early diastole, whereas in MB, this was driven by diminished accelerating energy arising from the upstream epicardial vessel in early systole. 54% of patients with MB versus 29% reference and 38% CMD had epicardial endothelial dysfunction.

Conclusions: MBs result in impaired coronary perfusion efficiency during exercise, which is due to diminished accelerating wave energy in early systole compared to the reference group. Additionally, there is a high prevalence of endothelial dysfunction; both mechanisms may cause ischaemia and represent distinct treatment targets.

6.2. Introduction

Angina with nonobstructive coronary arteries (ANOCA) is a common clinical problem and comprises several distinct pathophysiological entities, including coronary microvascular disease (CMD), coronary artery spasm and myocardial bridging (MB). Myocardial bridging due to intramyocardial passage of varying lengths of an epicardial artery is a common anatomical variant, found in up to 30% of patients on computed tomography coronary angiography (CTCA) imaging[138], predominantly in the left anterior descending (LAD) artery at the mid vessel. The intramyocardial segment of the vessel is known as the tunnelled segment. Historically, MBs have been considered a benign entity as myocardial perfusion predominantly occurs during diastole and MBs are thought to only alter vessel calibre during systole. However, growing evidence suggests that MBs are not always benign and have been associated with chronic intermittent angina as well as acute ischaemic presentations. Several mechanisms have been postulated, including delayed decompression of the tunnelled segment in diastole leading to luminal narrowing akin to obstructive coronary artery disease (CAD)[38], predisposition to coronary artery spasm[139], increased propensity to atherosclerotic CAD proximal to the bridged segment due to perturbed wall shear stress[139] and the venturi effect leading to reduced septal blood flow[140]. However, whilst a handful of studies have assessed the physiological response to adenosine and dobutamine stress, using pressure as a surrogate of flow, coronary flow during exercise has not been specifically and systematically evaluated in patients with MBs. Our study aimed to characterise the mechanisms that lead to myocardial ischaemia in patients with ANOCA and MB (**MB group**) during physical exercise using wave intensity analysis to describe patterns of cardiac-coronary coupling. We will compare these findings to those in patients with ANOCA but no MB, in turn classified as patients with coronary microvascular disease (**CMD group**) and normal coronary flow reserve (**reference group**), respectively. It is hoped that a better understanding of the mechanisms causing

ischaemia may allow development of stratified therapies for this underserved patient population.

Clinical assessment of myocardial bridges' ischaemic potential presently relies on measuring pressure-derived indices during intravenous dobutamine infusion[64–66,139]. The rationale for using dobutamine as a stressor, as opposed to adenosine, is that dobutamine is a positive chronotropic and inotropic agent that better mimics the conditions (i.e., physical exercise) that may predispose certain MBs to lead to ischaemia; adenosine, on the other hand leads to maximal vasodilatation, which may not be impaired in patients with MBs, and therefore may lead to under-recognition of an ischaemic substrate. During physical exercise, myocardial contractility is enhanced, which may lead to delayed decompression of the tunnelled segment, which is purported to be the predominant mechanism leading to ischaemia in these patients[38]. However, the intracoronary effects of dobutamine and physical exercise have never been compared. My second aim was to explore the differences in cardiac coronary coupling in response to adenosine, physical exercise and dobutamine, in patients with MBs, to identify if dobutamine is a suitable surrogate of physical exercise in the invasive assessment of patients. We compared coronary perfusion efficiency (accelerating energy/total energy flux) and changes in specific wave energies in response to these three stressors.

6.3. Methods

The classic angiographic finding of a MB is the systolic narrowing or "milking" of the vessel. This is associated with a step-down and step-up demarcating the affected coronary segment with either complete or partial decompression in diastole. A significant "milking effect" is present when there is a visual ≥70% reduction in the minimal luminal diameter during systole

and persistent ≥35% reduction in minimal luminal diameter during mid- to late-diastole[141]; however, these anatomical definitions do not take into account the coronary physiological changes that occur during systole and diastole in these patients. CTCA is useful for classifying the course of the artery as normal (within the epicardial fat), superficial intramyocardial, or deep intramyocardial[141].

6.3.1. Study population

We enrolled consecutive patients presenting with typical angina and myocardial bridge in the left anterior descending artery, identified on prior CTCA imaging or invasive coronary angiography between October 2021 – August 2023 (**MB group**). [140140]Additionally, we enrolled consecutive patients presenting with typical angina without a myocardial bridge between December 2013 – July 2018 (**CMD** and **reference groups**). Inclusion criteria were angina, preserved left ventricular ejection fraction (>50%) and nonobstructive coronary arteries (fractional flow reserve (FFR)>0.80). Exclusion criteria were chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/m^2), significant valvular disease, history of acute coronary syndrome, previous revascularisation, and cardiomyopathy. For the cardiac coronary coupling comparison between adenosine, exercise and dobutamine, only those patients with MBs who had undergone assessment with all three stressors were included for analysis. All patients provided written informed consent in accordance with the protocols approved by the UK National Research Ethics Service (20/LO/1294 and 17/LO/0203).

6.3.2 Intracoronary physiology assessment

Coronary haemodynamic measurements were recorded under resting conditions, during intravenous adenosine-mediated hyperaemia (140 mcg/kg/min) and continuously during bicycle exercise, using a specially adapted supine ergometer (Ergosana, Bitz, Germany)

attached to the cardiac catheter laboratory table. Exercise began at a workload of 30 W and increased every 2 minutes by 20 W. Where lower limb muscle fatigue restricted increasing workloads, resistance was fixed at the maximum tolerated level and exercise continued until exhaustion (**Figure 8 and Figure 14**). We assessed patients' coronary endothelial function using graded intracoronary infusions of acetylcholine (18 mcg/mL at 1 mL/min for 2 min followed by 2 mL/min for 2 min). In patients with MBs, we additionally infused intravenous dobutamine at 10mcg/kg/min, 20mcg/kg/min and 30mcg/kg/min for 2 minutes each. Patients were asked to withhold any negative chronotropic agents for 24 hours beforehand.

6.3.3. Analysis of Coronary Physiological Data

Coronary flow reserve (CFR) was derived as hyperaemic APV/basal APV and coronary microvascular disease (CMD) was defined as CFR<2.5[68]. Hyperaemic (minimal) microvascular resistance (hMR) was calculated as P_d/APV during hyperaemia. The steady state Pd/Pa ratio during peak exercise was termed exercise Pd/Pa and exercise flow reserve was calculated as the ratio of volumetric coronary blood flow (CBF) during peak exercise compared to resting CBF. A reduction in coronary luminal diameter by ≥20% in response to intracoronary acetylcholine infusion was defined as epicardial endothelial dysfunction[139]; 20% encompasses the recognised limits of precision for quantitative coronary angiography (QCA). Acetylcholine flow reserve (AChFR), a marker of microvascular endothelial function, was calculated as the ratio of volumetric CBF in response to acetylcholine infusion compared to resting CBF[50,142]. As exercise and acetylcholine infusion can lead to both vasodilatation and vasoconstriction of the epicardial arteries, volumetric CBF incorporating vessel diameter was calculated during the assessment of exercise and acetylcholine flow reserve, respectively. For exercise flow reserve, a cine image was acquired straight after supine bicycle exercise and this was used for QCA calculations pertaining to the peak exercise volumetric CBF.

Wave intensity was calculated as the product of the time-derivatives of distal coronary pressure (dPd/dt) and flow velocity (dU/dt), as dPd/dt x dU/dt, with wave separation performed as previously described[25]. For each patient, 5 dominant waves were identified and included in our analysis: (1) backward compression wave (BCW), causing flow deceleration due to compression of microvasculature during isovolumetric contraction; (2) forward compression wave, causing flow acceleration due to increased aortic pressure in early systole; (3) forward expansion wave, causing flow deceleration associated with the fall in aortic pressure in late systole; (4) backward expansion wave (BEW), causing flow acceleration due to decompression of the microvasculature in early diastole; (5) late forward compression wave, causing flow acceleration due to augmentation of the aortic pressure during aortic valve closure in diastole. Perfusion efficiency was calculated as the percentage of accelerating wave energies in relation to the total wave energies (%), using areas under the respective curves. The late forward compression wave appears to be an important wave energy in patients with MB and, therefore, was incorporated in the calculation of perfusion efficiency in all three groups (MB, CMD and reference) in this study.

6.3.4. Computed tomography coronary angiography imaging

All CTCA scans were acquired by retrospective ECG-gated spiral scan mode using single- and dual-source CT systems. Patients had 400 mcg sublingual nitroglycerin and metoprolol administered with a heart rate goal <60 beats/min before image acquisition. Reconstructed CTCA images were re-evaluated on an external workstation by 2 experienced radiologists. Diastolic datasets were reviewed. Multi- and curved-planar reformations were used for the assessment of the MB in 2 planes: 1 parallel and 1 perpendicular to the vessel's course.

MBs were graded according to the criteria proposed by Kim et al[143]: LAD within the interventricular groove and in myocardium contact (1=partial coverage), full encasement of LAD but without visibly overlying myocardium (2=unroofed) and full encasement of LAD with visibly overlying myocardium (3=full coverage). The MB location was determined by measuring the distance from the LAD ostium to the bridge's entrance. The MB length was measured in millimeters (mm) along the vessel axis from the disappearance of the epicardial fat plane proximally to its re-emergence distally. We calculated the MB muscle index (MMI) as MB length (mm) × MB coverage grade[139].

6.3.5. Statistical analyses

Sample size was estimated for the primary outcome measure, exercise perfusion efficiency. Assuming a distribution of 1:1, 21 patients in the MB group and 21 patients in the reference group provide 80% power (α=0.05) to detect a minimum difference in change in exercise perfusion efficiency (exercise – resting perfusion efficiency) of 8% (predicted SD, 10%) between the MB and reference groups. Normality of data was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous data are presented as mean$\pm$SD, unless specified otherwise, and compared using the independent sample's Student *t*-test. Non-normally distributed data are presented as median (interquartile range) and compared using the Mann-Whitney test (unpaired analyses) or the Wilcoxon matched-pairs signed rank test (paired analyses). Categorical variables are presented as n (%) and compared using the chi-squared test. Continuous endpoints were compared with the two-sample t test of the difference between groups; the findings are reported as the difference in mean change between study groups with 95% confidence intervals (CIs) and p values. Binary logistic regression was performed using univariate and multivariate analysis and reported as standardised coefficients (95% CI). Biologically plausible variables were assessed in the univariate model, and those that correlated

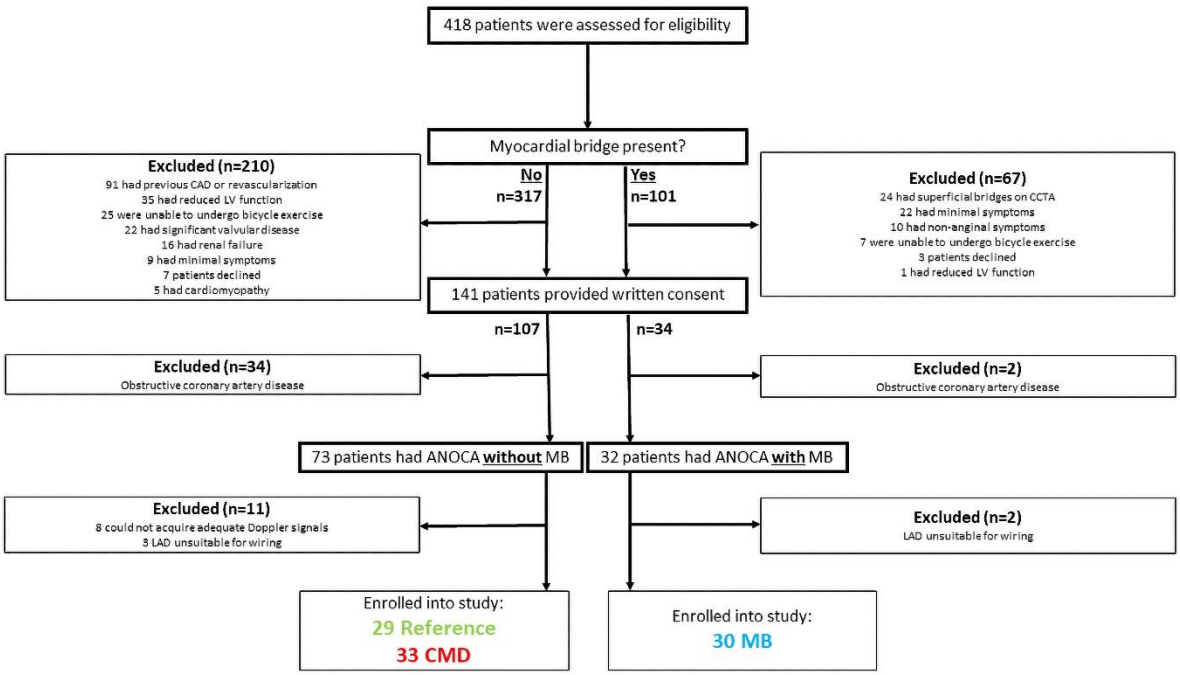
were included in the multivariate model. Receiver operating characteristic curves were used to identify the optimal MMI threshold that predicted ≥20% reduction in vessel calibre in response to acetylcholine infusion (epicardial endothelial dysfunction). All randomised patients were included in these analyses. All analyses were performed using SPSS Statistics 27 (IBM, NY, USA) and GraphPad Prism software version 9.0 (GraphPad software, San Diego, CA, USA). We deemed a p value less than 0.05 to be significant.

