ISCHAEMIA WITH NO OBSTRUCTIVE CORONARY ARTERY DISEASE (INOCA): INSIGHTS INTO ASSESSMENT AND OBSTRUCTIVE SLEEP APNOEA ASSOCIATION

By

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Dedication

This thesis is dedicated to my dearest mother Ooi Hooi Eng, late father Ng Ah Bah @ Ooi Gim Chooi, my beautiful and loving wife Lien Sea Tee and to my incredible children Bao Yun Grace Ooi and Yu Ze Alexander Ooi.

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Abstract

The evolution of myocardial ischaemia started with clinicopathological correlation of typical angina symptoms, traditionally, with flow-limiting atherosclerotic coronary artery disease (CAD). Notwithstanding, ischaemia with no obstructive coronary artery disease (INOCA) is increasingly recognised as a separate phenomenon, that impacts cardiac-related morbidity and financial burden on health services.[1-6] Obstructive sleep apnoea (OSA) is recognized to be a risk factor of cardiovascular disease through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances.[7-12] This thesis evaluates OSA as a risk factor of INOCA and explore the prospect of assessing INOCA non-invasively via advanced echocardiography.

Literatures review in chapter 1 provide comprehensive understanding of CAD, including the latest definition of chronic coronary syndrome (CCS), basic mechanisms of coronary ischaemia, the prevalence, prognosis and diagnosis of INOCA, with emerging invasive coronary physiology assessment and potential non-invasive technology. The current evidence illustrating the potential commonality in mechanisms and association of OSA with INOCA, is explored. Chapter 2 focused on more detail, of the association between OSA and structural and functional CAD. OSA is independently associated with CAD. The pathogenesis of atherosclerosis remains complex and poorly understood. CPAP therapy has been linked with reduction in major adverse cardiovascular events in a recent meta-analysis, albeit pivotal randomised controlled trials failed to demonstrate its significance. INOCA, a relatively new entity, remains unexplored in its association with OSA nor the effects of CPAP therapy.

In chapter 3, information of the prevalence and clinical predictors of OSA in patients with anginal symptoms who have undergone coronary angiogram in a South Australia registry is provided. This study shows INOCA to be an independent predictor of OSA; especially in those presenting with stable angina; in addition to established risk factors for OSA.

In chapter 4, we examined the relationship of obstructive sleep apnoea in patients experiencing angina with no obstructive coronary artery disease, with invasive coronary physiology study as a pilot study.

The next two chapters are aimed to determine the ability to evaluate INOCA non-invasively with advanced echocardiography, given the invasiveness of current diagnostic criteria requiring instrumentations of the apparently 'normal' coronary arteries. Chapter 5 demonstrates the importance of establishing the extent of myocardial ischaemia in the diagnosis, management and prognostication of coronary artery disease. Stress echocardiography modality is easily available, cost effective and radiation free. Myocardial ischaemia prediction, utilising non-invasive speckle tracking derived global longitudinal strain (GLS), providing a novel prospect.[13,14] Chapter 6 provides a pilot study information on correlation between GLS derived contractile reserve (CR) and the invasive coronary microvascular measures, specifically coronary flow reserve (CFR) and hyperaemic microvascular resistance (HMR), in INOCA. This study found that there were more CR at 5mcg/kg/min of dobutamine infusion between normal and abnormal coronary haemodynamic indices which suggest hibernating myocardium contractility recruitment. This was not seen on the higher dose of dobutamine. However, the results were limited by small numbers and echocardiographic technique remain at the mercy of suitable image quality.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chapter 2 – Obstructive Sleep Apnoea and Coronary Artery Disease

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Abbreviations

2D	Two-dimensional
3D	Three-dimensional
ACh	Acetylcholine
ACS	Acute coronary syndrome
ACTRN	Australia New Zealand Clinical Trials Registry number
AHI	Apnoea, hypopnoea index
APV	Average peak velocity
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
CI	Confidence intervals
CK	Creatinine kinase
CR	Contractile reserve
CS	Circumferential strain
CW	Constructive myocardial work
CAD	Coronary artery disease
CBF	Coronary artery blood flow
CCA	Calcium channel antagonists
CCS	Chronic coronary syndrome
CFR	Coronary flow reserve
CMD	Coronary microvascular disorder
CMR	Cardiac magnetic resonance
CTA	Computed tomographic angiography

CVD	Coronary vasomotor disorder
CPAP	Continuous positive airway pressure
CSFP	Coronary slow flow phenomena
CTCA	Computed tomography coronary angiography
CADOSA	Coronary Angiogram Database of South Australia
CASPAR	Coronary artery spasm in patients with acute coronary syndrome study
CorMicA	CORonary MICrovascular Angina study
COVADIS	Coronary Vasomotor Disorders International Study Group
DSE	Dobutamine stress echocardiography
EF	Ejection fraction
ECG	Electrocardiogram
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
ESC	European Society of Cardiology
f/s	Frames per second
FT	Feature tracking
FFR	Fractional flow reserve
FVC	Forced vital capacity
FEV1	Forced expiratory volume in 1 second
GAS	Global area strain
GLS	Global longitudinal strain
GWE	Global myocardial work efficiency
GWW	Global wasted myocardial work
HF	Heart failure

HMR	Hyperaemic microvascular resistance
HREC	Human Research Ethics Committee
hsTrop	High-sensitive troponin
IC	Intracoronary
IH	Intermittent hypoxia
IBM	International Business Machines Corporation
IHD	Ischemic heart disease
IMR	Index of microcirculatory resistance
ICSD	International Classification of Sleep Disorders
INOCA	Ischaemia with no obstructive coronary artery disease
iPOWER	ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease study
ISAACC	Impact of Sleep Apnoea syndrome in the evolution of Acute Coronary syndrome study
JCSA	Japanese Coronary Spasm Association
l	Length
LS	Longitudinal strain
LV	Left ventricle
LCx	Left circumflex coronary artery
LAD	Left anterior descending coronary artery
LBBB	Left bundle branch block
LDL-C	Low density lipoprotein cholesterol
MW	Myocardial work
MCE	Myocardial contrast echocardiography
MVA	Microvascular angina

MVD	Microvascular dysfunction
MVO_2	Myocardial oxygen consumption
MACE	Major adverse cardiac events
mol/L	Molar per litre
MACCE	Major adverse cardiac and cerebrovascular events
µg/min	Microgram per minute
µg/kg/min	Microgram per kilogram per minute
NCDR®	National Cardiovascular Data Registry
NOCAD	No obstructive coronary artery disease
NSTEMI	Non-ST elevation myocardial infarction
NO-cGMP	Nitric oxide - cyclic guanosine-3',-5'-monophosphase
OR	Odd ratios
OSA	Obstructive sleep apnoea
OCAD	Obstructive coronary artery disease
OSAS	Obstructive sleep apnoea syndrome
Pa	Mean aortic pressure
Pd	Distal coronary pressure
PET	Positron emission tomography
PCI	Percutaneous coronary intervention
PSL	Pressure-strain loop
PSG	Polysomnography
PVL	Pressure-volume loops
QCA	Quantitative coronary angiography
RS	Radial strain
RCA	Right coronary artery

RDI	Respiratory disturbance index
ROC	Receiver operating characteristic
RWMA	Regional wall motion abnormalities
RICCADSA	Randomized Intervention with CPAP in Coronary Artery Disease and Sleep Apnoea
STE	Speckle tracking echocardiography
SaO2	Oxygen Saturation
SAVE	Sleep Apnoea cardioVascular Endpoints trial
SPSS	Statistical Package for the Social Sciences
STEMI	ST elevation myocardial infarction
TDI	Tissue doppler imaging
TFC	Thrombolysis in myocardial infarction frame count
TIMI	Thrombolysis in myocardial infarction
TTDE	Transthoracic doppler echocardiography
U/kg	Unit per kilogram
v	Velocity
VSA	Vasospastic Angina
WISE	National Heart, Lung, and Blood Institute Women's Ischaemia Syndrome Evaluation study

CHAPTER 1

Literature Review

1.1 Introduction: Coronary ischaemia

The evolution of myocardial ischaemia started with clinicopathological correlation of typical angina symptoms with flow-limiting atherosclerotic coronary artery disease (CAD), traditionally in the form of obstructive coronary artery disease. Angina pectoris, chest discomfort described as tightness across the chest with or without radiation, often brought on by exertion, was first described by William Heberden in 1768.[15,16] Subsequently, William Osler (1849 – 1919) indicated that this is a syndrome rather than a disease itself and in 1912, James Herrick elaborated the obstructive coronary artery disease (OCAD) leading to the angina syndrome.[17]

Angina pectoris, which mechanisms are complex and not entirely understood, is a consequence of myocardial ischaemia. This result in the development of acidosis, reduced formation of adenosine triphosphate (ATP), the loss of the normal ATP sodium-potassium pump, myocardial membrane integrity, and the release of chemical substances stimulate the neural receptors leading to angina. Myocardial ischaemia has been extensively studied in literatures, its pathophysiology has been broadly categorised into myocardial oxygen demand and myocardial oxygen supply. [18-20]

Myocardial oxygen consumption (MVO_2) is determined by heart rate, systolic blood pressure as a clinical marker of afterload, the product of ventricular end-diastolic volume or preload and myocardial muscle mass as well as myocardial contractility. Myocardial oxygen supply is determined by coronary artery blood flow (CBF) delivered to the myocardium and oxygen carrying ability of the blood, haemoglobin and its unloading effects to the myocardial tissues which the oxygen extraction from the arterial blood is close to 60 – 70%. [20-22]

Under basal condition, the myocardium receives continuous phasic flow supply of oxygenated blood to generate the energy necessary for its contraction. Given the constant of oxygen extraction from the arterial blood, with increase in MVO2, this can be met by appropriate increase in CBF to meet adequate match between myocardial oxygen demand and supply. Myocardial ischaemia occurs as a result of the imbalance between the myocardial metabolic demand and CBF. The disruption of CBF can be caused by anatomical or dynamic functional abnormalities or extravascular influence of coronary circulation, at both the epicardial and/or the microcirculatory levels, leading to coronary ischaemia.[18,20-23]

1.2 Definition of chronic coronary syndromes

Chronic coronary syndrome (CCS), as defined the 2019 European Society of Cardiology (ESC) taskforce, encompasses the spectrum of stable ischemic heart disease (SIHD) and asymptomatic CAD with its underlying pathophysiology process to prognosticate respective outcomes, to facilitate further clarity on management approach on specific clinical scenario and stimulating further research in knowledge gaps.[24]

CCS depict the association of anginal symptoms with flow-limiting obstructive CAD, as a result of gradual development of atherosclerotic plaque in the epicardial arteries. Notwithstanding, the pathophysiology of CAD may also be attributed to a range of mechanisms, as described below (Section 1.2.2), that is now recognized as ischaemia with no obstructive disease (INOCA).

The common clinical scenarios in patients with suspected or established CCS are:

a. Patients with suspected CAD and 'stable' anginal symptoms, and/or dyspnoea (angina equivalent).
b. Patients with new onset of Heart failure (HF) or left ventricular (LV) dysfunction and suspected CAD.
c. Asymptomatic and symptomatic patients with stabilized symptoms <1 year after an acute coronary syndrome (ACS), or patients with recent revascularization.
d. Asymptomatic and symptomatic patients >1 year after initial diagnosis or revascularization.
e. Patients with angina and suspected vasospastic or microvascular disease.
f. Asymptomatic subjects in whom CAD is detected at screening.

The 2019 ESC taskforce has defined the above scenarios as a CCS but each of them involves different risks for future cardiovascular events, i.e. death or myocardial infarction (MI) and acknowledging the dynamic risk nature of specific condition. Insufficient control of cardiovascular risk factors, or unsuccessful revascularisation would lead to the development of an ACS, while, this risk may decrease as a consequence of an appropriate evidence based secondary prevention strategy.

1.3 Mechanisms of coronary ischaemia

The coronary arterial system is a continuous structure with different properties in respect to functions, providing CBF to the myocardium. However, its anatomical border cannot be clearly demarcated but broadly categorised into 3 compartments. The proximal

compartment consists of epicardial arteries and intramyocardial branches (diameter >500μm), intermediate compartment represented by pre-arteriolar vessels (diameter of 100μm to 500μm) and distal compartment by arterioles (diameter <100μm). The intermediate and distal compartment together with capillaries, constitute the coronary microcirculations.[25]

The epicardial coronary arteries run along the surface of the heart before branching into intra-mural vessels. These are thick wall vessels comprising of three tunicae of the adventitia, media, and intima. The intra-mural branches have thinner walls and lack a vasa vasorum. They provide capacitance function with little resistance to CBF. Ninety percent of CBF occurs in diastole with accumulation of elastic energy in the epicardial arteries during systole, followed by conversion of the elastic energy into kinetic energy contributing to the prompt reopening of the intramyocardial vessels after being compressed during systole.[26]

The pre-arteriolar vessels with a diameter range between 100μm to 500μm, is characterized by a quantifiable pressure decline along their length. They maintain pressure at the origin of downstream arterioles within a narrow range during changes in coronary perfusion pressure or flow, but not under the influence of diffusible myocardial metabolites. Proximal segment with diameter of 150 to 500μm responds predominantly to changes in flow, while distal segment with diameter of 100 to 150μm is more sensitive to pressure changes.[27] The arterioles are characterized by a significant drop in pressure along their course, the site where their vasomotor tone is influenced by myocardial metabolites produced from surrounding cardiac myocytes, regulating CBF.[27-29]

The coronary microvasculature regulates the vascular tone and rapidly changes diameter in response to underlying myocardial oxygen demand, promptly increasing CBF with functional hyperaemia. This is driven by the strict crosstalk between these vessels and

surrounding contracting cardiomyocytes. The effect of metabolic vasodilatation will lead to reduction in arteriolar resistance and increased CBF, followed by upstream vascular adaptations.[30,31]

Abnormalities along the coronary arterial conduit would contribute to the development of myocardial ischaemia leading to angina pectoris. They can be in the form of flow-limiting obstruction such as fixed obstructive atherosclerotic coronary artery disease or dynamic coronary artery vasospasm within the epicardial segments.[18,32] Furthermore, structural impairment of coronary microcirculation to increase CBF or functional impairment with coronary microvascular spasm, altering the coronary microcirculation limiting oxygen supply to myocardium depending on the demand, can lead to myocardial ischaemia and is termed coronary microvascular dysfunction.[27,33]

1.4 Ischaemia with obstructive disease

In obstructive coronary artery disease, myocardial ischaemia can manifest either by an acute coronary syndrome (ACS) or chronic coronary syndrome (CCS).

In the event of an ACS, the clinical presentation is often in an emergency setting, by sudden onset of angina symptoms at rest, corresponding with ST segment deviations on 12 lead electrocardiogram (ECG) and/or a rise in cardiac biomarkers such as Troponin or Creatinine Kinase (CK). A rise and/or fall in cardiac biomarkers and associated symptoms ECG changes may range from [ST elevation (constitutes-elevation myocardial infarction (STEMI)] or ST depression [non-ST elevation myocardial infarction (NSTEMI)]. In the absence of cardiac biomarker abnormality with new onset crescendo angina symptom (often within 2 weeks of

progressive angina symptoms onset), this has been termed unstable angina. This is usually confirmed with invasive coronary angiography, i.e. coronary stenosis resulted from plaque rupture or erosion with platelet activation and thrombus formation, and obstructive coronary stenoses are often treated by percutaneous or surgical intervention to prevent further damage to myocardium. [34,35]

As regards chronic coronary syndrome, stable coronary artery disease with stenotic severity of greater than 70% has often been associated with angina and myocardial ischaemia, when an increase in myocardial oxygen demand was not able to be met by myocardial supply due to inadequate coronary perfusion.[36] The relationship between the degree of coronary stenosis of less than 70% and myocardial ischaemia is best demonstrated by functional testing, either non-invasively functional studies with stress echocardiography or myocardial perfusion studies or cardiac MRI, or direct assessment of haemodynamic significance during invasive coronary angiography for the pressure difference of the pre- and post-stenotic lesions.[24] Studies have previously shown that myocardial blood flow was not compromised at luminal stenosis of less than 50%.[36] Other coronary vasomotor disorders as will be described in next section.

The scope of this thesis excludes this cohort of patients.

1.5 Ischaemia with no obstructive disease (INOCA)

Since the advent of coronary angiography, it has been found that approximately 30% to 40% of invasive coronary angiograms performed for the evaluation of chest pain do not reveal significant coronary artery disease (CAD).[1-4] The aetiology to the patient's chest pain whether is cardiac or non-cardiac can be difficult to determine. It is increasingly recognized

that patients can experience angina despite having no obstructive coronary artery disease (NOCAD). Whilst these forms of angina may not necessarily be associated with significant increase in cardiac mortality, they conversely have high morbidity with at least 50% of patients continuing to experience chest pain despite medications 12 months after coronary angiography.[37] Apart from impaired quality of life, patients may have multiple re-presentations to emergency department or hospital admission of different centres, leading to multiple diagnostic investigations, not only increasing procedural related complication risks, as well as having a substantial financial burden on health services.[3,5,6]

1.5.1 Definition/Diagnosis of coronary vasomotor disorder (CVD): INOCA endotypes

Angina with NOCAD has not been well recognized until recently due to multiple factors, ranging from medical professional's scepticism towards the diagnosis, lack of available standardised nomenclature in a very heterogenous group of patients, associated risks of invasive diagnostic techniques available to assess coronary physiology and lack of dedicated treatment available. In 2013, Coronary Vasomotor Disorders International Study Group (COVADIS) was established by an international group of Cardiologists to develop global standards in the diagnosis of angina with NOCAD, termed as Coronary Vasomotor Disorders, involving both large and microvessels within the heart. This will advance the unmet needs in this population, from diagnostic criteria standardization, safety experience of invasive diagnostics methodology, patients' outcomes and harness research into future therapy.[38,39]

Vasospastic and microvascular forms of angina are potentially life-threatening conditions which can be associated with myocardial infarction and malignant arrhythmias. In

Japan where there is believed to be a high prevalence of this condition, patients routinely undergo provocative testing for spasm in the absence of obstructive coronary artery disease. In Western countries, the prevalence of spasm is thought to be lower and thus provocative testing is not routinely performed. Although advocated in suspected cases, the prevalence of microvascular dysfunction and coronary endothelial function testing is not routinely performed.

A number of provocative approaches have been established over the years to evaluate coronary physiology which in turn facilitates a more comprehensive coronary artery assessment in patients with angina and NOCAD. Such protocols will allow for a better understanding of coronary microcirculation, as well as, evidence of epicardial vasospasm. Following a diagnostic coronary angiogram without any evidence of obstructive coronary artery disease, further coronary haemodynamic assessments should ensue. This will allow classification of different types of coronary vasomotor disorder, i.e. vasospastic angina (VSA), coronary microvascular angina (MVA), or mixed type.

1.5.1.1 Coronary Artery Vasospastic Angina (VSA)

Coronary artery vasospastic angina refers to abnormal vasomotor reactivity, in particular vasoconstriction of coronary artery of >90% luminal stenosis, leading to myocardial ischaemia with angina symptoms. Variant angina resulting from coronary artery spasm was first proposed in 1959 by Prinzmetal et al. Sones et al documented coronary artery spasm angiographically during a variant angina attack. Rogmanoli et al has shown that prolonged occlusive coronary artery spasm results in acute myocardial infarction.

The pathophysiology of coronary artery spasm is complex and multifactorial. The autonomic nervous system, inflammation, endothelial dysfunction, oxidative stress, and

genetic mutations have been found to be associated with coronary artery spasm. The differences in the incidence of coronary artery spasm in different countries is also well documented. The Japanese population appear to be at high risk of developing coronary artery spasm compared to western populations. Pristipino et al. demonstrated with a head-to-head controlled comparison of patients with acute ST elevation myocardial infarct that Japanese patients were more likely to have inducible spasm than their Caucasian counterparts. The specific reasons for these racial differences are unknown. These differences between Japanese and Caucasian patients are postulated as a result of low prevalence of fixed coronary stenoses and diffuse coronary hyperreactivity.

More recently, Sato et al indicated that there are ethnic differences in clinical profiles and long-term prognosis of contemporary coronary artery spasm patients. The prevalence of significant organic stenosis defined as ≥70% luminal narrowing by coronary angiography was similar between the 2 ethnic groups. More spasm provocative testing was performed in Japanese patients (Japanese/Caucasians, 1266/98). Pharmacological provocation testing with intracoronary Acetyl-Choline boluses was most commonly used in both cohorts. Importantly, the features of provoked spasm patterns were almost identical between the 2 ethnicities. However, multivessel coronary spasm was documented more frequently in Japanese than in Caucasians.

Most experience and the largest contribution to understanding this syndrome comes from studies in the Japanese population over the last 30 years, given its higher prevalence leading to routine clinical use of provocative testing as part of angina work-up during coronary angiography. It is important to recognize that the diagnosis of coronary artery spasm depends on coronary angiography and provocation test in patients with angina and no obstructive coronary artery disease. The Coronary Vasomotor Disorders International Study Group

(COVADIS) has published a guide for international standardisation of diagnostic criteria for vasospastic angina as below:[40]

Vasospastic angina diagnostic criteria elements:

1. Nitrate-responsive angina—during spontaneous episode, with at least one of the following:
    a. Rest angina—especially between night and early morning
    b. Marked diurnal variation in exercise tolerance—reduced in morning
    c. Hyperventilation can precipitate an episode
    d. Calcium channel blockers (but not b-blockers) suppress episodes
2. Transient ischaemic ECG changes—during spontaneous episode, including any of the following in at least two contiguous leads:
    a. ST segment elevation ≥0.1 mV
    b. ST segment depression ≥0.1 mV
    c. New negative U waves
3. Coronary artery spasm—defined as transient total or subtotal coronary artery occlusion (>90% constriction) with angina and ischaemic ECG changes either spontaneously or in response to a provocative stimulus (typically acetylcholine, ergot, or hyperventilation)

1.5.1.2 Microvascular Angina (MVA)

Microvascular angina refers to abnormal regulation of the coronary microcirculatory vasculature, often termed as coronary microvascular disorder (CMD), in the absence of other heart disease such as hypertrophic cardiomyopathy, leading to angina. MVA is a heterogenous clinical syndrome with its pathophysiology remains poorly understood.[27,41,42] CMD can result from combination of impaired microcirculatory vasodilatation, caused by endothelium-

dependent and endothelium-independent mechanisms and increased microcirculatory vasoconstriction caused by various stimuli, such as acetylcholine, catecholamines etc.[27]

Clinical presentation of patients with MVA due to CMD is variable and may present with typical angina pectoris, atypical symptoms, or angina-equivalent symptoms. Although CMD can also occur in asymptomatic subjects.[24] Typically, patients with MVA often present with exertion-related retrosternal chest discomfort or pain, and/or dyspnoea, and could persist after the exercise has ceased. These patients may experience episodes of chest pain at rest with variable duration, atypical features in both character and duration, i.e. tightening discomfort or stabbing like pain and prolonged in duration. MVA caused by CMD appear to be less responsive to nitrates therapy.[43,44] There is an increased female prevalence, especially in postmenopausal women.[5,45,46]

Diagnosis remains challenging given the inability of assessing these coronary microvessels at coronary angiography. MVA diagnosis is often hypothetical with exclusion of other possible causes of chest discomfort. This ranged from cardiac to non-cardiac causes. Cardiac causes would include exclusion of myopericardial diseases without OCAD, myocardial ischaemia from OCAD or abnormal cardiac nociception. Non-cardiac causes can range from gastro-oesophageal disorders, musculoskeletal or psychosomatic causes.[27,47,48] A definitive diagnosis of MVA will require the documentation of coronary microcirculatory functional assessment.[27,39]

The Coronary Vasomotor Disorders International Study Group (COVADIS) has published recommendation for international standardisation of diagnostic criteria for suspecting MVA as below:[39]

1. Symptoms of myocardial ischaemia:

    a. Effort and/or rest angina

    b. Angina equivalents (i.e. shortness of breath)

2. Absence of obstructive CAD (<50% diameter reduction or fractional flow reserve (FFR) >0.80) by:

    a. Coronary computed tomographic angiography (CTA)

    b. Invasive coronary angiography

3. Objective evidence of myocardial ischaemia:

    a. Ischaemic ECG changes during an episode of chest pain

    b. Stress-induced chest pain and/or ischaemic ECG changes in the presence or absence of transient/reversible abnormal myocardial perfusion and/or wall motion abnormality

4. Evidence of impaired coronary microvascular function:

    a. Impaired coronary flow reserve (cut-off values depending on methodology use between ≤2.0 and ≤2.5)

    b. Coronary microvascular spasm, defined as reproduction of symptoms, ischaemic ECG shifts but no epicardial spasm during acetylcholine testing.

    c. Abnormal coronary microvascular resistance indices [e.g. index of microcirculatory resistance (IMR) >25].

    d. Coronary slow flow phenomenon, defined as thrombolysis in myocardial infarction (TIMI) frame count >25.

A definitive diagnosis of MVA is made if all four criteria are met. Suspected MVA is diagnosed if symptoms of ischaemia are present (criteria-1) with no obstructive coronary artery disease (criteria-2) but are positive for only one of (a) objective evidence of myocardial ischaemia (criteria-3), or (b) evidence of impaired coronary microvascular function (criteria-4) alone.

1.5.2 Evaluation of INOCA

1.5.2.1 Invasive functional coronary angiography

Comprehensive invasive coronary haemodynamic evaluation includes the assessment of resting angiographic contrast flow, coronary microvascular hyperaemic function, coronary endothelial function and provocative coronary spasm testing. The diagnostic testing provides information on coronary vasomotor functions and classify into relevant endotypes after ruling out obstructive coronary artery disease:[4,39,40,49]

1. Microvascular angina
2. Vasospastic angina
3. Mixed MVA and VSA
4. Noncardiac chest pain
5. Non-flow-limiting coronary artery disease.

1.5.2.1.1 Thrombolysis in Myocardial Infarction Frame Count (TFC)

The TIMI frame count (TFC) method was first introduced in the early 1990s to provides a quantitative assessment of the number of cine-frames required for contrast to reach standardised distal landmarks in assessing coronary artery blood flow. This was based on angiograms acquired at 30 frames per second on 35 mm radiographic film. The first frame is defined by a column of contrast extending across >70% of the arterial lumen with antegrade motion. The last frame counted is that in which contrast enters (but not necessarily fills) a distal landmark. These landmarks are as follows: the first branch of the posterolateral artery in the right coronary artery; the distal branch of the lateral left ventricular wall artery furthest from

the coronary ostium in the circumflex system; and the distal bifurcation known as the ‘‘whale’s tail’’ in the left anterior descending artery.

The TFC for the left anterior descending coronary artery (LAD) can be corrected by a factor (1.7) to take account of the longer distance to the TIMI landmark. This ratio was obtained by dividing the mean TFC of the LAD by the mean TFC of the circumflex (LCx) and the right coronary artery (RCA) for normal arteries. In addition, a conversion factor of 2.4, 2 and 1.2 can be used to convert the frame rate values when filmed at 12.5, 15 and 25 f/s, respectively to adjust for the 30 frames/sec acquisition speed used in the original cine angiographic studies. In a study of 78 consecutive normal coronary arteries of 78 patients in the absence of acute myocardial infarction, mean corrected TFC for normal coronary arteries was 21 ± 3.1 frames, yielding a 95% confidence interval for normal flow of 15 to 27 frames. This technique is well-validated to assess epicardial blood flow.[50]

Although TFC is a measure of epicardial flow, it is dependent on resistive components in the microvasculature. The TFC is an inverted index of coronary flow velocity that correlates with Doppler-derived average peak velocity (APV).[51] Sun et al has demonstrated this as a simple technique that may be useful in the diagnosis of microvascular spasm during acetylcholine (ACh) provocative testing without requiring coronary sinus catheterization or myocardial lactate production measurement.[52,53]

1.5.2.1.2 Coronary Flow Reserve (CFR)

CFR is defined as the ratio of the maximal hyperaemic flow down a coronary vessel to the resting flow. This can be measured invasively by coronary thermodilution technique using a pressure–temperature sensor guidewire (PressureWire X, Abbott Vascular, Santa Clara, CA, USA with Coroventis CoroFlow cardiovascular system, Sweden) or a Doppler technique

(ComboWire XT or Flowire, Philips Volcano Corporation, San Diego, CA, USA). The ComboWire XT connects to the ComboMap system (Philips, Eindhoven).

Left anterior descending (LAD) coronary artery is the site of choice. Depending on local experience and system availability in place, PressureWire X is placed in distal third of the vessel or distal LAD, while ComboWire XT is positioned in proximal LAD. The usual approach to inducing steady-state hyperaemia is by the use of intravenous adenosine (140 μg/kg/min) to achieve endothelium-independent vasodilation.[54]

CFR can be calculated using thermodilution, as resting mean transit time divided by hyperaemic mean transit time.[49,55,56] The prognostic value of thermodilution-based CFR have used a cut-off value of 2.0.[39,57-60] When utilising the Doppler flow velocity technique, CFR is derived from hyperaemic flow velocity divided by resting flow velocity.[61] The prognostic impact of CFR based on Doppler flow velocity have used a CFR cut-off of 2.5 or lower.[6,39,62,63]

1.5.2.1.3 Coronary Microvascular Resistance

Coronary microvascular resistance can be calculated using either thermodilution or Doppler technique by combining pressure and flow measurements. The index of microvascular resistance (iMR) is calculated as the product of distal coronary pressure at maximal hyperaemia multiplied by the hyperaemic mean transit time.[64,65] Increased IMR (≥25) is representative of microvascular dysfunction.[66,67]

The hyperaemic microvascular resistance (HMR) index is a Doppler-based index, calculated by dividing intracoronary pressure by hyperaemic flow velocity. In a previous study of patients with angina and non-obstructed coronary arteries, HMR>1.9 [odds ratio: 15.6 (95%

confidence interval 2.1-114.0), P=0.007] was an independent predictor of recurrent chest pain.[3,68,69]

1.5.2.1.4 Coronary Vasoreactivity Testing

The most established approach for vasoreactivity testing is by intracoronary acetylcholine administration assessing for coronary endothelial function and coronary artery provocation testing.

Coronary endothelial function can be assessed with 2 doses of intracoronary acetylcholine (IC-ACh) infusions 10^{-6} mol/L (0.18 μg/min) and 10^{-5} mol/L (1.8 μg/min) administered over 2 minutes respectively.[70,71] Epicardial coronary artery endothelial dysfunction is defined on the basis of absence of a vasodilation to the ACh infusion.[72] Microvascular endothelial dysfunction is defined as a coronary blood flow increase <50% from baseline[73]. Coronary blood flow response is calculated from the Doppler-derived time-velocity integral and vessel diameter by the following equation: $coronary\ blood\ flow = \pi\left(\frac{average\ peak\ velocity}{2}\right).\left(\frac{vessel\ diameter}{2}\right)^2$. Abnormalities in coronary endothelial function have been associated with an increased risk of cardiovascular events, especially in women. Furthermore, improving coronary endothelial function with L-arginine also improves chest pain symptoms, supporting a clinical relationship[74].

Coronary spasm provocation testing is performed using incremental bolus doses of IC-ACh at 25, 50 and 100mcg for left coronary system administered over 20 seconds, with coronary angiography performed within one minute of each injection. If no inducible coronary artery spasm, right coronary system will be induced at 25 and 50mcg IC-ACh bolus. Coronary

artery spasm is diagnostic upon inducing occlusive/sub-occlusive coronary artery spasm > 90% stenosis). reversed with intra-coronary nitro-glycerine bolus.[2,75-77] Microvascular spasm is diagnosed if ACh administration induces chest pain, and ischaemic ECG changes but in the absence of coronary artery constriction (< 90%) stenosis.[5,40] Induced coronary artery spasm with ACh has been validated for the vasospastic angina syndrome[78] and is a predictor of cardiovascular events.[79] This has been shown to predict recurrent chest pain at 12 months.[37]

1.5.2.2 Non-Invasive assessment

There is no robust simple and effective non-invasive assessment for vasospastic angina, except for abnormal ECG changes for myocardial ischaemia and an exclusion of obstructive coronary artery disease on coronary angiography.[80,81] The dynamic nature of coronary artery spasm has made this challenging for non-invasive evaluation.

As for microvascular angina, different modalities have been explored. These include transthoracic doppler echocardiography (TTDE), myocardial contrast echocardiography (MCE), positron emission tomography (PET) and cardiac magnetic resonance (CMR).

TTDE assessing coronary blood flow velocity can be evaluated at baseline and during hyperaemia by pulsed-wave Doppler echocardiography, with the sample volume placed on the colour signal in the mid- or distal tract of the left anterior descending coronary artery (LAD). This technique requires extensive training and assessment is only confined to LAD but no other coronary arteries.[82-86]

MCE-derived myocardial blood flow and myocardial blood volume can be assessed through intravenously injected, echogenic, gas-filled microbubbles that are similar in size and

rheological properties to red blood cells and are detected in the myocardium by high-intensity ultrasound pulses. Myocardial perfusion abnormalities during dipyridamole infusion in patients with otherwise normal wall motion seems to be a marker of CMD.[87,88]

CMR derived myocardial perfusion obtained through the first-pass kinetics of T1-enhancing extracellular gadolinium-based contrast media. The contrast medium, diffusing from the microvasculature into the interstitial space, results in an increase in signal intensity that is proportional to the perfusion and blood volume of the tissue, the extravascular compartment size, and capillary permeability. A delayed signal increase and persistently hypointense regions are indicative of reduced perfusion. This technique is promising but limited to local availability. Further validation study is underway for this group of patients conducted by CorMicA CMR sub-study.[89]

PET provides accurate, and reproducible quantification of regional myocardial blood flow, by means of continuous monitoring of the radioactivity emitted by an intravenously administered tracer, in the circulation and the myocardium. Despite being well validated with coronary microvascular dysfunction and utilised in CMD research, this technique is limited by ionizing radiation, expense and lack of availability.[90]

1.6 Coronary Vasomotor Disorder Associated Risk Factors

1.6.1 Coronary Artery Vasospasm

Prevalence of VSA varies widely in different countries. As mentioned earlier the reported frequency historically appears higher in the Japanese than in the western populations.[91]. In a Japanese cohort of patients with angina, the prevalence has been shown to be as high as 40%, whereas in a similar Taiwanese population the prevalence was 19.3% and Caucasians 7.5%.[92-95] However, a recent study in Germany by Ong et al. with 921 consecutive white patients with angina and no obstructive coronary arteries demonstrated a diagnosis of VSA during provocative testing with acetylcholine was as high as 33.4%.[5] CorMicA study in Scotland also showed a total of 37% of INOCA has a diagnosis of VSA; isolated VSA of 17% and mixed VSA and MVA of 20%.[4] These findings highlighted the importance of VSA diagnostic investigation and the prevalence in Caucasians may not be as low as previously thought.