6.4. Results

6.4.1. Baseline characteristics

Of 418 patients screened, 141 were found to meet clinical eligibility criteria. Once these patients underwent invasive physiological assessment, 105 were found to have ANOCA, of which 73 did not have a MB and 32 did have a MB. After excluding patients on account of poor Doppler signals or inability to pass the Combowire into the distal LAD artery, 92 patients were recruited into the study: 30 in the MB group, 33 in the CMD group, and 29 in the reference group (**Figure 35**). The groups were well matched for age and cardiovascular risk factors. There were more women in the CMD and control groups, compared with the MB group (**Table 24**). Twelve patients in the MB group underwent a CCTA scan before coronary angiography; in this cohort, the tunnelled segment length, depth, and MMI were 29±9mm, 2±1mm, and 79±30, respectively.

Figure 35. Study screening and recruitment numbers for the in-lab mechanistic study.



CAD: coronary artery disease; LV: left ventricular; CTCA: computerised tomography coronary angiography; MB: myocardial bridge; CMD: coronary microvascular disease

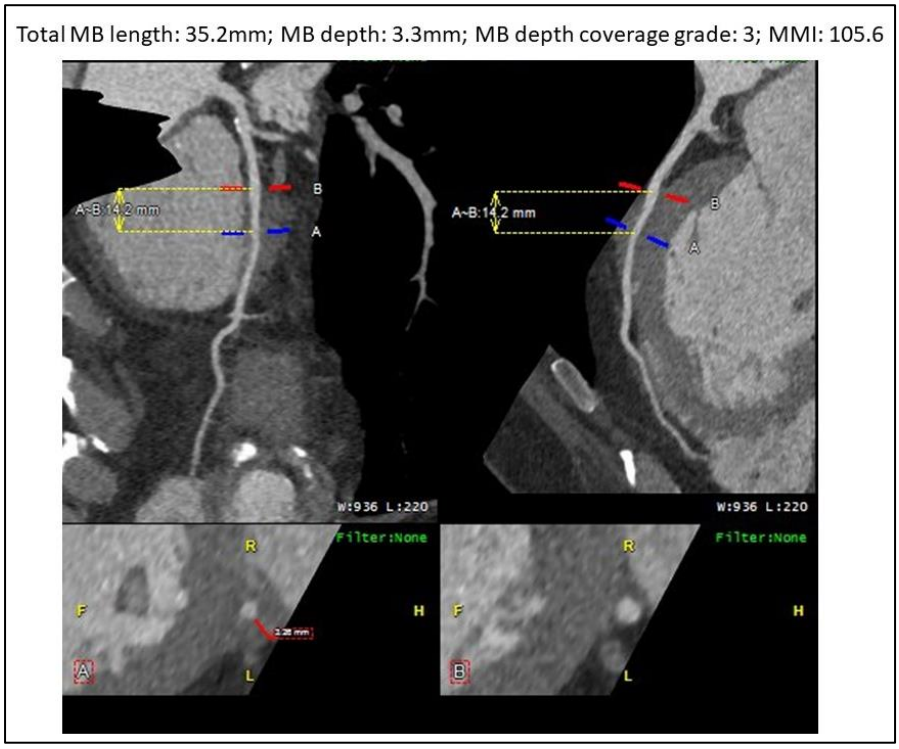
Table 24. Patient demographics for the in-lab mechanistic study.

	MB (n=30)	**CMD (n=33)**	**Reference (n=29)**	**P value**
Age, years	58±10	57±11	56±8	0.916
Female, n(%)	11 (37)	30 (88)	19 (68)	<0.001
Hypertension, n(%)	14 (47)	19 (56)	17 (61)	0.494
Diabetes mellitus, n(%)	8 (27)	9 (27)	5 (18)	0.595
Hyperlipidaemia, n(%)	18 (60)	14 (41)	15 (54)	0.377
Smoker, n(%)	7 (23)	7 (21)	11 (39)	0.285

Twelve patients in the MB group underwent a CTCA scan before coronary angiography; in this cohort, the tunnelled segment length, depth, and MMI were 29±9mm, 2±1mm, and 79±30,

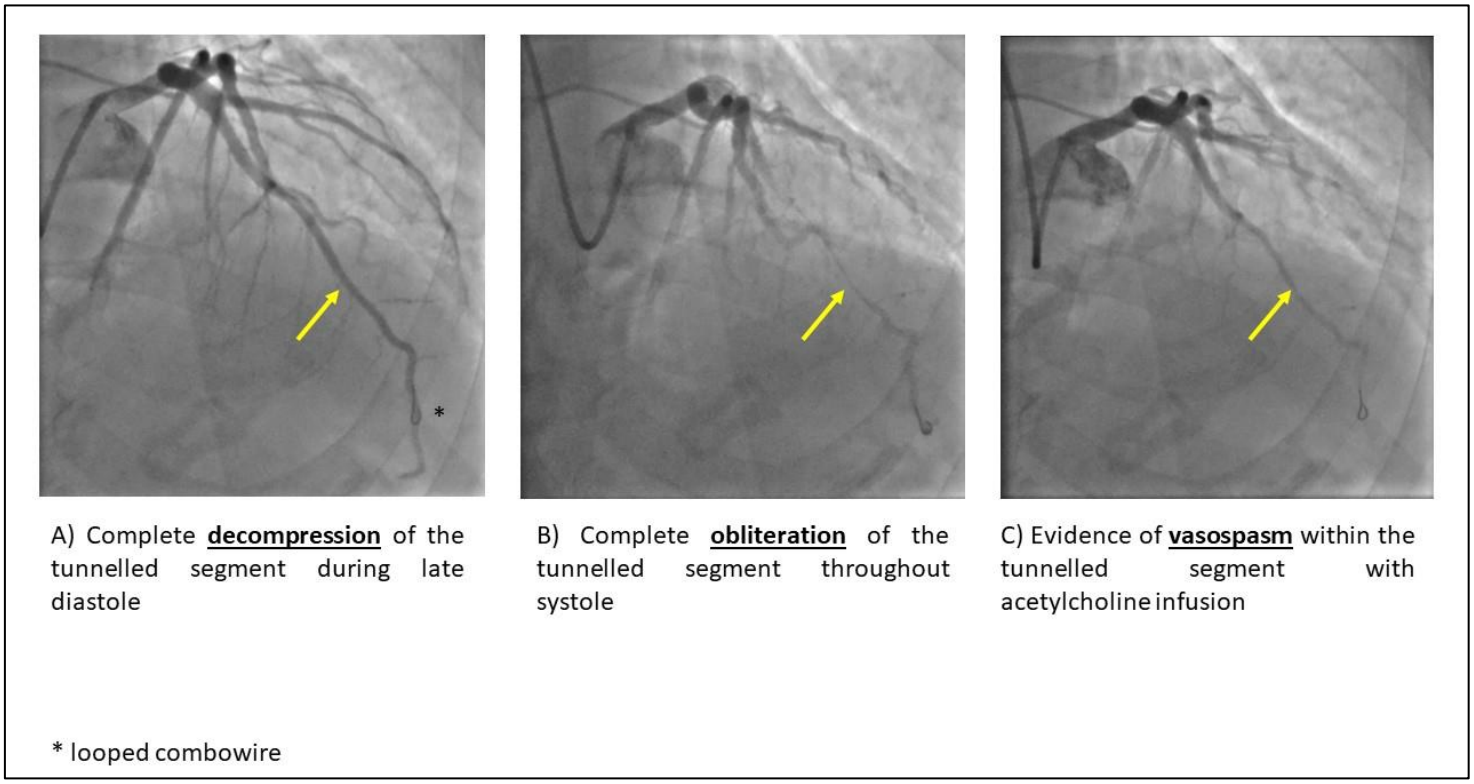
respectively. An example of a myocardial bridge on CTCA and coronary angiography is shown in **Figure 36** and **Figure 37**, respectively.

Figure 36. Computed tomography coronary angiography derived measurements in a patient with MB.



MMI: myocardial bridge muscle index

Figure 37. Example of a myocardial bridge during coronary angiography. A) Complete decompression of the tunnelled segment during mid-late diastole; B) Complete obliteration of the tunnelled segment throughout systole; C) Evidence of vasospasm within the tunnelled segment (throughout cardiac cycle) with acetylcholine infusion.



6.4.2. Whole Cardiac Cycle Physiology Measurements

Epicardial physiology measurements confirmed the absence of obstructive coronary disease in all patients (as per eligibility criteria), but the MB group had lower Pd/Pa and fractional flow reserve values than the reference group. CFR in the MB group was 2.5±0.5, which was lower than the reference group (3.2±0.6) but higher than the CMD group (2.0±0.3; P<0.001); 14 patients (47%) in the MB group had a CFR <2.5. Minimal microvascular resistance was similar between the MB and reference groups. Patients with a MB had the lowest exercise Pd/Pa (0.89

[0.87–0.93]; *P*<0.001 versus both CMD and reference groups). The MB and CMD groups had similar exercise flow reserve (1.5 [1.2–1.9] versus 1.4 [1.2–1.6]; *P*=0.150), both were lower than the reference group (**Table 25**).

Table 25. Coronary physiology measurements in patients recruited to the in-lab mechanistic study.

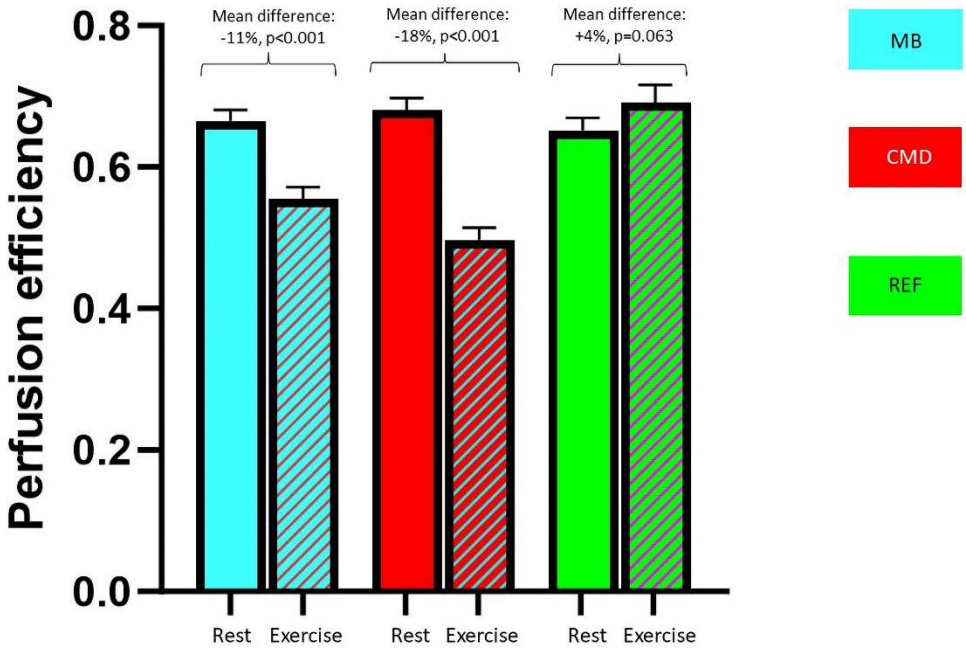
	MB (n=30)	**CMD (n=33)**	**Reference (n=29)**	**P value**		
				MB vs CMD	**MB vs Reference**	**CMD vs Reference**
Resting and hyperaemic physiology measurements						
Pd/Pa	0.93±0.05	0.95±0.03	0.96±0.03	0.041	0.017	0.377
FFR	0.86±0.05	0.92±0.04	0.94±0.05	<0.001	<0.001	0.241
CFR	2.5±0.5	2.0±0.3	3.2±0.6	<0.001	<0.001	<0.001
hMR, $mmHg.cm^{-1}.s^{-1}$	1.9±0.5	2.5±0.8	2.1±0.6	0.002	0.294	0.023
Exercise physiology measurements						
Pd/Pa	0.89 (0.87, 0.93)	0.95 (0.92, 0.97)	0.95 (0.92, 0.98)	<0.001	<0.001	0.575
Flow reserve	1.5 (1.2, 1.9)	1.4 (1.2, 1.6)	1.8 (1.5, 2.0)	0.150	0.046	<0.001
Peak RPP, mmHg.bpm	17849±5785	20928±5111	17356±4046	0.029	0.710	0.004

FFR: Fractional Flow Reserve; CFR: Coronary Flow Reserve; hMR: hyperaemic microvascular resistance; RPP: rate pressure product

6.4.3. Phasic Cycle Physiology Measurements

By wave intensity analysis, the resting perfusion efficiencies were similar in the MB, CMD, and reference groups (66±9%, 68±10%, and 65±9%; *P*=0.513). In the reference group, perfusion efficiency was numerically enhanced during exercise (from 65±9% to 69±13%; *P*=0.063). In contrast, perfusion efficiency was attenuated during exercise in the MB group (from 66±9% to 55±9%; *P*<0.001) and in the CMD group (from 68±10% to 50±10%; *P*<0.001; **Figure 38**). The difference in change in perfusion efficiency between the MB and reference groups (i.e., delta perfusion efficiency in the reference group and delta perfusion efficiency in the MB group) was 15% (95% CI, 9%–22%; *P*<0.001).

Figure 38. Coronary perfusion efficiency during exercise in patients with MB, CMD and reference group.

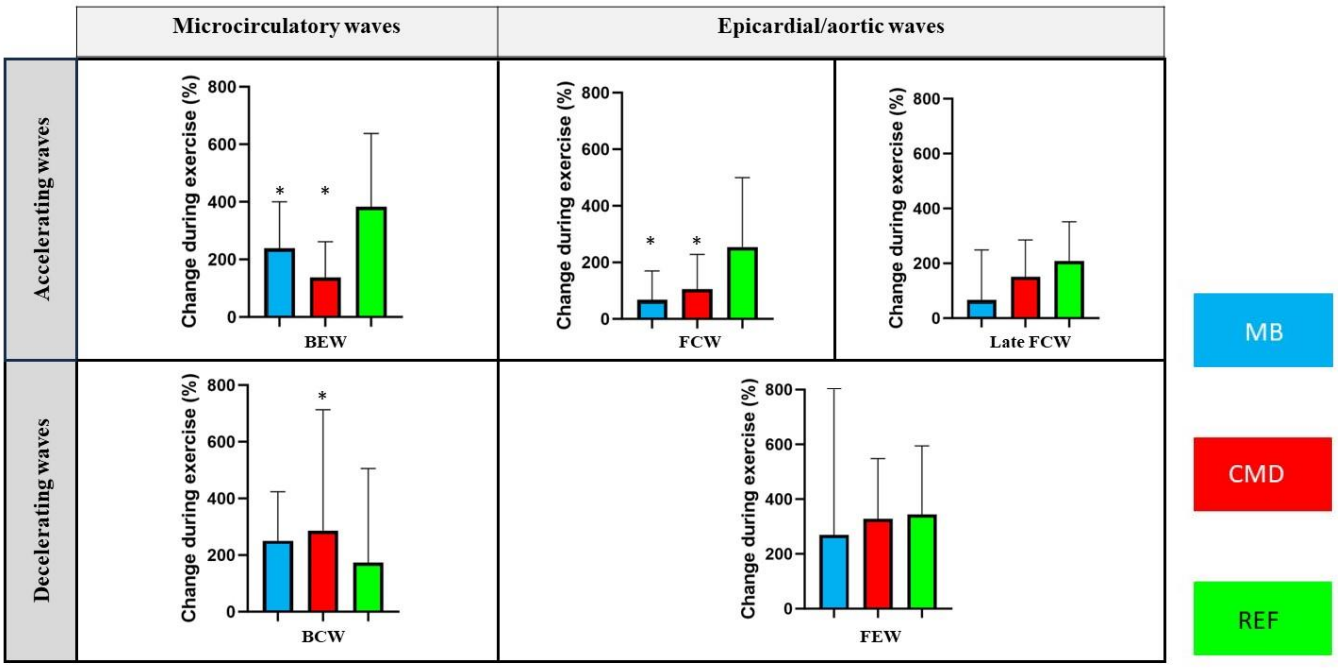


MB: myocardial bridge; CMD: coronary microvascular disease

Data are presented as mean±SEM.

The reduced perfusion efficiency during exercise in the MB group was predominantly driven by the diminution of proximally originating accelerating wave energy during early systole (increase in forward compression wave during exercise was 68% in MB versus 254% in the reference group, *P*<0.001; **Figure 39**). On the other hand, the reduced perfusion efficiency during exercise in the CMD group was driven by perturbations in the microcirculation-derived wave energies: diminished accelerating wave energy during early diastole (increase in backward expansion wave during exercise was 137% in CMD versus 383% in the reference group, *P*<0.001; **Figure 39**).

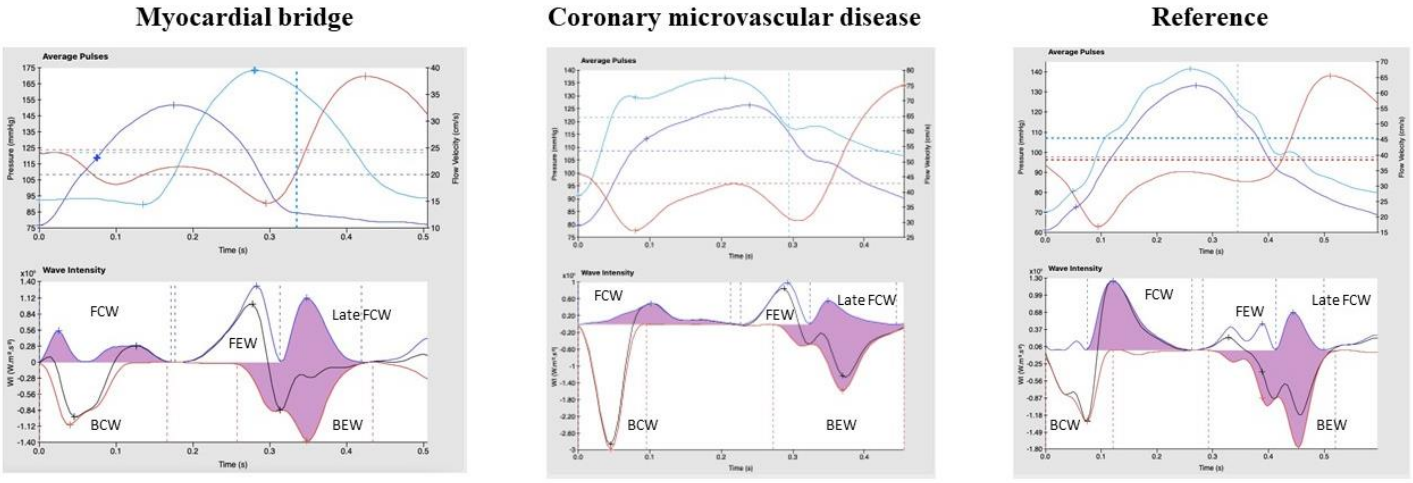
Figure 39. Changes in Coronary Wave Energies During exercise in patients with MB, CMD and reference group.



∗Significant difference compared to the reference group (p<0.05); Data are presented as median (interquartile range). BCW: backward compression wave; FCW: forward compression wave; FEW: forward expansion wave; BEW: backward expansion wave; late FCW: late forward compression wave.

Typical coronary pressure and flow waveforms, with corresponding wave intensity analysis profiles during peak exercise, are shown in **Figure 40**.

Figure 40. Examples of wave intensity analysis profiles during peak exercise in a patient with MB (left), CMD (middle) and reference group (right).



Ensemble averaged aortic pressure (top, light blue), distal coronary pressure (top, dark blue), flow velocity (top, red) and wave intensity analysis (bottom). BCW: backward compression wave; FCW: forward compression wave; FEW: forward expansion wave; BEW: backward expansion wave; late FCW: late forward compression wave.

6.4.4. Coronary endothelial function

Twenty-four patients in the MB, 24 in the CMD, and 14 in the reference groups underwent assessment of coronary endothelial function. Fifty-four percent of patients with MB, compared with 29% in the reference group, had epicardial endothelial dysfunction (p=0.126). By the binary definition, 83% of patients with a MB, compared with 64% in the reference group, had microvascular endothelial dysfunction (p=0.183). The prevalence of endothelial dysfunction was similar between the MB and CMD groups. MMI was independently associated with epicardial endothelial function (standardised coefficient, −0.723; p=0.047), and the model of MMI and impaired coronary perfusion efficiency during exercise accounted for 44% of epicardial endothelial function in patients with a MB (**Table 26**). None of the biologically plausible variables were associated with microvascular endothelial function (**Appendix Table 4**).

Table 26. Linear regression analysis testing the association between biologically plausible variables and epicardial endothelial function in patients with myocardial bridges.

	Standardised coefficients (95% CI)	**P value**
Univariable		
Age	0.012 (-1.153, 1.216)	0.957
Gender	-0.196 (-34.658, 13.069)	0.358
Hypertension	0.227 (-10.588, 34.256)	0.286
Diabetes Mellitus	0.337 (-4.541, 42.989)	0.108
CFR	-0.103 (-26.036, 16.138)	0.631

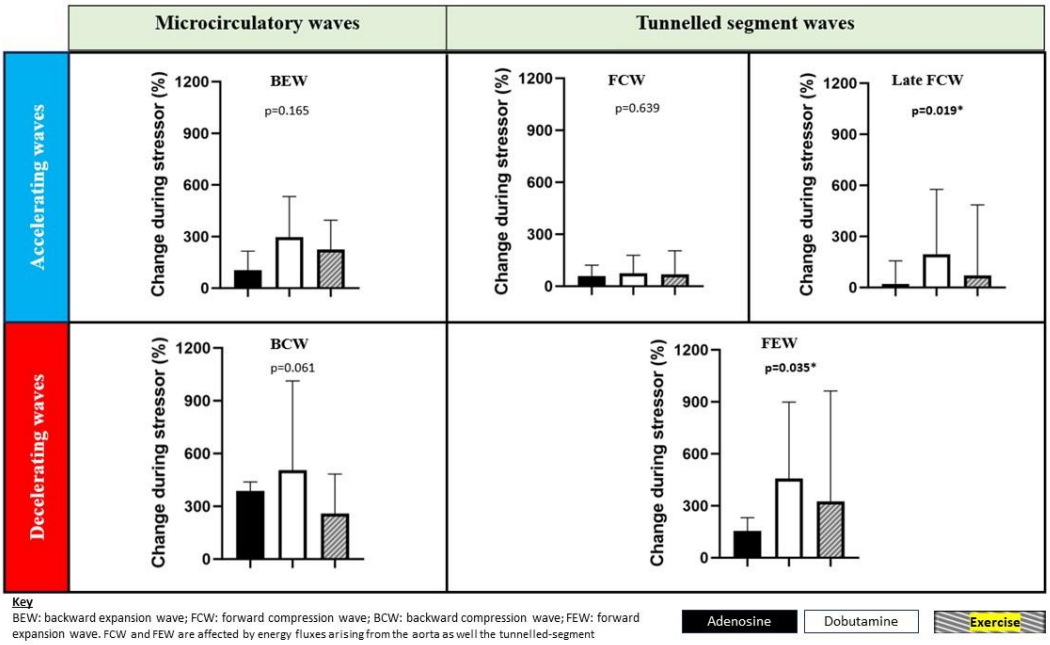
Impaired perfusion efficiency during exercise	-0.390 (-50.908, 1.125)	0.060
MMI	-0.757 (-1.018, -0.132)	0.018
Multivariable (R^2 0.440)		
Impaired coronary perfusion efficiency during exercise	-0.085 (-46.744, 36.711)	0.779
MMI	-0.723 (-1.086, -0.010)	0.047

CFR: Coronary Flow Reserve; MMI: myocardial bridge muscle index

6.4.5. Comparison of intracoronary physiology responses to adenosine, dobutamine and exercise

Twenty-five patients with MB underwent intracoronary physiology assessment during adenosine, dobutamine and exercise sequentially. Microvascular resistances during adenosine, dobutamine and exercise were 2.0±0.7, 3.4±1.4 and 4.3±1.3 $mmHg.cm^{-1}.s^{-1}$ respectively ($p<0.001$). There was a reduction in coronary perfusion efficiency in response to adenosine (64±8% to 51±12%), dobutamine (65±9% to 53±13%) and exercise (66±10% to 56±9%) (all $p<0.001$), with no between stressor differences in delta perfusion efficiency (ANOVA $p=0.641$). The impact of each stressor on the wave energies arising from the tunnelled segment was disparate, whereas no such difference (between stressors) was observed in the wave energies arising from the microcirculation. There was no difference in tunnelled-segment derived delta wave energies between dobutamine and exercise (**Figure 41**).

Figure 41. Changes in Coronary Wave Energies with adenosine, dobutamine and exercise in patients with MB.



FCW: forward compression wave; FEW: forward expansion wave; BCW: backward compression wave; BEW: backward expansion wave; late FCW: late forward compression wave

6.5. Discussion

To our knowledge, this is the first study to compare intracoronary blood flow and pressure responses during exercise between patients with myocardial bridges (MB), CMD and normal CFR. Our main findings are i) patients with MB have diminished coronary perfusion efficiency during exercise, whereas those in the reference group (no MB and normal CFR) have enhanced perfusion efficiency, ii) the diminished perfusion efficiency in MB is predominantly driven by reduction of the accelerating cardiac wave energy arising from the upstream epicardial vessel during early systole; this is in contrast to patients with CMD, where the main driver of perfusion inefficiency is perturbation of the microcirculation-derived wave energies, iii) patients with

MB have a high prevalence of endothelial dysfunction and iv) dobutamine is an adequate surrogate of physical exercise in the intracoronary assessment of patients with a myocardial bridge.

Seminal work from the 1990s first reported the potential of certain MBs to cause ischaemia, with delayed decompression in early diastole, when most of myocardial perfusion occurs, being the purported predominant mechanism[38,144]. Tachycardiac pacing, in patients with MBs, led to incomplete decompression of the tunnelled segment in early diastole as well as reducing the diastolic perfusion time. Others have since reported that certain MBs can lead to perturbed diastole-specific pressure indices in response to dobutamine in the catheter laboratory[64,65,139], which may be associated with inducible ischaemia on noninvasive stress imaging[66].

However, the mechanisms of ischaemia in patients with MB remains incompletely understood. It is now possible to carry out detailed invasive coronary physiology evaluation in humans during exercise[25,80]. Furthermore, the technique of wave intensity analysis (WIA) represents a powerful tool to study the intimate relationship between cardiac contraction and coronary blood flow (CBF), referred to as cardiac-coronary coupling. Coronary WIA provides directional, quantitative, and temporal information that has been used to study various human disease processes and may lead to a better understanding of the coronary physiological changes that occur during physical exercise in patients with MBs.

6.5.1. Coronary perfusion efficiency

We have demonstrated that perfusion efficiency, which is the proportion of accelerating cardiac wave energies / total cardiac wave energies (%), is enhanced during physical exercise in

patients with nonobstructive coronary arteries, normal microvascular function and absence of a MB (whom we termed the reference group). This is achieved by accentuation of the accelerating wave energy during early diastole, i.e., the backward expansion wave. This wave arises due to decompression of the microvasculature during early diastole, which leads to a suction effect and is the dominant driver of myocardial perfusion in healthy hearts. Paradoxically, perfusion efficiency decreases during exercise in both patients with MB and CMD. However, the mechanisms leading to diminished perfusion efficiency appear to be distinct between these groups. In CMD patients, the pathophysiology arises from the microcirculation (manifest as a reduction in the accelerating diastolic backward expansion wave and accentuation of the systolic decelerating backward compression wave), whereas in patients with MB, it is the impact of the tunnelled segment on epicardial vessel haemodynamics that manifests as a reduction in the early systolic accelerating forward compression wave. Finally, MB patients also have an increased propensity to develop atherosclerotic plaque, which acts as another potential substrate for ischaemia; in our study, this is indicated by the lower FFR and exercise Pd/Pa in this patient group compared to both the reference and CMD groups.