VSA is more prevalent in men than women and tend to be younger between the age of 40 and 70 years. The prevalence tends to decrease after the age of 70 years.[4,5,94] Cigarette smoking is a major risk factor for VSA.[4,5,96]

1.6.2 Microvascular Angina

The association between traditional cardiovascular risk factors and microvascular angina is not well established given the diagnostic criteria was recently standardised by COVADIS group.[39]

Coronary microvascular dysfunction defined by the impaired measure of coronary flow reserve (CFR) was shown to be associated with cigarette smoking, hypercholesterolaemia and type 2 diabetes. Cigarette smoking exerts its endothelial oxidative stress effects leading to reduction in CFR.[97-99] The pathogenic role of low density lipoprotein cholesterol (LDL-C) was demonstrated with its correlation with abnormal CFR, whilst total cholesterol level did not show any significant association.[100-102] Progression of type 2 diabetes, specifically with insulin resistance, was associated with worsening of CFR. Similar to those with obstructive coronary artery disease, the annual cardiovascular mortality is similar between diabetes without coronary artery disease and those with coronary artery disease without diabetes.[103-106]

Traditional cardiovascular risk factors, age, diabetes, hypertension and dyslipidaemia were associated with coronary microvascular dysfunction in the ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease (iPower) and National Heart, Lung, and Blood Institute Women's Ischaemia Syndrome Evaluation (WISE) study, which evaluated coronary microvascular function in women with angina and no obstructive coronary artery disease.[107,108] Inflammation was shown to be associated with CMD in this group of women suffering from angina. Rheumatological conditions such as systemic lupus erythematosus and rheumatoid arthritis were associated with CMD and angina.[109-112]

Given the lack of any large study to date, an international, multicentre, observational and prospective registry study has begun in order to assess the clinical characteristics and long-term prognosis of MVA in the current era. Differential assessment of the different endotypes of microvascular angina, such as impaired CFR, impaired iMR or microvascular spasm's

associations will be extremely helpful in understanding this heterogeneous condition and guide future therapeutics options.[113]

1.6.3 Obstructive sleep apnoea (OSA) as a risk factor

Obstructive sleep apnoea (OSA) is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. Diagnosis was based on "Australasian Sleep Association Guidelines for Sleep Studies in Adults" recommendation [114]

The apnoea, hypopnoea index (AHI) has been used to diagnose patients as having OSA and to classify the severity of OSA based on the Chicago criteria classification.[115]

- Normal: AHI <5 events per hour of sleep.
- Mild OSA: AHI 5 to 15 events per hour of sleep.
- Moderate OSA: AHI 15 to 30 events per hour of sleep.
- Severe OSA: AHI >30 events per hour of sleep.

A 2016 Australian online survey reported 8% of participants having a diagnosis of OSA.[116] These patients suffer from poor sleep quality and impaired quality of life.[12] OSA is recognized to be a risk factor of hypertension, ischaemic heart disease, cardiac arrhythmias and heart failure through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances. [7-11]

1.6.3.1 OSA in the pathogenesis of ischaemic heart disease

The pathophysiological mechanisms by which OSA can contribute to the development of coronary atherosclerosis remains incompletely understood. Current literature suggest a complex interaction between multiple factors including intermittent hypoxia (IH), sleep fragmentation, and intra-thoracic pressure swings leading to altered cardiopulmonary vascular hemodynamics.[117-119] Of the these, IH (i.e. repetitive cycles of desaturation and reoxygenation that characterize OSA) and its downstream maladaptive responses has the most robust evidence for OSA's role in atherogenesis. These include:

1) Sympathetic overactivity, endothelial dysfunction, and predilection to resistant hypertension: Several studies have shown marked increase of circulating catecholamines and sympathetic over-activation in patients with OSA, which has been linked with resistant hypertension in both animal and human observational studies [120-123]. This is predominately mediated by hypoxia-mediated chemoreceptor activation as well as oxidative stress-associated endothelial dysfunction.[124,125] Correction of IH using continuous positive airway pressure (CPAP) therapy has been shown to significantly reduce blood pressure in a meta-analysis of 5 randomised trials, suggesting a plausible causal relationship.[126]

2) Chronic inflammation: IH promotes systemic inflammation via several cascades including activation of nuclear factor kappa B [127], increased expression of pro-inflammatory cytokines and chemokines, recruitment of macrophages in the vascular and adipose tissue and elevated serum levels of inflammatory markers [128]; all of which are important mediators in the formation of pre-atherosclerotic lesion and subsequent CAD [129]. CPAP treatment has been shown to reduce the heightened inflammatory state seen in OSA, but its clinical utility in preventing the development of atherosclerosis remains unclear [130].

3) Generation of reactive oxygen species: Patients with OSA have been shown to have elevated production of reactive oxidative species and decrease in antioxidant defence [131]. The consequent increased oxidative stress promotes inflammation, endothelial dysfunction as well as metabolic disorders, which aggregate with OSA and development of CAD [124, 15].

4) Metabolic dysregulation: This is characterized by lipid dysfunction, insulin resistance and type 2 diabetes and subsequent coronary atherosclerosis [132].

5) Others: disturbance in lipid metabolism [133] and promotion of pro-coagulable state [134].

1.6.3.2 Effect of OSA on vascular function

The potential mechanisms affecting vascular function leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, neuronal and/or inflammatory basis. The hypotheses are linked to hypoxia related sympathetic surge, oxidative stress, inflammation and more recently, endothelial dysfunction.[9,135-142] Inflammation and endothelial dysfunction has been postulated to have a pathogenic role in coronary microvascular dysfunction (MVD).[143]

The latter, in particular, has been demonstrated in peripheral haemodynamics study of patients with moderate to severe OSA population utilizing flow-mediated dilatation pulse wave analysis to assess the change in augmentation index in response to salbutamol (an endothelium dependent vasodilator), assessing the endothelial function. Yim-yeh et al. found that the effect of OSA on endothelial function is similar to that of diabetes mellitus.[142] Furthermore, in a rat

model intermittent hypoxia is associated with coronary vascular dysfunction and vasoconstriction via RhoA activation.[144]

1.6.3.3 Evidence for the association and effect of OSA and INOCA

The association between OSA and coronary artery disease has been extensively evaluated, while understanding of the relationship between obstructive sleep apnoea and angina with no obstructive coronary artery disease is limited.[7-11] The pathophysiology of angina with NOCAD is not necessarily due to the presence of atherosclerotic plaques but functional abnormalities of coronary flow.

Two recent studies have invasively studied the coronary haemodynamics of patients with angina and NOCAD and its relationship to OSA. Both are performed in patients of Asian ethnicity. The first was a Chinese ethnicity at Nanjing who studied 1038 consecutive patients presenting with features of cardiac syndrome X, found a high prevalence of OSA in this population. This study demonstrated a strong correlation between the severity of OSA as measured by the AHI index and a reduction in coronary flow reserve and elevated high sensitivity C reactive protein.[145] Another small Japanese study (n=42) by Tamura et al also found that patient with moderate to severe OSA is associated with inducible epicardial vasospasm.[146] Japanese ethnicity has been demonstrated to have a high prevalence of VSA, so extrapolation to the Australian population is unclear.

More importantly, there were several clinical studies, utilizing non-invasive modalities such as myocardial contrast echocardiography and magnetic resonance perfusion techniques, showing abnormalities in the coronary microvascular perfusion in moderate to severe sleep

apnoea patients and improved after 3 months of CPAP therapy.[147,148] Thus far, there has been no invasive coronary haemodynamics studies that have been evaluated in patients with significant OSA with or without a chest pain syndrome.

1.7 Prevalence of INOCA

Up to 50% of patients who undergo elective coronary angiography for stable chest pain symptoms with an abnormal functional study were found to have no obstructive coronary artery disease.[1,149] The recent CORonary MICrovascular Angina study (CorMicA) study supports this finding with 47% found to have no obstructive coronary artery disease during coronary angiography for angina assessment. Amongst these patients who underwent further invasive coronary physiology study and acetylcholine provocative testings, 52% had isolated MVA, 17% had isolated VSA and 20% had both, whilst 11% had non-cardiac chest pain.[4]

Another consecutive study of acetylcholine provocative evaluation during coronary angiography of patients with angina and NOCAD in Germany demonstrated up to 57.6% of patients were diagnostic for either VSA or MVA. 28.6% were inconclusive while 13.8% did not demonstrate any abnormality. However, evaluation of coronary microcirculatory function with either CFR or iMR/HMR was not conducted.[5] Coronary artery spasm in patients with acute coronary syndrome (CASPAR) study demonstrated that coronary artery spasm can be a frequent cause of ACS.[150]

1.8 Prognosis of INOCA

Angina in the presence of no obstructive coronary arteries has previously been thought to have a relatively benign prognosis. However, evidence may point to the contrary with an increase in morbidity and mortality in this subset of patients.[151-154] Most studies to date have been of small numbers, whilst definitions can be heterogenous.

Both men and women with angina who had impaired CFR despite NOCAD appear to have worse clinical outcomes. In a recent review by Bairey-Merz et al, almost two thirds of women undergoing clinically indicated coronary angiography for suspected CAD in the original WISE cohort had ischaemia with no obstructive CAD. During follow-up, they had a risk rate for major adverse cardiac events (death, nonfatal MI, nonfatal stroke, and hospitalization for heart failure) of >2.5% yearly by 5 years, as well as elevated rates of hospital readmission and repeat angiography. At 10 years, cardiovascular death or myocardial infarction occurred in 6.7% of those with no evident angiographic CAD and in 12.8% of those with NOCAD. In the WISE database, invasively assessed CFR <2.32 was the best predictor of adverse outcomes, with a 5-year major adverse cardiac events rate of 27% versus 9.3% for women with a CFR ≥2.32 (P=0.01). Similar outcome findings have been reported using non-invasive assessment of CFR with transthoracic echo Doppler or PET.

Following recent standardisation of diagnosis by COVADIS group, this will enable future research and therapeutics development.[38,39,113]

1.8.1 Vasospastic Angina Prognosis

Data on VSA prognosis is limited to smaller population studies given that the true prevalence may be under-represented or established if the first occurrence of an event was sudden cardiac death.[155,156] CASPAR study showed that ACS patients without culprit lesion and proof of coronary spasm have an excellent prognosis for survival and coronary events after 3 years compared with patients with obstructive ACS. However, persistent angina represents a challenging problem leading to repeated coronary angiography.[150,157] The Japanese Coronary Spasm Association (JCSA) conducted an international, prospective, and multi-centre registry study for VSA patients. A total of 1339 Japanese and 118 Caucasians were enrolled based on the same diagnostic criteria for VSA. Survival rate free from major adverse cardiac events (MACE) was slightly but significantly higher in Japanese than in Caucasians (86.7 vs. 76.6% at 5years, P<0.001).[92]

More recently, Ong et al demonstrated that the overall prognosis of patients with coronary artery spasm was encouraging, although, they are at increased risk for myocardial infarction, repeat angiography and recurrent angina. With a median follow-up period of 7.2 years of 736 patients, the study showed 55 deaths (7.5%), 8 nonfatal myocardial infarctions (1.4%), and 12 strokes (2.2%) occurred during the follow-up period. Recurrent symptoms were reported by 64% of patients, and repeat coronary angiography was performed in 12% of cases. Multivariate analysis revealed epicardial spasm as a predictor of nonfatal myocardial infarction (hazard ratio: 14.469; 95% confidence interval: 1.735 to 120.646) and repeat angiography (hazard ratio: 1.703; 95% confidence interval: 1.062 to 2.732), whereas patients with microvascular spasm more often had recurrent angina at follow-up (hazard ratio: 1.311; 95% confidence interval: 1.013 to 1.697).[158]

1.8.2 Coronary Microvascular Dysfunction prognosis

Meta-analysis by Brainin et al, the incidence of all-cause death and non-fatal MI in patients with non-obstructive coronary artery disease was much higher (1.32/100 person-years) than in those with angiographically normal epicardial vessels (0.52/100 person-years). When functional testing was compared, proven myocardial ischaemia by non-invasive imaging techniques (stress echocardiography or nuclear imaging) was associated with a higher incidence of events (1.52/100 person-years) compared to ischaemia detected by exercise electrocardiographic stress testing 0.56/100 person-years.[154]

More recent systemic review and meta-analysis by Gdowski et al showed that CMD is associated with a nearly 4-fold increase in mortality and a 5-fold increase in major adverse cardiac events.[159] This further supports the importance of diagnosing CMD and future therapeutics research.

1.9 Management

There is no robust data on the most effective management strategy of INOCA. Studies to date were small with heterogenous definition, design and methodology. Future larger trials with a consistent definition as outlined by COVADIS are underway.

1.9.1 Lifestyle Management

Lifestyle management in INOCA should probably be supported using currently available ischaemic heart disease secondary prevention guidelines. Although no evidence of obstructive coronary artery disease, in cases with evidence of atherosclerosis or endothelial dysfunction addressing the modifiable cardiovascular risk factors may reduce future risk of cardiovascular events and possibly improved angina symptoms and quality of life.[160,161] For example, smoking cessation was shown to reduce angina symptoms and cardiovascular events in VSA patients, while aerobic exercise training improved angina burden.[162]

Currently there is no evidence for dietary intervention specific to this cohort of patients with angina. However, patients may have co-existing cardiovascular risk factors.[163]

The uncertain nature and limited understanding on INOCA evolution poses possible detrimental effects on mental health of affected individuals. This may aggravate their perceptions towards their angina syndrome, adversely impacting on quality of life worsening morbidity and contributing to adverse social impacts. Future understanding in this condition would be important in tailoring better psychological supports framework.[164]

1.9.2 Pharmacotherapy

As mentioned in the risk factors section 1.5, the traditional cardiovascular disease risk factors may associate with either coronary microvascular dysfunction or vasospastic angina.

ACE-inhibitor therapy and statins have benefit in MVA with evidence of coronary microvascular dysfunction. In women with signs and symptoms of ischaemia without obstructive CAD, ACE-inhibitor therapy improves microvascular function with associated reduction in angina.[165] The LDL-C was correlated with abnormal CFR and improved with statin therapy in studies.[100-102,166-168] Statin was also shown to supress acetylcholine induce coronary artery spasm.[169] Often, these patients may have underlying atherosclerosis which would benefit from secondary cardiovascular risk reduction management.[170]

1.9.2.1 Antianginals in vasospastic angina

Calcium channel antagonists (CCA) are the most effective first line therapy in treating more than 90% of VSA patients by reducing anginal symptoms, suppress inducible coronary spasm and preventing major adverse cardiovascular events.[171-173] High doses of calcium channel antagonists, either non-dihydropyridine and dihydropyridine, may be required alone or in combination.[162,174] CCA exert its effect via blocking the slow calcium currents that are responsible for electrical activation and contraction of vascular smooth muscle cells, leading to potent coronary artery vasodilatation effects.[175]

Nitrates as nitric oxide donor, induce coronary artery smooth muscle cell relaxation via the nitric oxide - cyclic guanosine-3',-5'-monophosphase (NO-cGMP) signalling pathway. Nitrate has been shown to reduce anginal symptoms, together with good long-term efficacy but no reduction in MACE.[162,176-178]

Nicorandil, *N*-[2-(Nitro-oxy) ethyl]-3-pyridine carboxamide, a balanced vasodilator acts via dual mechanism of nitrate like effects as NO donor and potassium channel opener. The potassium channel opener results in vascular smooth cell hyperpolarization and closure of L-

type voltage gated calcium channels, leading to dilatation of both coronary microvessels and peripheral resistance arteries. Nicorandil has been effective and extensively used in Japan for VSA.[177-184]

In refractory VSA, typically defined as unresponsive to both CCBs and nitrates, the current available therapies are largely empirical with limited available robust evidence. Fasudil, a specific Rho-kinase inhibitor, has been shown to be effective reducing VSA symptoms, via the inhibition of Rho-kinase pathway which pays an important role in pathophysiology of coronary artery spasm.[185-188] Cilostazol, a selective inhibitor of phosphodiesterase 3, which increases intracellular cyclic adenosine monophosphate (cAMP) concentrations and the reduction in cytosolic calcium concentration promote vasodilatation, improving angina symptoms on top of conventional therapy.[189-192]

Invasive approaches to drug refractory VSA were proposed, i.e. percutaneous coronary intervention with stent angioplasty. This is only recommended in patients with VSA and significant stenosis but the efficacy of coronary stenting to prevent VSA remains unclear with the dynamic nature of coronary spasm. A focal stenosis would be feasible, whilst a more diffuse coronary spasm would be impossible.[193,194]

1.9.2.2 Antianginals in microvascular angina

Effective evidence-based pharmacotherapy in MVA remains limited but studies on future therapies are emerging.[195]

Third generation β-adrenoreceptor antagonists, such as nebivolol and carvedilol, have additional endothelium-dependent vasodilatation properties towards coronary vasculature but

their molecular mechanism remains unclear.[196] Recently, there were reports suggesting the effectiveness of antianginal effects of nebivolol in doses up to 5mg per day.[197] Although, the precise mechanism remains unknown, the antianginal effects may not just be reduction in heart rate or reduction in sympathetic tone. Earlier studies with propranolol and acebutolol were negative, while, atenolol may have some favourable effects.[198-200]

CCAs, were shown to have some improvement in angina symptoms and exercise stress test parameters in patients with CMD but diltiazem did not show any improvement in CFR.[201-204] It has therefore been suggested that CCA may be better suited for management of acetylcholine induced microvascular spasm endotype of microvascular angina.[170]

Mibefradil, a unique calcium T-channel antagonist, was highly effective in coronary slow flow phenomenon.[205] However, mibefradil was voluntarily withdrawn from the market worldwide by its manufacturer, Rosche Laboratories of Nutley, N.J., following withdrawal of FDA approval given the increasing drug interaction with lethal effects.

Multiple studies on nitrates in MVA has been disappointing with hyporesponsive to nitrates, possibly due to the resistance nature of microvessels with less nitric oxide influence in comparison to epicardial conduit vessel. Redox stress may also play a role.[43] However, nicorandil has been shown to be effective in small studies by improvement in scintigraphy results and significantly prolongation of exercise duration.[206,207]

Ranolazine exerts its antianginal effect via inhibition of the late sodium channel current, leading to a reduction of sodium-dependent calcium channel activity resulting in less intracellular calcium in cardiomyocytes during ischaemia.[208] A pilot study showed

improvement in angina symptoms but only a trend towards an improvement of CMR derived myocardial perfusion reserve index.[209] Another confirmatory study has shown that ranolazine improved anginal symptoms and quality of life, and increased exercise capacity.[210] The most recent study with baseline stratified CFR showed an improvement with ranolazine on both symptoms and CMR derived myocardial perfusion.[211]

1.10 Gaps in knowledge

INOCA has often been dismissed as 'false positive' in the current 'stenotic centric' era in managing coronary artery disease. There are no large prevalence studies to date but international registry collaborations have been established, and hopefully, there will be more understanding towards the nature of this condition. Underlying pathophysiology remains poorly understood which also reflects the lack of effective targeted therapy in addressing INOCA.

Diagnostic criteria have been unified by COVADIS study group and hopefully will be clinically and scientifically robust enough to remain the universal diagnostic criteria for many years. However, limited uptake of provocation testing prevails in most busy cardiovascular intervention suites exacerbated by a lack of physician awareness of the prevalence and diagnostic, therapeutic and prognostic significance of INOCA. The lack of demand for provocation testing has resulted in, limited and in fact reduced technology advancement in this area. ComboWire XT wire manufacturing was discontinued in 2020 and we are only left with PressureWire X. Non-invasive diagnostic techniques are either not easily accessible due to cost (CMR) or has ionising radiation exposure (PET). Echocardiography with emerging speckle

tracking technique may be a potential non-invasive means of diagnosing MVA but lack large validation studies.

Clinical determinants towards INOCA remain poorly understood with larger studies required. The evolution and prognostic implication of the INOCA remains unknown, while emerging evidence suggest that this may not be as uncommon or benign as we originally thought. Without accurate data on the life trajectory of INOCA or evidence to support the importance of this condition in the lexicon of cardiac diseases, it remains challenging to increase awareness within the Cardiology, general medical and pharmaceutical fraternity. As has occurred in several other rare medical conditions, a patient-led advocacy towards better treatment and recognition may be required.

1.11 Scope of thesis

Ischaemia with no obstructive coronary artery leading to angina pectoris was first described in 1959 by Printzmetal et al. in the form of coronary artery spasm, followed by further understanding of coronary microcirculation dysfunction over the last 20 years, as a contributing factor leading to angina syndrome. However, there remains significant areas that require further evaluation, particularly as regards to diagnosis, mechanism of ischaemia, causative/associative factors and therapy. The course of evaluating this group of patients is often limited due to lack of widespread adoption with experience, inadequate available empirical therapeutics leading to poor quality of life, increased healthcare cost burden and more recently increasing recognition of its major cardiovascular adverse even (MACE) and heart failure.

This thesis will provide novel data in the area of assessing potential association with obstructive sleep apnoea and diagnostic process in coronary microcirculatory disorders. The specific objectives are:

1. Observe the prevalence of obstructive sleep apnoea in patients with angina with no obstructive coronary artery disease.

2. Evaluate the association of obstructive sleep apnoea on coronary haemodynamics.

3. Assess for correlation of non-invasive derived measures by the echocardiography technique of speckle tracking and invasive coronary haemodynamic measures of Coronary Flow Reserve (CFR), and Hyperaemic Microvascular Resistance (HMR) in patients with angina and no obstructive coronary artery disease.

1.11.1 Chapter 2: Obstructive Sleep Apnoea and Coronary Artery Disease

This review is aimed at understanding the clinical connect between obstructive sleep apnoea and coronary artery disease.

1.11.2 Chapter 3: Predictors of Obstructive Sleep Apnoea (OSA) Population in the Coronary Angiogram Database of South Australia (CADOSA)

This study assessed the prevalence and clinical predictors of OSA in patients with anginal symptoms who have undergone coronary angiogram in South Australia.

1.11.3 Chapter 4: The Association of Obstructive Sleep Apnoea (OSA) in Ischaemia with No Obstructive Coronary Artery Disease (INOCA) – A Pilot Study.

This study examined the relationship of obstructive sleep apnoea in patients experiencing angina with no obstructive coronary artery disease.

1.11.4 Chapter 5: The Evolution of Ischaemia Detection with Echocardiography

This review is aimed at understanding the clinical connection between myocardial ischaemia and tissue changes by firstly, describing the historical and clinicopathophysiological perspective of deformation imaging and clinical advances of tissue imaging in echocardiography.

1.11.5 Chapter 6: Speckle tracking derived contractile reserve and microvascular functions in angina with no obstructive coronary artery disease. – A Pilot Study.

The objective of this study is to determine if speckle tracking derived contractile reserve correlates with invasive coronary microvascular measures, specifically CFR and HMR, in patients with angina and no obstructive coronary artery disease.

CHAPTER 2

Obstructive Sleep Apnoea and Coronary Artery Disease

This chapter provides a review of the association between Obstructive sleep apnoea and coronary artery disease (CAD). OSA patients not only suffer from poor sleep quality, reduced quality of life and cardiovascular comorbidities, but OSA is also independently associated with CAD. CAD is the term associated with an inadequate supply of blood to the myocardium due to obstruction of the epicardial coronary arteries, usually from atherosclerosis. The pathogenesis of atherosclerosis remains complex and poorly understood. CPAP therapy has been linked with reduction in major adverse cardiovascular events, however, recent pivotal randomised controlled trials failed to demonstrate its significance. INOCA is a relatively new entity with recent standardisation of its endotypes. INOCA association with OSA or the effects of CPAP therapy remains unexplored but both mechanistic commonality revolves in endothelial dysfunction.

2.1 Statement of Authorship

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review and wrote manuscript and acted as corresponding author.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sharmalar Rajendran		
Contribution to the Paper	Supervised development of work, helped in literature review and manuscript evaluation.		
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2.2 Introduction

Obstructive sleep apnoea (OSA) is a common sleep-related breathing disorder, which is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. As a result, patients with OSA suffer from poor sleep quality and reduced quality of life.[12] OSA is recognized to be a risk factor of hypertension, coronary artery disease, cardiac arrhythmias and heart failure through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances. [7-11] This review focuses on the evidence to date on the association between obstructive sleep apnoea and coronary artery disease.

2.3 Definition of OSA

The presence and severity of OSA are primarily defined using the apnoea hypopnea index (AHI), or the respiratory disturbance index (RDI), which quantifies the frequency of obstructive events per hour of sleep or recording time. However, according to the third edition of the International Classification of Sleep Disorders (ICSD), the diagnosis of OSA requires either signs/symptoms or an associated medical or psychiatric disorder (e.g. hypertension, coronary artery disease, atrial fibrillation, congestive heart failure, stroke, diabetes, cognitive dysfunction, or mood disorder) coupled with ≥5 apnoea and/or hypopnea events per hour of sleep during PSG. Alternatively, AHI ≥15 events per hour satisfies the criteria for OSA in the absence of symptoms.[212]

2.4 Definition of Coronary Artery Disease

Coronary artery disease (CAD) is defined, most commonly, structurally, by the development of atherosclerosis (either obstructive or non-obstructive) of the epicardial coronary arteries. Patients may have acute (unstable) or chronic (stable) disease. It is increasingly recognized and accepted that functional CAD, which comprises the spectrum of coronary vasomotor disorders, namely functional vasospasm and/or microvascular disorders, plays an important role in myocardial ischaemia with no obstructive disease (INOCA).[213]

2.4.1 Structural CAD

In acute coronary syndrome (ACS), the clinical presentation is often in an emergency setting, by sudden onset of angina symptoms at rest, corresponding with ST segment deviations on 12 lead electrocardiogram (ECG) and/or a rise in cardiac biomarkers such as Troponin or Creatinine Kinase (CK). A rise and/or fall in cardiac biomarkers and associated symptoms ECG changes may range from [ST elevation (constitutes-elevation myocardial infarction (STEMI)] or ST depression [non-ST elevation myocardial infarction (NSTEMI)]. In the absence of cardiac biomarker abnormality with new onset crescendo angina symptom (often within 2 weeks of progressive angina symptoms onset), this has been termed unstable angina. This is usually confirmed with invasive coronary angiography demonstrating obstructive coronary stenoses, causes such as from plaque rupture or erosion with platelet activation and thrombus formation, which are often treated by percutaneous or surgical intervention to prevent further damage to myocardium. [34,35]

Chronic coronary syndrome (CCS), as defined the 2019 European Society of Cardiology (ESC) taskforce, encompasses the spectrum of stable ischemic heart disease (SIHD) and asymptomatic CAD.[24] CCS, traditionally, depicts the association of anginal symptoms with flow-limiting obstructive CAD, often with stenotic severity of greater than 70% when an increase in myocardial oxygen demand was not able to be met by myocardial supply due to inadequate coronary perfusion.[36] Studies have previously shown that myocardial blood flow was not compromised at luminal stenosis of less than 50%.[36] This is usually a result of gradual development of atherosclerotic plaque in the epicardial arteries. The diagnosis of CCS can be confirmed by stress testing or, if that is ambivalent, an anatomic assessment of the coronary arteries, either with coronary computed tomography angiography (CTA) or an invasive coronary angiogram.

2.4.2 Functional CAD

Notwithstanding, the pathophysiology of CAD may also be attributed to a range of other mechanisms, including the currently accepted classification of coronary vasomotor disorders, leading to INOCA. In 2013, Coronary Vasomotor Disorders International Study Group (COVADIS) was established by an international group of Cardiologists to develop global standards in the diagnosis of angina with no obstructive coronary artery disease, termed as Coronary Vasomotor Disorders, involving both macro- and micro-circulation within the heart. This will advance the unmet needs in this population, from diagnostic criteria standardization, safety experience of invasive diagnostics methodology, patients' outcomes and harness research into future therapy.[38,39] Vasospastic and microvascular forms of angina are potentially life-threatening conditions which can be associated with myocardial infarction and malignant arrhythmias.

2.4.1.1 Definition Box

Obstructive sleep apnoea (OSA) – pathophysiological process characterised by obstructive of upper airway during sleep, resulting in repetitive breathing pauses accompanied by oxygen desaturation and arousal from sleep.

Obstructive sleep apnoea syndrome (OSAS) – pathophysiological process of OSA resulting in diurnal sleepiness, leading to cognitive impairment or cardiovascular morbidity.

Acute coronary syndrome (ACS) – a clinical syndrome applied to patients in whom there is a suspicion or confirmation of acute myocardial ischaemia or infarction, typically caused by three traditional types such as non-ST-elevation myocardial infarction (NSTEMI), ST-elevation MI (STEMI), and unstable angina.

Chronic coronary syndrome (CCS) – synonymous with stable ischaemic heart disease (SIHD), based on a classic history of angina pectoris in the presence of either risk factors for or known atherosclerotic cardiovascular disease.

2.5 OSA in the pathogenesis of CAD

Detailed reviews of the pathophysiological mechanisms by which OSA can contribute to the development of coronary atherosclerosis have been published elsewhere.[117-119] In brief, current literature suggest a complex interaction between multiple factors including intermittent hypoxia (IH), sleep fragmentation, and intra-thoracic pressure swings, leading to altered cardiopulmonary vascular hemodynamics.[214] IH, in particular, (i.e. repetitive cycles of desaturation and reoxygenation that characterize OSA) and its downstream maladaptive responses has the most robust evidence for OSA's role in atherogenesis. These include:

2.5.1 Sympathetic overactivity, endothelial dysfunction, and predilection to resistant hypertension

Several studies have shown marked increase of circulating catecholamines and sympathetic over-activation in patients with OSA, which has been linked with resistant hypertension in both animal and human observational studies.[120-123] This is predominately mediated by hypoxia-mediated chemoreceptor activation as well as oxidative stress-associated endothelial dysfunction.[124,125] Correction of IH using CPAP has been shown to significantly reduce blood pressure in a meta-analysis of 5 randomised trials, suggesting a plausible causal relationship.[126]

2.5.2 Chronic inflammation

IH promotes systemic inflammation via several cascades including activation of nuclear factor kappa B [127], increased expression of pro-inflammatory cytokines and chemokines, recruitment of macrophages in the vascular and adipose tissue and elevated serum levels of inflammatory markers [128]; all of which are important mediators in the formation of pre-atherosclerotic lesion

and subsequent CAD.[129] CPAP treatment has been shown to reduce the heightened inflammatory state seen in OSA, but its clinical utility in preventing the development of atherosclerosis remains unclear.[130]

2.5.3 Generation of reactive oxygen species

Patients with OSA have been shown to have elevated production of reactive oxidative species and decrease in antioxidant defence.[131] The consequent increased oxidative stress promotes inflammation, endothelial dysfunction as well as metabolic disorders, which aggregate with OSA and development of CAD.[124]

2.5.4 Metabolic dysregulation

This is characterized by lipid dysfunction, insulin resistance and type 2 diabetes and subsequent coronary atherosclerosis. Ip and colleagues investigated the relationship between sleep-disordered breathing and insulin resistance. OSA subjects were more insulin resistant, as indicated by higher levels of fasting serum insulin, μU/ml, [7.8 (5.2-11.7) vs 5.4 (3.5-8.9), p=0.001) and HOMA-IR, [1.8 (1.2-3.0) vs 1.3 (0.8-2.1), p<0.001). Stepwise multiple linear regression analysis showed OSA is independently associated with insulin resistance.[132]

2.5.5 Others

Disturbance in lipid metabolism [133] and promotion of pro-coagulable state.[134] Chronic intermittent hypoxia induced by OSA is associated with generation of sterol regulatory element binding protein-1 (SREBP-1) and stearoyl coenzyme A desaturase-1 (SCD-1), peroxidation of lipids, HDL dysfunction, increased total cholesterol level, and sympathetic dysfunction. These factors create a proinflammatory milieu responsible for development of dyslipidaemia and propagation of atherosclerosis and CVD in OSA. [133] Phillips and colleagues showed in

randomised, placebo-controlled crossover trial, treatment of OSA with CPAP reduced the early morning level of vWF, and nocturnal levels of FVIII and FV. These findings suggest that CPAP may reduce cardiovascular risk in OSA, in part through reducing risk of thrombosis.[134]

2.6 Effect of OSA on vascular function

The potential mechanisms affecting vascular function leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, neuronal and/or inflammatory basis. The hypotheses are linked to hypoxia related sympathetic surge, oxidative stress, inflammation and more recently, endothelial dysfunction.[9,135-142] Inflammation and endothelial dysfunction has been postulated to have a pathogenic role in coronary microvascular dysfunction (MVD).[143]

The latter, in particular, has been demonstrated in peripheral haemodynamics study of patients with moderate to severe OSA population utilizing flow-mediated dilatation pulse wave analysis to assess the change in augmentation index in response to salbutamol (an endothelium dependent vasodilator), assessing the endothelial function. Yim-yeh and colleagues found that the effect of OSA on endothelial function is similar to that of diabetes mellitus.[142] Furthermore, in a rat model intermittent hypoxia is associated with coronary vascular dysfunction and vasoconstriction via RhoA activation.[144]

2.7 Prevalence and association of OSA and cardiovascular risk factors/disease

OSA affects approximately 17% of the adult population, 24% in male and 9% in female, in the first Wisconsin Sleep Cohort Study. A 20 years follow-up study demonstrated that the prevalence is increasing, particularly with increasing obesity.[215] The HypnoLaus study showed a high prevalence of OSA in Switzerland population, affecting 23.4% in women and 49.7% in men have moderate to severe obstructive sleep apnoea (AHI ≥ 15). However, obstructive sleep apnoea syndrome is less frequent and affects about 13% of the male and 7% of the female population. The study found independent association with cardiovascular risk factors such as hypertension (OR 1.60, 95% CI 1.14-2.26; p=0.0292) and diabetes (OR 2.00, 95% CI 1.05-3.99); p=0.0467), with a mean BMI of 25.6 kg/m^2.[216] There is indeed much evidence linking OSA with cardiovascular risk factors such as diabetes, hypertension, as well as heart failure.[8,217-220]

Further, in the Sleep Heart Health Study, severe OSA was associated with a 68 % higher risk of coronary artery disease in men aged 40–70 years.[220] Consistent with the above, a long-term follow-up study, by Peker and colleagues, of 308 middle-aged OSA subjects over 7 years, showed that OSA diagnosis at baseline was linked with an almost five-fold increase in risk of development of CAD (angina pectoris or myocardial infarction requiring hospitalization) independent of traditional risk factors (95% CI 1.8-11.6; p=0.001).[221]

Moreover, the association of OSA with increased cardiovascular events, has been observed beyond that of stable coronary artery. In a recent systemic review by Yang and colleagues, OSA was linked with increased adverse outcomes of repeat revascularization and

heart failure in this pooled analysis of patients with newly diagnosed acute coronary syndrome.[222]

To date, a number of meta-analyses have demonstrated an increased risk of vascular outcomes, all-cause sudden death and all-cause mortality, in moderate and severe OSA.[223,224] The most recent systemic review also demonstrated a marginally significant dose-response relation between the severity of OSA and all-cause sudden death, with a relative risk for moderate OSA of 1.72 (95% CI:1.11 to 2.67, I^2=0%) and severe OSA was 2.87 (95% CI:1.7 to 4.85, I^2=0%) (p for interaction=0.05).[224]

2.8 Emerging evidence between OSA and INOCA

In 1994, a series of case reports suggested that patients with OSA may experience angina in the absence of obstructive coronary artery disease (now recognized as INOCA).[225] In these patients, the angina may arise from either functional vasospasm and microvascular disorders.