6.5.2. Coronary endothelial dysfunction

A handful of previous studies have investigated the prevalence of coronary endothelial dysfunction in patients with MB. In a study by Paraongkar et al, 85% of patients with MB had epicardial endothelial dysfunction using the same diagnostic criteria as our study[139], whereas Sara et al have reported 60% of patients with MB as having epicardial endothelial dysfunction and 58% as having microvascular endothelial dysfunction[142]. Interestingly, Sara et al reported co-localisation of epicardial endothelial dysfunction within the tunnelled segments, suggesting a pathophysiological link between the two. Alterations in wall shear stress (WSS) have been

purported to be the mechanism predisposing the tunnelled segments to epicardial endothelial dysfunction. Lifelong systolic compression is thought to lead to high WSS within the tunnelled segment and is thought to lead to local structural changes of the endothelial cells, leading to reduced nitric oxide production[145]. We report 54% prevalence of epicardial endothelial dysfunction and 83% of microvascular endothelial dysfunction in the MB group, which was similar to the CMD group and numerically higher than the reference group. Myocardial bridge muscle index (MMI) was independently associated with epicardial endothelial dysfunction. Forsdahl et al have previously reported that MMI>31 on CTCA predicts *dobutamine diastolic FFR* <0.76 with good diagnostic accuracy[146]. *Dobutamine diastolic FFR* <0.76, in turns, predicts the presence of inducible ischaemia on exercise stress echocardiography[66]. These findings have significant clinical implications, as an ischaemic substrate can now be identified in patients with MB directly from the initial CTCA scan, which may obviate the need for noninvasive stress imaging and invasive coronary angiography in the future.

6.5.3. Comparison of the effects of adenosine, dobutamine and physical exercise on cardiac coronary coupling in patients with myocardial bridges

Dobutamine is the preferred agent of choice for interrogating the physiological significance of myocardial bridges. This is based on the finding that delayed and incomplete decompression of the tunnelled segment during early diastole, which is when the majority of myocardial perfusion occurs, is the mechanism leading to ischaemia in patients with MBs during tachycardia pacing[38]. Schwarz et al first demonstrated that, during tachycardia pacing, there was a delay in diastolic decompression of the tunnelled segment in 14 patients with MB[38]. This delay in diastolic luminal gain was associated with altered flow physiology, with a characteristic ‘helmet-spike’ pattern of Doppler flow profile. This is due to high flow velocity secondary to narrowed lumen in early diastole, followed by rapid deceleration of flow once the

tunnelled segment starts to decompress and the lumen size starts to increase; this is finally followed by a plateau of the flow profile, which is due to a static luminal size during the late diastolic period when the tunnelled segment has fully decompressed[38]. The subendocardium is particularly vulnerable to myocardial ischaemia in patients with MBs due to the differential perfusion compared to the subepicardium, which occurs due to the time delay in subendocardial perfusion when compared to subepicardial perfusion. Conceptually, when the subepicardial perfusion reaches its peak, the subendocardial perfusion reaches less than half of that. Any process that impedes the rapid early diastolic perfusion and diastolic perfusion time exacerbates this normal delay in subendocardial perfusion; therefore, predisposing the myocardium to subendocardial ischaemia[147]. Therefore, an agent that mimics the positive inotropic and chronotropic effects of physical exercise, like dobutamine, is postulated to be the ideal stressor for the assessment of physiological significance of MBs[64]. Based on this, certain centres use intracoronary physiology assessment during dobutamine infusion to delineate the physiological significance of MBs[65,66,139], and even refer patients for surgical unroofing of the tunnelled segment depending on the physiology measurements[148]. However, much to our surprise, there is a paucity of studies comparing the effects of physical exercise and dobutamine on intracoronary physiology in patients with a MB. In our study, we have demonstrated that adenosine, dobutamine and exercise have different effects on coronary microvascular resistance and cardiac coronary coupling in patients with myocardial bridges. Our findings provide novel mechanistic insights into the pathophysiology of ANOCA in combination with MB and suggest that dobutamine (but not adenosine) could be used as a surrogate for physical exercise during intracoronary physiological assessment as these two stressors have a similar impact on the tunnelled segment derived wave energies in patients with MBs.

6.5.4. Clinical implications

It is now being increasingly recognised that not all myocardial bridges are benign anatomical variants. We have demonstrated, for the first time, that coronary perfusion efficiency is impaired during exercise in patients with myocardial bridges. Furthermore, our data suggests that the diminution of coronary perfusion efficiency during exercise is due to attenuation of accelerating wave energy during early systole arising from the upstream epicardial vessel. We have also demonstrated a high prevalence of both epicardial and microvascular endothelial dysfunction in patients with MB. These different ischaemic substrates may respond to disparate therapeutic strategies. Statins and endothelin receptor antagonists[137] may ameliorate endothelial dysfunction, whereas spasm within the tunnelled segment may respond well to calcium channel blockers, long-acting nitrates and nicorandil[79]. The management of atheroma that usually develops 10-20mm proximal to the mouth of the tunnelled segment may necessitate the use of statins to reduce the risk of plaque progression and rupture, and the treatment of attenuated forward compression wave may require a mechanical solution, such as percutaneous coronary intervention (generally avoided due to high risk of complications and suboptimal results) and surgical intervention (surgical deroofing or myectomy). Developing therapies that target the ischaemic mechanism, as identified in our study, is appealing and may lead to better patient-centric outcomes. Finally, we have demonstrated that dobutamine is a suitable surrogate of physical exercise in the interrogation of vessels with MBs, which gives further impetus to its use in the clinical setting.

6.5.5. Study Limitations

This is a relatively small, single-centre study, which has some limitations and caveats. First, patients had a high suspicion of an ischaemic substrate based on pretest probabilities and,

therefore, our findings should not be extrapolated to MBs that are incidentally discovered or are found in patients with symptoms atypical of angina. Second, all investigations were performed in the LAD artery, potentially limiting the applicability of the findings in other vessels, although MBs mostly involve the LAD artery. Third, we did not assess the bridged vessels with intravascular imaging, which may have uncovered another mechanism of ischaemia in the form of proximal vessel atherosclerosis as has been demonstrated in previous studies; however, the finding of reduced panyclic pressure indices during adenosine-mediated vasodilatation and exercise is suggestive of the presence of atherosclerotic coronary artery disease in our patients.

6.6. Conclusions

Patients with ANOCA and MB exhibit impaired coronary perfusion efficiency during exercise, which is predominantly due to diminution of the accelerating wave energy in early systole; this is likely secondary to the upstream epicardial vessel. This mechanism is distinct to that leading to coronary perfusion inefficiency in patients with CMD. Furthermore, patients with MB have a very high prevalence of endothelial dysfunction, which correlates with the myocardial bridge muscle index on CTCA. Future studies are warranted to assess if patients with MB may benefit from distinct pharmacological or mechanical therapies.

Chapter 7

Synthesis

7.1. Summary

In this thesis, I have demonstrated the following:

First, in patients with typical and limiting angina with nonobstructive coronary arteries, ischaemia during an exercise electrocardiogram treadmill test (ETT) was always indicative of an underlying ischaemic substrate secondary to abnormalities in the coronary microvascular function (either endothelium-*independent* and/or endothelium-*dependent* microvascular dysfunction). Acetylcholine flow reserve (AChFR) was the strongest predictor of ischaemic ECG changes on ETT. Adding coronary microvascular disease (CMD) to the reference standard resulted in there being no false positive ETTs. Our data challenge the dogma that ETTs have a high false positive rate. With hindsight and advancement in our understanding of coronary vasomotor disorders, we speculate that this widely held belief is because of the erroneous use of obstructive coronary artery disease as the reference against which noninvasive tests were historically validated. In patients with typical limiting angina and nonobstructive coronary arteries, a positive ETT may be an excellent rule-in diagnostic test for CMD. However, this needs to be tested in a diagnostic pathway trial.

Second, in an otherwise phenotypically similar group of patients, CFR was the strongest predictor of an improvement in exercise time in response to anti-ischaemic therapy, with a distinct gain-and-loss of function noted with and without therapy, respectively, in patients with impaired CFR. Those with a normal CFR did not derive any objective or subjective benefit from anti-ischaemic therapy. CFR$\leq$2.5 had the best accuracy at predicting response to therapy.

Third, further stratification of patients according to minimal microvascular resistance, into functional and structural CMD, and acetylcholine flow reserve, into sole coronary endothelial

dysfunction, added incremental value in predicting response to therapy. Those with structural CMD may derive greater benefit in their exercise response to amlodipine compared with ranolazine, whereas those with functional CMD had an equally good response to both anti-ischaemic therapies. Patients with sole coronary endothelial dysfunction demonstrated a numerical increment in exercise time in response to anti-ischaemic therapy, whereas no such effect was seen in the reference group.

Fourth, patients with myocardial bridging (MB) and CMD had attenuation of their coronary perfusion efficiency (PE) during exercise, whereas the reference group had a numerical increase in PE. The mechanisms driving this attenuation in PE were disparate between patients with MB and CMD. The predominant mechanism, in patients with MB, was diminution of the accelerating forward compression wave in early systole, which was likely related to the upstream epicardial vessel. In patients with CMD, the predominant mechanism was perturbation of the microcirculation derived energies (augmentation of the decelerating backward compression wave and attenuation of the accelerating backward expansion wave). There was a high prevalence of epicardial and microvascular endothelial dysfunction in patients with MB; the former being associated with myocardial bridge muscle index (the product of the length and depth of the tunnelled segment). Both the impaired coronary perfusion efficiency during exercise and endothelial dysfunction represent distinct therapeutic targets in this patient group.

7.2. Future directions

7.2.1. What is the utility of exercise electrocardiogram treadmill testing in patients with angina and nonobstructive coronary arteries?

Given the 100% specificity and positive predictive value of ETTs in detecting an ischaemic substrate in patients with angina and nonobstructive coronary arteries, it is tempting to speculate that an ETT can become the investigation of choice to rule-in a diagnosis of CMD in patients with typical and limiting angina who have nonobstructive coronary arteries on computed tomography coronary angiography. This diagnostic pathway trial will need to be tested against the current standard of care in a diagnostic trial design, such as PRECISE[149]; the main benefits of this completely noninvasive pathway would be low cost and widespread availability, which are important factors for both patients and healthcare economics. If awarded the academic clinical lectureship, this will be one of the main trials that I will be involved with.

7.2.2. CFR predicts response to anti-ischaemic therapy in patients with angina and nonobstructive coronary arteries….what next?

We have demonstrated that CFR predicts response to anti-ischaemic therapy. This has important implications for the future of therapeutic trials in patients with ANOCA. The ideal trial in the future would be a phenotype blinded, placebo-controlled, randomised controlled trial that includes patients with normal and impaired CFR. The definition of CMD also needs to be precise using the CFR≤2.5 cutoff. Ideally, such a study should include both patient-centric and physiological endpoints to provide a mechanistic link with clinically relevant outcomes.

7.2.3. Deep endotyping in the catheter laboratory *may* allow further therapy stratification….what next?

We have demonstrated that whilst patients with functional CMD responded equally well to both amlodipine and ranolazine, those with structural CMD responded preferentially to amlodipine. However, the mechanism of improvement in these patients was not clear from our study. Future studies should consider including positron emission tomography at baseline and in response to therapy in order to assess for changes in resting myocardial oxygen demand, which is purported to be the mechanism leading to ischaemia in patients with functional CMD. It is purported that coronary-peripheral uncoupling is a key mechanism leading to ischaemia in patients with structural CMD and reversal of this may lead to ischaemia attenuation and improvement in patient-centric outcomes. Other agents that reduce myocardial work during exercise, by promoting a peripheral vasodilatory response, such as phosphodiesterase inhibitors, nitrites and endothelin-receptor antagonists, should be tested in future trials. Serial left ventricular pressure-volume loops, or non-invasive monitoring of the Buckberg index, can be considered as a mechanistic outcome measure in future trials of patients with structural CMD in order to understand whether peripheral vasodilating agents lead to a reduction in LV afterload and myocardial stress. Finally, our results suggest that patients with sole coronary endothelial dysfunction *may* derive benefit from anti-ischaemic therapy. Future, larger scale studies should focus on assessing response to mechanistically plausible therapy in this patient cohort. A recent pilot study has demonstrated that an intracoronary infusion of CD34+ cells was associated with improvement in AChFR over 6 months' time. Other agents, such as L-arginine, which have shown promise in small-scale, single centre, non-randomised studies should be explored further in this patient group.

7.2.4. Therapy trial in patients with myocardial bridges

There is a paucity of data guiding therapy in patients with ANOCA and myocardial bridges. One of the early seminal studies by Schwarz et al in 1996 demonstrated the acute beneficial effects of intravenous beta blockers on coronary haemodynamics in these patients[144]. There have been no randomised controlled trials investigating the effects of anti-ischaemic therapy in this patient group. Furthermore, there is no randomised evidence to suggest that surgical deroofing is beneficial in this patient cohort. The two disparate substrates for ischaemia (coronary perfusion inefficiency during exercise and coronary endothelial dysfunction) in this patient group represent distinct therapeutic targets. A physiology-blinded randomised controlled trial including a negative inotropic and chronotropic agent, like diltiazem, against a placebo with both patient-centric and physiology-based outcome measures would provide much needed data in this field. Diltiazem would be the optimal drug choice given that it is both a negative inotropic agent, which may rectify the delayed decompression of the tunnelled segment, and an anti-spasm agent, which will rectify the heightened propensity to spasm within the tunnelled segment. Nebivolol, with its negative inotropic activity and NO-derived vasodilating effect, may be a suitable alternative.