Two previous studies have invasively studied the coronary haemodynamics of patients with angina and INOCA and its relationship to OSA. Both are performed in patients of Asian ethnicity. The first was a Chinese ethnic at Nanjing who studied 1038 consecutive patients presenting with features of cardiac syndrome X, found a high prevalence of OSA in this population. This study demonstrated a strong correlation between the severity of OSA as measured by the AHI index and a reduction in coronary flow reserve and elevated high sensitivity C reactive protein.[145] Another small Japanese study (n=42) by Tamura and

colleagues also found that patient with moderate to severe OSA is associated with inducible epicardial vasospasm.[146] Japanese ethnicity has been demonstrated to have a high prevalence of vasospasm, so extrapolation to the a Caucasian population is unclear.

Additionally, there were several clinical studies, utilizing non-invasive modalities such as myocardial contrast echocardiography and magnetic resonance perfusion techniques, showing abnormalities in the coronary microvascular perfusion in moderate to severe sleep apnoea patients and improved after 3 months of CPAP therapy.[147,148]

Our own data, from the Coronary Angiogram Database of South Australia (CADOSA) of consecutive patients undergoing coronary angiography for investigation of chest pain, 11% of patients had OSA. The presence of no obstructive disease in symptomatic stable angina patients was an independent predictor of OSA. These observations, supports the potential link between functional abnormalities in coronary circulation beyond obstructive plaques, to the underlying mechanisms of OSA (as described in the pathogenesis section).

In this journal, we report the first invasive coronary haemodynamic evaluation in an Australian cohort. In keeping with the evidence above, there was a significant correlation between abnormal coronary microvascular function and OSA in symptomatic patients with INOCA.

2.9 Overview of OSA treatment on CAD

Observational and retrospective studies have demonstrated the relationship of OSA and CAD. However, whether CPAP therapy prevents major cardiovascular events is uncertain. There are 3 well designed randomised controlled studies to date, CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnoea (SAVE) trial, Randomized Intervention with CPAP in Coronary Artery Disease and Sleep Apnoea (RICCADSA) study and Impact of Sleep Apnoea syndrome in the evolution of Acute Coronary syndrome (ISAACC) study, to address this issue.

SAVE study randomly assigned 2717 eligible adults between 45 and 75 years of age who had moderate-to-severe obstructive sleep apnoea and coronary or cerebrovascular disease to receive CPAP treatment plus usual care (CPAP group) or usual care alone (usual-care group). The primary composite end point was death from cardiovascular causes, myocardial infarction, stroke, or hospitalization for unstable angina, heart failure, or transient ischemic attack. Most of the participants were men who had moderate-to-severe obstructive sleep apnoea and minimal sleepiness. In the CPAP group, the mean duration of adherence to CPAP therapy was 3.3 hours per night, and the mean apnoea-hypopnea index (the number of apnoea or hypopnea events per hour of recording) decreased from 29.0 events per hour at baseline to 3.7 events per hour during follow-up. After a mean follow-up of 3.7 years, a primary end-point event had occurred in 229 participants in the CPAP group (17.0%) and in 207 participants in the usual-care group (15.4%) (hazard ratio with CPAP, 1.10; 95% confidence interval, 0.91 to 1.32; P=0.34). No significant effect on any individual or other composite cardiovascular end point was observed. CPAP significantly reduced snoring and daytime sleepiness and improved health-related quality of life and mood. Therapy with CPAP plus usual care, as compared with

usual care alone, did not prevent cardiovascular events in patients with moderate-to-severe obstructive sleep apnoea and established cardiovascular disease.[10]

There are several factors should be considered in interpreting SAVE study. There was a low adherence with CPAP therapy (3 hours). Potentially, a larger number may have been required to be sufficiently powered to detect a change in cardiovascular outcomes, particularly with a longer duration of follow-up. In terms of patient demographics and study generalizability, the study did not utilise polysomnography study to diagnose OSA, and most participants were men, of Asian descent, with a mean body mass index of 28 kg/m^2, and low levels of sleepiness at baseline.

The RICCADSA trial, a single-centre randomized controlled trial from Sweden, looking at the effect of positive airway pressure on cardiovascular outcomes in coronary artery disease patients with non-sleepy obstructive sleep apnoea. Peker and colleagues randomized patients with OSA (AHI ≥ 15) with newly revascularized CAD to either auto-titration CPAP or no CPAP therapy. Their primary endpoint was repeat revascularization, myocardial infarction, stroke, or cardiovascular mortality. They found no statistically significant difference between groups in primary endpoint (HR 0.80 (95% CI, [0.46, 1.41], p = .449). However, an adjusted on-treatment analysis that similar to a prior observational study,[226,227] showed a significant beneficial effect of CPAP was seen in patients who used the device for equal to or more than 4 h/night. The issue of poor compliance has been an on-going limitation of CPAP trials. Therefore, the actual percentage of sleep per night an individual is using his or her CPAP and treating the OSA is of importance in an intention-to-treat analysis. This was a key limitation to the CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnoea (SAVE) trial as mentioned above.[228]

The ISAACC study, a multicentre, open-label, parallel-group, randomized controlled trial, evaluating the effect of obstructive sleep apnoea (OSA) and its treatment with continuous positive airway pressure (CPAP) on the clinical evolution of patients with ACS at 15 hospitals in Spain. A total of 2834 patients with ACS had respiratory polygraphy, of whom 2551 (90·01%) were recruited. 1264 (49·55%) patients had OSA and were randomly assigned to the CPAP group (n=633) or the usual-care group (n=631). 1287 (50·45%) patients did not have OSA, of whom 603 (46·85%) were randomly assigned to the reference group. Patients were followed up for a median of 3·35 years (IQR 1·50-5·31). The prevalence of cardiovascular events was similar in the CPAP and usual-care group (98 events [16%] vs 108 events [17%]; hazard ratio [HR] 0·89 [95% CI 0·68-1·17]; p=0·40) during follow-up. Mean time of adherence to CPAP treatment was 2·78 h/night (SD 2·73). The prevalence of cardiovascular events was similar between patients in the reference group (90 [15%] events) and those in the usual-care group (102 (17%) events) during follow-up (1·01 [0·76-1·35]; p=0·93). In this study, the presence of OSA was not associated with an increased prevalence of cardiovascular events and treatment with CPAP did not significantly improve prognosis. Again, adherence towards CPAP therapy remains a limitation with very low compliance of therapy (2.78 hours/night), especially without sleep unit involvement.[229]

The commonality of the lack of benefit of CPAP therapy observed in the 3 RCT likely be due to low compliance of therapy of less than 4 hours/night of CPAP therapy. This is a well-known issue with CPAP therapy compliance. A recent updated meta-analysis of 9 studies, including the above mentioned RCTs, enrolling 2590 participants with OSA and CAD showed there was significant association of CPAP with reduced risk of MACE (RR, 0.73, 95% CI [0.55, 0.96]). Similarly, the same result was found in all-cause death (RR, 0.66, 95% CI, [0.46, 0.94]) and cardiovascular death (RR, 0.495, 95% CI [0.292, 0.838]). A significant beneficial

effect of CPAP was seen in patients used the device for equal to or more than 4 h/night. However, a null result with respect to MI, stroke or repeat revascularisation. The latest data suggested that CPAP usage, compared to usual care, was associated with reduced risks of cardiovascular outcomes or death in patients with OSA and CAD.[230]

In view of conflicting evidence to date of OSA treatment on CAD, further larger studies with minimum CPAP therapy of greater than 4 hours/night to address this issue.

2.10 Conclusion

OSA is an important public health concern, with its increasing prevalence, and is significantly associated with coronary artery disease, independent of traditional cardiovascular risk factors. The potential mechanisms of OSA affecting vascular function leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, and/or neuronal basis, linked to hypoxic-related sympathetic surge, oxidative stress, inflammation and/or endothelial dysfunction. INOCA is a relatively new entity with novel evidence of haemodynamic association with OSA but the prognostic impact of OSA treatment on both this endotype and obstructive CAD remains unresolved.

CHAPTER 3

Predictors of Obstructive Sleep Apnoea (OSA) Population in the Coronary Angiogram Database of South Australia (CADOSA)

OSA is increasingly recognized to be a risk factor for cardiovascular disease. The pathogenesis of atherosclerosis remains complex and poorly understood. CPAP therapy has been linked with reduction in major adverse cardiovascular events, however, recent pivotal randomised controlled trials failed to demonstrate its significance. This chapter assessed the prevalence and clinical predictors of OSA in patients with anginal symptoms who have undergone coronary angiogram in South Australia. Therefore, providing insights towards the relationship of OSA with presence of angina with no obstructive coronary artery disease.

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Statement of Authorship

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Contribution to the Paper	Performed literature search, critical appraisal / review. analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author on behalf of Professor John Beltrame.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sharmalar Rajendran		
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Contribution to the Paper	Performed literature search, critical appraisal / review. analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author on behalf of Professor John Beltrame.		
Overall percentage (%)	80%		
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Contribution to the Paper	Performed literature search, critical appraisal / review. analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author on behalf of Professor John Beltrame.		
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3.2 Introduction

Obstructive sleep apnoea (OSA) is a common sleep-related breathing disorder which is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. A 2016 Australian online survey reported 8% of participants having a diagnosis of OSA.[116] These patients suffer from poor sleep quality and impaired quality of life.[12] OSA is recognized to be a risk factor of hypertension, ischaemic heart disease, cardiac arrhythmias and heart failure through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances. [7-11]

In 1994, a series of case reports suggested that patients with OSA may experience angina in the absence of obstructive coronary artery disease (NOCAD).[225] In these patients, the angina may arise from either coronary microvascular dysfunction (CMD) or coronary vasospastic angina (VSA). Furthermore, the association between OSA and CMD or VSA was demonstrated in patients of Chinese and Japanese ethnicity.[145,146] Whether this relationship extends to other ethnicities requires further evaluation.

To determine if a relationship exists between OSA & NOCAD in an Australian cohort, this study assessed the predictors of OSA in consecutive patients undergoing coronary angiography for the assessment of chest pain in South Australia.

3.3 Methods

3.3.1 Clinical Data Source

The Coronary Angiogram Database of South Australia (CADOSA) is a comprehensive clinical quality registry with data specification/definitions that are compatible with the NCDR® CathPCI® Registry. The registry prospectively collects data on patient characteristics, clinical features, angiographic and procedural details, and in-hospital outcomes of consecutive patients undergoing diagnostic angiography or PCI at participating hospitals. Standard data definitions are used at all participating sites to ensure uniformity and comparability. Approximately 5% of the CADOSA registry data is routinely audited against the clinical record, to ensure data accuracy.

3.3.2 Study Cohort

Consecutive patients undergoing coronary angiography for suspected ischaemic heart disease, all of whom had chest pain. Data captured by 3 South Australian public/university affiliated hospitals participating in the CADOSA registry from 2015-2018 were included in the analysis. Patients with principal cardiac diagnoses of valvular heart disease, pulmonary hypertension, cardiomyopathy, pre-operative evaluation before non-cardiac surgery, and without angina symptoms were excluded.

3.3.3 Study Definitions

Sleep Apnoea was documented in the registry based upon patient self-report, either obtained direct from the patient via CADOSA data collectors or via abstraction from the medical record. No obstructive coronary artery disease (NOCAD) was defined as the absence of any obstructive coronary artery stenosis (i.e. ≥ 50%) by the interventional cardiologist performing the invasive coronary angiogram.

3.3.4 Statistical Methods

Baseline admissions for patients with chest pain but without valvular disease were determined and data from these admissions used.

Covariates were determined using Hosmer and Lemeshow's purposeful selection of variables method. Variables were selected by univariate analysis. Independent t tests were used for comparison of continuous variables whereas Chi-square test was used for comparison of numbers (proportions). Two-sided p values < 0.05 were considered to indicate statistical significance.

Any variable having a univariate test with a p-value cut-off of 0.25 or less were selected. All variables were then entered into a logistic regress model using STATA 15.0 (StataCorp 2017). In an iterative process, covariates were removed if they were non-significant and a non-confounder. Significance was evaluated at the 0.05 level, and confounding was defined as a

change in any remaining parameter estimate of 15% or greater. At the end of this iterative process, the model contained significant predictors and confounders.

At this point any variable not selected for the original multivariate model was then added in one at a time, to identify variables that may not be significantly related to the outcome variable but make an important contribution in the presence of other variables. Variables that were significant at the 0.1 level were then put in the model, and the model was again iteratively reduced but only for the variables that were added at the end.

3.4 Results

Amongst the 9,885 consecutive patients undergoing diagnostic angiography for the investigation of chest pain between 1st January 2015 and 31st December 2018, 1089 [11%, 95% CI 10.4%-11.7%] were documented as having OSA. Table A summarises the demographic, comorbidities and the clinical features of these patients with and without OSA, as well as detailing the results of the univariate analyses.

3.4.1 Demographic, comorbidities and clinical features of those with and without OSA

All comparisons of baseline variables between 2 cohorts found to be statistically important differences, except age [64 ± 10.4 vs 64 ± 12.6, p=0.946]. Patients with OSA were

more likely to be male [75.3% vs 65.5%, p=<0.001], have no obstructive coronary artery disease (NOCAD) on angiography [29.1% vs 26.3%, p=0.051], non-smoker [16.3% vs 24.3%, p=<0.001], and have conventional cardiovascular risk factors (hypertension [83.3% vs 67.2%, p<0.001], dyslipidaemia [80.6% vs 65.7%, p<0.001] and diabetes mellitus [50.2% vs 29.3%, p<0.001]) or prior cerebrovascular disease [11.8% vs 7.0%, p<0.001], depression [37.9% vs 21.6%, p<0.001], chronic lung disease [17.3% vs 9.7%, p<0.001], asthma [24.8% vs 14.3%, p<0.001], peripheral arterial disease [10.1% vs 6.7%, p<0.001] and atrial fibrillation/flutter [17.8% vs 11.6%, p<0.001], as compared with patients without OSA.

Patients with OSA were more likely to have experienced prior cardiovascular events of myocardial infarction [31.1% vs 22.2%, p<0.001], prior percutaneous coronary intervention [29.7% vs 20.6%, p<0.001], prior heart failure diagnosis [7.8% vs 3.6%, p<0.001] and prior coronary artery bypass grafting [11.2% vs 8.2%, p=0.001], as compared with patients without OSA.

Patients with OSA were less likely to have acute coronary syndrome presentation [60.6% vs 71.9%, p<0.001] prior to coronary angiography, presenting as stable angina, in comparison with patients without OSA.

3.4.2 Independent Predictors of OSA in patients with chest pain undergoing coronary angiography

Table B presents the final multivariate logistic regression model for predictors of OSA in patients with chest pain undergoing coronary angiography. The Hosmer-Lemeshow test was

not significant [H-L Goodness of Fit=5386, p=0.634], indicating that the final model is a good fit. The c-statistic for the model is 0.7118.

Accordingly, risk factors such as male gender [OR 2.22, 1.86-2.65, p<0.001], diabetes mellitus [OR 1.84, 1.58-2.14, p<0.001], depression [OR 1.81, 1.55-2.12, p<0.001], prior heart failure [OR 1.63, 1.22-2.18, p=0.001], hypertension [OR 1.61, 1.32-1.95, p=<0.001], asthma [OR 1.61, 1.34-1.93, p<0.001], not a current smoker [OR 1.60, 1.30-1.96, p<0.001], dyslipidaemia [OR 1.46, 1.22-1.76, p<0.001], chronic lung disease [OR 1.40, 1.12-1.73, p=0.003], cerebrovascular disease [OR 1.36, 1.07-1.73, p=0.012] and atrial fibrillation/flutter [OR 1.30, 1.06-1.60, p=0.012] were clinical characteristics independently associated with OSA. Additionally, non-obstructive coronary artery disease (NOCAD) [OR 1.30, 1.10-1.55, p=0.003] and non-acute coronary syndrome presentation [OR 1.45, 1.25-1.69, p<0.001] were predictive for OSA.

Finally, stable angina (32.1% vs 22.7%) and NOCAD (29.1% vs 26.3%, P=0.051) were tended more common in patients with OSA versus no OSA.

3.5 Discussion

In a cohort of patients undergoing coronary angiography for the investigation of suspected ischaemic chest pain, OSA was observed in 11% and associated with previously described OSA risk factors (male gender, hypertension, dyslipidaemia, diabetes, depression), as well as the novel finding of the presence of NOCAD was predictive (particularly in those with a stable angina pattern), This sub-group of patients may have coronary vasomotor

disorders, either from coronary microvascular dysfunction (CMD) or Vasospastic angina (VSA).

Traditional cardiovascular risk factors such as male gender, hypertension and diabetes were demonstrated to be associated with OSA in the general population.[7-11] We have also noted these to be independent risk factors in our cohort of patients undergoing coronary angiography for chest pain syndromes. In addition, dyslipidaemia, the presence of cerebrovascular disease, atrial fibrillation/flutter and depression were other cardiovascular risk factors that were predictors of OSA in our cohort. Metabolic syndrome has been previously associated with OSA and many of our demonstrated predictors would be consistent with this finding.[7] However, the CADOSA registry does not document waist circumference and BMI, so the prevalence of metabolic syndrome cannot be accurately calculated. Interestingly, current smoking was not a predictor of OSA in our cohort but similar to the general population, asthma was an independent predictor of OSA.

The association between OSA and coronary artery disease has been extensively evaluated, while understanding of the relationship between obstructive sleep apnoea and angina with no obstructive coronary artery disease is limited.[7-11] The pathophysiology of angina with NOCAD is not due to the presence of atherosclerotic plaques but functional abnormalities of coronary flow.

Two recent studies have invasively studied the coronary haemodynamics of patients with angina and NOCAD and its relationship to OSA. Both are performed in patients of Asian ethnicity. The first was a Chinese ethnic at Nanjing who studied 1038 consecutive patients presenting with features of cardiac syndrome X, found a high prevalence of OSA in this

population. This study demonstrated a strong correlation between the severity of OSA as measured by the AHI index and a reduction in coronary flow reserve and elevated high sensitivity C reactive protein.[145] Another small Japanese study (n=42) by Tamura et al also found that patient with moderate to severe OSA is associated with inducible epicardial vasospasm.[146] Japanese ethnicity has been demonstrated to have a high prevalence of VSA, so extrapolation to the Australian population is unclear.[92]

The potential mechanisms leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, neuronal and/or inflammatory basis. The hypotheses are linked to hypoxic related sympathetic surge, oxidative stress, inflammation and more recently, endothelial dysfunction.[9,135-142] Inflammation and endothelial dysfunction has been postulated to have a pathogenic role in coronary microvascular dysfunction (MVD).[143]

The latter, in particular, has been demonstrated in peripheral haemodynamics study of patients with moderate to severe OSA population utilizing flow-mediated dilatation pulse wave analysis to assess the change in augmentation index in response to salbutamol (an endothelium dependent vasodilator), assessing the endothelial function. Yim-yeh et al. found that the effect of OSA on endothelial function is similar to that of diabetes mellitus.[142] Furthermore, in a rat model intermittent hypoxia is associated with coronary vascular dysfunction and vasoconstriction via RhoA activation.[144]

More importantly, there were several clinical studies, utilizing non-invasive modalities such as myocardial contrast echocardiography and magnetic resonance perfusion techniques, showing abnormalities in the coronary microvascular perfusion in moderate to severe sleep

apnoea patients and improved after 3 months of CPAP therapy.[147,148] Thus far, there has been no invasive coronary haemodynamics studies that have been evaluated in patients with significant OSA with or without a chest pain syndrome.

3.6 Study Limitations

The interpretation of the above study findings should be considered in the context of the following limitations. Firstly, OSA was based on prior diagnosis and the data collection was based on patient self-reporting. It is well recognised that the incidence of obstructive sleep apnoea and the prevalence is generally underdiagnosed but would be impacted by diagnostic criteria. Secondly, there was no dedicated comprehensive coronary haemodynamic study nor provocative testing for coronary vasospasm and an assumption was made that these patients may possibly has coronary vasomotor disorder. Thirdly, this study does not provide any insights into the mechanism underlying this potentially important association. Finally, reporting bias may occur as patient may not necessarily be tested for OSA without prior cardiac condition, in comparison to patients with established cardiac diagnosis.

3.7 Conclusion

In addition to established risk factors for OSA, this study found NOCAD to be independent predictor of OSA; especially in those presenting with a stable angina presentation. This suggests that coronary vasomotor disorders may be associated with OSA, although further detailed studies are required.

3.8 Tables

Table A summarises the demographic, comorbid clinical characteristics and the cardiac clinical features of CADOSA patients with and without OSA. This table depict the results of the univariate analyses used to determine the selection of variables for the model.

Clinical Characteristics	**OSA (n = 1089)**		**No OSA (n = 8796)**		**p-value**
	n	**%**	**n**	**%**	
Age (mean ± SD)	64 ± 10.4		64 ± 12.6		0.946
Male	820	75.3	5757	65.5	<0.001
No CAD	317	29.1	2317	26.3	0.051
Current Smoker	177	16.3	2135	24.3	<0.001
Hypertension	895	83.3	5819	67.2	<0.001
Dyslipidaemia	857	80.6	5638	65.7	<0.001
Diabetes Mellitus	541	50.2	2564	29.3	<0.001
Depression	390	37.9	1863	21.6	<0.001
Chronic Lung Disease	179	17.3	848	9.7	<0.001
Asthma	256	24.8	1250	14.3	<0.001
Peripheral Arterial Disease	103	10.1	578	6.7	<0.001
Atrial Fibrillation/Flutter	182	17.8	984	11.6	<0.001
Prior MI	320	31.1	1901	22.2	<0.001
Prior HF	80	7.8	306	3.6	<0.001
Prior Valve Surgery	26	2.5	145	1.7	0.065
Prior PCI	314	29.7	1784	20.6	<0.001
Prior CABG	120	11.2	715	8.2	0.001
Presentation for Coronary Angiography Indication					
Acute Coronary Syndrome	660	60.6	6326	71.9	<0.001
STEMI	101	9.3	1831	20.8	
NSTEMI	270	24.8	2462	28.0	
Unstable Angina	289	26.5	2033	23.1	
Stable Angina	350	32.1	1995	22.7	
Non ischaemic symptoms	79	7.3	475	5.4	

Table B presents the final multivariate logistic regression model for predictors of OSA in patients with chest pain undergoing coronary angiography. The Hosmer-Lemeshow test was not significant, indicating that the final model is a good fit. The c-statistic for the model is 0.7118.

Predictors	**OR**	**95% CI**	**p-value**
Male	2.22	1.26 – 2.65	<0.001
No CAD	1.30	1.10 – 1.55	0.003
Not a Current Smoker	1.60	1.30 – 1.96	<0.001
Hypertension	1.61	1.32 – 1.95	<0.001
Dyslipidaemia	1.46	1.22 – 1.76	<0.001
Diabetes Mellitus	1.84	1.58 – 2.14	<0.001
Cerebrovascular Disease	1.36	1.07 – 1.73	0.012
Depression	1.81	1.55 – 2.12	<0.001
Chronic Lung Disease	1.40	1.12 – 1.73	0.003
Asthma	1.61	1.34 – 1.93	<0.001
Atrial fibrillation/flutter	1.30	1.06 – 1.60	0.012
Prior HF	1.63	1.22 – 2.18	0.001
Not admitted with ACS	1.45	1.25 – 1.69	<0.001

3.9 Appendix

Predictors of Obstructive Sleep Apnoea (OSA) Population in the Coronary Angiogram Database of South Australia (CADOSA)

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Abstract: Obstructive sleep apnoea (OSA) is increasingly recognized to be a risk factor for cardiovascular disease. This study assessed the prevalence and clinical predictors of OSA in patients undergoing coronary angiography. Consecutive patients undergoing coronary angiography in South Australian public hospitals

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This study was funded by The South Australian Cardiovascular Research Development Program (SACVRDP), Heart Foundation and University of Adelaide.
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from 2015 to 2018 were included. Clinical details for consecutive patients undergoing coronary angiography in South Australian public hospitals were captured by the Coronary Angiogram Database of South Australia (CADOSA) registry staff, with OSA identified by patient report. Among the 9,885 patients undergoing coronary angiography for the investigation of chest pain, 11% (n = 1,089) were documented as having OSA. Independent clinical predictors of OSA included male gender (OR 2.22, 1.86-2.65, $P < 0.001$), diabetes mellitus (OR 1.84, 1.58-2.14, $P < 0.001$), depression (OR 1.81, 1.55-2.12, $P < 0.001$), prior heart failure (OR 1.63, 1.22-2.18, $P = 0.001$), hypertension (OR 1.61, 1.32-1.95, $P \leq 0.001$), asthma (OR 1.61, 1.34-1.93, $P < 0.001$), not a current smoker (OR 1.60, 1.30-1.96, $P < 0.001$), dyslipidaemia (OR 1.46, 1.22-1.76, $P < 0.001$), non-acute coronary syndrome presentation (OR 1.45, 1.25-1.69, $P < 0.001$), chronic lung disease (OR 1.40, 1.12-1.73, $P = 0.003$), cerebrovascular disease (OR 1.36, 1.07-1.73, $P = 0.012$), non-obstructive coronary artery disease (NOCAD) (OR 1.30, 1.10-1.55, $P = 0.003$) and atrial fibrillation/flutter (OR 1.30, 1.06-1.60, $P = 0.012$). Finally, stable angina (32.1% vs 22.7%) and NOCAD (29.1% vs 26.3%, $P = 0.051$) were trended more common in patients with OSA versus no OSA. In addition to established risk factors for OSA, this study found NOCAD to be independent predictor of OSA; especially in those presenting with a stable angina presentation. This suggests that coronary vasomotor disorders may be associated with OSA, although further detailed studies are required. (Curr Probl Cardiol 2021;00:100846.)

Introduction

Obstructive sleep apnoea (OSA) is a common sleep-related breathing disorder which is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. A 2016 Australian online survey reported 8% of participants having a diagnosis of OSA.[1] These patients

suffer from poor sleep quality and impaired quality of life.[2] OSA is recognized to be a risk factor of hypertension, ischaemic heart disease, cardiac arrhythmias and heart failure through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances.[3-7]

In 1994, a series of case reports suggested that patients with OSA may experience angina in the absence of obstructive coronary artery disease (NOCAD).[8] In these patients, the angina may arise from either coronary microvascular dysfunction (CMD) or coronary vasospastic angina (VSA). Furthermore, the association between OSA and CMD or VSA was demonstrated in patients of Chinese and Japanese ethnicity.[9,10] Whether this relationship extends to other ethnicities requires further evaluation.

To determine if a relationship exists between OSA and NOCAD in an Australian cohort, this study assessed the predictors of OSA in consecutive patients undergoing coronary angiography for the assessment of chest pain in South Australia.

Methods

Clinical Data Source

The Coronary Angiogram Database of South Australia (CADOSA) is a comprehensive clinical quality registry with data specification/definitions that are compatible with the NCDR CathPCI Registry. The registry prospectively collects data on patient characteristics, clinical features, angiographic and procedural details, and in-hospital outcomes of consecutive patients undergoing diagnostic angiography or PCI at participating hospitals. Standard data definitions are used at all participating sites to ensure uniformity and comparability. Approximately 5% of the CADOSA registry data is routinely audited against the clinical record, to ensure data accuracy.

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Consecutive patients undergoing coronary angiography for suspected ischaemic heart disease, all of whom had chest pain. Data captured by 3 South Australian public/university affiliated hospitals participating in the CADOSA registry from 2015 to 2018 were included in the analysis. Patients with principal cardiac diagnoses of valvular heart disease, pulmonary hypertension, cardiomyopathy, pre-operative evaluation before non-cardiac surgery, and without angina symptoms were excluded.

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Sleep Apnoea was documented in the registry based upon patient self-report, either obtained direct from the patient via CADOSA data collectors or via abstraction from the medical record. No obstructive Coronary Artery Disease (NOCAD) was defined as the absence of any obstructive coronary artery stenosis (ie, $\geq$ 50%) by the interventional cardiologist performing the invasive coronary angiogram.

Statistical Methods

Baseline admissions for patients with chest pain but without valvular disease were determined and data from these admissions used.

Covariates were determined using Hosmer and Lemeshow's purposeful selection of variables method. Variables were selected by univariate analysis. Independent t tests were used for comparison of continuous variables whereas Chi-square test was used for comparison of numbers (proportions). Two-sided P values < 0.05 were considered to indicate statistical significance.

Any variable having a univariate test with a P-value cut-off of 0.25 or less were selected. All variables were then entered into a logistic regress model using STATA 15.0 (StataCorp 2017). In an iterative process, covariates were removed if they were non-significant and a non-confounder. Significance was evaluated at the 0.05 level, and confounding was defined as a change in any remaining parameter estimate of 15% or greater. At the end of this iterative process, the model contained significant predictors and confounders.

At this point any variable not selected for the original multivariate model was then added in one at a time, to identify variables that may not be significantly related to the outcome variable but make an important contribution in the presence of other variables. Variables that were significant at the 0.1 level were then put in the model, and the model was again iteratively reduced but only for the variables that were added at the end.

Results

Amongst the 9,885 consecutive patients undergoing diagnostic angiography for the investigation of chest pain between 1st January 2015 and 31st December 2018, 1089 (11%, 95% CI 10.4%-11.7%) were documented as having OSA. Table 1 summarises the demographic, comorbidities and the clinical features of these patients with and without OSA, as well as detailing the results of the univariate analyses.

TABLE 1. Results of the univariate analyses used to determine the selection of variables for the model

Clinical characteristics	OSA (n = 1089)		No OSA (n = 8796)		*P*-value
	n	%	n	%	
Age (mean ± SD)	64 ± 10.4		64 ± 12.6		0.946
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Depression	390	37.9	1863	21.6	<0.001
Chronic lung disease	179	17.3	848	9.7	<0.001
Asthma	256	24.8	1250	14.3	<0.001
Peripheral arterial disease	103	10.1	578	6.7	<0.001
Atrial fibrillation/flutter	182	17.8	984	11.6	<0.001
Prior MI	320	31.1	1901	22.2	<0.001
Prior HF	80	7.8	306	3.6	<0.001
Prior valve surgery	26	2.5	145	1.7	0.065
Prior PCI	314	29.7	1784	20.6	<0.001
Prior CABG	120	11.2	715	8.2	0.001
Presentation for coronary angiography indication					
Acute Coronary Syndrome	660	60.6	6326	71.9	<0.001
STEMI	101	9.3	1831	20.8	
NSTEMI	270	24.8	2462	28.0	
Unstable angina	289	26.5	2033	23.1	
Stable angina	350	32.1	1995	22.7	
Non-ischaemic symptoms	79	7.3	475	5.4	

Demographic, Comorbidities and Clinical Features of Those With and Without OSA

All comparisons of baseline variables between 2 cohorts found to be statistically important differences, except age (64 ± 10.4 vs 64 ± 12.6, $P = 0.946$). Patients with OSA were more likely to be male (75.3% vs 65.5%, $P \leq 0.001$), have no obstructive coronary artery disease (NOCAD) on angiography (29.1% vs 26.3%, $P = 0.051$), non-smoker (16.3% vs 24.3%, $P \leq 0.001$), and have conventional cardiovascular risk factors (hypertension [83.3% vs 67.2%, $P < 0.001$], dyslipidaemia [80.6% vs 65.7%, $P < 0.001$] and diabetes mellitus [50.2% vs 29.3%, $P < 0.001$]) or prior cerebrovascular disease (11.8% vs 7.0%, $P < 0.001$), depression (37.9% vs 21.6%, $P < 0.001$), chronic lung disease (17.3% vs 9.7%, $P < 0.001$), asthma (24.8% vs 14.3%, $P < 0.001$),

peripheral arterial disease (10.1% vs 6.7%, $P < 0.001$), and atrial fibrillation/flutter (17.8% vs 11.6%, $P < 0.001$), as compared with patients without OSA.

Patients with OSA were more likely to have experienced prior cardiovascular events of myocardial infarction (31.1% vs 22.2%, $P < 0.001$), prior percutaneous coronary intervention (29.7% vs 20.6%, $P < 0.001$), prior heart failure diagnosis (7.8% vs 3.6%, $P < 0.001$) and prior coronary artery bypass grafting (11.2% vs 8.2%, $P = 0.001$), as compared with patients without OSA.

Patients with OSA were less likely to have acute coronary syndrome presentation (60.6% vs 71.9%, $P < 0.001$) prior to coronary angiography, presenting as stable angina, in comparison with patients without OSA.

Independent Predictors of OSA in Patients With Chest Pain Undergoing Coronary Angiography

Table 2 presents the final multivariate logistic regression model for predictors of OSA in patients with chest pain undergoing coronary angiography. The Hosmer-Lemeshow test was not significant (H-L Goodness of Fit=5386, $P = 0.634$), indicating that the final model is a good fit. The c-statistic for the model is 0.7118.

Accordingly, risk factors such as male gender (OR 2.22, 1.86-2.65, $P < 0.001$), diabetes mellitus (OR 1.84, 1.58-2.14, $P < 0.001$), depression (OR 1.81, 1.55-2.12, $P < 0.001$), prior heart failure (OR 1.63, 1.22-2.18, $P = 0.001$), hypertension (OR 1.61, 1.32-1.95, $P \leq 0.001$), asthma (OR

TABLE 2. Multi-variable predictors of OSA in patients with chest pain undergoing coronary angiography

Predictors	OR	95% CI	*P*-value
Male	2.22	1.26-2.65	<0.001
No CAD	1.30	1.10-1.55	0.003
Not a Current Smoker	1.60	1.30-1.96	<0.001
Hypertension	1.61	1.32-1.95	<0.001
Dyslipidaemia	1.46	1.22-1.76	<0.001
Diabetes mellitus	1.84	1.58-2.14	<0.001
Cerebrovascular disease	1.36	1.07-1.73	0.012
Depression	1.81	1.55-2.12	<0.001
Chronic lung disease	1.40	1.12-1.73	0.003
Asthma	1.61	1.34-1.93	<0.001
Atrial fibrillation/flutter	1.30	1.06-1.60	0.012
Prior HF	1.63	1.22-2.18	0.001
Not admitted with ACS	1.45	1.25-1.69	<0.001

1.61, 1.34-1.93, $P < 0.001$), not a current smoker (OR 1.60, 1.30-1.96, $P < 0.001$), dyslipidaemia (OR 1.46, 1.22-1.76, $P < 0.001$), chronic lung disease (OR 1.40, 1.12-1.73, $P = 0.003$), cerebrovascular disease (OR 1.36, 1.07-1.73, $P = 0.012$) and atrial fibrillation/flutter (OR 1.30, 1.06-1.60, $P = 0.012$) were clinical characteristics independently associated with OSA. Additionally, non-obstructive coronary artery disease (NOCAD) (OR 1.30, 1.10-1.55, $P = 0.003$) and non-acute coronary syndrome presentation (OR 1.45, 1.25-1.69, $P < 0.001$) were predictive for OSA.

Finally, stable angina (32.1% vs 22.7%) and NOCAD (29.1% vs 26.3%, $P = 0.051$) were trended more common in patients with OSA versus no OSA.

Baseline Clinical Features

Table 1 summarises the demographic, comorbid clinical characteristics and the cardiac clinical features of CADOSA patients with and without OSA. Table 2 presents the final multivariate logistic regression model for predictors of OSA. The Hosmer-Lemeshow test was not significant, indicating that the final model is a good fit. The c-statistic for the model is 0.7118.

Discussion

In a cohort of patients undergoing coronary angiography for the investigation of suspected ischaemic chest pain, OSA was observed in 11% and associated with previously described OSA risk factors (male gender, hypertension, dyslipidaemia, diabetes, depression), as well as the novel finding of the presence of NOCAD was predictive (particularly in those with a stable angina pattern), This sub-group of patients may have coronary vasomotor disorders, either from coronary microvascular dysfunction (CMD) or Vasospastic angina (VSA).

Traditional cardiovascular risk factors such as male gender, hypertension and diabetes were demonstrated to be associated with OSA in the general population.[3-7] We have also noted these to be independent risk factors in our cohort of patients undergoing coronary angiography for chest pain syndromes. In addition, dyslipidaemia, the presence of cerebrovascular disease, atrial fibrillation/flutter and depression were other cardiovascular risk factors that were predictors of OSA in our cohort. Metabolic syndrome has been previously associated with OSA and many of our demonstrated predictors would be consistent with this finding.[3] However, the CADOSA registry does not document waist circumference and BMI, so the prevalence of metabolic syndrome cannot be accurately

calculated. Interestingly, current smoking was not a predictor of OSA in our cohort but similar to the general population, asthma was an independent predictor of OSA.

The association between OSA and coronary artery disease has been extensively evaluated, while understanding of the relationship between obstructive sleep apnoea and angina with no obstructive coronary artery disease is limited.[3-7] The pathophysiology of angina with NOCAD is not due to the presence of atherosclerotic plaques but functional abnormalities of coronary flow.

Two recent studies have invasively studied the coronary haemodynamics of patients with angina and NOCAD and its relationship to OSA. Both are performed in patients of Asian ethnicity. The first was a Chinese ethic at Nanjing who studied 1038 consecutive patients presenting with features of cardiac syndrome X, found a high prevalence of OSA in this population. This study demonstrated a strong correlation between the severity of OSA as measured by the AHI index and a reduction in coronary flow reserve and elevated high sensitivity C reactive protein.[9] Another small Japanese study (n = 42) by Tamura et al also found that patient with moderate to severe OSA is associated with inducible epicardial vasospasm.[10] Japanese ethnicity has been demonstrated to have a high prevalence of VSA, so extrapolation to the Australian population is unclear.[11]

The potential mechanisms leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, neuronal and/or inflammatory basis. The hypotheses are linked to hypoxic related sympathetic surge, oxidative stress, inflammation and more recently, endothelial dysfunction.[5,12-19] Inflammation and endothelial dysfunction has been postulated to have a pathogenic role in coronary microvascular dysfunction (MVD).[20]

The latter, in particular, has been demonstrated in peripheral haemodynamics study of patients with moderate to severe OSA population utilizing flow mediated dilatation pulse wave analysis to assess the change in augmentation index in response to salbutamol (an endothelium dependent vasodilator), assessing the endothelial function. Yim-yeh et al found that the effect of OSA on endothelial function is similar to that of diabetes mellitus.[19] Furthermore, in a rat model intermittent hypoxia is associated with coronary vascular dysfunction and vasoconstriction via RhoA activation.[21]

More importantly, there were several clinical studies, utilizing non-invasive modalities such as myocardial contrast echocardiography and magnetic resonance perfusion techniques, showing abnormalities in the coronary microvascular perfusion in moderate to severe sleep apnoea patients and improved after 3 months of CPAP therapy.[22,23] Thus far,

there has been no invasive coronary haemodynamics studies that have been evaluated in patients with significant OSA with or without a chest pain syndrome.

Study Limitations

The interpretation of the above study findings should be considered in the context of the following limitations. At first, every patient report of a formal previous diagnosis of OSA, accordingly, the data collection was based on patient self-reporting. It is well recognised that the incidence of obstructive sleep apnoea and the prevalence is generally underdiagnosed but would be impacted by diagnostic criteria. Second, there was no dedicated comprehensive coronary haemodynamic study nor provocative testing for coronary vasospasm and an assumption was made that these patients may possibly has coronary vasomotor disorder. Third, this study does not provide any insights into the mechanism underlying this potentially important association. Finally, reporting bias may occur as patient may not necessarily be tested for OSA without prior cardiac condition, in comparison to patients with established cardiac diagnosis.

Conclusion

In addition to established risk factors for OSA, this study found NOCAD to be independent predictor of OSA; especially in those presenting with a stable angina presentation. This suggests that coronary vasomotor disorders may be associated with OSA, although further detailed studies are required.

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CHAPTER 4

The Association of Obstructive Sleep Apnoea (OSA) in Ischaemia with no obstructive coronary artery disease (INOCA) – a pilot study.

In the predictors of obstructive sleep apnoea (OSA) population in the coronary angiogram database of South Australia (CADOSA) cohort of patients undergoing coronary angiography for the investigation of suspected ischaemic chest pain, 11 % of these patients have a diagnosis of OSA. They were associated with previously described OSA risk factors (male gender, hypertension, dyslipidaemia, diabetes, depression), as well as the novel finding of the presence of ischaemia with no obstructive coronary artery disease (INOCA) was predictive (particularly in those with a stable angina pattern). This sub-group of patients may have coronary vasomotor disorders, either from coronary microvascular dysfunction (CMD) or Vasospastic angina (VSA).[231] This chapter examined the relationship of obstructive sleep apnoea in patients experiencing angina with no obstructive coronary artery disease, with invasive coronary physiology study as a pilot study.

4.1 Statement of Authorship

Statement of Authorship

Title of Paper	The Association of Obstructive Sleep Apnea in Ischemia with No Obstructive Coronary Artery Disease - A Pilot Study.
Publication Status	☐ Published ☒ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review, study design and methodology, applied for ethics and governance approval, participants' recruitment, data collection and collations, analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Statement of Authorship

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Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Contribution to the Paper	Supervised development of work and manuscript evaluation.
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Contribution to the Paper	Performed literature search, critical appraisal / review, study design and methodology, applied for ethics and governance approval, participants' recruitment, data collection and collations, analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review, study design and methodology, applied for ethics and governance approval, participants' recruitment, data collection and collations, analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

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4.2 Introduction

Ischaemic heart disease does not always require significant structural coronary artery disease (CAD) to manifest as angina. There is an association between angina with significant morbidity and the presence of ischaemia with no obstructive coronary artery disease (INOCA) on coronary angiography. The underlying mechanism in this cohort may include coronary vasospastic angina (VSA), (either epicardial or microvascular) and/or coronary microvascular disorders (CMD). CMD is defined as abnormal coronary microvascular resistance (either arteriolar or pre-arteriolar) that is clinically evident as an inappropriate coronary blood flow response, impaired myocardial perfusion and/or myocardial ischaemia that cannot be accounted for by abnormalities in the epicardial coronary arteries (traditionally coined as coronary syndrome X).[2,3,38,162,232-235]

Obstructive sleep apnoea (OSA) is a common sleep-related breathing disorder which is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. A 2016 Australian online survey reported 8% of participants having a diagnosis of OSA.[116] These patients suffer from poor sleep quality and impaired quality of life.[12] OSA is recognized to be a risk factor for hypertension, ischaemic heart disease, cardiac arrhythmias and heart failure through a number of postulated mechanisms that encompasses hemodynamic, autonomic and inflammatory disturbances. [7-11]

In 1994, a series of case reports suggested that patients with OSA may experience angina in the absence of obstructive coronary artery disease.[225] More recently, the association between OSA and CMD or VSA was demonstrated in patients of Chinese and Japanese

ethnicity.[145,146] Japanese ethnicity has been demonstrated to have a high prevalence of VSA, so extrapolation to the Australian population is unclear.[92]

South Australian registry (CADOSA) data has demonstrated that among patients undergoing coronary angiography for the investigation of suspected ischaemic chest pain, OSA was prevalent in 11% of patients. In addition to previously described OSA risk factors (male gender, hypertension, dyslipidaemia, diabetes, depression), the novel finding of the presence of INOCA, was independently predictive of OSA, particularly in those with a stable angina pattern. This sub-group of patients may have functional coronary vasomotor disorders, either from coronary microvascular dysfunction (CMD) or Vasospastic angina (VSA).[231]

This pilot study assessed the association and predictors of OSA in patients who have been diagnosed with coronary vasomotor disorders, which included proven epicardial or microvascular spasm, abnormal invasive coronary flow reserve (CFR) and hyperaemic microvascular resistance (HMR), alone or in combination.

4.3 Methods

4.3.1 Study Design

We prospectively screened and recruited participants with ischaemia with no obstructive coronary artery disease (INOCA) to an observational study (Australia New Zealand Clinical Trials Registry number, ACTRN12618000149268 and ACTRN12618000227291). Those who met the inclusion criteria and gave informed consent to participate in invasive

coronary diagnostic studies, and a type 2 ambulatory sleep study after independent assessment by a Sleep Physician or had a previous diagnosis of obstructive sleep apnoea but not currently on treatment were included.

Inclusion criteria were clinical diagnosis of angina, persistent angina, coronary angiography demonstrating normal and no obstructive coronary artery disease (<50% diameter stenosis) and patients able and willing to give appropriate informed consent.

Exclusion criteria included severe respiratory disease defined as severe chronic obstructive pulmonary disease (FEV1/FVC<70% and FEV1 <50% predicted), resting SaO2 <90%, contra-indication to CPAP use, active CPAP treatment of OSA, increased risk of a sleep-related accident and/or excessive daytime sleepiness, defined by any one of the following: driver occupation (e.g. truck, taxi), 'fall asleep' accident or 'near miss' accident in previous 12 months or a high Epworth Sleepiness Scale score (>15). The Central Adelaide Local Health Network Human Research Ethics Committee approved the study (reference HREC/17/TQEH/156 and HREC/17/TQEH/177).

4.3.2 Study cohort

The study cohort were participants who had angina and normal coronaries following the invasive coronary haemodynamic studies and were screened or had a previous diagnosis for OSA that was currently untreated. In those without a previous diagnosis of OSA, the type 2 ambulatory sleep study was performed within 4 weeks of the coronary procedure after clinical assessment by a consultant sleep physician.

4.3.2.1 Ischaemia with no obstructive coronary artery disease (INOCA) definition

Angina like symptoms with or without signs of myocardial ischaemia on functional testing and the absence of any obstructive coronary artery stenosis (≤50% luminal diameter stenosis and fractional flow reserve ≥0.80 during coronary angiography)[36,236].

4.3.2.2 Interventional diagnostic procedure in angina with INOCA for assessment of coronary vasoactivity

The interventional diagnostic procedure consisted of assessment of coronary circulation vasorelaxation using invasive coronary physiology at rest and with hyperaemia and assessment of the propensity of coronary circulation to excessive vasoconstriction using intra-coronary acetylcholine (microvascular and/or epicardial vasospasm). The left anterior descending coronary artery was the target vessel because it subtends the largest myocardial mass.[3]

6F-guiding catheter was used to engage the left main coronary artery. A temporary pacing electrode was advanced into right ventricle via femoral venous approach and set to a threshold of 50bpm. Combowire (dual sensor guide wire) was calibrated and advanced in the proximal to mid left anterior descending artery (LAD), ensuring a good Doppler signal waveform. ECG, pressure and flow velocity waveforms were recorded using the ComboMap system (Volcano Corporation San Diego, CA, USA). Systemic, coronary haemodynamics, 12 lead ECG were monitored throughout the protocol and the procedure was covered with intravenous heparin (50–70 U/kg). Patients underwent a comprehensive invasive coronary

haemodynamic evaluation that included the assessment of resting angiographic contrast flow, coronary microvascular hyperaemic function, coronary endothelial function and provocative coronary spasm testing. The details of these components are outlined below:

(1) *Resting angiographic contrast flow* assessment was undertaken for the diagnosis of the Coronary slow flow phenomena (CSFP), based upon the contrast opacification rate; that is requiring ≥3 beats to opacify the vessel (equivalent of TIMI-2 flow). The TIMI frame count was also calculated to provide a more quantitative measure of angiographic flow.

(2) *Coronary microvascular hyperaemic function* was assessed using coronary flow reserve (CFR) and hyperaemic microvascular resistance index (HMR) measured by a combined pressure/Doppler wire (Combowire, Volcano Corporation, San Diego, CA, USA). This procedure has been previously described in the literature [237,238]. Following pressure equalization in the aorta and then placement of the Combowire in the LAD, baseline resting coronary physiology measurements including mean aortic and distal pressure (Pa & Pd) and average peak velocity (APV) were recorded over a 5-minute period and then reassessed following hyperaemic stimulus with intravenous adenosine (140mcg/kg/min) via a femoral venous line infused over two minutes. HMR was calculated as the distal coronary pressure divided by APV at maximal hyperaemia and the HMR >1.9 was used to define coronary microvascular dysfunction[68]. CFR was calculated as a ratio of peak and baseline APV and the value of < 2.5 was considered as an impaired flow reserve.[62]

(3) *Coronary endothelial function* was assessed with low dose intracoronary acetylcholine (IC-ACh) infusions 10^{-6} mol/L (0.18 μg/min) and 10^{-5} mol/L (1.8 μg/min) administered over 2 minutes respectively[70,71]. Combowire remained in-situ and Doppler measurement of peak velocity was obtained at the end of each acetylcholine infusion before contrast injection. Post

acetylcholine cine images were obtained for each concentration for quantitative coronary angiography (QCA) assessment (see below). We ensured that coronary flow returned to baseline before each infusion. Epicardial coronary artery endothelial dysfunction was defined on the basis of absence of a vasodilation (including vasoconstriction) to the ACh infusion i.e. no change or reduction in coronary diameter in response to the ACh infusion[72]. Microvascular endothelial dysfunction was defined as a coronary blood flow increase <50% from baseline[73]. Coronary blood flow response was calculated from the Doppler-derived time-velocity integral and vessel diameter by the following equation: $coronary\ blood\ flow = \pi\left(\frac{average\ peak\ velocity}{2}\right).\left(\frac{vessel\ diameter}{2}\right)^2$. Vessel diameter was calculated at the mid-LAD segment, just distal to the Doppler wire tip.

(4) <u>*Coronary spasm provocation testing*</u> was performed using incremental bolus doses of IC-ACh (25, 50 and 100mcg) administered over 20 seconds in left coronary system, with coronary angiography was performed within one minute of each injection. Provocative testing was concluded following either the induction of occlusive/sub-occlusive coronary artery spasm, or by attaining the maximal ACh dose without inducing coronary spasm. Right coronary artery (RCA) was assessed for inducible spasm using 25 & 50 mcg IC-ACh boluses, if no spasm was documented in left system. The provocation test was considered diagnostic for *vasospastic angina* if ACh administration induced (i) chest pain, (ii) ischaemic ECG changes, defined as transient ST-segment depression or elevation ≥0.1 mV in at least 2 contiguous leads on 12 lead continuous monitoring[2,75-77], and (iii) ≥90% vasoconstriction in epicardial artery.[38] A diagnosis of '*microvascular spasm*' was made if ACh administration induced (i) chest pain, and (ii) ischaemic ECG changes as above, in (iii) the absence of coronary artery constriction (< 90%). *Basal epicardial coronary artery tone* was determined by the change in coronary artery diameter from baseline images in response to 150mcg of intracoronary nitroglycerine

administered into the right and left coronary arteries, at the end of the procedure.

4.3.2.3 Angina with INOCA and impaired coronary microvascular function definition

In patients with INOCA, impaired coronary microvascular function was defined as a hyperaemic microvascular resistance index (HMR) >1.9 and/or coronary flow reserve (CFR) <2.5.[62,68]

4.3.2.4 Sleep study

A type 2 ambulatory sleep study has a high degree of certainty in the diagnosis of obstructive sleep apnoea as compared to in-laboratory polysomnography to diagnose obstructive sleep apnoea.[114] The Alice PDx (Philips Respironics, Murrayville, PA) sleep study device was used which had a minimum of seven channels recording EEG, EOG, chin EMG, heart rate, nasal flow respiratory effort (thoracic and abdominal bands), oxygen saturation and limb EMG. Sleep staging and calculation of an AHI was performed using the software Sleepware G3 (Phillips Respironics, Murrayville, PA).

4.3.2.5 Obstructive Sleep Apnoea definition

Obstructive sleep apnoea (OSA) is caused by recurrent collapse of the upper airway during sleep, leading to intermittent airway obstruction and absence of airflow. Diagnosis was

based on “Australasian Sleep Association Guidelines for Sleep Studies in Adults” recommendation [114]

The apnoea, hypopnoea index (AHI) has been used to diagnose patients as having OSA and to classify the severity of OSA. OSA severity in our cohort were based on the Chicago criteria classification.[115]

- Normal: AHI <5 events per hour of sleep.
- Mild OSA: AHI 5 to 15 events per hour of sleep.
- Moderate OSA: AHI 15 to 30 events per hour of sleep.
- Severe OSA: AHI >30 events per hour of sleep.

4.3.3 Statistical Methods

Statistical analyses were performed using SPSS version 25 (IBM Inc, Armonk, NY, USA). Normally distributed continuous data was expressed as mean ± standard deviation and tested with unpaired t-tests between groups. Skewed distributions were expressed as median and inter-quartile and means tested using Mann-Whitney U. A multivariate logistic regression analysis was performed to estimate odd ratios (ORs) with 95% confidence intervals (CIs). In multivariate analyses, we included variables that were considered significant (p value <0.2 on univariate analysis) and a priori selected potential confounders. Variables in the multivariable models represented the most common confounding or mediating factors of the association between OSA and abnormal haemodynamics in patients with INOCA. A p value < 0.05 was considered to be statistically significant.

4.4 Results

Of the 42 patients with angina screened with coronary angiography, 2 were excluded from further study due to the presence of obstructive coronary artery disease. A total of 40 participants of INOCA underwent interventional diagnostic procedure for coronary vasomotor disorder diagnosis. Out of these, 10 participants declined sleep study. Twenty-one participants underwent sleep study for OSA diagnosis, and a further 9 participants had a prior formal diagnosis of OSA not currently being treated.

In our study cohort (n = 30) (Figure 1), 87% (n=26) of the participants had a diagnosis of OSA. Accordingly, 11 with mild severity, 7 with moderate severity and 8 with severe OSA. No OSA was found in 4 participants. 73.3% (n=22) had abnormal coronary microvascular function (Table A).

In the 30 patients who had a sleep study, the diagnostic coronary haemodynamic evaluation demonstrated 2 with abnormal HMR, 7 with abnormal CFR, 4 with epicardial spasm, 1 with both epicardial spasm and abnormal HMR, 8 with both abnormal CFR and epicardial spasm and 4 with abnormal HMR, CFR and epicardial spasm. Only 4 had no objective abnormalities (Diagram A).

4.4.1 Demographic and comorbid clinical characteristics of the study cohort

Table B provides demographic and comorbid clinical characteristic for the study population cohorts classified into AHI < 15, (no and mild OSA) versus AHI ≥15 (moderate

OSA and above). In comparisons of baseline variables between 2 cohorts, age and BMI were found to be statistically different. Patient with OSA was more likely to be older [61.4±8.7 vs 49.9±9.7, p=0.002] and higher BMI [30.3±6.6 vs 35.4±6.0, p=0.036].

4.4.2 The clinical symptom characteristics of the study cohort

Table C summarises the clinical symptom characteristics of the study cohort between patients with AHI < 15, (no and mild OSA) versus AHI ≥15 (moderate and severe OSA). There was no statistical difference between symptoms characteristic of angina.

4.4.3 Predictor of abnormal coronary microvascular function

Figure 2 presents the multivariate logistic regression model for predictors of OSA in patients with angina and NOCAD with abnormal coronary microvascular function. OSA [OR 53.95, 1.41-2065.01, p=0.032] was a significant independent predictor of abnormal coronary microvascular function.

4.5 Discussion

In this study of South Australian patients with INOCA, functional coronary vasomotor disorders by invasive spasm challenge and coronary haemodynamic assessment were highly prevalent, consistent with previous evidence.[3] Additionally, this study is the first to show that the presence of OSA was the only significant independent predictor of abnormal coronary microvascular function in patients with INOCA. These observations are in keeping with recently published CADOSA registry data showing an increase in the prevalence of obstructive sleep apnoea in a similar cohort with the presence of INOCA, being independently predictive of OSA, particularly in those with a stable angina pattern.[116,231]

The association between OSA and structural coronary artery disease has been extensively evaluated, while understanding of the relationship between obstructive sleep apnoea and angina with no obstructive coronary artery disease is limited.[7-11] The pathophysiology of INOCA is not necessarily due to the presence of atherosclerotic plaques but functional abnormalities of coronary flow. There have been several clinical studies, utilizing non-invasive modalities such as myocardial contrast echocardiography and magnetic resonance perfusion techniques, showing abnormalities in the coronary microvascular perfusion in moderate to severe sleep apnoea patients improved after 3 months of CPAP therapy.[147,148]

Two recent studies have also invasively studied the coronary haemodynamics of patients with INOCA and its relationship to OSA. Both are performed in patients of Asian ethnicity whereas our cohort was 90% Caucasian ethnicity. The first was a Chinese cohort at Nanjing where in 1038 consecutive patients presenting with features of cardiac syndrome X,

the study found a high prevalence of OSA. This study demonstrated a strong correlation between the severity of OSA as measured by the AHI index and a reduction in coronary flow reserve and elevated high sensitivity C reactive protein.[145] Second was a small Japanese study (n=42) by Tamura et al found that patient with moderate to severe OSA is associated with inducible epicardial vasospasm.[146] Japanese ethnicity has been demonstrated to have a high prevalence of VSA, so extrapolation to the Australian population is unclear.

The potential mechanisms of OSA leading to adverse cardiovascular pathophysiology are complex and likely related to changes on a haemodynamic, autonomic, neuronal and inflammatory basis. The hypotheses are linked to hypoxic related sympathetic surge, oxidative stress, inflammation and endothelial dysfunction.[9,135-142] Inflammation and endothelial dysfunction has been postulated to have a pathogenic role in coronary microvascular dysfunction (MVD).[143] In a peripheral haemodynamics study of patients with moderate to severe OSA utilizing flow-mediated dilatation pulse wave analysis to assess endothelial function by means of the change in augmentation index in response to salbutamol (an endothelium dependent vasodilator) Yim-yeh et al. found that the deleterious effect of OSA on endothelial function is similar to that of diabetes mellitus.[142] Furthermore in a rat model, intermittent hypoxia was associated with coronary vascular dysfunction and vasoconstriction via RhoA activation.[144]

Thus far, there has been no invasive coronary haemodynamics studies that have been evaluated in patients with significant OSA with or without a chest pain syndrome.

4.6 Study Limitations

The interpretation of our study findings should be considered in the context of the following limitations. Firstly, about a third of patients' diagnosis of OSA relied upon a previous diagnosis of OSA not using the Alice PDx system but using a variety of Tier 1 or 2 sleep study modalities. Despite this, all previously performed sleep study reports were retrieved from their Sleep specialist and reassessed for confirmation. They were not on CPAP therapy at the time of coronary angiography due to non-compliance or inability to tolerate CPAP therapy. Secondly, this study while showing an important association between OSA and abnormal coronary haemodynamics, does not provide any insights into the mechanism underlying this observed phenomenon. Thirdly, the number of patients with pure epicardial spasm was too small to draw a meaningful conclusion between OSA and epicardial spasm. Finally, previous studies have demonstrated in the general population an association between OSA and traditional cardiovascular risk factors including male sex, hypertension and diabetes.[7-11] With no healthy control in our study cohort, we were unable to demonstrate a meaningful difference to reflect this in our study cohort being limited without a healthy control cohort.

4.7 Conclusion

This study has provided important observations of a high prevalence of abnormal coronary vasomotor function in patients with INOCA and the presence of OSA was a significant independent predictor of functional coronary artery disease. Thus, OSA may play a significant pathophysiological role in the coronary vasomotor mechanism of angina in this subgroup and screening of OSA should be considered. The benefit of treatment of OSA in this

subset remains unknown but these results suggest a randomised controlled trial of nocturnal CPAP for this cohort is worth pursuing. Generally, patients with INOCA are resistant to conventional angina management, so alternative treatment options are urgently required. Also, further mechanistic studies evaluating the pathophysiological relationship in this sub-group of patients is important to understand the likely complex relationship between these two conditions.

4.8 Figures

Figure 1 Study Overview

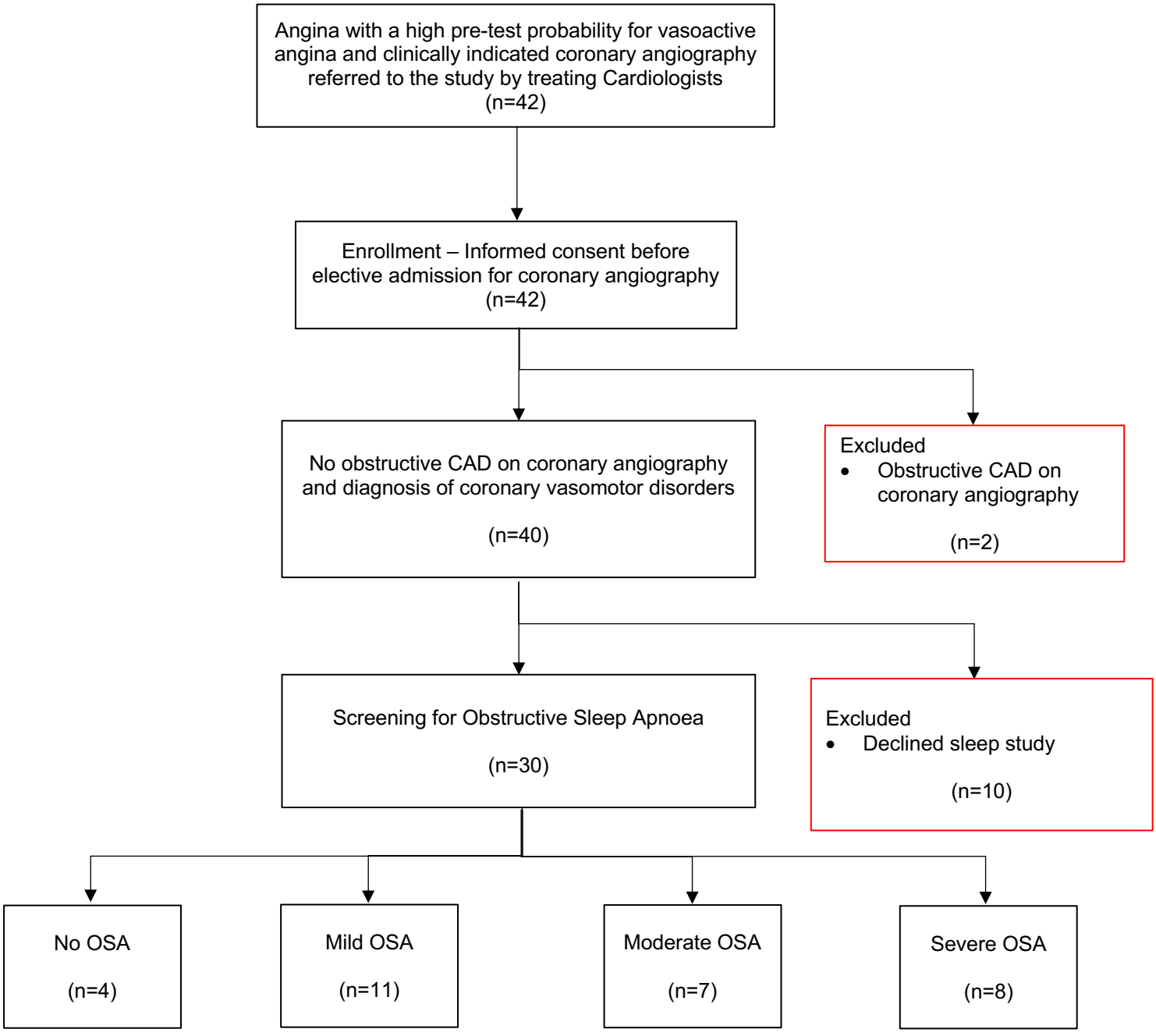
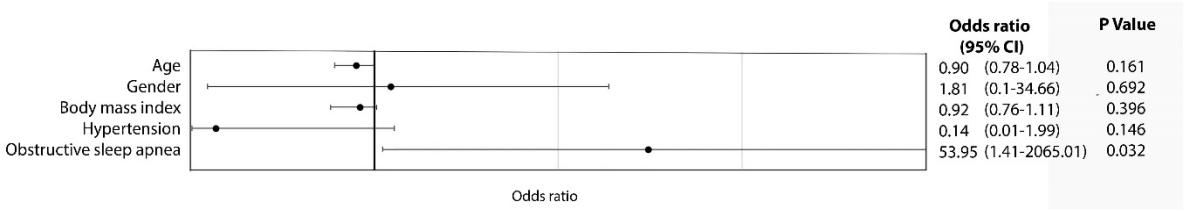


Figure 2 – Predictor of abnormal coronary microvascular function.



4.9 Tables

Table A - INOCA and coronary microvascular function

Coronary Microvascular Function	N (%)
Normal	8 (26.7)
Abnormal	22(73.3)

Table B – Demographic and comorbid clinical characteristics of the study cohort.