Of note, the Combowire, which has been used exclusively for all my studies, is no longer available. Whilst this will not have an impact on my future plans, as all of the aforementioned trials can be run using continuous thermodilution-derived measures, it means that we may lose the ability to quantify wave energies under different stressors. This may make identifying disease mechanisms in the catheter laboratory more challenging. Whether we can utilise continuous thermodilution derived rate of change of temperature as a surrogate for rate of change of flow velocity (as assessed using the Doppler wire), to characterise wave energies, is an enticing concept that I wish to explore further.

7.3. Conclusions

My study findings add to the growing field of coronary physiology assessment in patients with angina and nonobstructive coronary arteries. We have demonstrated that ischaemia on an ETT is always attributable to an ischaemic substrate secondary to microcirculatory abnormalities. This knowledge can be used to create novel diagnostic pathways with widespread availability. It also questions the appropriateness of using obstructive coronary artery disease as the reference standard to compare the diagnostic accuracy of noninvasive stress imaging. We have demonstrated that an impaired CFR identifies patients who respond to anti-ischaemic therapy, in an otherwise phenotypically similar group of patients with ANOCA. This is a significant step forward as currently the management of these patients is empirical and inadequate. Our findings stress the importance of routine coronary physiology assessment in patients with typical and limiting angina with nonobstructive coronary arteries. Finally, we have demonstrated, for the first time, that patients with myocardial bridges have impaired coronary perfusion efficiency during exercise, which is driven by disparate underlying pathobiology compared to that in patients with coronary microvascular disease. This represents a potential therapeutic target in these patients.

Appendix

Appendix Table 1. Primary and secondary outcomes in ChaMP-CMD (intention-to-treat analyses).

	Amlodipine		**Ranolazine**	
	CMD	**Reference**	**CMD**	**Reference**
Exercise time (seconds)				
Increment	89 (95% CI 69 to 117)	13 (95% CI -14 to 40)	80 (95% CI 53 to 108)	5 (95% CI -27 to 37)
Difference in change between groups	76 (95% CI 34 to 118)		75 (95% CI 31 to 120)	
P value	<0.001		0.001	
SAQ summary score				
Increment	7 (95% CI 3 to 11)	6 (95% CI 2 to 10)	11 (95% CI 6 to 16)	7 (95% CI 2 to 12)
Difference in change between groups	1 (95% CI -5 to 7)		4 (95% CI -3 to 12)	
P value	0.768		0.254	

Appendix Table 2. Additional ETT parameters in patients recruited into ChaMP-CMD.

	Baseline			**Amlodipine**			**Ranolazine**		
	CMD	**Reference**	**P value**	**CMD**	**Reference**	**P value**	**CMD**	**Reference**	**P value**
Angina during ETT, n(%)	44 (80)	23 (77)	0.710	30 (61)	15 (58)	0.766	25 (49)	14 (58)	0.451
Reason for stopping ETT, n(%)			0.459			0.198			0.433
Angina	29 (53)	17 (57)		16 (33)	8 (31)		16 (31)	12 (52)	
Dyspnoea	15 (27)	4 (13)		7 (15)	8 (31)		17 (33)	7 (30)	
Fatigue	10 (18)	8 (27)		18 (38)	10 (38)		14 (28)	3 (13)	
Presyncope	1 (2)	1 (3)		3 (6)	0		3 (6)	1 (4)	
Musculoskeletal pain	0	0		4 (8)	0		1 (2)	0	
Time to angina, seconds	205±98	187±111	0.490	297±161	251±146	0.362	**260±122**	**183±88**	**0.028**

ETT: exercise ECG treadmill test

Appendix Table 3. Within group differences in change in exercise time in patients with structural and functional CMD recruited into ChaMP-CMD (intention-to-treat analyses).

	Structural CMD		**Functional CMD**	
	AML	**RNL**	**AML**	**RNL**
Exercise time (seconds)				
Mean increment	74 (95% CI 25 to 124)	42 (95% CI 8 to 76)	97 (95% CI 62 to 132)	101 (95% CI 63 to 139)
Difference in change within group	39 (95% CI -1 to 79)		-2 (95% CI -29 to 25)	
P value	0.058		0.865	

AML: amlodipine; RNL: ranolazine

Appendix Table 4. Linear regression analysis testing the association between biologically plausible variables and microvascular endothelial dysfunction in patients with myocardial bridges.

	Standardised coefficients (95% CI)	**P value**
Univariate		
Age	0.401 (-0.002, 0.033)	0.080
Gender	0.289 (-0.171, 0.705)	0.217
Hypertension	0.050 (-0.357, 0.438)	0.833
Diabetes Mellitus	-0.218 (-0.612, 0.231)	0.355
CFR	-0.308 (-0.580, 0.121)	0.186
Impaired coronary perfusion efficiency during exercise	0.063 (-0.432, 0.557)	0.794
MMI	0.124 (-0.006, 0.004)	0.701

CFR: Coronary Flow Reserve; MMI: myocardial bridge muscle index

References

1. Patel MR, Peterson ED, Dai D, Brennan JM, Redberg RF, Anderson HV, Brindis RG, Douglas PS. Low Diagnostic Yield of Elective Coronary Angiography. *New England Journal of Medicine* 2010;**362**:886–895.

2. Marinescu MA, Löffler AI, Ouellette M, Smith L, Kramer CM, Bourque JM. Coronary microvascular dysfunction, microvascular angina, and treatment strategies. *JACC Cardiovasc Imaging* 2015;**8**:210–222.

3. Bruyne B De, Oldroyd KG, Pijls NHJ. Microvascular (Dys)Function and Clinical Outcome in Stable Coronary Disease. *J Am Coll Cardiol* 2016;**67**:1170–1172.

4. Duncker DJ, Koller A, Merkus D, Canty JM. Regulation of Coronary Blood Flow in Health and Ischemic Heart Disease. *Prog Cardiovasc Dis* 2015;**57**:409–422.

5. Sinha A, Rahman H, Webb A, Shah AM, Perera D. Untangling the pathophysiologic link between coronary microvascular dysfunction and heart failure with preserved ejection fraction. *Eur Heart J* 2021;**42**:4431–4441.

6. Seddon M, Melikian N, Dworakowski R, Shabeeh H, Jiang B, Byrne J, Casadei B, Chowienczyk P, Shah AM. Effects of Neuronal Nitric Oxide Synthase on Human Coronary Artery Diameter and Blood Flow In Vivo. *Circulation* 2009;**119**:2656–2662.

7. Laughlin MH, Tomanek RJ. Myocardial capillarity and maximal capillary diffusion capacity in exercise-trained dogs. *J Appl Physiol* 1987;**63**:1481–1486.

8. Gute D, Fraga C, Laughlin MH, Amann JF. Regional changes in capillary supply in skeletal muscle of high-intensity endurance-trained rats. *J Appl Physiol* 1996;**81**:619–626.

9. KOCH-WESER J BJ. THE INFLUENCE OF THE INTERVAL BETWEEN BEATS ON MYOCARDIAL CONTRACTILITY. *Pharmacol Rev* 1963:601–652.

10. Poliner LR, Dehmer GJ, Lewis SE, Parkey RW, Blomqvist CG, Willerson JT. Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. *Circulation* 1980;**62**:528–534.

11. Sonnenblick EH, Braunwald E, Williams JF, Glick G. Effects of exercise on myocardial force-velocity relations in intact unanesthetized man: relative roles of changes in heart rate, sympathetic activity, and ventricular dimensions. *Journal of Clinical Investigation* 1965;**44**:2051–2062.

12. NELSON RR, GOBEL FL, JORGENSEN CR, WANG K, WANG Y, TAYLOR HL. Hemodynamic Predictors of Myocardial Oxygen Consumption During Static and Dynamic Exercise. *Circulation* 1974;**50**:1179–1189.

13. Duncker DJ, Zon NS Van, Crampton M, Herrlinger S, Homans DC, Bache RJ. Coronary pressure-flow relationship and exercise: contributions of heart rate, contractility, and alpha 1-adrenergic tone. *American Journal of Physiology-Heart and Circulatory Physiology* 1994;**266**:H795–H810.

14. Parks CM, Manohar M. Transmural coronary vasodilator reserve and flow distribution during severe exercise in ponies. *J Appl Physiol* 1983;**54**:1641–1652.

15. Berne RM RR. *Handbook of Physiology. The Cardiavascular System. The Heart.* . 1979.

16. Duncker DJ, Ishibashi Y, Bache RJ. Effect of treadmill exercise on transmural distribution of blood flow in hypertrophied left ventricle. *American Journal of Physiology-Heart and Circulatory Physiology* 1998;**275**:H1274–H1282.

17. Hoef TP van de, Meuwissen M, Piek JJ. Fractional flow reserve and beyond. *Heart* 2013;**99**:1699–1705.

18. Gdowski MA, Murthy VL, Doering M, Monroy-Gonzalez AG, Slart R, Brown DL. Association of Isolated Coronary Microvascular Dysfunction With Mortality and Major Adverse Cardiac Events: A Systematic Review and Meta-Analysis of Aggregate Data. *J Am Heart Assoc* 2020;**9**.

19. Tavella R, Cutri N, Tucker G, Adams R, Spertus J, Beltrame JF. Natural history of patients with insignificant coronary artery disease. *Eur Heart J Qual Care Clin Outcomes* 2016;**2**:117–124.

20. Jespersen L, Hvelplund A, Abildstrom SZ, Pedersen F, Galatius S, Madsen JK, Jorgensen E, Kelbaek H, Prescott E. Stable angina pectoris with no obstructive coronary artery disease is associated with increased risks of major adverse cardiovascular events. *Eur Heart J* 2012;**33**:734–744.

21. AlBadri A, Bairey Merz CN, Johnson BD, Wei J, Mehta PK, Cook-Wiens G, Reis SE, Kelsey SF, Bittner V, Sopko G, Shaw LJ, Pepine CJ, Ahmed B. Impact of Abnormal Coronary Reactivity on Long-Term Clinical Outcomes in Women. *J Am Coll Cardiol* 2019;**73**:684–693.

22. Ong P, Camici PG, Beltrame JF, Crea F, Shimokawa H, Sechtem U, Kaski JC, Bairey Merz CN. International standardization of diagnostic criteria for microvascular angina. *Int J Cardiol* 2018;**250**:16–20.

23. Taqueti VR, Carli MF Di. Coronary Microvascular Disease Pathogenic Mechanisms and Therapeutic Options. *J Am Coll Cardiol* 2018;**72**:2625–2641.

24. Rahman H, Demir OM, Khan F, Ryan M, Ellis H, Mills MT, Chiribiri A, Webb A, Perera D. Physiological Stratification of Patients With Angina Due to Coronary Microvascular Dysfunction. *J Am Coll Cardiol* 2020;**75**:2538–2549.

25. Rahman H, Ryan M, Lumley M, Modi B, McConkey H, Ellis H, Scannell C, Clapp B, Marber M, Webb A, Chiribiri A, Perera D. Coronary Microvascular Dysfunction Is Associated With Myocardial Ischemia and Abnormal Coronary Perfusion During Exercise. *Circulation* 2019;**140**:1805–1816.

26. Seddon MD, Chowienczyk PJ, Brett SE, Casadei B, Shah AM. Neuronal Nitric Oxide Synthase Regulates Basal Microvascular Tone in Humans In Vivo. *Circulation* 2008;**117**:1991–1996.

27. Sezer M, Kocaaga M, Aslanger E, Atici A, Demirkiran A, Bugra Z, Umman S, Umman B. Bimodal Pattern of Coronary Microvascular Involvement in Diabetes Mellitus. *J Am Heart Assoc* 2016;**5**.

28. Lerman A, Holmes DR, Bell MR, Garratt KN, Nishimura RA, Burnett JC. Endothelin in Coronary Endothelial Dysfunction and Early Atherosclerosis in Humans. *Circulation* 1995;**92**:2426–2431.

29. Ford TJ, Rocchiccioli P, Good R, McEntegart M, Eteiba H, Watkins S, Shaukat A, Lindsay M, Robertson K, Hood S, Yii E, Sidik N, Harvey A, Montezano AC, Beattie E, Haddow L, Oldroyd KG, Touyz RM, Berry C. Systemic microvascular dysfunction in microvascular and vasospastic angina. *Eur Heart J* 2018;**39**:4086–4097.

30. Ford TJ, Corcoran D, Padmanabhan S, Aman A, Rocchiccioli P, Good R, McEntegart M, Maguire JJ, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sattar N, Hsu L-Y, Arai AE, Oldroyd KG, Touyz RM,

Davenport AP, Berry C. Genetic dysregulation of endothelin-1 is implicated in coronary microvascular dysfunction. *Eur Heart J* 2020;**41**:3239–3252.

31. Förstermann U, Münzel T. Endothelial Nitric Oxide Synthase in Vascular Disease. *Circulation* 2006;**113**:1708–1714.

32. Hasdai D, Gibbons RJ, Holmes DR, Higano ST, Lerman A. Coronary Endothelial Dysfunction in Humans Is Associated With Myocardial Perfusion Defects. *Circulation* 1997;**96**:3390–3395.

33. AlBadri A, Leong D, Bairey Merz CN, Wei J, Handberg EM, Shufelt CL, Mehta PK, Nelson MD, Thomson LE, Berman DS, Shaw LJ, Cook-Wiens G, Pepine CJ. Typical angina is associated with greater coronary endothelial dysfunction but not abnormal vasodilatory reserve. *Clin Cardiol* 2017;**40**:886–891.

34. Suwaidi J Al, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction. *Circulation* 2000;**101**:948–954.

35. AlBadri A, Bairey Merz CN, Johnson BD, Wei J, Mehta PK, Cook-Wiens G, Reis SE, Kelsey SF, Bittner V, Sopko G, Shaw LJ, Pepine CJ, Ahmed B. Impact of Abnormal Coronary Reactivity on Long-Term Clinical Outcomes in Women. *J Am Coll Cardiol* 2019;**73**:684–693.

36. Çiçek D, Kalay N, Müderrisoğlu H. Incidence, clinical characteristics, and 4-year follow-up of patients with isolated myocardial bridge: a retrospective, single-center, epidemiologic, coronary arteriographic follow-up study in southern Turkey. *Cardiovascular Revascularization Medicine* 2011;**12**:25–28.

37. Hostiuc S, Negoi I, Rusu MC, Hostiuc M. Myocardial Bridging: A Meta-Analysis of Prevalence. *J Forensic Sci* 2018;**63**:1176–1185.

38. Schwarz ER, Klues HG, Dahl J vom, Klein I, Krebs W, Hanrath P. Functional characteristics of myocardial bridging: A combined angiographic and intracoronary Doppler flow study. *Eur Heart J* 1997;**18**:434–442.

39. DOWNEY HF, CRYSTAL GJ, BASHOUR FA. Asynchronous transmural perfusion during coronary reactive hyperaemia. *Cardiovasc Res* 1983;**17**:200–206.

40. Seitz A, Gardezy J, Pirozzolo G, Probst S, Athanasiadis A, Hill S, Mahrholdt H, Bekeredjian R, Sechtem U, Ong P. Long-Term Follow-Up in Patients With Stable Angina and Unobstructed Coronary Arteries Undergoing Intracoronary Acetylcholine Testing. *JACC Cardiovasc Interv* 2020;**13**:1865–1876.

41. Kugiyama K, Yasue H, Okumura K, Ogawa H, Fujimoto K, Nakao K, Yoshimura M, Motoyama T, Inobe Y, Kawano H. Nitric Oxide Activity Is Deficient in Spasm Arteries of Patients With Coronary Spastic Angina. *Circulation* 1996;**94**:266–272.

42. Toyo-oka T, Aizawa T, Suzuki N, Hirata Y, Miyauchi T, Shin WS, Yanagisawa M, Masaki T, Sugimoto T. Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation* 1991;**83**:476–483.

43. Ford TJ, Rocchiccioli P, Good R, McEntegart M, Eteiba H, Watkins S, Shaukat A, Lindsay M, Robertson K, Hood S, Yii E, Sidik N, Harvey A, Montezano AC, Beattie E, Haddow L, Oldroyd KG, Touyz RM, Berry C. Systemic microvascular dysfunction in microvascular and vasospastic angina. *Eur Heart J* 2018;**39**:4086–4097.

44. Satoh S, Tomoike H, Mitsuoka W, Egashira S, Tagawa H, Kuga T, Nakamura M. Smooth muscles from spastic coronary artery segments show hypercontractility to

histamine. *American Journal of Physiology-Heart and Circulatory Physiology* 1990;**259**:H9–H13.

45. Kadokami T, Shimokawa H, Fukumoto Y, Ito A, Takayanagi T, Egashira K, Takeshita A. Coronary Artery Spasm Does Not Depend on the Intracellular Calcium Store but Is Substantially Mediated by the Protein Kinase C–Mediated Pathway in a Swine Model With Interleukin-1β In Vivo. *Circulation* 1996;**94**:190–196.

46. Kuga T, Shimokawa H, Hirakawa Y, Kadokami Y, Arai Y, Fukumoto Y, Kuwata K, Kozai T, Egashira K, Takeshita A. Increased Expression of L-Type Calcium Channels in Vascular Smooth Muscle Cells at Spastic Site in a Porcine Model of Coronary Artery Spasm. *J Cardiovasc Pharmacol* 2000;**35**:822–828.

47. Kandabashi T, Shimokawa H, Miyata K, Kunihiro I, Kawano Y, Fukata Y, Higo T, Egashira K, Takahashi S, Kaibuchi K, Takeshita A. Inhibition of Myosin Phosphatase by Upregulated Rho-Kinase Plays a Key Role for Coronary Artery Spasm in a Porcine Model With Interleukin-1β. *Circulation* 2000;**101**:1319–1323.

48. Pepine CJ, Anderson RD, Sharaf BL, Reis SE, Smith KM, Handberg EM, Johnson BD, Sopko G, Bairey Merz CN. Coronary Microvascular Reactivity to Adenosine Predicts Adverse Outcome in Women Evaluated for Suspected Ischemia. *J Am Coll Cardiol* 2010;**55**:2825–2832.

49. Murthy VL, Naya M, Taqueti VR, Foster CR, Gaber M, Hainer J, Dorbala S, Blankstein R, Rimoldi O, Camici PG, Carli MF Di. Effects of Sex on Coronary Microvascular Dysfunction and Cardiac Outcomes. *Circulation* 2014;**129**:2518–2527.

50. Rahman H, Demir OM, Ryan M, McConkey H, Scannell C, Ellis H, Webb A, Chiribiri A, Perera D. Optimal Use of Vasodilators for Diagnosis of Microvascular Angina in the Cardiac Catheterization Laboratory. *Circ Cardiovasc Interv* 2020;**13**.

51. Boerhout CB, Waard G de W de, Lee JM, Mejia-Renteria H, Lee SH, Jung J-H, Hoshino M, Echavarria-Pinto M, Meuwissen M, Matsuo H, Madera-Cambero M, Eftekhari A, Effat ME, Murai T, Marques K, Appelman Y, Doh J-H, Christiansen EC, Banerjee R, Nam C-W, Niccoli G, Nakayama M, Tanaka N, Shin E-S, Beijk MB, Knaapen P, Escaned J, Kakuta T, Koo B-K, Piek JP, Hoef T van de H van de. Prognostic value of structural and functional coronary microvascular dysfunction in patients with non-obstructive coronary artery disease; from the multicentre international ILIAS registry. *EuroIntervention* 2022;**18**:719–728.

52. Rogers IS, Tremmel JA, Schnittger I. Myocardial bridges: Overview of diagnosis and management. *Congenit Heart Dis* 2017;**12**:619–623.

53. Nishizaki M. Life-threatening arrhythmias leading to syncope in patients with vasospastic angina. *J Arrhythm* 2017;**33**:553–561.

54. Nakamura M, Takeshita A, Nose Y. Clinical characteristics associated with myocardial infarction, arrhythmias, and sudden death in patients with vasospastic angina. *Circulation* 1987;**75**:1110–1116.

55. Sato K, Kaikita K, Nakayama N, Horio E, Yoshimura H, Ono T, Ohba K, Tsujita K, Kojima S, Tayama S, Hokimoto S, Matsui K, Sugiyama S, Yamabe H, Ogawa H. Coronary Vasomotor Response to Intracoronary Acetylcholine Injection, Clinical Features, and Long-term Prognosis in 873 Consecutive Patients With Coronary Spasm: Analysis of a Single-Center Study Over 20 Years. *J Am Heart Assoc* 2013;**2**.

56. Takagi Y, Takahashi J, Yasuda S, Miyata S, Tsunoda R, Ogata Y, Seki A, Sumiyoshi T, Matsui M, Goto T, Tanabe Y, Sueda S, Sato T, Ogawa S, Kubo N, Momomura S, Ogawa H, Shimokawa H. Prognostic Stratification of Patients With Vasospastic Angina. *J Am Coll Cardiol* 2013;**62**:1144–1153.

57. Beltrame JF, Crea F, Kaski JC, Ogawa H, Ong P, Sechtem U, Shimokawa H, Bairey Merz CN. International standardization of diagnostic criteria for vasospastic angina. *Eur Heart J* 2015:ehv351.

58. Yasue H, Takizawa A, Nagao M, Nishida S, Horie M, Kubota J, Omote S, Takaoka K, Okumura K. Long-term prognosis for patients with variant angina and influential factors. *Circulation* 1988;**78**:1–9.

59. Ishii M, Kaikita K, Sato K, Tanaka T, Sugamura K, Sakamoto K, Izumiya Y, Yamamoto E, Tsujita K, Yamamuro M, Kojima S, Soejima H, Hokimoto S, Matsui K, Ogawa H. Acetylcholine-Provoked Coronary Spasm at Site of Significant Organic Stenosis Predicts Poor Prognosis in Patients With Coronary Vasospastic Angina. *J Am Coll Cardiol* 2015;**66**:1105–1115.

60. Shiomi M, Ishida T, Kobayashi T, Nitta N, Sonoda A, Yamada S, Koike T, Kuniyoshi N, Murata K, Hirata K, Ito T, Libby P. Vasospasm of Atherosclerotic Coronary Arteries Precipitates Acute Ischemic Myocardial Damage in Myocardial Infarction–Prone Strain of the Watanabe Heritable Hyperlipidemic Rabbits. *Arterioscler Thromb Vasc Biol* 2013;**33**:2518–2523.

61. Montone RA, Niccoli G, Fracassi F, Russo M, Gurgoglione F, Cammà G, Lanza GA, Crea F. Patients with acute myocardial infarction and non-obstructive coronary arteries: safety and prognostic relevance of invasive coronary provocative tests. *Eur Heart J* 2017.

62. Everaars H, Waard GA de, Driessen RS, Danad I, Ven PM van de, Raijmakers PG, Lammertsma AA, Rossum AC van, Knaapen P, Royen N van. Doppler Flow Velocity and Thermodilution to Assess Coronary Flow Reserve. *JACC Cardiovasc Interv* 2018;**11**:2044–2054.

63. Hakeem A, Cilingiroglu M, Leesar MA. Hemodynamic and intravascular ultrasound assessment of myocardial bridging: Fractional flow reserve paradox with dobutamine versus adenosine. *Catheterization and Cardiovascular Interventions* 2010;**75**:229–236.

64. Escaned J, Cortés J, Flores A, Goicolea J, Alfonso F, Hernández R, Fernández-Ortiz A, Sabaté M, Bañuelos C, Macaya C. Importance of diastolic fractional flow reserve and dobutamine challenge in physiologic assessment of myocardial bridging. *J Am Coll Cardiol* 2003;**42**:226–233.

65. Tarantini G, Barioli A, Nai Fovino L, Fraccaro C, Masiero G, Iliceto S, Napodano M. Unmasking Myocardial Bridge–Related Ischemia by Intracoronary Functional Evaluation. *Circ Cardiovasc Interv* 2018;**11**.

66. Aleksandric SB, Djordjevic-Dikic AD, Dobric MR, Giga VL, Soldatovic IA, Vukcevic V, Tomasevic M V., Stojkovic SM, Orlic DN, Saponjski JD, Tesic MB, Banovic MD, Petrovic MT, Juricic SA, Nedeljkovic MA, Stankovic G, Ostojic MC, Beleslin BD. Functional Assessment of Myocardial Bridging With Conventional and Diastolic Fractional Flow Reserve: Vasodilator Versus Inotropic Provocation. *J Am Heart Assoc* 2021;**10**.

67. Ong P, Athanasiadis A, Borgulya G, Vokshi I, Bastiaenen R, Kubik S, Hill S, Schäufele T, Mahrholdt H, Kaski JC, Sechtem U. Clinical Usefulness, Angiographic Characteristics, and Safety Evaluation of Intracoronary Acetylcholine Provocation Testing Among 921 Consecutive White Patients With Unobstructed Coronary Arteries. *Circulation* 2014;**129**:1723–1730.

68. Perera D, Berry C, Hoole SP, Sinha A, Rahman H, Morris PD, Kharbanda RK, Petraco R, Channon K. Invasive coronary physiology in patients with angina and non-obstructive coronary artery disease: a consensus document from the coronary

microvascular dysfunction workstream of the British Heart Foundation/National Institute for Health Research Partnership. *Heart* 2023;**109**:88–95.

69. Okumura K, Yasue H, Matsuyama K, Goto K, Miyag H, Ogawa H, Matsuyama K. Sensitivity and specificity of intracoronary injection of acetylcholine for the induction of coronary artery spasm. *J Am Coll Cardiol* 1988;**12**:883–888.

70. Beijk MA, Vlastra W V., Delewi R, Hoef TP van de, Boekholdt SM, Sjauw KD, Piek JJ. Myocardial infarction with non-obstructive coronary arteries: a focus on vasospastic angina. *Netherlands Heart Journal* 2019;**27**:237–245.

71. Guidelines for Diagnosis and Treatment of Patients With Vasospastic Angina (Coronary Spastic Angina) (JCS 2013). *Circulation Journal* 2014;**78**:2779–2801.

72. Sueda S, Kohno H. The acetylcholine administration time plays the key role for provoked spasm in the spasm provocation test. *J Cardiol* 2017;**70**:141–146.

73. Sueda S, Kohno H, Miyoshi T, Sakaue T, Sasaki Y, Habara H. Maximal acetylcholine dose of 200 μg into the left coronary artery as a spasm provocation test: comparison with 100 μg of acetylcholine. *Heart Vessels* 2015;**30**:771–778.

74. Konst RE, Damman P, Pellegrini D, Hartzema-Meijer MJ, Uden BJC van, Jansen TPJ, Brandsma J, Vart P, Gehlmann H, Maas AHEM, Royen N van, Elias-Smale SE. Vasomotor dysfunction in patients with angina and nonobstructive coronary artery disease is dominated by vasospasm. *Int J Cardiol* 2021;**333**:14–20.

75. Newman CM, Maseri A, Hackett DR, El-Tamimi HM, Davies GJ. Response of angiographically normal and atherosclerotic left anterior descending coronary arteries to acetylcholine. *Am J Cardiol* 1990;**66**:1070–1076.

76. Reynolds HR, Bairey Merz CN, Berry C, Samuel R, Saw J, Smilowitz NR, Souza AC do AH de, Sykes R, Taqueti VR, Wei J. Coronary Arterial Function and Disease in Women With No Obstructive Coronary Arteries. *Circ Res* 2022;**130**:529–551.