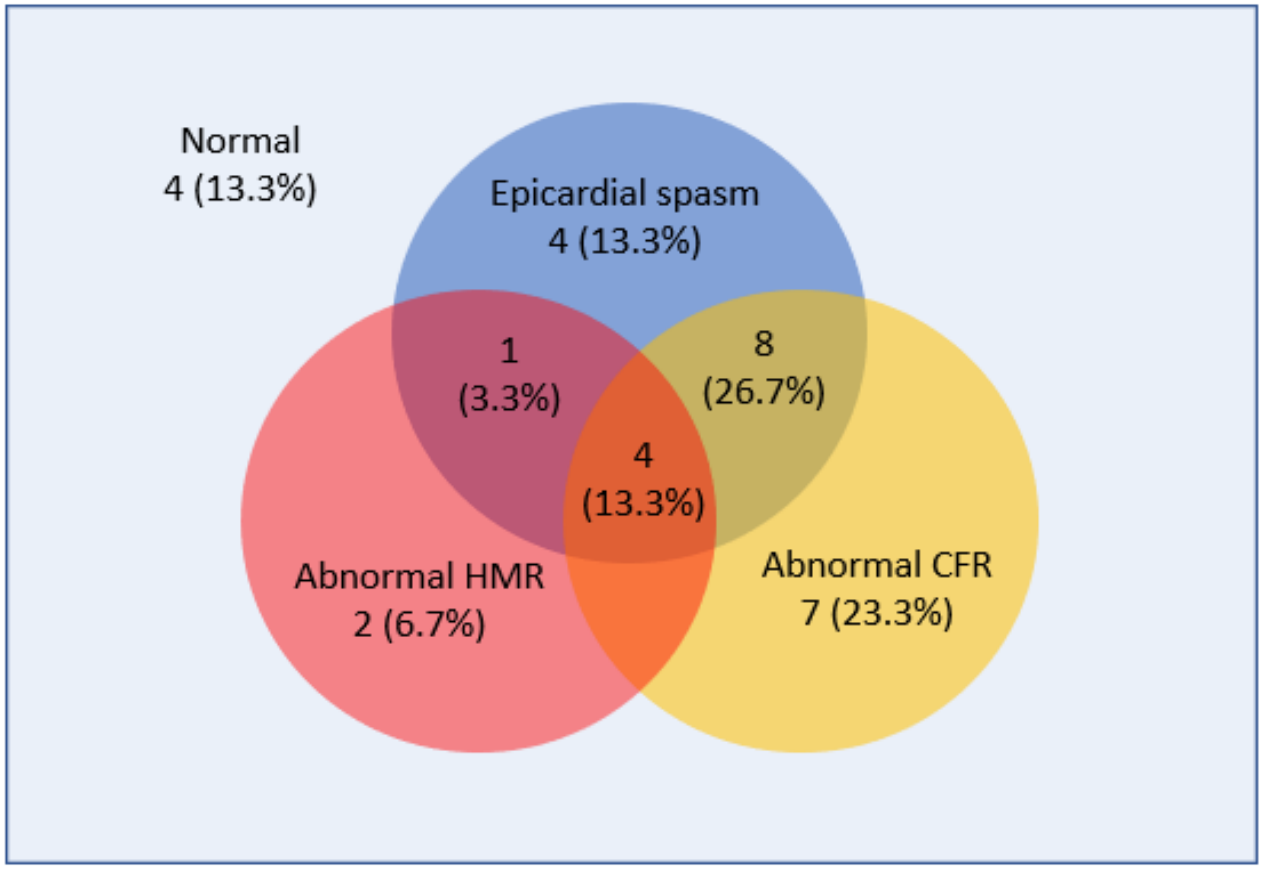
Baseline Demography	No/mild OSA (n=15)	Moderate and severe OSA (n=15)	All	P value
Age (mean ± SD)	49.9 ± 9.7	61.4 ± 8.7	55.6 ± 10.8	0.002
Gender -female (n, %) - Post menopause	13 (86.7) 8 (61.5)	11 (73.3) 11 (100)	24 (80.0) 19 (79.1)	0.651
Ethnicity - Caucasian - Indigenous - Asian	 13 (86.7) 2 (13.3) 0 (0)	 14 (93.3) 0 (0) 1 (6.7)	 27 (90.0) 2 (6.7) 1 (3.3)	0.789
Obesity	8 (53.3)	13 (86.7)	21 (70.0)	0.109
BMI	30.3 ± 6.6	35.4 ± 6.0	32.9 ± 6.7	0.036
Hypertension	6 (40.0)	9 (60.0)	15 (50.0)	0.273
Diabetes	2 (13.3)	3 (20.0)	5 (16.7)	1.000
Dyslipidaemia	9 (60.0)	10 (66.7)	19 (63.3)	0.705
Family history of ischemic heart disease	9 (60.0)	7 (46.7)	16 (53.3)	0.464
Active smoker	2 (13.3)	2 (13.3)	4 (13.3)	0.705
Depression/anxiety	8 (53.3)	7 (46.7)	15 (50)	0.715
Migraine	7 (46.7)	3 (20.0)	10 (33.3)	0.121
Atrial fibrillation	0 (0)	2 (13.3)	2 (6.7)	0.483
GORD	8 (53.3)	10 (66.7)	18 (60)	0.456
Medication - ACE/ARB - Beta-blocker - CCB – dihydropyridine - CCB – Non DHP - Nitrates - SSRI - NSAID - Antiplatelet - Anticoagulation - Statins - HRT	 6 (40.0) 1 (6.7) 3 (20.0) 8 (53.3) 9 (60.0) 7 (46.7) 1 (6.7) 2 (13.3) 0 (0) 8 (53.3) 1 (6.7)	 5 (33.3) 2 (13.3) 3 (20.0) 8 (53.3) 8 (53.3) 6 (40.0) 2 (13.3) 5 (33.3) 2 (13.3) 9 (60.0) 2 (14.3)	 11 (36.7) 3 (10.0) 6 (20.0) 16 (53.3) 17 (56.7) 13 (43.3) 3 (10.0) 7 (23.3) 2 (6.7) 17 (56.7) 3 (10.3)	 0.705 1.000 1.000 1.000 0.713 0.713 1.000 0.390 0.483 0.713 0.598

Table C – Clinical symptom characteristics of the study cohort.

Variables	No/mild OSA (n=15)	Moderate and severe OSA (n=15)	All	P value
Symptom				
- Angina on exertion	11 (73.3)	12 (80.0)	23 (76.7)	1.000
- Angina at rest	15 (100)	13 (86.7)	28 (93.3)	0.483
- Angina with emotional stress	6 (40.0)	5 (33.3)	11 (36.7)	0.705
- Nocturnal angina	9 (60.0)	7 (46.7)	16 (53.3)	0.464
- Angina on exposure to cold	3 (20.0)	6 (40.0)	9 (30.0)	0.427
Duration of angina				0.751
- Less than 20 mins	6 (40.0)	8 (53.3)	14 (46.7)	
- 20 mins- 1 hr	8 (53.3)	3 (20.0)	11 (36.7)	
- >1 hr	1 (6.7)	4 (26.7)	5 (16.7)	
Frequency of angina				
- First presentation	0 (0)	1 (3.3)	1 (3.3)	
- Once a month	2 (13.3)	2 (13.3)	4 (13.3)	
- Weekly	1 (6.7)	2 (13.3)	3 (10.0)	
- > once a week	12 (80.0)	10 (66.7)	22 (73.3)	

4.10 Diagrams

Diagram A – Interventional diagnostic procedure outcomes



HMR – Hyperaemic Microvascular Resistance

CFR – Coronary Flow Reserve

CHAPTER 5

The Evolution of Ischaemia Detection with Echocardiography

Myocardial ischaemia detection has come a long way since the clinical symptom was scientifically described several centuries ago and matched with pathophysiological correlation. Most cases are atherosclerotic macro-vessel obstruction or thrombosis and to a lesser degree various other forms of obstruction including dynamic changes in both small and large vascular beds. With the identification of non-linearity of ischemic cascade and hence concepts of ischemic constellation, it is increasingly clear that circumstances can determine which aspect of the cascade is noted and altering the probability of significant ischaemia. Myocardial deformation and perfusion imaging adds a novel perspective of myocardial performance under stress. This chapter is aimed at understanding the clinical connect between ischaemia and tissue changes by firstly, describing the historical and clinicopathophysiological perspective of deformation imaging and secondly clinical advances of tissue imaging, the body of evidence currently available for this methodology and finally, the feasibility and barriers to clinical translation.

5.1 Statement of Authorship

Statement of Authorship

Title of Paper	The Evolution of Ischemia Detection with Echocardiography.
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ooi EL, Chapman M, Iyngkaran P, Rajendran S, Mahadavan G. The Evolution of Ischemia Detection with Echocardiography. Curr Cardiovasc Imaging Rep 2020;13:16.

Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review and wrote manuscript and acted as corresponding author on behalf of Dr Mahadavan.		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Matthew Chapman		
Contribution to the Paper	Helped with literature review and manuscript evaluation.		
Signature		Date	24-01-2022

Name of Co-Author	Pupalan Iyngkaran		
Contribution to the Paper	Helped with literature review and manuscript evaluation. NB// My contribution was the minimum required. Agree Dr Ooi has made the most significant contributions.		
Signature		Date	24/01/2022

Please cut and paste additional co-author p

Statement of Authorship

Title of Paper	The Evolution of Ischemia Detection with Echocardiography.
Publication Status	☒ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ooi EL, Chapman M, Iyngkaran P, Rajendran S, Mahadavan G. The Evolution of Ischemia Detection with Echocardiography. Curr Cardiovasc Imaging Rep 2020;13:16.

Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review and wrote manuscript and acted as corresponding author on behalf of Dr Mahadavan.		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sharmalar Rajendran		
Contribution to the Paper	Supervised development of work, helped in literature review and manuscript evaluation.		
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Name of Co-Author	Gnanadevan Mahadavan		
Contribution to the Paper	Supervised development of work, helped in literature review and manuscript evaluation.		
Signature		Date	20/01/2022

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5.2 Introduction

The evolution of ischaemia started with clinicopathological correlation of typical angina symptoms with atherosclerotic coronary artery disease. From this understanding dynamic testing was introduced to reproduce and quantitate inducible ischaemia presenting as subjective clinical symptoms or objective changes in haemodynamics and electrical (electrocardiography – ECG) or contractile function (imaging). The cumulative findings generate a probability largely from the initial works of Diamond and Forster et al. Protocols based on these provide a probability of successfully detecting and excluding significant coronary artery disease (CAD).[15,16,239-243]

Stress echocardiography is potentially the most readily available modality of non-invasive imaging as well as being cost effective and free of ionizing radiation. The problem with stress echocardiography has been the moderate sensitivity of the test, despite the high specificity and it's prognostically impressive negative predictive value.

A normal stress echo is associated with an annual risk of 0.4-0.9% for cardiac mortality or acute myocardial infarction based on a total of over 11,000 patients.[244] However, it would be prudent to concentrate on improving the sensitivity of stress echocardiography to make it an ideal stand-alone first-line modality for identifying, prognosticating and tailoring treatment for stable coronary artery disease, and in conjunction, improving imaging surrogates for the coronary arterial bed, such as calcium scoring and peripheral imaging.

The two technological advances in the last decade have been the evolution of tissue deformation imaging and the use of contrast for perfusion with flash imaging Myocardial contrast echocardiography (MCE).

The introduction of tissue deformation imaging has thus raised the question of what role greater information on myocardium has in diagnosis and management of ischemic heart disease (IHD)? To answer this, we have to detail the gaps in current screening for IHD across the spectrum of care from acute to stable. The acute setting is a crowded field with high-sensitive troponin (hsTrop), abnormal rest ^{99m}technitium-sestamibi (MIBI) myocardial perfusion imaging (MPI) and computed tomography coronary angiography (CTCA), however, the role for deformation and perfusion imaging in regional centres are less clear and could be worthy of exploration. In the stable setting, improving the predictive capabilities for ischaemia earlier on the ischemic cascade to improve sensitivity with; a. low workloads and exercise duration; b. various compensatory states (ischemic preconditioning, coronary collaterals); c. pre-existing abnormalities (e.g. left bundle branch block (LBBB), wall motion defects); d. and finally improving the quantification of ischemic zone or myocardium at risk for established disease are considerations to enhance surveillance. Caveats are age, gender, loading conditions and angle. These variables alone can raise inter- and intra- personal technical issue, in addition to the variables in observer interpretations.[245] Thus, any new strategy must improve from the existing cost efficacy equation among heterogeneous patient populations.

5.3 Cardiac contractility and the evolution of strain

Cardiomyocytes (cardiac muscle cell) is differentiated from skeletal and smooth muscle by its short, branched, striated and single nucleus with involuntary (intrinsic rhythm) and a feedback regulated contractile capacity. Cumulative global function is possible through intercalated discs, specialised junctions that anchor and provide communication via gap junctions, resulting in connectivity of electrical and mechanical flux and synchronised cardiac

haemodynamic performance. Many physiological and disease processes alter this equation, by affecting performance of each fibre based on events and feedback encountered; and the greater the magnification of this process the greater 'noise' will be generated. A completed electrical cycle will excite 3 layers of cardiac muscle fibres resulting in 3 vectors of myocardial deformation, longitudinal, radial, and circumferential.[246,247]

5.3.1 General principles of deformation imaging techniques

Tissue imaging utilises Doppler and speckle tracking to detect actual or relative changes (rate) in velocity (v), length (l) to provide 'strain' and muscle rotational motion (d) as 'twist' or 'torsion', surrogates for myocardial systolic mechanics.[245-249]

Assessment of cardiac contractile function with traditional parameters (e.g. ejection fraction, EF) is limited by volumetric assessment and is suboptimal in providing accurate regional function assessments and changes reproducible. Tissue deformation or strain allows study of change from relaxed to contractile states in longitudinal strain (LS), circumferential strain (CS), or radial strain (RS) directions, both globally and regionally; and is load dependant similar to EF. Imaging techniques share the similar concept of identifying specific patterns or features and following it over time to provide data from specific analytical algorithms (tissue tracking). Techniques have evolved from echocardiographic derived velocity of circumferential fibre shortening, cardiac magnetic resonance (CMR) tissue tagging, tissue Doppler echocardiography, to presently used speckle tracking echocardiography (STE), and feature tracking (FT).[249] Tissue tracking analysis (deformation) using algorithms to detect actual or relative changes (rate) in velocity (v), length (l) to provide 'strain' and muscle

rotational motion (d) as ‘twist’ or ‘torsion’, surrogates for myocardial systolic mechanics.[245-249]

5.3.3 Principles and Specific modalities of tissue tracking and strain imaging

Specific modalities of tissue tracking, and strain imaging are well described.[245-250] Strain originates from description of 3D deformation of a cube over a specified time interval in six components (numbers or value). The myocardium is divided into numerous cubes, and three numbers are assigned for shortening along three external orthogonal axes (x, y, z) and three giving the skew in the x–y, x–z, and y–z planes. This concept is simplified to an internal three cardiac axes coordinates mentioned above (LS, CS, RS). First generation strain in 90’s utilised TDI however limitations included angle dependency.

Fixed factors e.g. age, sex, blood pressure (load) and operator factors e.g. foreshortening (underestimate), angle dependency in radial strain, poor lateral spatial resolution need to be factored.[245]

5.4 Speckle tracking – Two-dimensional (2D) and Three-dimensional (3D)

Speckle tracking would be considered the second generation of strain imaging. The advantage of this generation was the fact that strain measurements were angle independent but still required images of high frame rate and good quality. Whilst gaining traction in systemic

conditions such as with the diagnosis of amyloidosis and chemotherapy related cardiotoxicity, it's utility in assessing regional dysfunction is more limited. The main issue is the heterogeneity of strain measurements in different regions and the difficulty in tracking the speckles at higher heart rates during exercise. The technique is also sensitive to acoustic shadowing and reverberations resulting in underestimation of true deformation.

There have been a number of studies that have utilised 2D speckle tracking the latest being a study by Roushdy et al in 2017.[250] In a prospective 80 patient cohort, speckle tracking at peak stress for global longitudinal strain during dobutamine stress echocardiography (DSE) showed an increased sensitivity of 77% and specificity of 84% in detecting ischaemia, as well as reducing false positive results and improving detection of multi-vessel ischaemia. The cut-off value for Global longitudinal strain of > -16.9% was determined by performing receiver operating characteristic (ROC) analysis but the results were not validated in the validation group.

There was a smaller study that suggested speckle tracking during Dobutamine stress echocardiography requires expertise, as a group of experts performed better than fellows with interpretation of peak stress ischaemia from generation of bull's eye maps for peak longitudinal strain and post systolic shortening.[251]

Another major innovation in echocardiography are 3D matrix transducers with probe dimensions similar to 2D transducers whilst maintaining a relatively high frame rate of more

than 25 frames per second with single beat acquisition. This has resulted in image quality similar to 2D images but with the added benefits of 3D acquisition of avoiding the geometrical assumptions of left ventricle (LV) volumes inherent in 2D imaging. This technology has been combined with the other major development in echocardiography which has been the evolution of deformation imaging, in particular speckle tracking. A recent study by Dogdus et al. evaluated the utility of both these technological advances in stress echocardiography.[252] In this study, 120 patients who had coronary angiography after a positive non-invasive test were evaluated with a 3D assessment of deformation using a number of parameters, including global longitudinal strain (GLS) and global area strain (GAS) at rest. Two groups were created according to Gensini score based on the invasive angiography: noncritical stenosis (Gensini: 0–19) (n = 84) and critical stenosis (Gensini ≥ 20) (n = 36). Global longitudinal strain and all other strain parameters were significantly worse in patients with the critical CAD group compared with the noncritical CAD group. Receiver operator characteristic analyses were performed to find out ideal strain cut-off values to detect severe coronary artery disease defined as Gensiniscore≥20. A GLS value of ≥ 10 has 88.9% sensitivity and 92.9% specificity; A GAS value of ≥ 21 has 97.2% sensitivity and 88.1% specificity to detect critical CAD.

However, speckle tracking has been known to be afterload-dependant and in some circumstances may not be reflective of myocardial contractility. In addition, subsequent measurements may predispose to differences in blood pressure affecting accurate analysis and potentiate misinterpretation of LV contractile state.

5.5 Myocardial Work

Myocardial work (MW) is a novel non-invasive echocardiographic approach that assess regional myocardial work by LV pressure-strain loop (PSL) analysis via echocardiographic software, thus incorporating both strain and afterload with non-invasively estimated pressure from brachial cuff blood pressure. This technique has been validated with invasive LV pressure-volume loops (PVL) and regional myocardial metabolism with glucose turnover measured by positron emission tomography (PET).[253]

The invasive LV PVL provides haemodynamic parameters of contractility, elastance, power, energetics, and efficiency. For any given contractile state of the LV, it linearly correlates to myocardial oxygen consumption.[254,255]

Speckle tracking imaging is assessed utilising three apical images, typical frame rates are in the vicinity of 60-90 frames per second. After calculation of GLS, values of brachial systolic blood pressure and timing of valvular events are introduced to available software. The latest vendor-specific software is (EchoPAC V.202,GE). A non-invasive LV pressure curve adjusted via utilisation of the timings of aortic and mitral valve opening and closing events. The following parameters are generated:

1. Global MW index (MW) (mmHg %) (GWI): total work within the area of the left ventricular pressure volume loop (LV PSL). Where global values are calculated as averages of all segments.

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3. Global wasted MW (WW) (mmHg %): work performed by the LV that does not contribute to LV ejection. Classified as lengthening of myocytes and any additional shortening during the isovolumic relaxation phase.
4. MW Efficiency (GWE %): calculated via constructive MW divided by the sum of CW and WW. (These values will not be affected by peak LV pressure).

Myocardial ischaemia prediction, utilising non-invasive MW has been promising with greater sensitivity and specificity in comparison to GLS.[13,14] In a prospective registry of 115 patients, who were referred for coronary angiography, also underwent echocardiography and myocardial work assessment within 3 hours before invasive procedure. Pre-existing significant valvular or myocardial diseases were excluded in this study. While both LV GLS and global MW were significantly lower in all patients with significant CAD, despite the lack of resting regional wall motion abnormalities (RWMAs), global MW was superior to GLS with an area under the curve (AUC) of 0.786 and 0.693 respectively ($P<0.001$). According to the AUC, the optimal cut-off for global MW for the detection of CAD was 1810mmHg% with a sensitivity of 92% and a specificity of 51%. (In contrast, the optimal cut-off for GLS was 16.7% with a sensitivity of 89.3% and specificity of 61.8%.) Moreover, MW was significantly reduced in both single and multivessel CAD while the reduction of GLS was only significant in multivessel CAD.[13] Novel results have been described by Boe et al. in non-ST elevation acute coronary syndrome patients. They found regional MW of less than 1700mmHg % in more than

four adjacent segments was again more significantly better than GLS and ejection fraction in determining an acute coronary occlusion with a sensitivity of 81% and specificity of 82%.[13,14]

In summary, MW is superior to GLS in predicting the presence and extent of CAD as well as assessing early subclinical ischemic myocardial dysfunction, despite the absence of resting RWMAs. This is a useful additional risk stratification tool for early coronary intervention. Further, the accounting for afterload by MW reduces the likelihood of false-positive results in hypertensive patients with suspected CAD.

5.6 Myocardial contrast echocardiography

There are a number of contrast agents approved in clinical practice for opacification of the left ventricular cavity and enhancement of endocardial definition. This has improved the assessment of wall motion abnormalities.

Perfusion stress echocardiography with flash imaging for destruction and replenishment of contrast (myocardial contrast echocardiography), has certainly shown to have improved the sensitivity of stress echocardiography. This technique utilizes microbubbles that remain in the intravascular space and the contrast intensity reflects the concentration of the microbubbles in the myocardium. A homogeneous opacification of the myocardium after destruction of the microbubbles during high-mechanical index (flash imaging), is regarded as normal perfusion and absence of CAD. If the replenishment rate is reduced, this is suggestive of significant CAD.

In a study of 1252 patients undergoing dipyridamole stress echocardiography and perfusion imaging, the event free survival when there were no perfusion or wall motion abnormalities over a 25-month follow-up was in the order of 97.9%. When there were perfusion abnormalities but no wall motion abnormalities, the event free survival in that period reduced by almost 10%.[256]

The popularity of this technique is limited by the lack of progress in the perfusion software and flash imaging on the latest generation echo machines. The level of imaging on the earlier generation echo machines has not been reproduced, and most of those machines are not available or have been decommissioned in most echo labs.

5.7 Conclusion

Establishing the extent of ischaemia is critical in the diagnosis, management and prognostication of coronary artery disease. Stress echocardiography is a modality that is easily available, cost effective and radiation free, in establishing ischaemia. The evolution of stress echocardiography as described above has certainly improved the sensitivity of the test which has been its Achilles heel in the past. However, there still remains a lot of progress to be made before the use of these techniques become widespread in its clinical application and they all remain at the mercy of image quality especially post exercise.

5.8 Figures

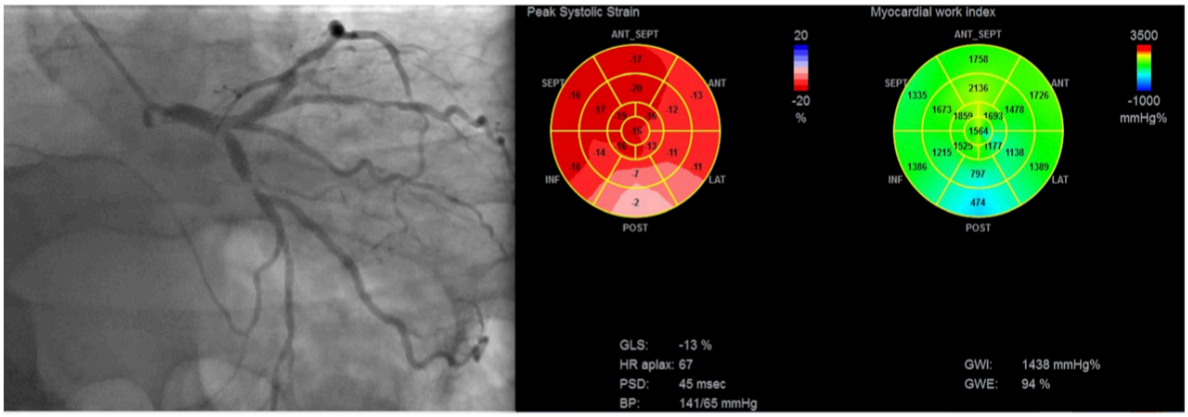

Fig. 1 There is reduced sensitivity for detecting left circumflex (LCx) ischemia by utilising wall motion abnormality. Below is an example of LCx ischemia detected by using three-dimensional (3D) speckle tracking and myocardial work

5.9 Appendix

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ECHOCARDIOGRAPHY (G. DWIVEDI, SECTION EDITOR)

The Evolution of Ischemia Detection with Echocardiography

Eng Lee Ooi [1,2] · Matthew Chapman [2] · Pupalan Iyngkaran [3] · Sharmalar Rajendran [1,2,4] · Gnanadevan Mahadavan [1,2,4]

Published online: 28 April 2020

Abstract
Purpose of Review This review is aimed at understanding the clinical connect betweenischemia and tissue changes by firstly, describing the historical and clinicopathophysiologicalperspective of deformation imaging and secondly clinical advances of tissue imaging; nextthe body of evidence currently available for this methodology; and finally, the feasibility andbarriers to clinical translation. There will also be a brief review of the feasibility and currentuse of contrast for perfusion with flash imaging Myocardial contrast echocardiography (MCE) for the detection of ischemia.
Recent Findings Myocardial ischemia detection has come a long way since the clinicalsymptom was scientifically described several centuries ago and matched withpathophysiological correlation. The majority of cases are atherosclerotic macro-vesselobstruction or thrombosis and lesser degree various other forms of obstruction includingdynamic changes in both small and large vascular beds. On a statistical level most lifethreatening cases are atherosclerotic and correlated physiologically with an ischemiccascade. With the identification of non-linearity of ischemic cascade and hence concepts ofischemic constellation, it is increasingly clear that circumstances can determine which aspectof the cascade is noted and altering the probability of significant ischemi a.
Summary Myocardial deformation and perfusion imaging adds a novel perspective of myocardial performance under stress.

Keywords Perfusion and deformation imaging · Myocardial ischemia · Strain imaging · Echocardiography and stress echocardiography

Introduction

The evolution of ischemia started with the clinicopathological correlation of typical angina symptoms with atherosclerotic coronary artery disease. From this understanding, dynamic testing was introduced to reproduce and quantitate inducible ischemia presenting as subjective clinical symptoms or objective changes in haemodynamics and electrical (electrocardiography (ECG)) or contractile function (imaging). The cumulative findings generate a probability largely from the initial works of Diamond and Forster et al. Protocols based on these provide a probability of successfully detecting and excluding significant coronary artery disease (CAD) [1–7].

Stress echocardiography is potentially the most readily available modality of non-invasive imaging as well as being cost-effective and free of ionising radiation. The problem with stress echocardiography has been the moderate sensitivity of the test, despite the high specificity and its prognostically impressive negative predictive value.

A normal stress echo is associated with an annual risk of 0.4–0.9% for cardiac mortality or acute myocardial infarction based on a total of over 11,000 patients [8]. However, it would be prudent to concentrate on improving the sensitivity of stress echocardiography to make it an ideal stand-alone first-line modality for identifying, prognosticating and tailoring treatment for stable coronary artery disease, and in conjunction, improving imaging surrogates for the coronary arterial bed, such as calcium scoring and peripheral imaging.

The two technological advances in the last decade have been the evolution of tissue deformation imaging and the

This article is part of the Topical Collection on *Echocardiography*

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use of contrast for perfusion with flash imaging myocardial contrast echocardiography (MCE).

The introduction of tissue deformation imaging has thus raised the question of what role greater information on myocardium has in diagnosis and management of ischemic heart disease (IHD)? To answer this, we have to detail the gaps in current screening for IHD across the spectrum of care from acute to stable. The acute setting is a crowded field with high-sensitive troponin (hsTrop), abnormal rest ^{99m}technitium-sestamibi (MIBI) myocardial perfusion imaging (MPI) and computed tomography coronary angiography (CTCA); however, the role for deformation and perfusion imaging in regional centres are less clear and could be worthy of exploration. In the stable setting, improving the predictive capabilities for ischemia earlier on the ischemic cascade to improve sensitivity with (a) low workloads and exercise duration, (b) various compensatory states (ischemic preconditioning, coronary collaterals), (c) pre-existing abnormalities (e.g. left bundle branch block (LBBB), wall motion defects) and (d) and finally, improving the quantification of ischemic zone or myocardium at risk for established disease are considerations to enhance surveillance. Caveats are age, gender, loading conditions and angle. These variables alone raise inter- and intra-personal technical issue also well as variables in observer interpretations [9]. Thus, any new strategy must improve from the existing cost efficacy equation among heterogeneous patient populations.

Cardiac Contractility and the Evolution of Strain

Cardiomyocytes (cardiac muscle cell) are differentiated from skeletal and smooth muscle by its short, branched, striated and single nucleus with involuntary (intrinsic rhythm) and a feedback-regulated contractile capacity. Cumulative global function is possible through intercalated discs, specialised junctions that anchor and provide communication via gap junctions, resulting in connectivity of electrical and mechanical flux and synchronised cardiac haemodynamic performance. Many physiological and disease processes alter this equation by affecting performance of each fibre based on events and feedback encountered, and the greater the magnification of this process the greater 'noise' will be generated. A completed electrical cycle will excite 3 layers of cardiac muscle fibres resulting in 3 vectors of myocardial deformation, longitudinal, radial and circumferential [10, 11].

General Principles of Deformation Imaging Techniques

Tissue imaging utilises Doppler and speckle tracking to detect actual or relative changes (rate) in velocity (v), length (l) to provide 'strain' and muscle rotational motion (d) as 'twist' or 'torsion', surrogates for myocardial systolic mechanics [9–11, 12•, 13].

Assessment of cardiac contractile function with traditional parameters, e.g. ejection fraction (EF), is limited by volumetric assessment and is suboptimal in providing accurate regional function assessments and changes reproducible. Tissue deformation or 'strain' allows study of change from relaxed to contractile states in longitudinal strain (LS), circumferential strain (CS) or radial strain (RS) directions, both globally and regionally, and is load dependant similar to EF. Imaging techniques share the similar concept of identifying specific patterns or features and following it over time to provide data from specific analytical algorithms (tissue tracking). Techniques have evolved from echocardiographic-derived velocity of circumferential fibre shortening, cardiac magnetic resonance (cMR) tissue tagging and tissue Doppler echocardiography to presently used speckle tracking echocardiography (STE) and feature tracking (FT) [13]. Tissue tracking analysis (deformation) using algorithms to detect actual or relative changes (rate) in velocity (v), length (l) to provide 'strain' and muscle rotational motion (d) as 'twist' or 'torsion', surrogates for myocardial systolic mechanics [9–11, 12•, 13].

Principles and Specific Modalities of Tissue Tracking and Strain Imaging

Specific modalities of tissue tracking and strain imaging are well-described [9–11, 12•, 13, 14]. Strain originates from the description of 3D deformation of a cube over a specified time interval in six components (numbers or value). The myocardium is divided into numerous cubes, and three numbers are assigned for shortening along three external orthogonal axes (x, y, z) and three giving the skew in the x–y, x–z, and y–z planes. This concept is simplified to an internal three cardiac axes coordinates mentioned above (LS, CS, RS). First generation strain in 90's utilised TDI; however, limitations included angle dependency.

Fixed factors, e.g. age, sex, blood pressure (load) and operator factors e.g. foreshortening (underestimate), angle dependency in radial strain, and poor lateral spatial resolution, need to be factored [9].

Speckle Tracking—Two-Dimensional and Three-Dimensional

Speckle tracking would be considered the second generation of strain imaging. The advantage of this generation was the fact that strain measurements were angle independent but still required images of high frame rate and good quality. Whilst gaining traction in systemic conditions such as with the diagnosis of amyloidosis and chemotherapy-related cardiotoxicity,

its utility in assessing regional dysfunction is more limited. The main issue is the heterogeneity of strain measurements in different regions and the difficulty in tracking the speckles at higher heart rates during exercise. The technique is also sensitive to acoustic shadowing and reverberations resulting in underestimation of the true deformation.

There have been a number of studies that have utilised 2D speckle tracking, the latest being a study by Roushdy et al. in 2017 [14]. In a prospective 80 patient cohort, speckle tracking at peak stress for global longitudinal strain during dobutamine stress echocardiography (DSE) showed an increased sensitivity of 77% and specificity of 84% in detecting ischemia, as well as reducing false-positive results and improving detection of multi-vessel ischemia. The cut-off value for global longitudinal strain of $> -16.9\%$ was determined by performing receiver operating characteristic (ROC) analysis, but the results were not validated in the validation group.

There was a smaller study that suggested speckle tracking during dobutamine stress echocardiography requires expertise, as a group of experts performed better than fellows with interpretation of peak stress ischemia from generation of bull's eye maps for peak longitudinal strain and post-systolic shortening [15].

Another major innovation in echocardiography are 3D matrix transducers with probe dimensions similar to 2D transducers whilst maintaining a relatively high frame rate of more than 25 frames per second with single-beat acquisition. This has resulted in an image quality similar to 2D images but with the added benefits of 3D acquisition of avoiding the geometrical assumptions of the left ventricle (LV) volumes inherent in 2D imaging. This technology has been combined with the other major development in echocardiography which has been the evolution of deformation imaging, in particular speckle tracking. A recent study by Dogdus et al. evaluated the utility of both these technological advances in stress echocardiography [16••]. In this study, 120 patients who had coronary angiography after a positive non-invasive test were evaluated with a 3D assessment of deformation using a number of parameters, including global longitudinal strain (GLS) and global area strain (GAS) at rest. Two groups were created according to Gensini score based on the invasive angiography: non-critical stenosis (Gensini: 0–19) ($n = 84$) and critical stenosis (Gensini ≥ 20) ($n = 36$). Global longitudinal strain and all other strain parameters were significantly worse in patients with the critical CAD group compared with that of the non-critical CAD group. Receiver operator characteristic analyses were performed to find out ideal strain cut-off values to detect severe coronary artery disease defined as Gensini score ≥ 20. A GLS value of ≥ 10 has 88.9% sensitivity and 92.9% specificity; A GAS value of ≥ 21 has 97.2% sensitivity and 88.1% specificity to detect critical CAD.

However, speckle tracking has been known to be afterload-dependent and in some circumstances may not be reflective of myocardial contractility. In addition, subsequent measurements may predispose to differences in blood pressure affecting accurate analysis and potentiate misinterpretation of LV contractile state.

Myocardial Work

Myocardial work (MW) is a novel non-invasive echocardiographic approach that assesses regional myocardial work by LV pressure-strain loop (PSL) analysis via an echocardiographic software, thus incorporating both strain and afterload with non-invasively estimated pressure from brachial cuff blood pressure. This technique has been validated with invasive LV pressure-volume loops (PVL) and regional myocardial metabolism with glucose turnover measured by positron emission tomography (PET) [17].

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Perfusion stress echocardiography with flash imaging for destruction and replenishment of contrast (myocardial contrast echocardiography) has certainly shown to have improved the sensitivity of stress echocardiography. This technique utilises microbubbles that remain in the intravascular space, and the contrast intensity reflects the concentration of the microbubbles in the myocardium. A homogeneous opacification of the myocardium after destruction of the microbubbles during high-mechanical index (flash imaging) is regarded as normal perfusion and absence of CAD. If the replenishment rate is reduced, this is suggestive of significant CAD.

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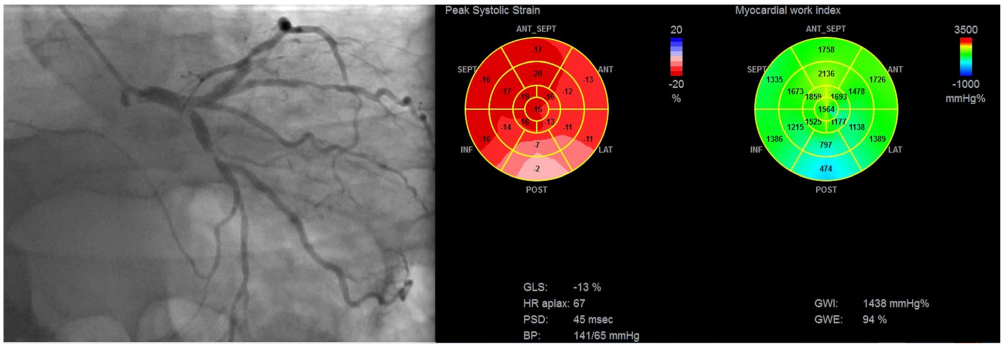

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Compliance with Ethical Standards

Conflict of Interest All authors declare no conflict of interest. The authors report no relationships with industry or other entities.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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Chapter 6

Speckle tracking derived contractile reserve and microvascular functions in ischaemia with no obstructive coronary artery disease – A Pilot Study.

The objective of this chapter is to determine if speckle tracking derived contractile reserve correlates with invasive coronary microvascular measures, specifically CFR and HMR, in patients with angina and no obstructive coronary artery disease.

6.1 Statement of Authoship

Statement of Authorship

Title of Paper	Speckle tracking derived contractile reserve and microvascular functions in ischaemia with no obstructive coronary artery disease – A Pilot Study.
Publication Status	☐ Published ☐ Accepted for Publication ☒ Submitted for Publication ☒ ~~Unpublished and Unsubmitted work written in manuscript style~~ **27/01/2022 - ELO**
Publication Details	Ooi EL, Rajendran S, Chapman M, Munarwar DA, Pati P, Tavella R, Beltrame J, Arstall M, Mahadavan G. Speckle tracking derived contractile reserve and microvascular functions in ischaemia with no obstructive coronary artery disease – A Pilot Study.

Submitted to The International Journal of Cardiovascular Imaging on 27/01/2022

Principal Author

Name of Principal Author (Candidate)	Eng Lee Ooi		
Contribution to the Paper	Performed literature search, critical appraisal / review, study design and methodology, applied for ethics and governance approval, participants' recruitment, data collection and collations, analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/01/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Sharmalar Rajendran		
Contribution to the Paper	Supervised development of work, review and critical appraisal of the project, helped with patients recruitment, conducted interventional diagnostic studies, and manuscript evaluation.		
Signature		Date	10/01/22

Name of Co-Author	Matthew Chapman		
Contribution to the Paper	Helped with conducting echocardiogram image acquisition, post-processing analysis (Speckle tracking) and data collection.		
Signature		Date	24-01-2022

Please cut and paste additional co-author panels here as required.

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Statement of Authorship

Title of Paper	Speckle tracking derived contractile reserve and microvascular functions in ischaemia with no obstructive coronary artery disease – A Pilot Study.
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Statement of Authorship

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6.2 Introduction

Ischaemic heart disease does not always require significant fixed obstructive coronary artery disease (CAD) to manifest as angina.[2,3,38,162,232-235] Ischaemia with no obstructive coronary artery disease (INOCA) is a relatively new entity, comprising the spectrum of vasospastic angina (VSA), coronary microvascular angina (MVA), or a combination of both. Microvascular angina (MVA) refers to abnormal regulation of the coronary microcirculatory vasculature in the absence of other heart disease such as hypertrophic cardiomyopathy, leading to angina. MVA is a heterogenous clinical syndrome with its pathophysiology of coronary microvascular dysfunction (CMD) remaining poorly understood.[27,41,42]

Clinical presentation of patients with MVA are variable and may present with typical angina pectoris, atypical symptoms, or angina-equivalent symptoms. However, CMD can also occur in asymptomatic subjects.[24] MVA caused by CMD appear to be less responsive to nitrates therapy.[43,44] There is an increased female prevalence, especially in postmenopausal women.[5,45,46] Perhaps, the false positive functional study phenomenon in females with chest pain, abnormal stress test with chest pain but normal or no obstructive coronary artery disease could be attributed to microvascular angina.

Diagnosis remains challenging given the inability of assessing these coronary microvessels at coronary angiography. MVA diagnosis is often hypothetical with exclusion of other possible causes of chest discomfort.[27,47,48] A definitive diagnosis of MVA will require the documentation of a coronary microcirculatory functional assessment which is often invasive with its procedural risk.[27,39] Different non-invasive modalities, such as transthoracic doppler echocardiography (TTDE), myocardial contrast echocardiography (MCE), positron

emission tomography (PET) and cardiac magnetic resonance (CMR), have been explored in assessing for MVA.[82-90] However, these modalities have its own pitfalls with availability, accessibility and costs.

Two-dimensional (2-D) speckle tracking, a second generation of strain imaging is gaining traction in systemic conditions such as with the diagnosis of amyloidosis and chemotherapy related cardiotoxicity.[257-261] Utilising this technique during DSE allows sensitive and accurate quantification of contractile reserve (CR) compared with visual wall motion assessment and has been shown to be of incremental value to visual assessment during dobutamine stress echocardiography (DSE).[262] A reduced left ventricular (LV) contractile reserve (CR) to inotropic stimuli has been demonstrated in conditions such as hypertension, valvular heart disease and assessment of stunned myocardium in ischaemia.[263-265] LV CR with low-dose DSE may be used to estimate IMR non-invasively with an impaired CR indicating coronary microvascular dysfunction.[266]

This pilot study explores the feasibility and the reproducibility of LV CR, measured via speckle-tracking imaging on low dose DSE and its correlation with invasive coronary microvascular function, coronary flow reserve (CFR) and hyperaemic microvascular resistance (HMR) in INOCA patients.

6.3 Methods

6.3.1 Study Cohort and Design

We prospectively screened and recruited participants with INOCA to an observational study (Australia New Zealand Clinical Trials Registry number, ACTRN12618000149268). Those who met the inclusion criteria and gave informed consent to participate in an invasive functional coronary diagnostic study, and 2-D speckle-tracking imaging on low dose dobutamine stress echocardiography (DSE) study within 2 weeks of their invasive procedure.

Inclusion criteria were a clinical diagnosis of angina, persistent angina with referral for elective coronary angiography demonstrating normal and no obstructive coronary artery disease (<50% diameter stenosis), permission to withhold calcium channel blockers and/or nitrates for at least 36 hours prior to diagnostic angiography from the referring cardiologist, and patients that were willing and able to give appropriate informed consent.

Exclusion criteria included other causes for chest pain [such as: obstructive coronary artery disease (any stenosis ≥ 50%), valvular heart disease, pulmonary embolus, myocarditis/pericarditis, cardiomyopathy (EF<50%)], the inability to withhold concurrent vasoactive medications, previous coronary artery bypass grafting, history of severe asthma, non-English speaking patients, those with permanent pacemakers or defibrillators, and those unable to give consent due to cognitive impairment, pregnancy and poor echocardiographic image quality for speckle tracking imaging, active cardiac arrhythmias or cardiomegaly due to the potential of these conditions to produce inaccurate and/or unreliable results. Severe comorbidities with severe disability or likelihood of death and significant memory, perceptual or behavioural disorders were also excluded. The Central Adelaide Local Health Network

Human Research Ethics Committee approved the study (reference HREC/17/TQEH/156). The definition of INOCA and the interventional diagnostic procedure for assessment of coronary vasoactivity in INOCA has been previously described in Chapter 4.2.2

6.3.2 Low dose dobutamine stress echocardiography

Two-dimensional transthoracic echocardiography was performed in the left lateral decubitus position with commercially available ultrasound equipment (M5S probe, Vivid e95; GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). Images were digitally stored on hard discs for offline analysis (EchoPAC, BT13; GE Healthcare Life Sciences). Images were acquired using standard protocol with DSE images acquisition at rest and at low-dose dobutamine infusion with 5mcg/kg/min, 10mcg/kg/min and 20mcg/kg/min, at 3 minutes mark of every 5 minutes of each infusion stage. Participant's beta-blocking agents, calcium channel blockers and/or nitrates were held for at least 36 hours prior to the low-dose DSE. The 2-D images of the LV were obtained with the highest possible frame rates in the apical four-chamber, two-chamber, and long-axis views. Three cardiac cycles were obtained for each view.

6.3.2.1 Two-dimensional speckle tracking analysis

Two-dimensional speckle tracking analyses were performed by an experienced independent observer blinded to the clinical history, coronary angiography and coronary physiology measurements. During 2-D speckle tracking analyses, the endocardial border was manually traced at end-systole and the region of interest width was adjusted to include the entire myocardium. The software then automatically tracks and accepts segments of good tracking quality and rejects poorly tracked segments, while allowing the observer to manually

override decisions based on visual assessments of tracking quality. The mean global longitudinal systolic strain was calculated from the three global longitudinal strain curves of the three apical views. The mean global peak negative systolic longitudinal strain was measured in the three apical views at rest and low-dose dobutamine stages**.** Contractile reserve was then calculated as the mean global peak negative systolic longitudinal strain at low-dose dobutamine minus the corresponding values at rest.

6.3.3 Interobserver and intra-observer reliability on GLS analysis

Strain measurements were repeated in 10 randomly selected patients at least four weeks apart by the same observer on the same echocardiographic images, and by a second observer to determine inter-observer and intra-observer reliability respectively.

6.3.4 Statistical Methods

Standard statistics were used to describe the baseline clinical characteristics, and procedural and clinical outcomes. Normally distributed continuous data are expressed as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate. Normal distribution data is tested with independent t-tests, while data with skewed distributions is tested using Mann-Whitney U. Discrete variables are presented as counts and percentages and were compared by chi-square or Fisher exact test.

Pearson's correlation was used to assess the relationship between the absolute or relative changes of GLS measurement and coronary hemodynamic parameters, for each level of dobutamine increase (baseline to 5 mcg, baseline to 10 mcg, baseline to 20 mcg, 5 mcg to 10 mcg, 10 mcg to 20 mcg). General linear model repeated measures analysis was performed to evaluate the change in the GLS in each level of dobutamine dose (baseline, 5 mcg, 10 mcg, and 20 mcg). A P-value (two-sided) of 0.05 was considered statistically significant. Inter-observer reliability and intra-observer reliability of GLS measurement were performed using Cohen's kappa. SPSS software (SPSS for Windows, version 25) was used for statistical analyses.

6.4 Results

Of the 42 patients with angina with a high pre-test probability of vasoactive angina screened with coronary angiography, 2 were excluded from further study due to the presence of obstructive coronary artery disease and 2 declined to perform the DSE. A total of 38 participants of angina with NOCAD underwent interventional diagnostic procedure for coronary vasomotor disorder diagnosis. Out of these, a further 8 were excluded due to loss to follow-up (n=3) and poor image quality (n=5). A final total of 30 participants were included in the final analysis.

6.4.1 Demographic and comorbid clinical characteristics of the study cohort

Table A showed the demographic and comorbid clinical characteristics of the total study cohort (n=30). Average age of 55.7 ± 11.4 years old and more females (73.3%) than males were recruited. 90% of patients were of Caucasian ethnicity. 66.7% were obese (BMI > 30).

6.4.2 The clinical symptom characteristics of the study cohort

Table B showed the clinical symptom characteristics of the study cohort demonstrating a degree of angina burden, with 73.3% experiencing greater than one episode of angina in a week.

6.4.3 Inter-observer and intra-observer reliability on GLS analysis

The GLS inter-observer reliability showed a satisfactory interclass correlation of 0.819, while, intra-observer reliability was acceptable with an interclass reliability of 0.914.

6.4.4 Relationship between contractile reserve and hyperaemic microvascular reserve (HMR)

Figure 1 to 3 depicts the association between contractile reserve (relative change in % and absolute change) and HMR.

At 5mcg/kg/min dobutamine infusion, there was a statistically significant positive association between CR (relative change in %) and HMR (standardized β -0.021 (95% confidence interval [CI] -0.04- -0.001), r = 0.382, p-value = 0.037) (Figure 1a). This is shown similarly with a positive weak association between CR (absolute change in GLS) and HMR (standardized β -0.129 (95%CI -0.235- -0.023), r = 0.427, p-value = 0.019) (Figure 1b).

However, at higher doses of dobutamine this positive association was not demonstrated. Specifically at 10mcg/kg/min dobutamine infusion, there was no association between CR and HMR. CR(relative change in %) and HMR (standardized β 0.005 (95%CI -0.011- -0.022), r = 0.125, p-value = 0.512) (Figure 2a) and CR (absolute change in GLS) and HMR (standardized β 0.033 (95%CI -0.067- -0.134), r = 0.127, p-value = 0.504) (Figure 2b).

Similarly at 20mcg/kg/min dobutamine infusion, there was no association between CR and HMR. CR(relative change in %) and HMR (standardized β 0.005 (95%CI -0.011- -0.022), r = 0.001, p-value = 0.905) (Figure 3a) and CR (absolute change in GLS) and HMR (standardized β 0.055 (95%CI -0.087- -0.098), r = 0.023, p-value = 0.905) (Figure 3b).

Differences of contractile reserve and HMR between each dose of dobutamine infusions were explored as shown in Figure 4 to 6.

At 5mcg/kg/min and 10mcg/kg/min dobutamine infusion, there was a statistically significant positive association between CR (relative change in %) and HMR (standardized β -0.021 (95%CI -0.006- -0.040), r = 0.462, p-value = 0.010) (Figure 4a). This was shown similarly with a statistically positive association between CR (absolute change in GLS) and HMR (standardized β -0.122 (95%CI -0.036- -0.208), r = 0.481, p-value = 0.007) (Figure 4b).

At 5mcg/kg/min and 20mcg/kg/min dobutamine infusion, there was a non-statistically significant trend towards a positive association between CR (relative change in %) and HMR (standardised β -0.349 (95%CI -17.213 - 0.344), r = 0.349, p-value = 0.059) (Figure 5a). This was shown similarly with a a non-statistically significant trend towards a positive association between CR (absolute change in GLS) and HMR (standardised β -0.350 (95%CI -0.052 – 3.065), r = 0.350, p-value = 0.058). (Figure 5b).

At 10mcg/kg/min and 20mcg/kg/min dobutamine infusion, there was no association between CR and HMR. CR (relative change in %) and HMR (standardised β 0.005 (95%CI -0.011- -0.022), r = 0.118, p-value = 0.529) (Figure 6a) and CR (absolute change in GLS) and HMR (standardised β 0.033 (95%CI -0.067- -0.134), r = 0.145, p-value = 0.441). (Figure 6b).

Figure 7 demonstrated the relationship between GLS at different dobutamine infusion rate at baseline, 5 mcg/kg/min, 10 mcg/kg/min and 20mcg/kg/min with LAD HMR. Abnormal HMR was defined as ≥ 2.0. Although not statistically significant (p=0.400), there was a trend of increase in contractile reserve at a low dose of dobutamine of 5mcg/kg/min, but a blunted response at the higher doses of 10 and 20mcg/kg/min.

6.4.5 Relationship between contractile reserve (CR) and coronary flow reserve (CFR)

Figure 8 to 10 depicts the association between contractile reserve (relative change in % and absolute change) and CFR.

At 5mcg/kg/min dobutamine infusion, there was a positive association between CR (relative change in %) and CFR (standardised β 0.027 (95%CI 0.004- 0.050), r = 0.414, p-value = 0.023) (Figure 8a). This is shown similarly with a positive association between CR (absolute change in GLS) and CFR (standardised β 0.162 (95%CI 0.035 -0.289), r = 0.442, p-value = 0.015) (Figure 8b).

As with HMR, the positive association between CR and CFR was lost at higher concentrations of dobutamine. During 10mcg/kg/min dobutamine infusion, there was no association between CR and CFR. CR (relative change in %) and CFR (standardised β -0.085 (95%CI -9.081 – 5.805), r = 0.085, p-value = 0.656) (Figure 9a) and CR (absolute change in GLS) and CFR (standardised β 0.049 (95%CI -1.062 – 1.370), r = 0.049, p-value = 0.797) (Figure 9b).

Similarly at 20mcg/kg/min dobutamine infusion, there was no association between CR and CFR. CR (relative change in %) and CFR (standardised β -0.127 (95%CI -11.268 – 5.672), r = 0.127, p-value = 0.504) (Figure 10a) and CR (absolute change in GLS) and CFR (standardised β 0.123 (95%CI -0.903 – 1.749), r = 0.123, p-value = 0.519) (Figure 10b).

Differences of contractile reserve and CFR between each doses of dobutamine infusions were explored as shown in Figure 11 to 13. Overall there was no association between these parameters.

In detail, at 5mcg/kg/min and 10mcg/kg/min dobutamine infusion, there was no association between CR (relative change in %) and CFR ((standardised β 0.301 (95%CI -1.136 – 11.103), r = 0.301, p-value = 0.106) (Figure 11a) and CR (absolute change in GLS) and CFR (standardised β -0.323 (95%CI -2.241 – 0.140), r = 0.323, p-value = 0.082) (Figure 11b).

Then at 5mcg/kg/min and 20mcg/kg/min dobutamine infusion, there was no association between CR (relative change in %) and CFR (standardised β -0.221 (95%CI -2.119 – 0.556), r = 0.221, p-value = 0.241) (Figure 12a) and CR (absolute change in GLS) and CFR (standardised β 0.199 (95%CI -3.596 – 11.533), r = 0.199, p-value = 0.292) (Figure 12b).

Again at 10mcg/kg/min and 20mcg/kg/min dobutamine infusion, there was no association between CR (relative change in %) and CFR (standardised β -0.128 (95%CI -5.239 – 2.621), r = 0.128, p-value = 0.501) (Figure 13a) and CR (absolute change in GLS) and CFR (standardised β 0.122 (95%CI -0.575 – 1.113), r = 0.122, p-value = 0.519) (Figure 13b).

Figure 14 demonstrated the relationship between GLS at different dobutamine infusion rate at baseline, 5 mcg/kg/min, 10 mcg/kg/min and 20mcg/kg/min with LAD CFR. Abnormal CFR was defined as ≤ 2.5. Although not statistically significant (p=0.393), there was a trend of increase in contractile reserve at a low dose of dobutamine of 5mcg/kg/min, but a blunted response at the higher doses of 10 and 20mcg/kg/min.

6.5 Discussion

In this pilot study in South Australian patients with INOCA, we explored different doses of low dose dobutamine stress echocardiography (5mcg/kg/min, 10mcg/kg/min and 20mcg/kg/min) and assessed the association between GLS derived contractile reserve and invasive coronary haemodynamic indices of CFR and HMR. We found that there was more GLS derived contractile reserve at 5mcg/kg/min of dobutamine infusion between normal and abnormal coronary haemodynamic indices which may suggest hibernating myocardium contractility recruitment during the infusion. It is possible that in this cohort of patients with INOCA who reported frequent, prolonged and often random episodes of spontaneous myocardial ischaemia (Table B) developed a resting hibernating state that was reversed at very low dose dobutamine. The consistent failure to have any response to higher doses of dobutamine indicated limited contractile reserve.

Furthermore, the inability to demonstrate a negative association between GLS and CFR or HMR at low dose dobutamine may be explained by an inability to be a stressor that induces sufficient myocardial ischaemia to alter GLS. Higher doses of dobutamine were not used in this study. Alternatively, the mechanism of inducing myocardial ischaemia in patients with this pathophysiology is not triggered by dobutamine.

Leung et al also demonstrated an impaired CR indicates coronary microvascular dysfunction with low-dose DSE derived LV CR via echocardiographic speckle tracking method to estimate IMR non-invasively.[266] Coronary microvascular dysfunction and impaired myocardial contractile reserve was demonstrated in women with angina and no obstructive coronary artery disease by Michelsen et al but without invasively derived microvascular indices

but with reduced coronary flow reserve on echocardiography. The GLS reserve was found to be significantly lower in women with CMD.[267]

The finding in our study is similar to the findings of Leung et al. The main difference is the slight increase in contractile reserve at a low dose of dobutamine of 5mcg/kg/min (a dose not used in the Leung study) , but a blunted response at the higher doses of 10 and 20mcg/kg/min. Our hypothesis is that there is a degree of hibernating myocardium that is recruited initially but the expected demonstration of contractile reserve in normal myocardium is impaired in this cohort with microvascular dysfunction as demonstrated by Leung et al.

Recently, Rahman et al demonstrated that in patients with angina and no obstructive coronary artery disease, diminished coronary flow reserve characterizes a cohort with inducible ischaemia and a maladaptive physiological response to exercise, during simultaneous coronary pressure and flow velocity measured using a dual sensor-tipped guidewire during rest, supine bicycle exercise, and adenosine-mediated hyperaemia.[268]

There is no robust simple and effective non-invasive assessment for vasospastic angina, except for abnormal ECG changes for myocardial ischaemia and an exclusion of obstructive coronary artery disease on coronary angiography.[80,81] The dynamic nature of coronary artery spasm has made this challenging for non-invasive evaluation. As for microvascular angina, different modalities have been explored. These include transthoracic doppler echocardiography (TTDE), myocardial contrast echocardiography (MCE), positron emission tomography (PET) and cardiac magnetic resonance (CMR). TTDE assessing coronary blood flow velocity can be evaluated at baseline and during hyperaemia by pulsed-wave Doppler echocardiography, with the sample volume placed on the colour signal in the mid- or distal tract of the left anterior

descending coronary artery (LAD). This technique requires extensive training and assessment is only confined to LAD but not other coronary arteries.[82-86]

MCE-derived myocardial blood flow and myocardial blood volume can be assessed through intravenously injected, echogenic, gas-filled microbubbles that are similar in size and rheological properties to red blood cells and are detected in the myocardium by high-intensity ultrasound pulses. Myocardial perfusion abnormalities during dipyridamole infusion in patients with otherwise normal wall motion seems to be a marker of CMD.[87,88]

CMR derived myocardial perfusion obtained through the first-pass kinetics of T1-enhancing extracellular gadolinium-based contrast media. The contrast medium, diffusing from the microvasculature into the interstitial space, results in an increase in signal intensity that is proportional to the perfusion and blood volume of the tissue, the extravascular compartment size, and capillary permeability. A delayed signal increase and persistently hypointense regions are indicative of reduced perfusion. This technique is promising but limited to local availability. A further validation study is underway for this group of patients conducted by the CorMicA CMR sub-study.[89]

PET provides accurate, and reproducible quantification of regional myocardial blood flow, by means of continuous monitoring of the radioactivity emitted by an intravenously administered tracer, in the circulation and the myocardium. Despite well validated with coronary microvascular dysfunction and utilised in CMD research, this technique is limited by ionizing radiation, expense and lack of availability.[90]

The utility of 2D speckle tracking was assessed in a study by Roushdy et al in 2017.[250] In a prospective 80 patient cohort of patients with possible angina, speckle tracking at peak stress for global longitudinal strain during dobutamine stress echocardiography (DSE) showed an increased sensitivity of 77% and specificity of 84% in detecting ischaemia due to obstructive epicardial coronary artery disease, Our study in patients with INOCA did not demonstrate the same result as for patients with obstructive coronary artery disease.

Further research to define a simple functional non-invasive protocol that may assist in the diagnosis of myocardial ischaemia for patients with angina with no obstructive coronary artery disease would have incremental value given the recent increasingly recognised condition, together with emerging endotypes specific stratified medical therapy.

6.6 Study Limitations

The interpretation of our study findings should be considered in the context of the following limitations. Firstly, speckle tracking has been known to be afterload-dependant and in some circumstances may not be reflective of myocardial contractility. Secondly, the echocardiographic acquisition was not conducted simultaneously with the invasive study, instead, within 2 weeks from initial assessment. Thirdly, there was no healthy control in our study cohort. Finally, the population size was small and indicates the need for further studies in a larger cohort of patients.

6.7 Conclusion

This study has demonstrated the complexity of the effect of microvascular dysfunction on myocardial contractile response to dobutamine in patients with INOCA.

6.8 Diagrams

Diagram 1 Study Overview of INOCA endotypes

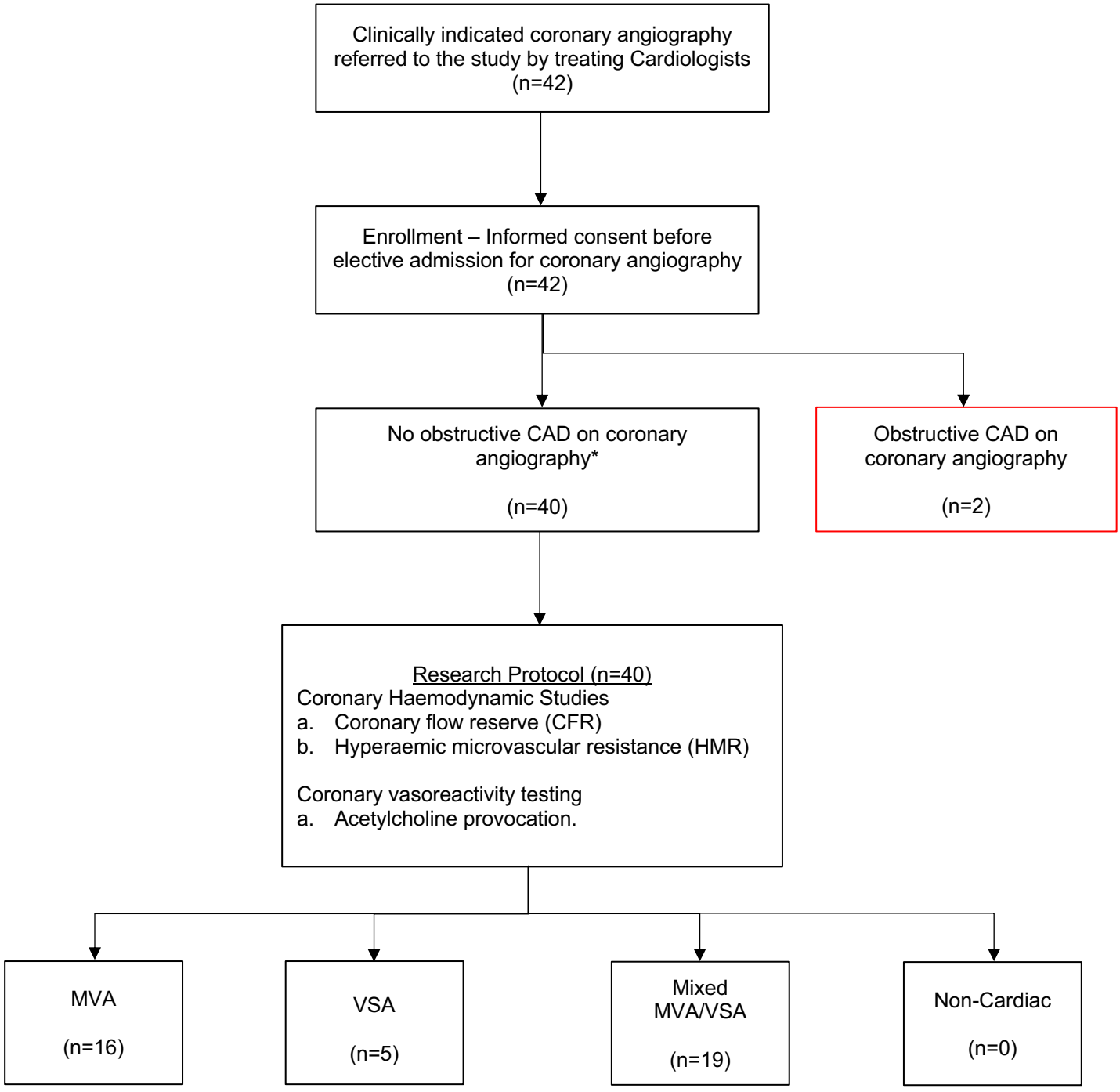
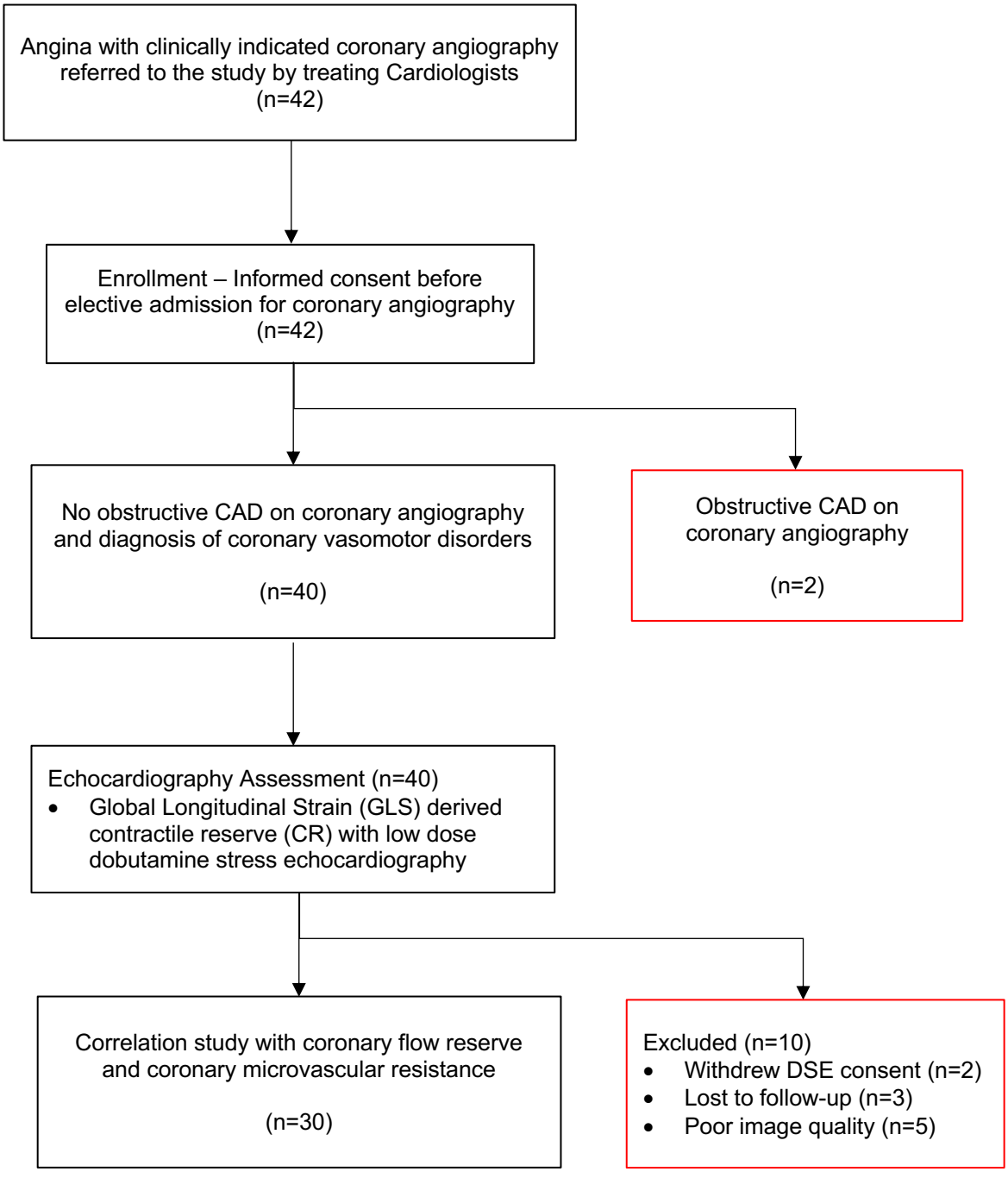


Diagram 2 Study Overview of Echocardiographic Correlation Study



6.9 Tables

Table A – Demographic and comorbid clinical characteristics of the study cohort. (n=30)

Baseline Demography	Mean ± SD Median (IQR)	N (%)
Age (mean ± SD)	55.7 ± 11.4	
Gender - female (n, %) Post menopause		22 (73.3) 16 (72.7)
Ethnicity - Caucasian - Indigenous - Asian		 27 (90.0) 2 (6.7) 1 (3.3)
Obesity		20 (66.7)
BMI (mean ± SD)	31.5 ± 5.9	
Hypertension		15 (50.0)
Diabetes		7 (23.3)
Dyslipidaemia		19 (63.3)
Active smoker Ex-smoker		4 (13.3) 10 (33.3)
Depression/anxiety		13 (43.3)
Migraine		8 (26.7)
Atrial fibrillation		1 (3.3)
GORD		19 (63.3)
Medication - ACE/ARB - Beta-blocker - CCB – dihydropyridine (DHP) - CCB – Non DHP - Nitrates - SSRI - Antiplatelet - Anticoagulation - Statins - HRT		 0 (33.3) 3 (10.0) 6 (20.0) 17 (56.7) 18 (60.0) 11 (36.7) 11 (36.7) 1 (3.3) 16 (53.3) 3 (10.0)

Table B – Clinical symptom characteristics of the study cohort.