77. Gibbons RJ, Balady GJ, Beasley JW, FAAFP, Bricker JT, Duvernoy WFC, Froelicher VF, Mark DB, Marwick TH, McCallister BD, Thompson PD, FACSM, Winters WL, Yanowitz FG. ACC/AHA Guidelines for Exercise Testing: Executive Summary. *Circulation* 1997;**96**:345–354.

78. Gibbons RJ, Balady GJ, Timothy Bricker J, Chaitman BR, Fletcher GF, Froelicher VF, Mark DB, McCallister BD, Mooss AN, O'Reilly MG, Winters WL, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Hiratzka LF, Jacobs AK, Russell RO, Smith SC. ACC/AHA 2002 Guideline Update for Exercise Testing: Summary Article. *Circulation* 2002;**106**:1883–1892.

79. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, Prescott E, Storey RF, Deaton C, Cuisset T, Agewall S, Dickstein K, Edvardsen T, Escaned J, Gersh BJ, Svitil P, Gilard M, Hasdai D, Hatala R, Mahfoud F, Masip J, Muneretto C, Valgimigli M, Achenbach S, Bax JJ, Neumann F-J, Sechtem U, Banning AP, Bonaros N, Bueno H, Bugiardini R, Chieffo A, Crea F, Czerny M, Delgado V, Dendale P, Flachskampf FA, Gohlke H, Grove EL, James S, Katritsis D, Landmesser U, Lettino M, Matter CM, Nathoe H, Niessner A, Patrono C, Petronio AS, Pettersen SE, Piccolo R, Piepoli MF, Popescu BA, Räber L, Richter DJ, Roffi M, Roithinger FX, Shlyakhto E, Sibbing D, Silber S, Simpson IA, Sousa-Uva M, Vardas P, Witkowski A, Zamorano JL, Achenbach S, Agewall S, Barbato E, Bax JJ, Capodanno D, Cuisset T, Deaton C, Dickstein K, Edvardsen T, Escaned J, Funck-Brentano C, Gersh BJ, Gilard M, Hasdai D, Hatala R, Mahfoud F, Masip J, Muneretto C, Prescott E, Saraste A, Storey RF, Svitil

P, Valgimigli M, Windecker S, Aboyans V, Baigent C, Collet J-P, Dean V, Delgado V, Fitzsimons D, Gale CP, Grobbee D, Halvorsen S, Hindricks G, Iung B, Jüni P, Katus HA, Landmesser U, Leclercq C, Lettino M, Lewis BS, Merkely B, Mueller C, Petersen S, Petronio AS, Richter DJ, Roffi M, Shlyakhto E, Simpson IA, Sousa-Uva M, Touyz RM, Benkhedda S, Metzler B, Sujayeva V, Cosyns B, Kusljugic Z, Velchev V, Panayi G, Kala P, Haahr-Pedersen SA, Kabil H, Ainla T, Kaukonen T, Cayla G, Pagava Z, Woehrle J, Kanakakis J, Tóth K, Gudnason T, Peace A, Aronson D, Riccio C, Elezi S, Mirrakhimov E, Hansone S, Sarkis A, Babarskiene R, Beissel J, Maempel AJC, Revenco V, Grooth GJ de, Pejkov H, Juliebø V, Lipiec P, Santos J, Chioncel O, Duplyakov D, Bertelli L, Dikic AD, Studenčan M, Bunc M, Alfonso F, Bäck M, Zellweger M, Addad F, Yildirir A, Sirenko Y, Clapp B. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J* 2020;**41**:407–477.

80. Lumley M, Williams R, Asrress KN, Arri S, Briceno N, Ellis H, Rajani R, Siebes M, Piek JJ, Clapp B, Redwood SR, Marber MS, Chambers JB, Perera D. Coronary Physiology During Exercise and Vasodilation in the Healthy Heart and in Severe Aortic Stenosis. *J Am Coll Cardiol* 2016;**68**:688–697.

81. Perera D. Cardiac-Coronary Coupling ∗. *J Am Coll Cardiol* 2016;**68**:1661–1663.

82. Davies JE, Whinnett ZI, Francis DP, Willson K, Foale RA, Malik IS, Hughes AD, Parker KH, Mayet J. Use of simultaneous pressure and velocity measurements to estimate arterial wave speed at a single site in humans. *American Journal of Physiology-Heart and Circulatory Physiology* 2006;**290**:H878–H885.

83. Davies JE, Whinnett ZI, Francis DP, Manisty CH, Aguado-Sierra J, Willson K, Foale RA, Malik IS, Hughes AD, Parker KH, Mayet J. Evidence of a Dominant Backward-

Propagating “Suction” Wave Responsible for Diastolic Coronary Filling in Humans, Attenuated in Left Ventricular Hypertrophy. *Circulation* 2006;**113**:1768–1778.

84. Lockie TPE, Rolandi MC, Guilcher A, Perera D, Silva K De, Williams R, Asrress KN, Patel K, Plein S, Chowienczyk P, Siebes M, Redwood SR, Marber MS. Synergistic Adaptations to Exercise in the Systemic and Coronary Circulations That Underlie the Warm-Up Angina Phenomenon. *Circulation* 2012;**126**:2565–2574.

85. Silva K De, Lumley M, Kailey B, Alastruey J, Guilcher A, Asrress KN, Plein S, Marber M, Redwood S, Perera D. Coronary and Microvascular Physiology During Intra-Aortic Balloon Counterpulsation. *JACC Cardiovasc Interv* 2014;**7**:631–640.

86. Sidhu BS, Claridge S, Gu H, Li Y, Gould J, Porter B, Elliott MK, Mehta V, Jackson T, Patterson T, Briceno N, Lee J, Redwood S, Adhya S, Niederer SA, Chowienczyk P, Rinaldi CA. The physiological effects of cardiac resynchronization therapy on aortic and pulmonary flow and dynamic and static components of systemic impedance. *Heart Rhythm O2* 2021;**2**:365–373.

87. Labazi H, Trask AJ. Coronary microvascular disease as an early culprit in the pathophysiology of diabetes and metabolic syndrome. *Pharmacol Res* 2017;**123**:114–121.

88. Gullu H, Caliskan M, Ciftci O, Erdogan D, Topcu S, Yildirim E, Yildirir A, Muderrisoglu H. Light cigarette smoking impairs coronary microvascular functions as severely as smoking regular cigarettes. *Heart* 2007;**93**:1274–1277.

89. Bairey Merz CN, Handberg EM, Shufelt CL, Mehta PK, Minissian MB, Wei J, Thomson LEJ, Berman DS, Shaw LJ, Petersen JW, Brown GH, Anderson RD, Shuster JJ, Cook-Wiens G, Rogatko A, Pepine CJ. A randomized, placebo-controlled trial of late Na

current inhibition (ranolazine) in coronary microvascular dysfunction (CMD): impact on angina and myocardial perfusion reserve. *Eur Heart J* 2016;**37**:1504–1513.

90. Koh J-S, Hung OY, Eshtehardi P, Kumar A, Rabah R, Raad M, Kumar S, Chaudhry S, Gupta S, Hosseini H, Brilakis E, Corban M, Sabbak N, Burnett GM, Liu C, Mehta PK, Quyyumi AA, Samady H. Microvascular Assessment of Ranolazine in Non-Obstructive Atherosclerosis. *Circ Cardiovasc Interv* 2020;**13**.

91. Jansen TPJ, Konst RE, Vos A de, Paradies V, Teerenstra S, Oord SCH van den, Dimitriu-Leen A, Maas AHEM, Smits PC, Damman P, Royen N van, Elias-Smale SE. Efficacy of Diltiazem to Improve Coronary Vasomotor Dysfunction in ANOCA. *JACC Cardiovasc Imaging* 2022;**15**:1473–1484.

92. Corban MT, Toya T, Albers D, Sebaali F, Lewis BR, Bois J, Gulati R, Prasad A, Best PJM, Bell MR, Rihal CS, Prasad M, Ahmad A, Lerman LO, Solseth ML, Winters JL, Dietz AB, Lerman A. IMPROvE-CED Trial: Intracoronary Autologous CD34+ Cell Therapy for Treatment of Coronary Endothelial Dysfunction in Patients With Angina and Nonobstructive Coronary Arteries. *Circ Res* 2022;**130**:326–338.

93. Henry TD, Bairey Merz CN, Wei J, Corban MT, Quesada O, Joung S, Kotynski CL, Wang J, Lewis M, Schumacher AM, Bartel RL, Takagi H, Shah V, Lee A, Sietsema WK, Losordo DW, Lerman A. Autologous CD34+ Stem Cell Therapy Increases Coronary Flow Reserve and Reduces Angina in Patients With Coronary Microvascular Dysfunction. *Circ Cardiovasc Interv* 2022;**15**.

94. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd

KG, Berry C. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina. *J Am Coll Cardiol* 2018;**72**:2841–2855.

95. Ford TJ, Stanley B, Sidik N, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, McCartney P, Corcoran D, Collison D, Rush C, Sattar N, McConnachie A, Touyz RM, Oldroyd KG, Berry C. 1-Year Outcomes of Angina Management Guided by Invasive Coronary Function Testing (CorMicA). *JACC Cardiovasc Interv* 2020;**13**:33–45.

96. Heggie R, Briggs A, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Collison D, Oldroyd K, Ford TJ, Berry C. Stratified medicine using invasive coronary function testing in angina: A cost-effectiveness analysis of the British Heart Foundation CorMicA trial. *Int J Cardiol* 2021;**337**:44–51.

97. Marroquin OC, Holubkov R, Edmundowicz D, Rickens C, Pohost G, Buchthal S, Pepine CJ, Sopko G, Sembrat RC, Meltzer CC, Reis SE. Heterogeneity of microvascular dysfunction in women with chest pain not attributable to coronary artery disease: Implications for clinical practice. *Am Heart J* 2003;**145**:628–635.

98. Montone RM, Rinaldi R, Buono MG Del, Gurgoglione F, Vecchia G La, Russo M, Caffè A, Burzotta F, Leone AM, Romagnoli E, Sanna T, Pelargonio G, Trani C, Lanza GA, Niccoli G, Crea F. Safety and prognostic relevance of acetylcholine testing in patients with stable myocardial ischaemia or myocardial infarction and non-obstructive coronary arteries. *EuroIntervention* 2022;**18**:e666–e676.

99. Reis SE, Holubkov R, Lee JS, Sharaf B, Reichek N, Rogers WJ, Walsh EG, Fuisz AR, Kerensky R, Detre KM, Sopko G, Pepine CJ. Coronary flow velocity response to

adenosine characterizes coronary microvascular function in women with chest pain and no obstructive coronary disease. *J Am Coll Cardiol* 1999;**33**:1469–1475.

100. Sen S, Petraco R, Mayet J, Davies J. Wave Intensity Analysis in the Human Coronary Circulation in Health and Disease. *Curr Cardiol Rev* 2014;**10**:17–23.

101. Al-Lamee R, Thompson D, Dehbi H-M, Sen S, Tang K, Davies J, Keeble T, Mielewczik M, Kaprielian R, Malik IS, Nijjer SS, Petraco R, Cook C, Ahmad Y, Howard J, Baker C, Sharp A, Gerber R, Talwar S, Assomull R, Mayet J, Wensel R, Collier D, Shun-Shin M, Thom SA, Davies JE, Francis DP, Al-Lamee R, Thompson D, Sen S, Tang K, Davies J, Keeble T, Kaprielian R, Malik IS, Nijjer SS, Petraco R, Cook C, Ahmad Y, Howard J, Shun-Shin M, Sethi A, Baker C, Sharp A, Ramrakha P, Gerber R, Talwar S, Assomull R, Foale R, Mayet J, Wensel R, Thom SA, Davies JE, Francis DP, Khamis R, Hadjiloizou N, Khan M, Kooner J, Bellamy M, Mikhail G, Clifford P, O'Kane P, Levy T, Swallow R. Percutaneous coronary intervention in stable angina (ORBITA): a double-blind, randomised controlled trial. *The Lancet* 2018;**391**:31–40.

102. Fox KM, Mulcahy D, Findlay I, Ford I, Dargie HJ. The Total Ischaemic Burden European Trial (TIBET): Effects of atenolol, nifedipine SR and their combination on the exercise test and the total ischaemic burden in 608 patients with stable angina. *Eur Heart J* 1996;**17**:96–103.

103. Frishman WH, Glasser S, Stone P, Deedwania PC, Johnson M, Fakouhi TD. Comparison of controlled-onset, extended-release verapamil with amlodipine and amlodipine plus atenolol on exercise performance and ambulatory ischemia in patients with chronic stable angina pectoris. *Am J Cardiol* 1999;**83**:507–514.

104. Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, Pepine CJ, Wang W, Nelson JJ, Hebert DA, Wolff AA. Anti-ischemic effects and long-term survival

during ranolazine monotherapy in patients with chronic severe angina. *J Am Coll Cardiol* 2004;**43**:1375–1382.

105. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. *The Lancet* 2010;**375**:2161–2167.

106. Spertus JA, Winder JA, Dewhurst TA, Deyo RA, Prodzinski J, McDonnell M, Fihn SD. Development and evaluation of the Seattle Angina questionnaire: A new functional status measure for coronary artery disease. *J Am Coll Cardiol* 1995;**25**:333–341.

107. Mozaffarian D, Bryson CL, Spertus JA, McDonell MB, Fihn SD. Anginal symptoms consistently predict total mortality among outpatients with coronary artery disease. *Am Heart J* 2003;**146**:1015–1022.

108. Chaitman BR. Effects of Ranolazine With Atenolol, Amlodipine, or Diltiazem on Exercise Tolerance and Angina Frequency in Patients With Severe Chronic Angina<SUBTITLE>A Randomized Controlled Trial</SUBTITLE> *JAMA* 2004;**291**:309.