Clinical Symptom Characteristics	N (%)
Symptom	
- Angina on exertion	22 (73.3)
- Angina at rest	28 (93.3)
- Angina with emotional stress	14 (46.7)
- Nocturnal angina	16 (53.3)
- Angina on exposure to cold	9 (30.0)
Duration of angina	
- Less than 20 mins	13 (43.3)
- 20 mins- 1 hr	13 (43.3)
- >1 hr	4 (13.3)
Frequency of angina	
- First presentation	1 (3.3)
- Once a month	4 (13.3)
- Weekly	3 (10.0)
- > once a week	22 (73.3)

6.10 Figures

Figure 1a Contractile reserve relative GLS change (%) vs HMR – Baseline to Dobutamine infusion of 5 mcg/kg/min (r=0.0382, p-value=0.037)

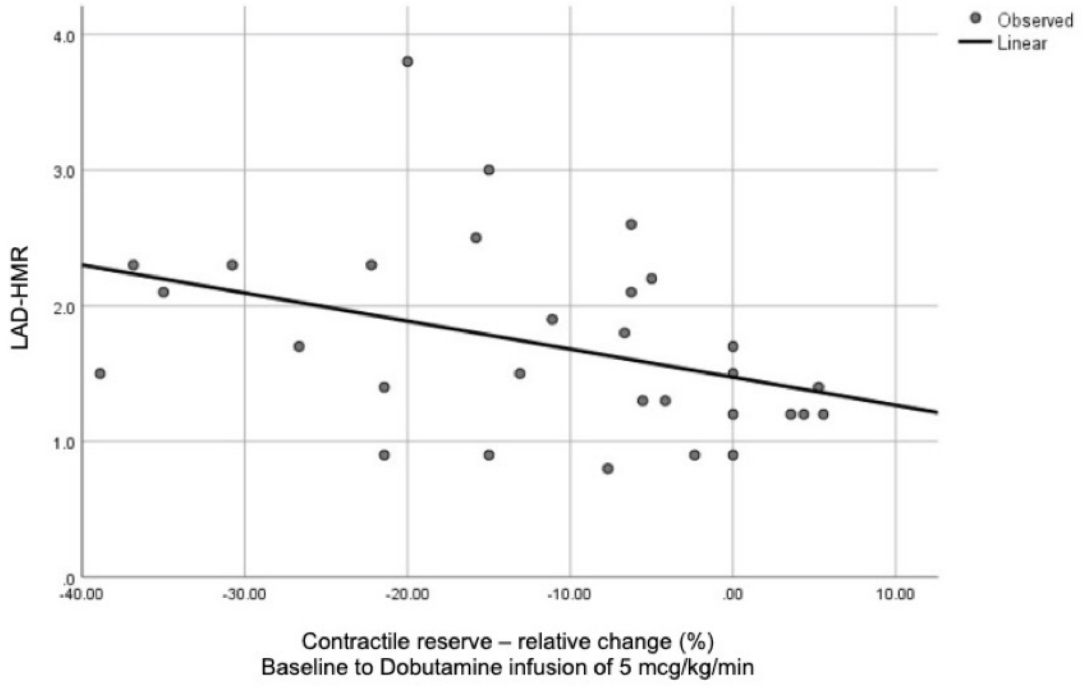


Figure 1b Contractile reserve absolute GLS change (%) vs HMR – Baseline to Dobutamine infusion of 5 mcg/kg/min (r = 0.427, p-value = 0.019)

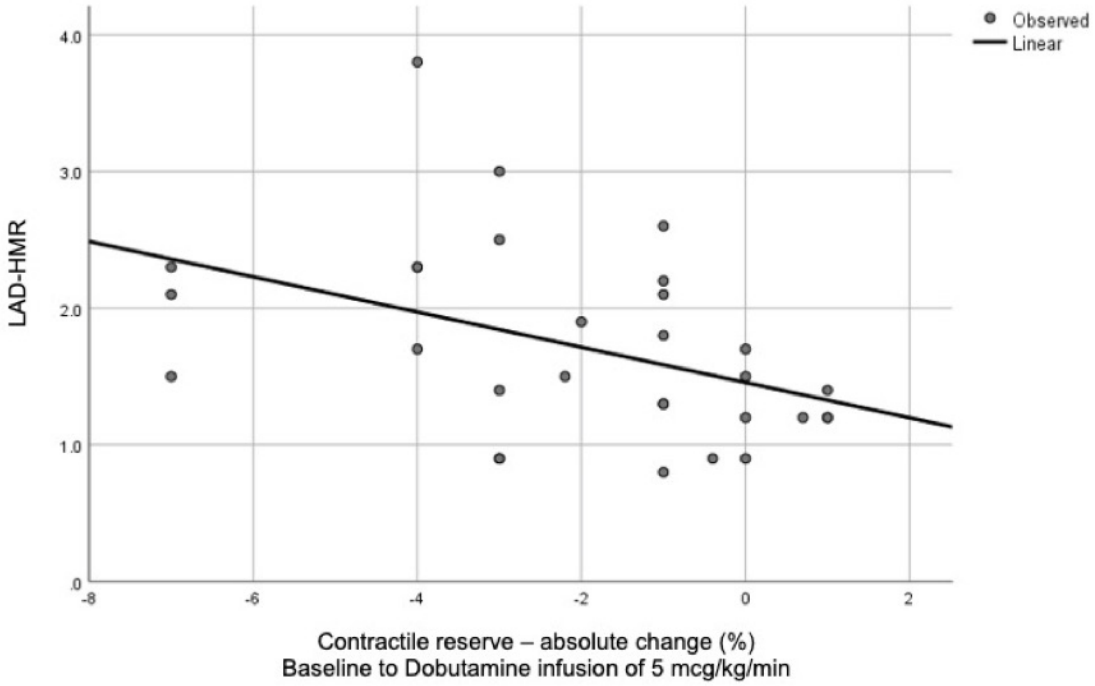


Figure 2a Contractile reserve relative GLS change (%) vs HMR – Baseline to 10 mcg/kg/min (r=0.125, p-value=0.512)

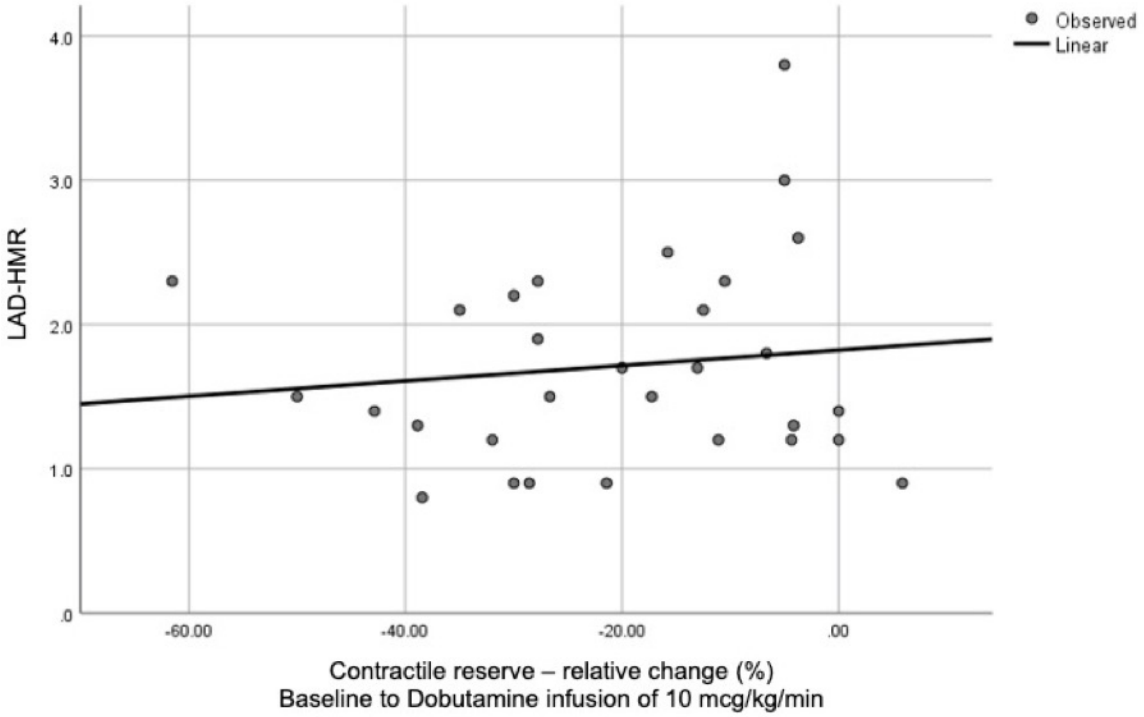


Figure 2b Contractile reserve absolute GLS change (%) vs HMR – Baseline to 10 mcg/kg/min (r=0.127, p-value=0.504)

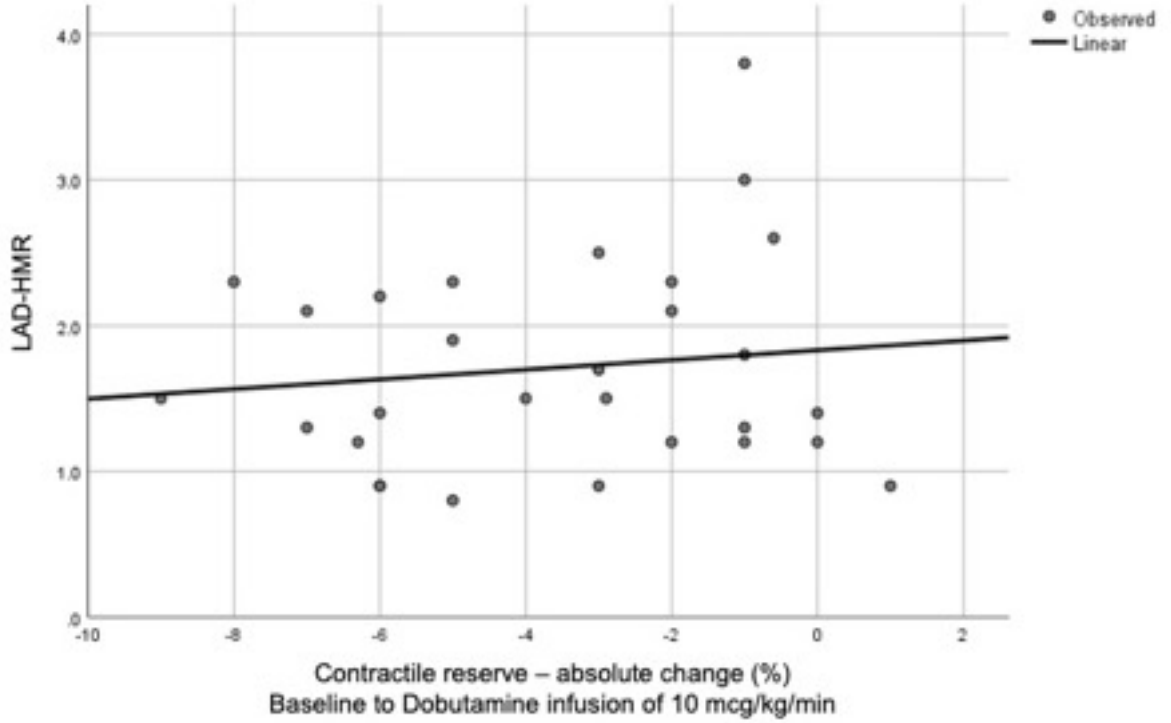


Figure 3a Contractile reserve relative GLS change (%) vs HMR – Baseline to 20 mcg/kg/min (r=0.001, p-value=0.905)

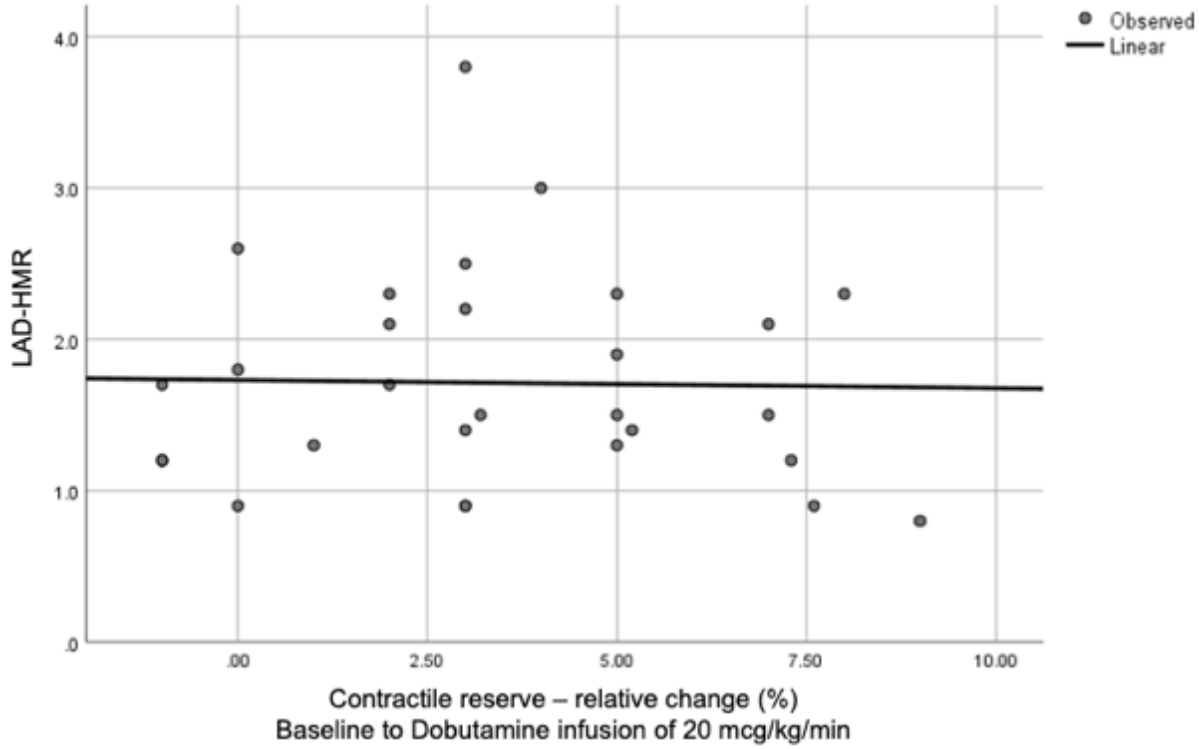


Figure 3b Contractile reserve absolute GLS change (%) vs HMR – Baseline to 20 mcg/kg/min (r=0.023, p-value=0.905)

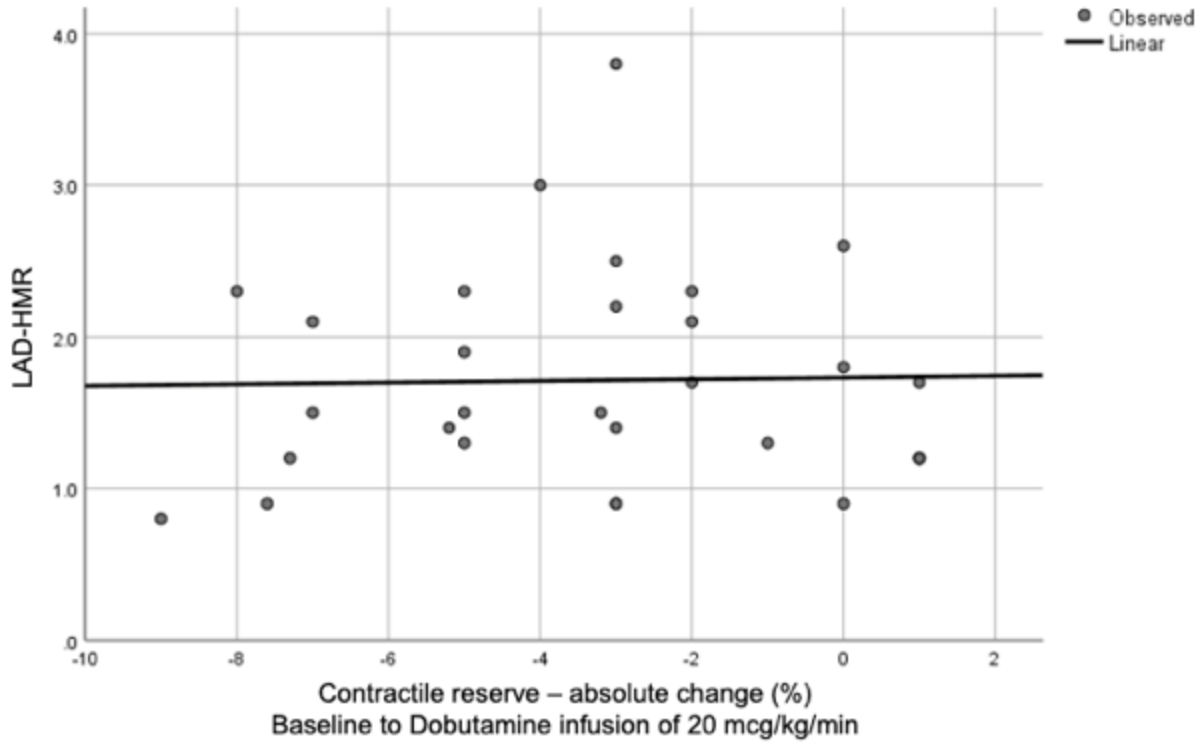


Figure 4a Contractile reserve relative GLS change (%) vs HMR – 5mcg/kg/min to 10 mcg/kg/min (r=0.462, p-value=0.010)

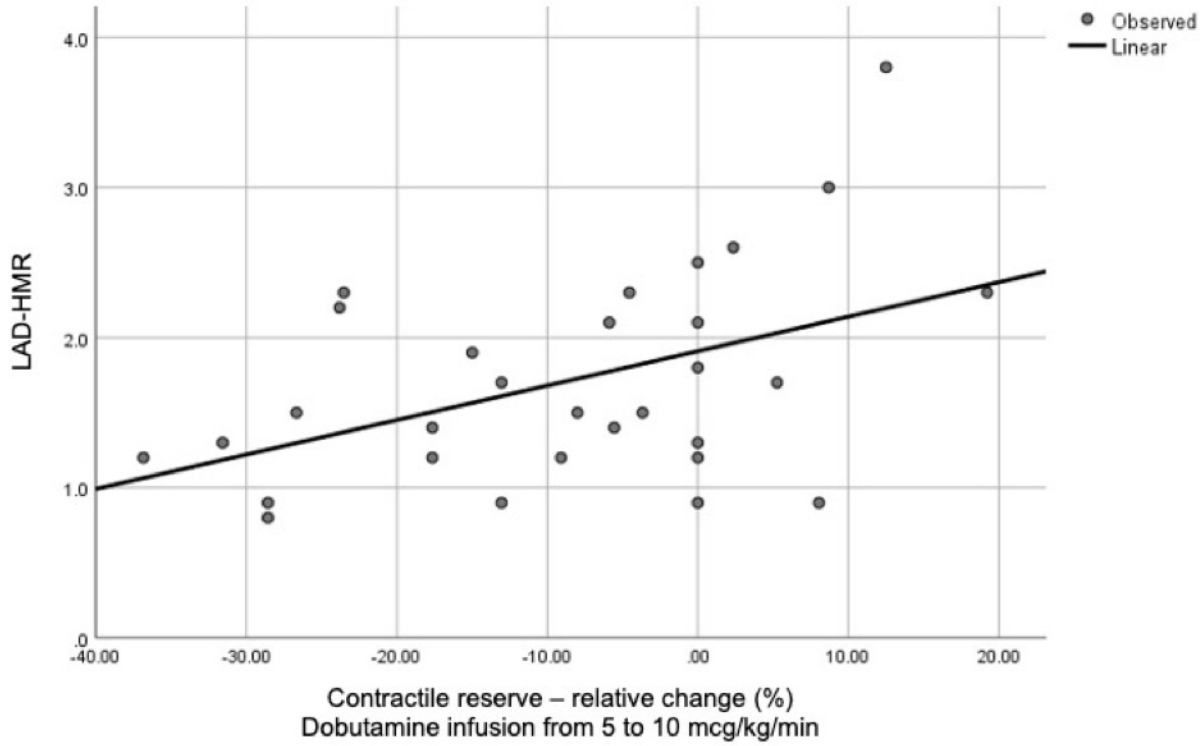


Figure 4b Contractile reserve absolute GLS change (%) vs HMR – 5mcg/kg/min to 10 mcg/kg/min (r=0.481, p-value=0.007)

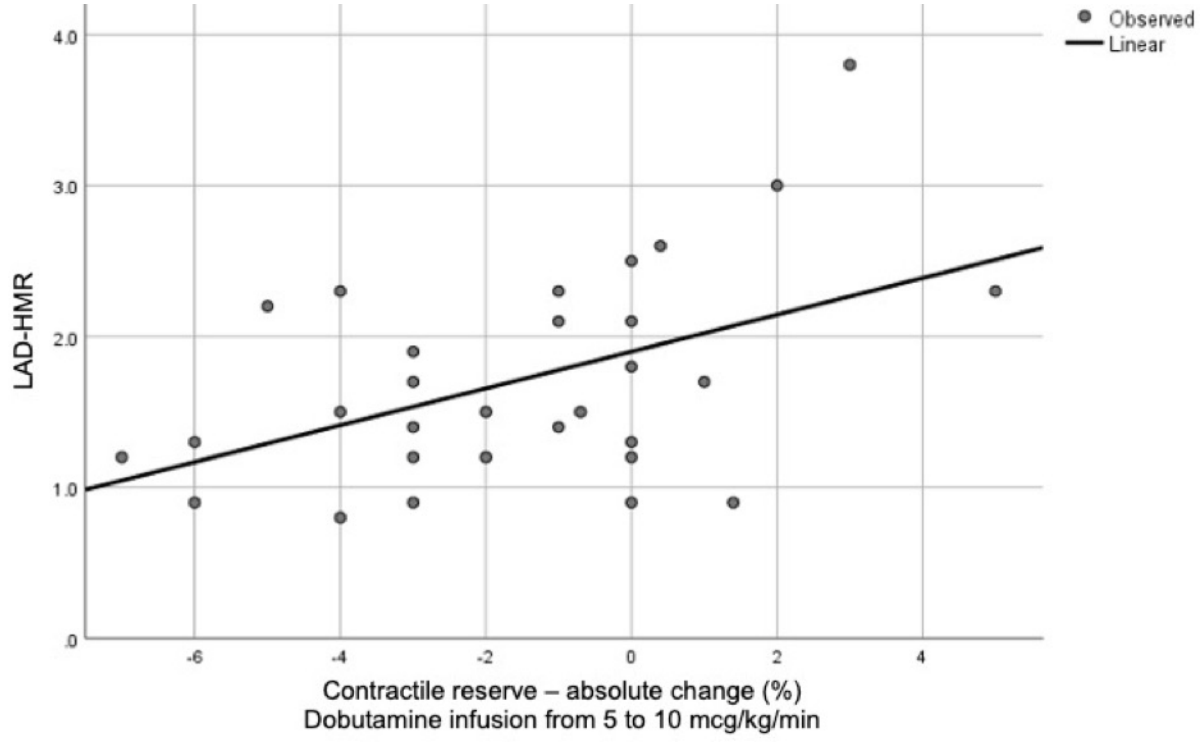


Figure 5a Contractile reserve relative GLS change (%) vs HMR – 5mcg/kg/min to 20 mcg/kg/min (r=0.349, p-value=0.059)

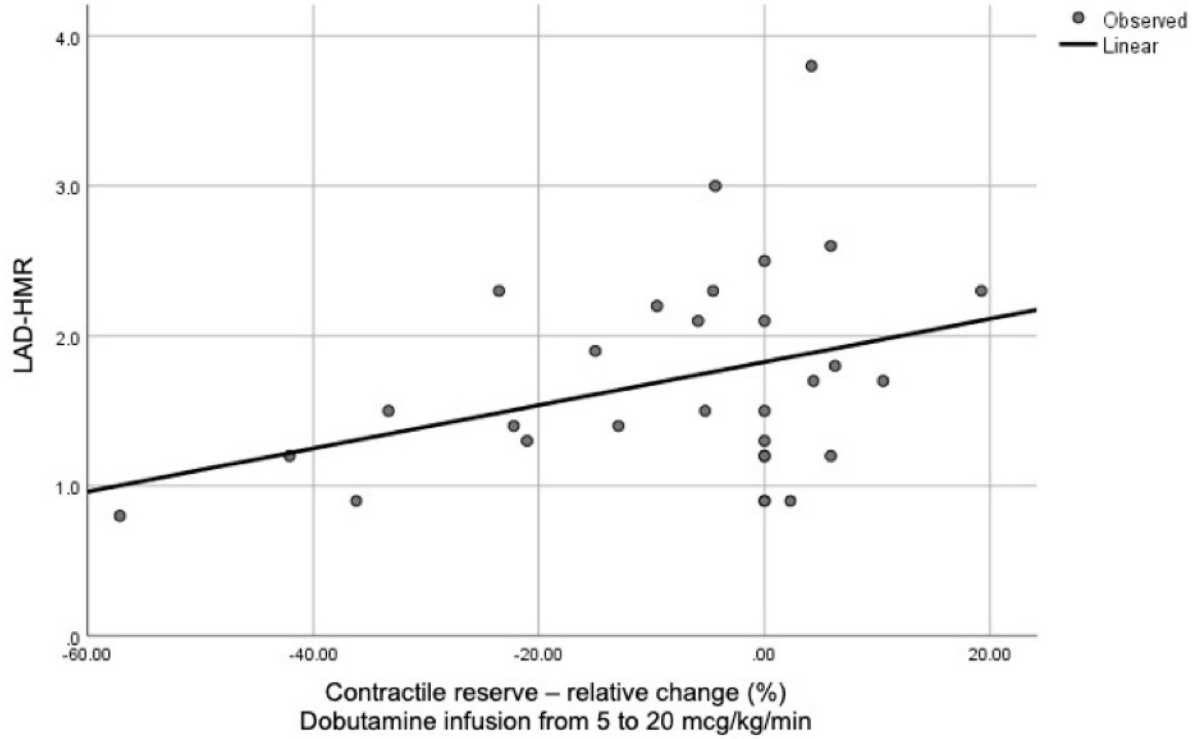


Figure 5b Contractile reserve absolute GLS change (%) vs HMR – 5mcg/kg/min to 20 mcg/kg/min (r=0.350, p-value=0.058)

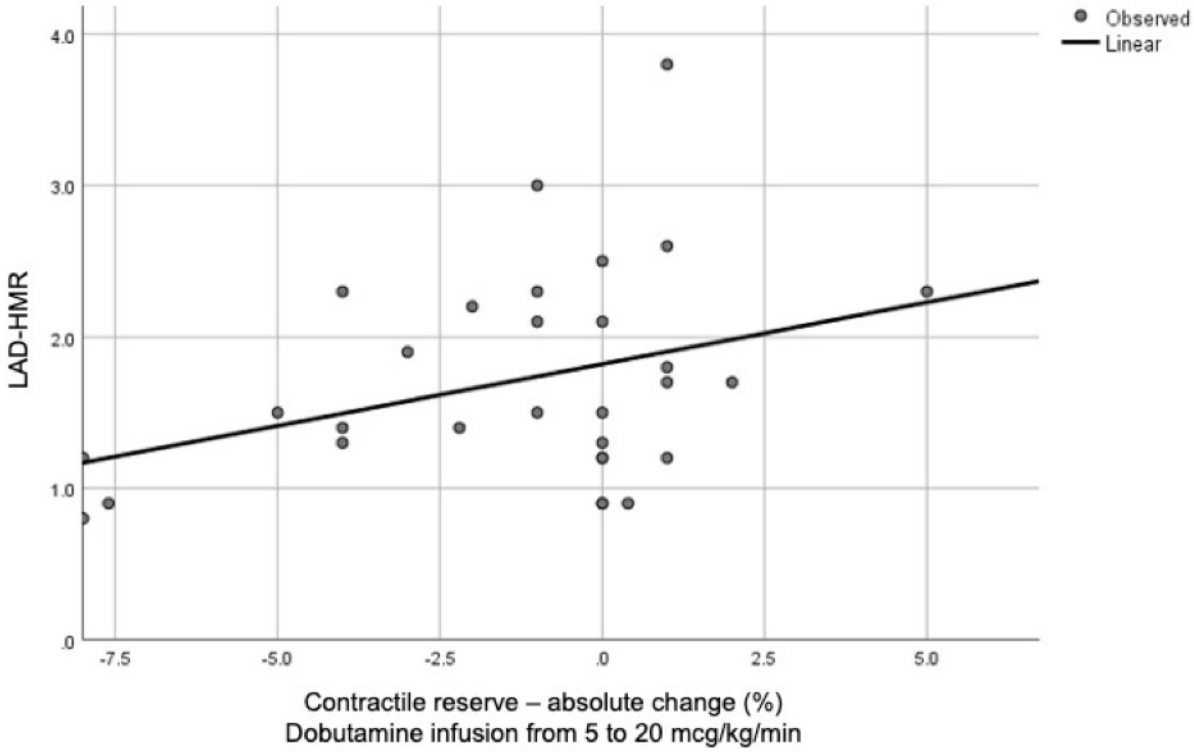


Figure 6a Contractile reserve relative GLS change (%) vs HMR – 10 mcg/kg/min to 20 mcg/kg/min (r=0.118, p-value=0.529)

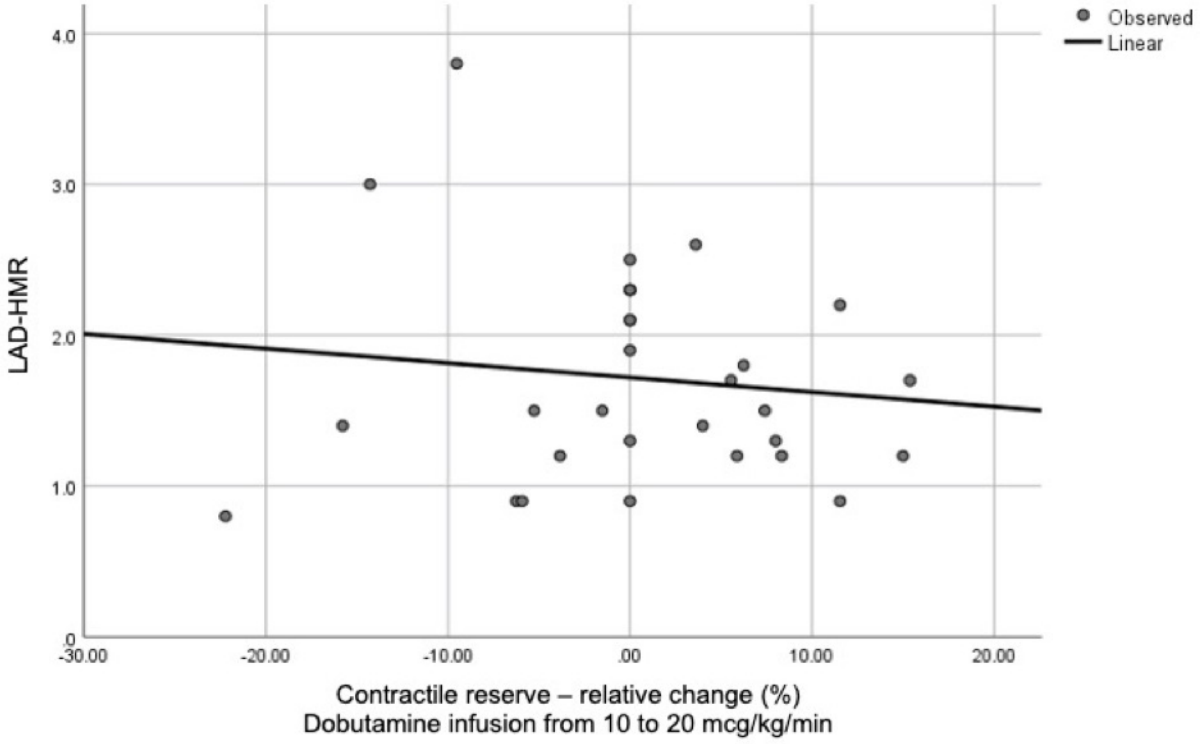


Figure 6b Contractile reserve absolute GLS change (%) vs HMR – 10 mcg/kg/min to 20 mcg/kg/min (r=0.145, p-value=0.441)