109. Modi BN, Rahman H, Arri S, Ellis H, Mills MT, Williams R, Asrress K, Clapp B, Redwood S, Perera D. Resting Coronary Flow Varies With Normal Cardiac Catheter Laboratory Stimuli. *Cardiovascular Revascularization Medicine* 2019;**20**:669–673.

110. Mileva N, Nagumo S, Mizukami T, Sonck J, Berry C, Gallinoro E, Monizzi G, Candreva A, Munhoz D, Vassilev D, Penicka M, Barbato E, Bruyne B De, Collet C. Prevalence of Coronary Microvascular Disease and Coronary Vasospasm in Patients With Nonobstructive Coronary Artery Disease: Systematic Review and Meta-Analysis. *J Am Heart Assoc* 2022;**11**.

111. Cassar A, Chareonthaitawee P, Rihal CS, Prasad A, Lennon RJ, Lerman LO, Lerman A. Lack of Correlation Between Noninvasive Stress Tests and Invasive Coronary Vasomotor Dysfunction in Patients With Nonobstructive Coronary Artery Disease. *Circ Cardiovasc Interv* 2009;**2**:237–244.

112. Malhotra R, Indrayan A. A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. *Indian J Ophthalmol* 2010;**58**:519.

113. Kaski JC, Collins P, Nihoyannopoulos P, Maseri A, Poole-Wilson PA, Rosano GMC. Cardiac syndrome X: Clinical characteristics and left ventricular function. *J Am Coll Cardiol* 1995;**25**:807–814.

114. Vandeloo B, Andreini D, Brouwers S, Mizukami T, Monizzi G, Lochy S, Mileva N, Argacha J-F, Boulle M De, Muyldermans P, Belmonte M, Sonck J, Gallinoro E, Munhoz D, Roosens B, Trabattoni D, Galli S, Seki R, Penicka M, Wyffels E, Mushtaq S, Nagumo S, Pardaens S, Barbato E, Bartorelli AL, Bruyne B De, Cosyns B, Collet C. Diagnostic performance of exercise stress tests for detection of epicardial and microvascular coronary artery disease: the UZ Clear study. *EuroIntervention* 2023;**18**:e1090–e1098.

115. Pargaonkar VS, Kobayashi Y, Kimura T, Schnittger I, Chow EKH, Froelicher VF, Rogers IS, Lee DP, Fearon WF, Yeung AC, Stefanick ML, Tremmel JA. Accuracy of non-invasive stress testing in women and men with angina in the absence of obstructive coronary artery disease. *Int J Cardiol* 2019;**282**:7–15.

116. Lopez DM, Divakaran S, Gupta A, Bajaj NS, Osborne MT, Zhou W, Hainer J, Bibbo CF, Skali H, Dorbala S, Taqueti VR, Blankstein R, Carli MF Di. Role of Exercise Treadmill Testing in the Assessment of Coronary Microvascular Disease. *JACC Cardiovasc Imaging* 2022;**15**:312–321.

117. Ford TJ, Yii E, Sidik N, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, McCartney P, Corcoran D, Collison D, Rush C, Stanley B, McConnachie A, Sattar N, Touyz RM, Oldroyd KG, Berry C. Ischemia and No Obstructive Coronary Artery Disease. *Circ Cardiovasc Interv* 2019;**12**.

118. Wei J, Mehta PK, Johnson BD, Samuels B, Kar S, Anderson RD, Azarbal B, Petersen J, Sharaf B, Handberg E, Shufelt C, Kothawade K, Sopko G, Lerman A, Shaw L, Kelsey SF, Pepine CJ, Merz CNB. Safety of Coronary Reactivity Testing in Women With No Obstructive Coronary Artery Disease. *JACC Cardiovasc Interv* 2012;**5**:646–653.

119. Takahashi T, Samuels BA, Li W, Parikh MA, Wei J, Moses JW, Fearon WF, Henry TD, Tremmel JA, Kobayashi Y. Safety of Provocative Testing With Intracoronary Acetylcholine and Implications for Standard Protocols. *J Am Coll Cardiol* 2022;**79**:2367–2378.

120. Kayikcioglu M. Benefits of statin treatment in cardiac syndrome-X. *Eur Heart J* 2003;**24**:1999–2005.

121. Tavella R, Cutri N, Tucker G, Adams R, Spertus J, Beltrame JF. Natural history of patients with insignificant coronary artery disease. *Eur Heart J Qual Care Clin Outcomes* 2016;**2**:117–124.

122. Senn StephenS. Cross-over Trials in Clinical Research. 2nd Edition, Chichester, UK: John Wiley & Sons, Ltd. *J R Stat Soc Ser A Stat Soc* 2002;**156**.

123. Jansen TPJ, Konst RE, Vos A de, Paradies V, Teerenstra S, Oord SCH van den, Dimitriu-Leen A, Maas AHEM, Smits PC, Damman P, Royen N van, Elias-Smale SE. Efficacy of Diltiazem to Improve Coronary Vasomotor Dysfunction in ANOCA. *JACC Cardiovasc Imaging* 2022;**15**:1473–1484.

124. Koh J-S, Hung OY, Eshtehardi P, Kumar A, Rabah R, Raad M, Kumar S, Chaudhry S, Gupta S, Hosseini H, Brilakis E, Corban M, Sabbak N, Burnett GM, Liu C, Mehta PK, Quyyumi AA, Samady H. Microvascular Assessment of Ranolazine in Non-Obstructive Atherosclerosis. *Circ Cardiovasc Interv* 2020;**13**.

125. Rouleau JL, Pfeffer MA, Stewart DJ, Isaac D, Sestier F, Kerut EK, Porter CB, Proulx G, Qian C, Block AJ. Comparison of vasopeptidase inhibitor, omapatrilat, and lisinopril on exercise tolerance and morbidity in patients with heart failure: IMPRESS randomised trial. *The Lancet* 2000;**356**:615–620.

126. Randomised, placebo-controlled trial of carvedilol in patients with congestive heart failure due to ischaemic heart disease. *The Lancet* 1997;**349**:375–380.

127. Reynolds HR, Picard MH, Spertus JA, Peteiro J, Lopez Sendon JL, Senior R, El-Hajjar MC, Celutkiene J, Shapiro MD, Pellikka PA, Kunichoff DF, Anthopolos R, Alfakih K, Abdul-Nour K, Khouri M, Bershtein L, Belder M De, Poh KK, Beltrame JF, Min JK, Fleg JL, Li Y, Maron DJ, Hochman JS. Natural History of Patients With Ischemia and No Obstructive Coronary Artery Disease. *Circulation* 2021;**144**:1008–1023.

128. Spertus JA, Jones PG, Maron DJ, O'Brien SM, Reynolds HR, Rosenberg Y, Stone GW, Harrell FE, Boden WE, Weintraub WS, Baloch K, Mavromatis K, Diaz A, Gosselin G, Newman JD, Mavromichalis S, Alexander KP, Cohen DJ, Bangalore S, Hochman JS, Mark DB. Health-Status Outcomes with Invasive or Conservative Care in Coronary Disease. *New England Journal of Medicine* 2020;**382**:1408–1419.

129. Al-Lamee R, Howard JP, Shun-Shin MJ, Thompson D, Dehbi H-M, Sen S, Nijjer S, Petraco R, Davies J, Keeble T, Tang K, Malik IS, Cook C, Ahmad Y, Sharp ASP, Gerber R, Baker C, Kaprielian R, Talwar S, Assomull R, Cole G, Keenan NG, Kanaganayagam G, Sehmi J, Wensel R, Harrell FE, Mayet J, Thom SA, Davies JE, Francis DP. Fractional

Flow Reserve and Instantaneous Wave-Free Ratio as Predictors of the Placebo-Controlled Response to Percutaneous Coronary Intervention in Stable Single-Vessel Coronary Artery Disease. *Circulation* 2018;**138**:1780–1792.

130. Mozaffarian D, Bryson CL, Spertus JA, McDonell MB, Fihn SD. Anginal symptoms consistently predict total mortality among outpatients with coronary artery disease. *Am Heart J* 2003;**146**:1015–1022.

131. Wouw J van de, Sorop O, Drie RWA van, Duin RWB van, Nguyen ITN, Joles JA, Verhaar MC, Merkus D, Duncker DJ. Perturbations in myocardial perfusion and oxygen balance in swine with multiple risk factors: a novel model of ischemia and no obstructive coronary artery disease. *Basic Res Cardiol* 2020;**115**:21.

132. Shabeeh H, Melikian N, Dworakowski R, Casadei B, Chowienczyk P, Shah AM. Differential role of endothelial versus neuronal nitric oxide synthase in the regulation of coronary blood flow during pacing-induced increases in cardiac workload. *American Journal of Physiology-Heart and Circulatory Physiology* 2013;**304**:H1277–H1282.

133. Nardone M, McCarthy M, Ardern CI, Nield LE, Toleva O, Cantor WJ, Miner SES. Concurrently Low Coronary Flow Reserve and Low Index of Microvascular Resistance Are Associated With Elevated Resting Coronary Flow in Patients With Chest Pain and Nonobstructive Coronary Arteries. *Circ Cardiovasc Interv* 2022;**15**.

134. Clarke B. Ranolazine Increases Active Pyruvate Dehydrogenase in Perfused Normoxic Rat Hearts: Evidence for an Indirect Mechanism. *J Mol Cell Cardiol* 1996;**28**:341–350.

135. Hayashida W, Eyll C van, Rousseau MF, Pouleur H. Effects of ranolazine on left ventricular regional diastolic function in patients with ischemic heart disease. *Cardiovasc Drugs Ther* 1994;**8**:741–747.

136. Lerman A, Burnett JC, Higano ST, McKinley LJ, Holmes DR. Long-term <scp>l</scp> -Arginine Supplementation Improves Small-Vessel Coronary Endothelial Function in Humans. *Circulation* 1998;**97**:2123–2128.

137. Reriani M, Raichlin E, Prasad A, Mathew V, Pumper GM, Nelson RE, Lennon R, Rihal C, Lerman LO, Lerman A. Long-Term Administration of Endothelin Receptor Antagonist Improves Coronary Endothelial Function in Patients With Early Atherosclerosis. *Circulation* 2010;**122**:958–966.

138. Grutta L La, Runza G, Re G Lo, Galia M, Alaimo V, Grassedonio E, Bartolotta TV, Malagò R, Tedeschi C, Cademartiri F, Maria M De, Cardinale AE, Lagalla R, Midiri M. Prevalence of myocardial bridging and correlation with coronary atherosclerosis studied with 64-slice CT coronary angiography. *Radiol Med* 2009;**114**:1024–1036.

139. Pargaonkar VS, Kimura T, Kameda R, Tanaka S, Yamada R, Schwartz JG, Perl L, Rogers IS, Honda Y, Fitzgerald P, Schnittger I, Tremmel JA. Invasive assessment of myocardial bridging in patients with angina and no obstructive coronary artery disease. *EuroIntervention* 2021;**16**:1070–1078.

140. Lin S, Tremmel JA, Yamada R, Rogers IS, Yong CM, Turcott R, McConnell M V., Dash R, Schnittger I. A Novel Stress Echocardiography Pattern for Myocardial Bridge With Invasive Structural and Hemodynamic Correlation. *J Am Heart Assoc* 2013;**2**.

141. Sternheim D, Power DA, Samtani R, Kini A, Fuster V, Sharma S. Myocardial Bridging: Diagnosis, Functional Assessment, and Management. *J Am Coll Cardiol* 2021;**78**:2196–2212.

142. Sara JDS, Corban MT, Prasad M, Prasad A, Gulati R, Lerman LO, Lerman A. Prevalence of myocardial bridging associated with coronary endothelial dysfunction in

patients with chest pain and non-obstructive coronary artery disease. *EuroIntervention* 2020;**15**:1262–1268.

143. Kim PJ, Hur G, Kim SY, Namgung J, Hong SW, Kim YH, Lee WR. Frequency of Myocardial Bridges and Dynamic Compression of Epicardial Coronary Arteries. *Circulation* 2009;**119**:1408–1416.

144. Schwarz ER, Klues HG, Dahl J vom, Klein I, Krebs W, Hanrath P. Functional, angiographic and intracoronary doppler flow characteristics in symptomatic patients with myocardial bridging: Effect of short-term intravenous beta-blocker medication. *J Am Coll Cardiol* 1996;**27**:1637–1645.

145. Ishikawa Y, Ishii T, Asuwa N, Masuda S. Absence of atherosclerosis evolution in the coronary arterial segment covered by myocardial tissue in cholesterol-fed rabbits. *Virchows Archiv* 1997;**430**:163–171.

146. Forsdahl SH, Rogers IS, Schnittger I, Tanaka S, Kimura T, Pargaonkar VS, Chan FP, Fleischmann D, Tremmel JA, Becker H-C. Myocardial Bridges on Coronary Computed Tomography Angiography ― Correlation With Intravascular Ultrasound and Fractional Flow Reserve ―. *Circulation Journal* 2017;**81**:1894–1900.

147. Gould KL, Johnson NP. Myocardial Bridges: Lessons in Clinical Coronary Pathophysiology. *JACC Cardiovasc Imaging* 2015;**8**:705–709.

148. Boyd JH, Pargaonkar VS, Scoville DH, Rogers IS, Kimura T, Tanaka S, Yamada R, Fischbein MP, Tremmel JA, Mitchell RS, Schnittger I. Surgical Unroofing of Hemodynamically Significant Left Anterior Descending Myocardial Bridges. *Ann Thorac Surg* 2017;**103**:1443–1450.

149. Nanna MG, Vemulapalli S, Fordyce CB, Mark DB, Patel MR, Al-Khalidi HR, Kelsey M, Martinez B, Yow E, Mullen S, Stone GW, Ben-Yehuda O, Udelson JE, Rogers C, Douglas PS. The prospective randomized trial of the optimal evaluation of cardiac symptoms and revascularization: Rationale and design of the PRECISE trial. *Am Heart J* 2022;**245**:136–148.