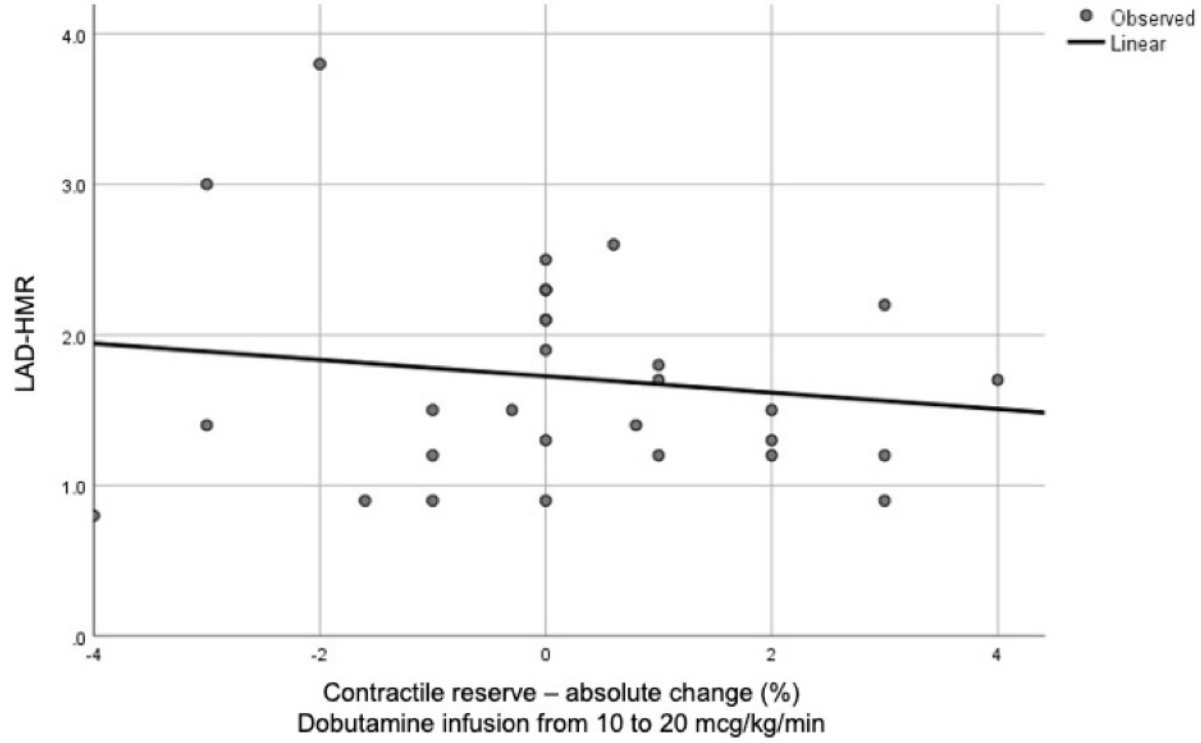


Figure 7: The relationship between GLS at different dobutamine infusion rate (baseline, 5, 10 and 20mcg/kg/min) with LAD HMR. Abnormal LAD HMR was defined as ≥ 2.0. (p=0.400)

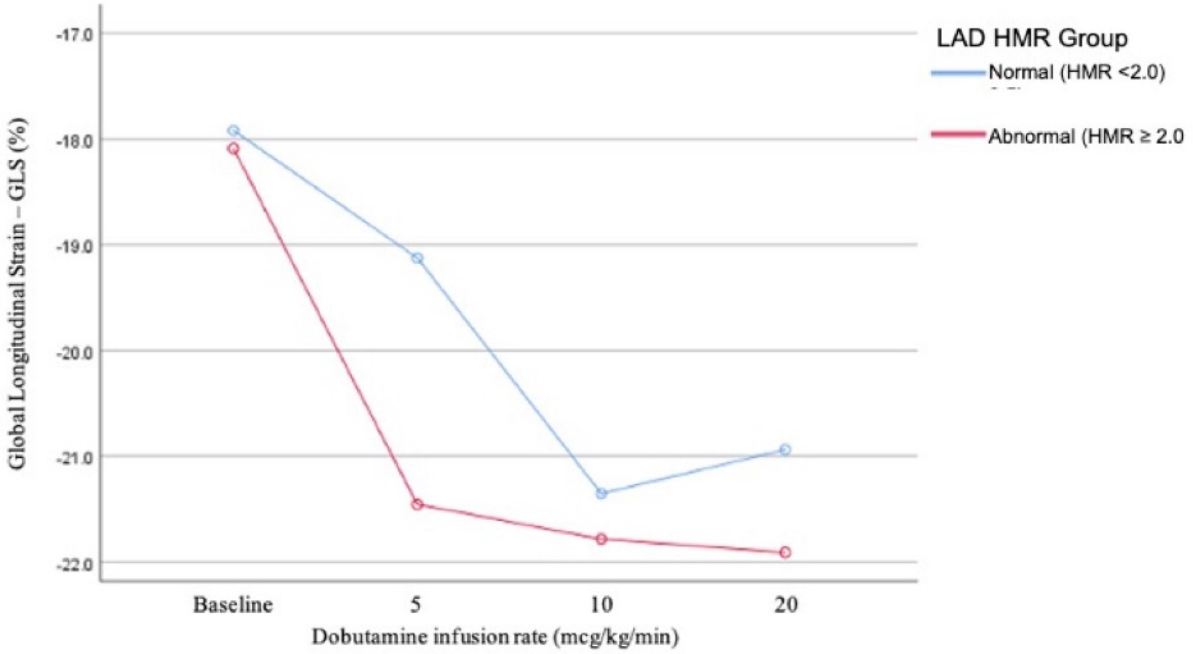


Figure 8a Contractile reserve relative GLS change (%) vs CFR – baseline to 5 mcg/kg/min (r=0.414, p-value=0.023)

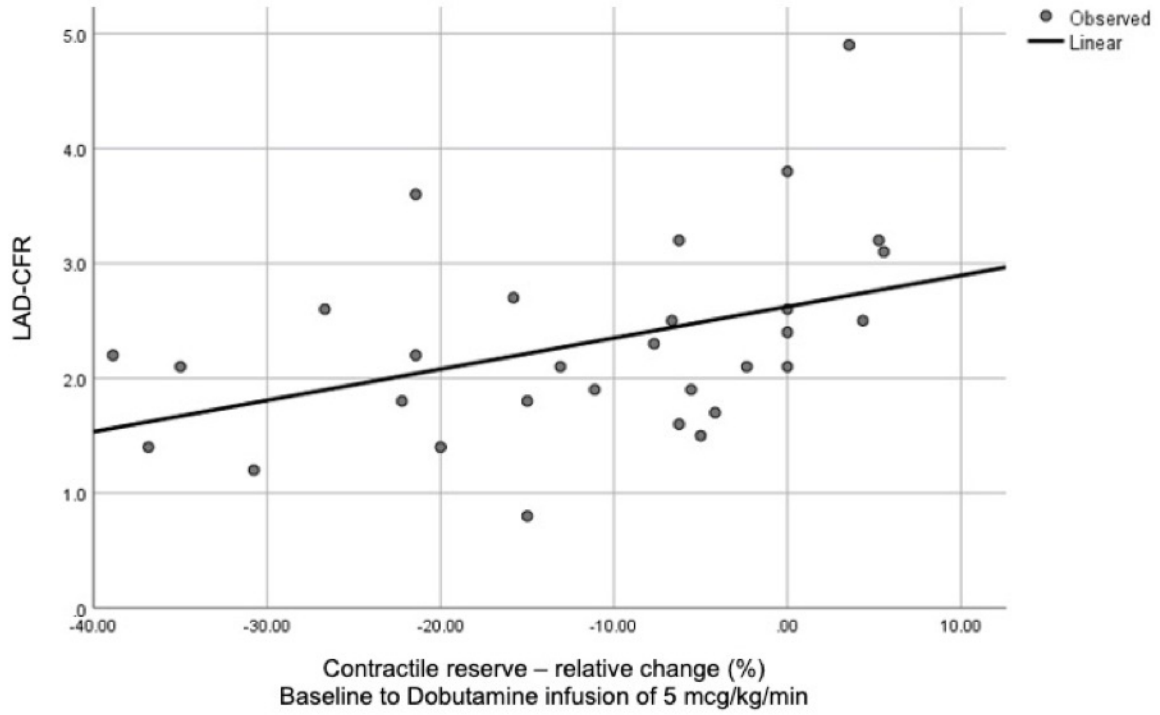


Figure 8b Contractile reserve absolute GLS change (%) vs CFR – Baseline to 5 mcg/kg/min (r = 0.442, p-value = 0.015)

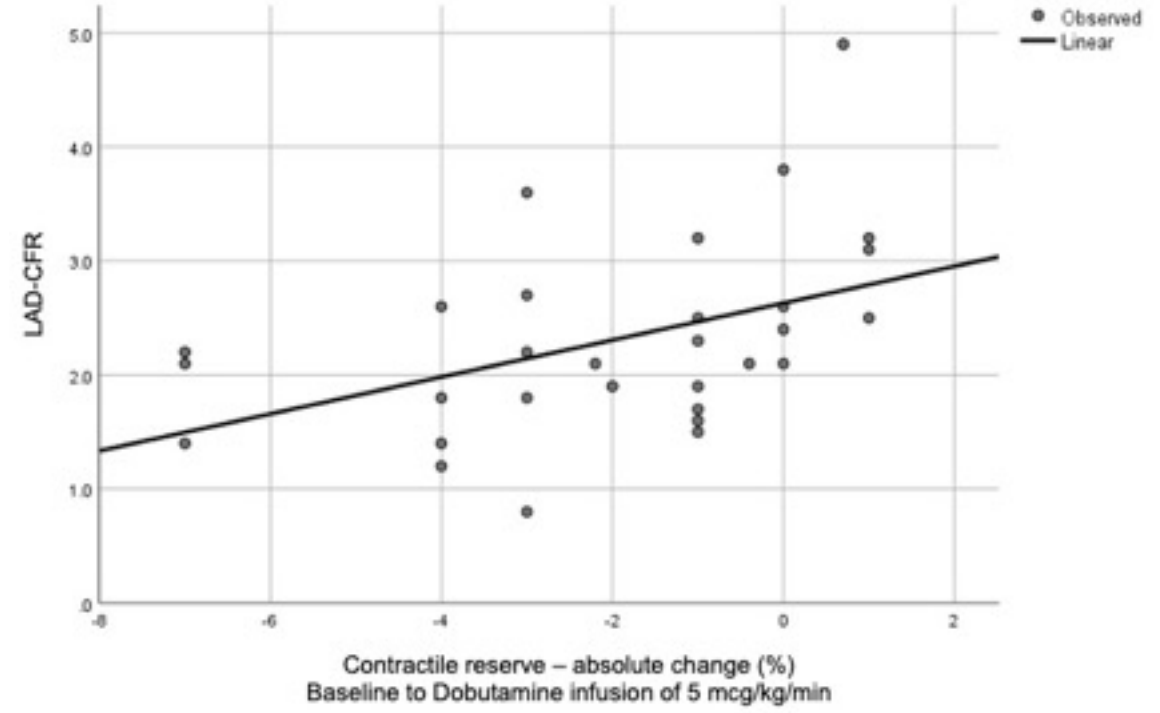


Figure 9a Contractile reserve relative GLS change (%) vs CFR – baseline to 10 mcg/kg/min (r=0.083, p-value=0.656)

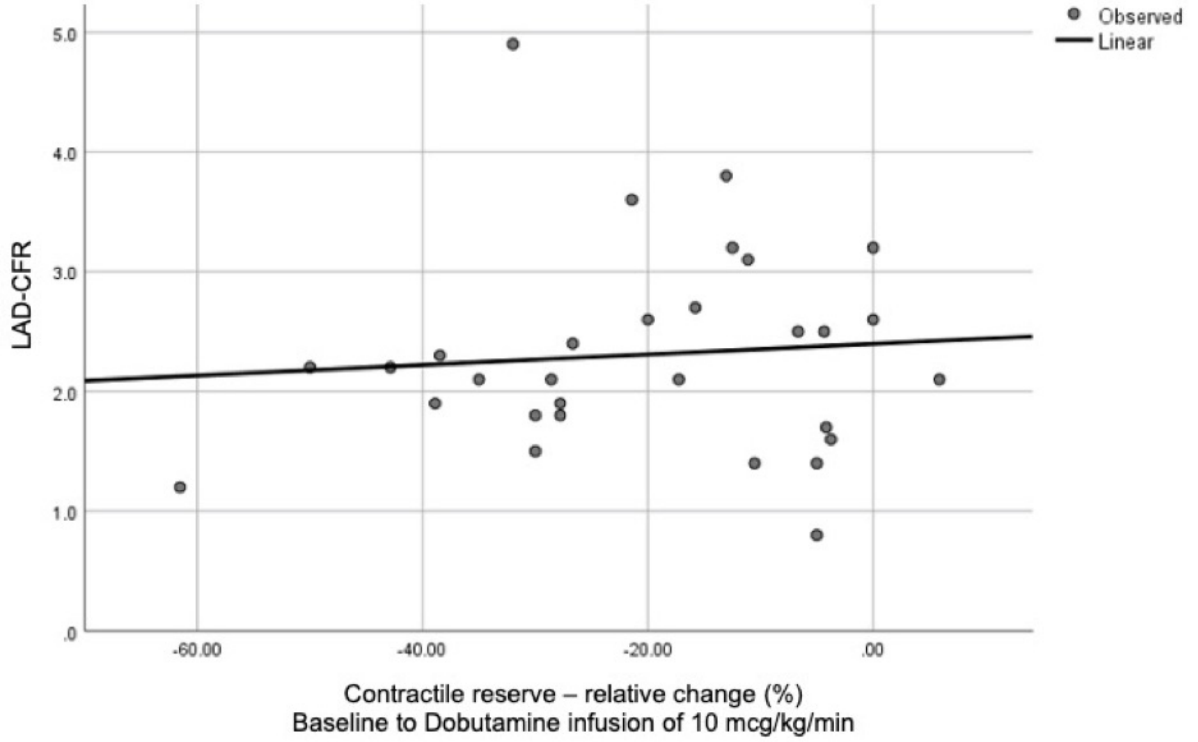


Figure 9b Contractile reserve absolute GLS change (%) vs CFR – baseline to 10 mcg/kg/min (r=0.044, p-value=0.797)

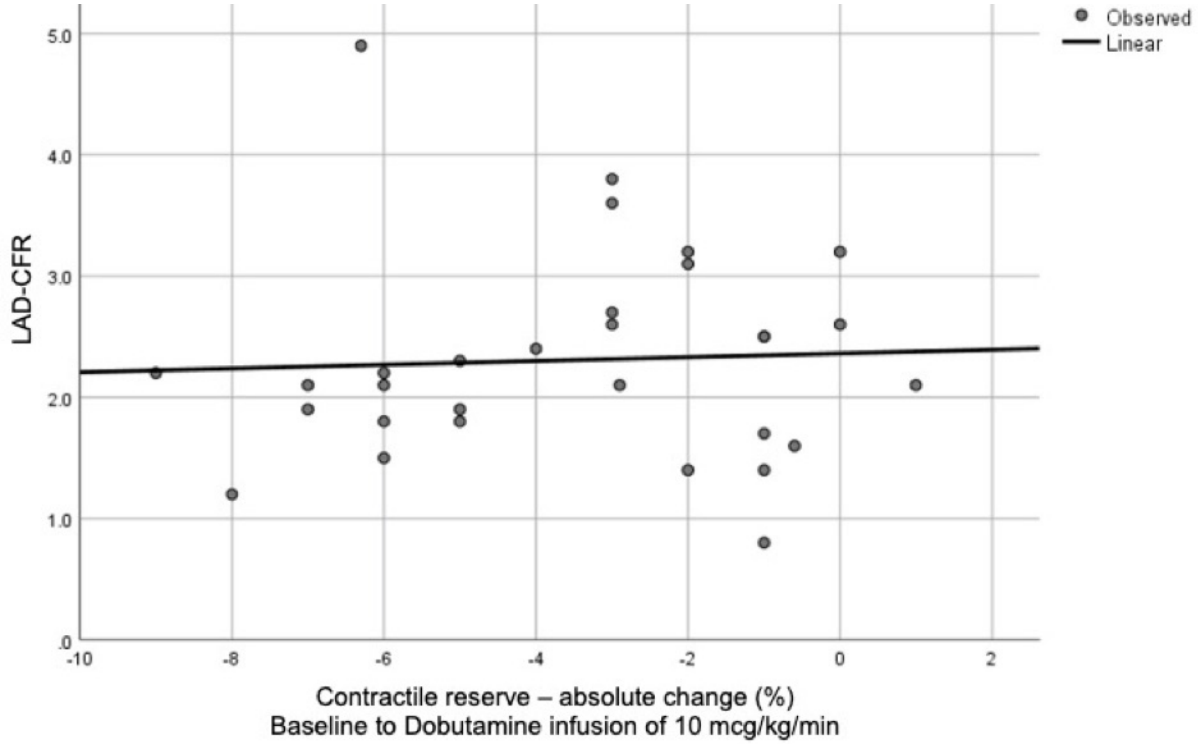


Figure 10a Contractile reserve relative GLS change (%) vs CFR – baseline to 20 mcg/kg/min (r=0.127, p-value=0.504)

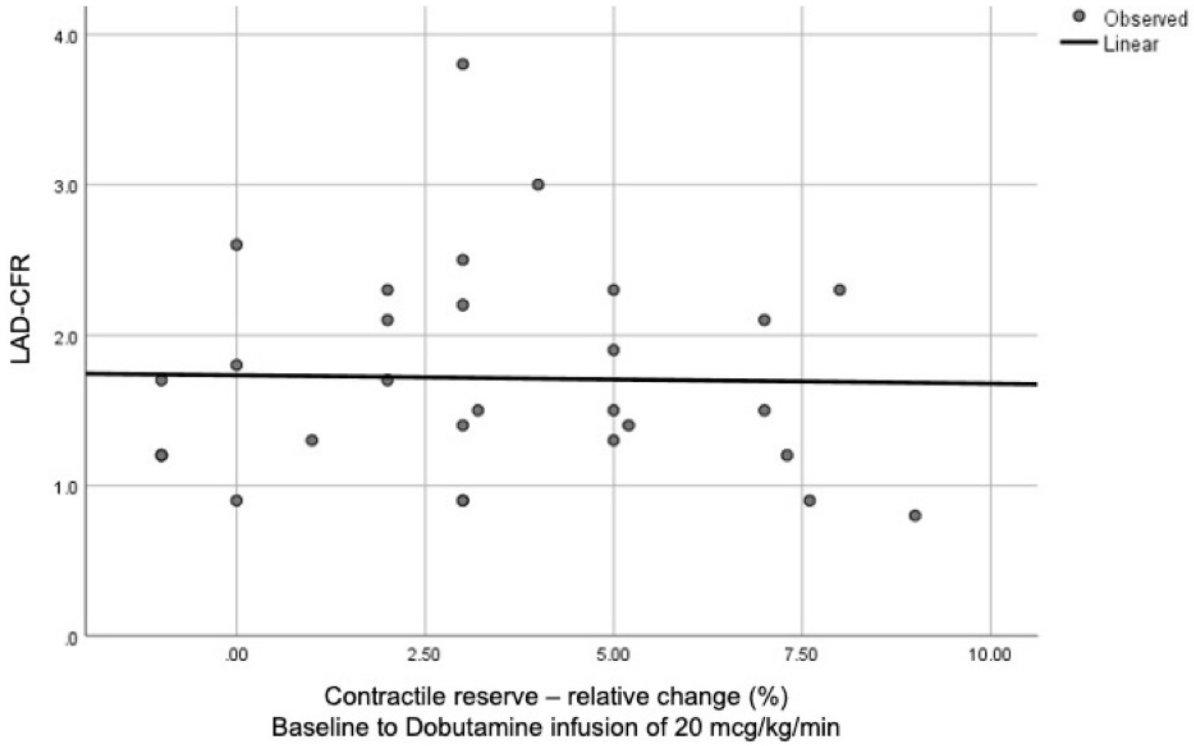


Figure 10b Contractile reserve absolute GLS change (%) vs CFR – baseline to 20 mcg/kg/min (r=0.122, p-value=0.519)

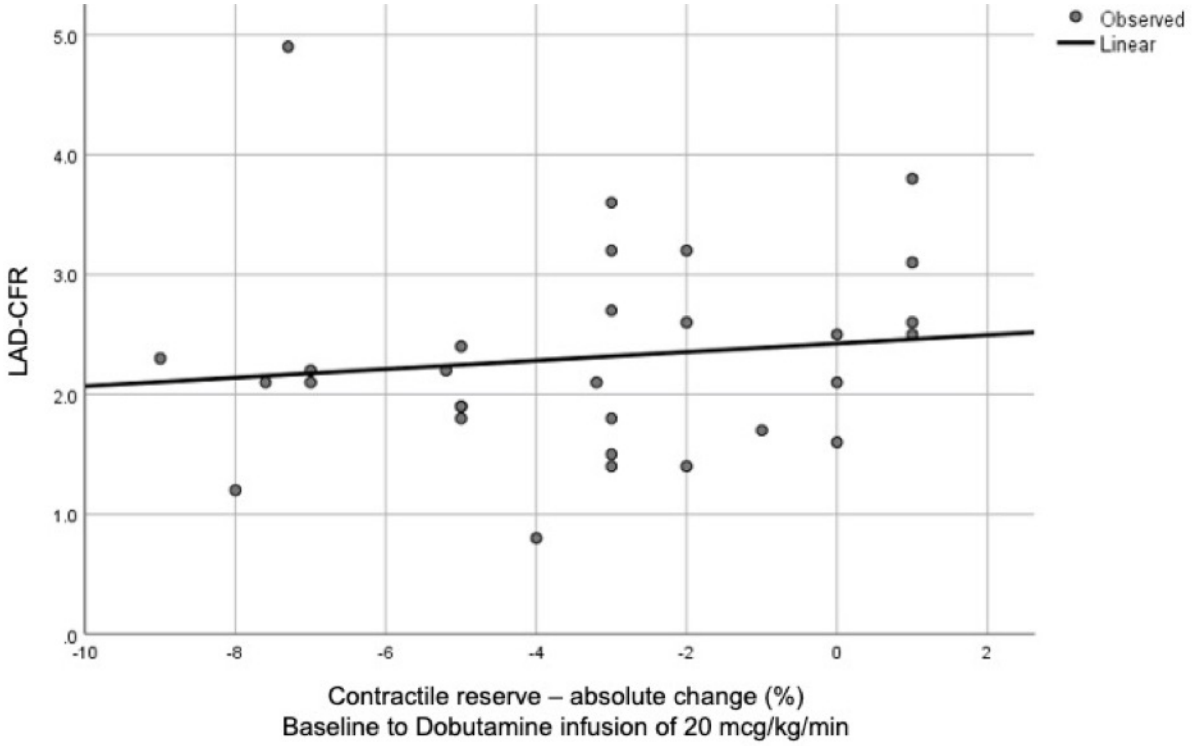


Figure 11a Contractile reserve relative GLS change (%) vs CFR – 5mcg/kg/min to 10 mcg/kg/min (r=0.300, p-value=0.106)

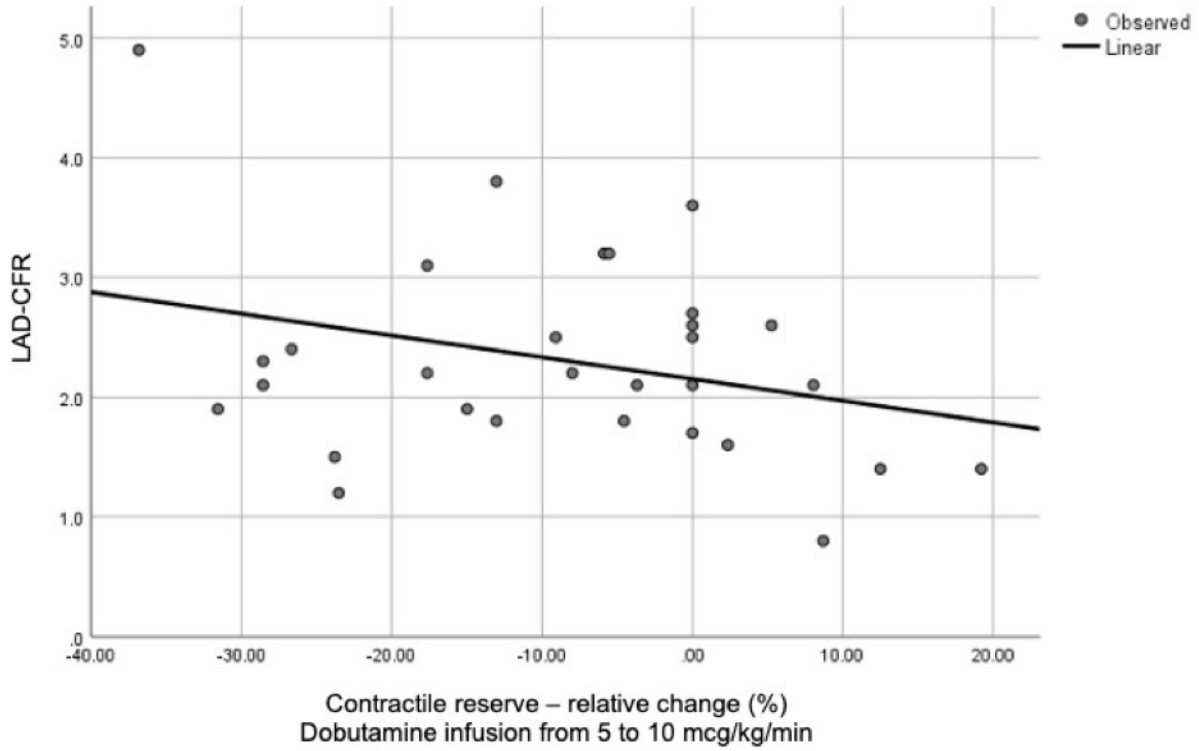


Figure 11b Contractile reserve absolute GLS change (%) vs CFR – 5mcg/kg/min to 10 mcg/kg/min (r=0.322, p-value=0.082)

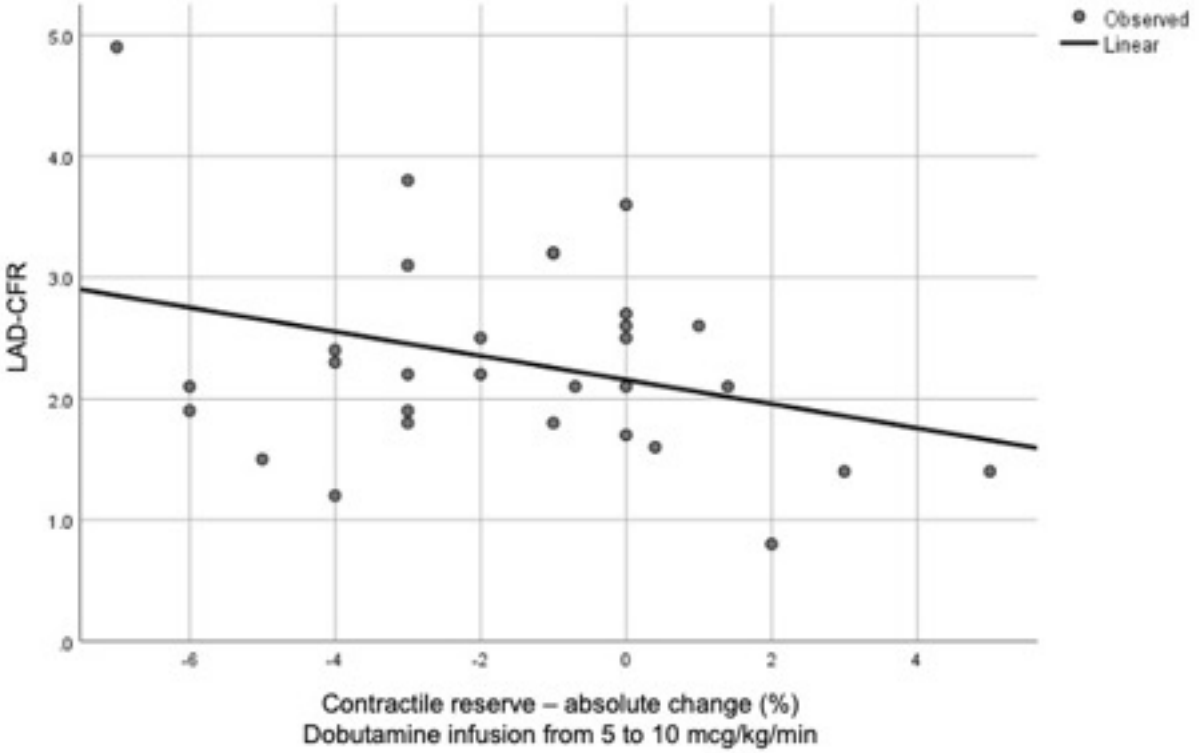


Figure 12a Contractile reserve relative GLS change (%) vs CFR – 5mcg/kg/min to 20 mcg/kg/min (r=0.200, p-value=0.292)

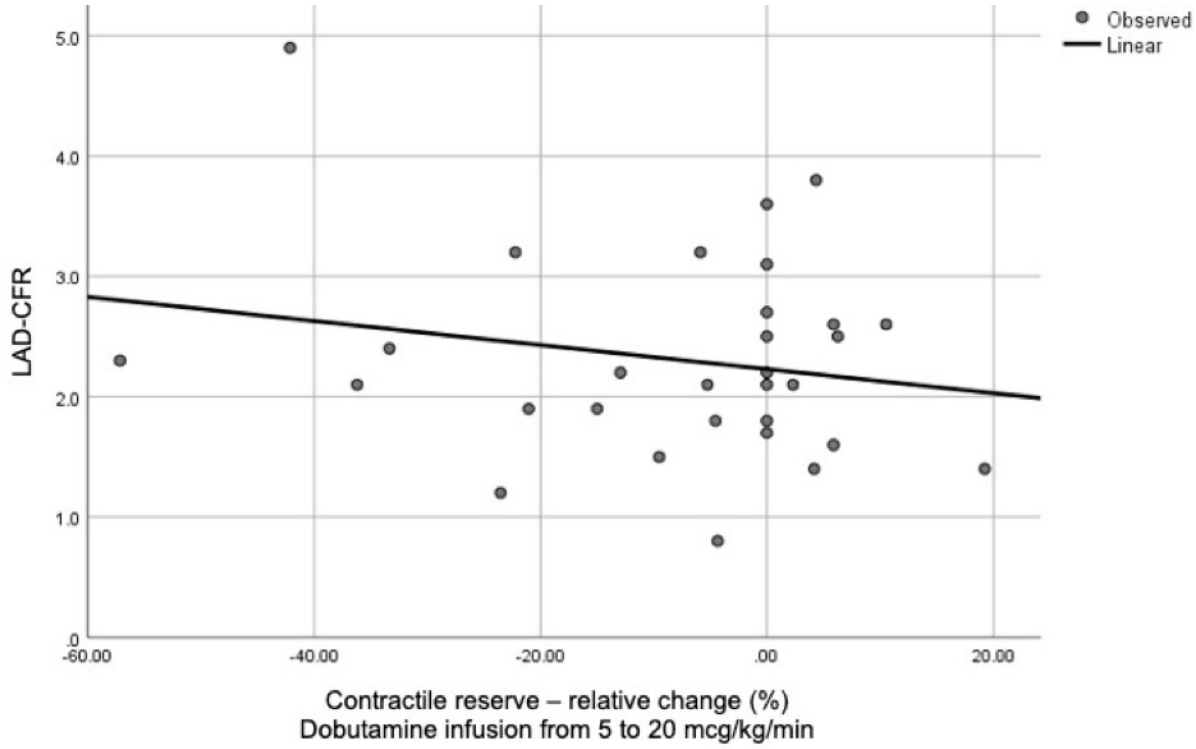


Figure 12b Contractile reserve absolute GLS change (%) vs CFR – 5mcg/kg/min to 20 mcg/kg/min (r=0.221, p-value=0.241)

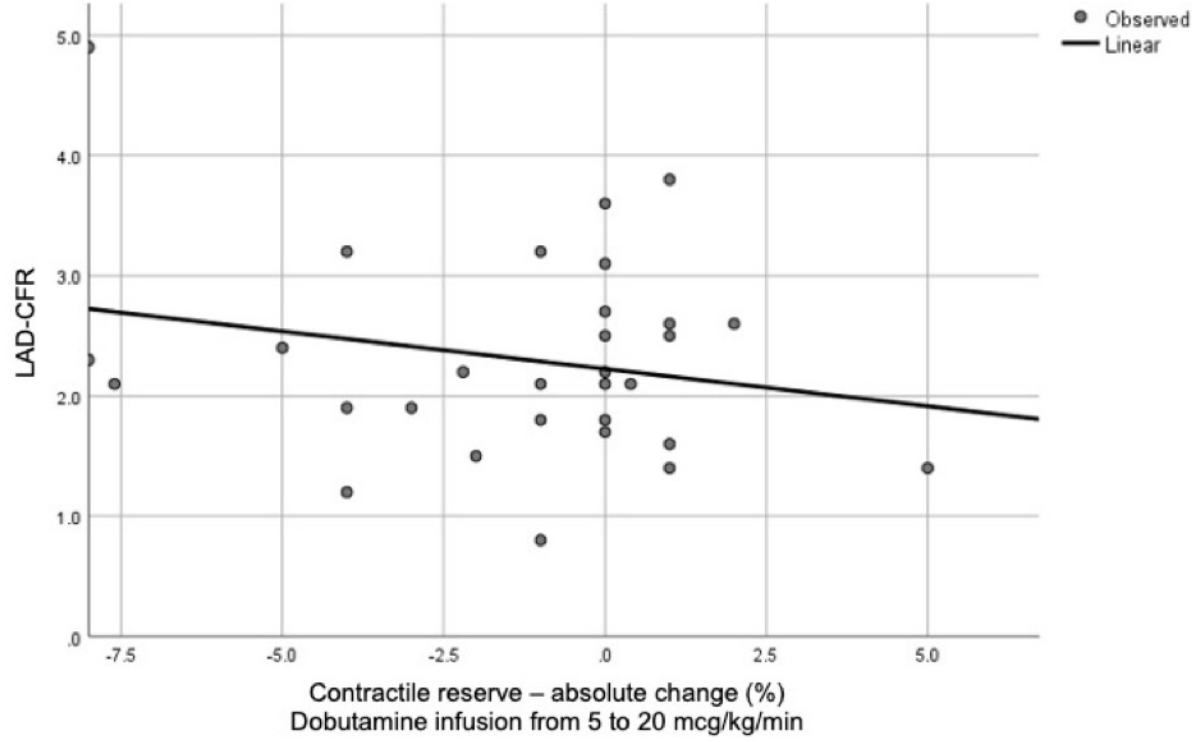


Figure 13a Contractile reserve relative GLS change (%) vs CFR – 10 mcg/kg/min to 20 mcg/kg/min (r=0.126, p-value=0.501)

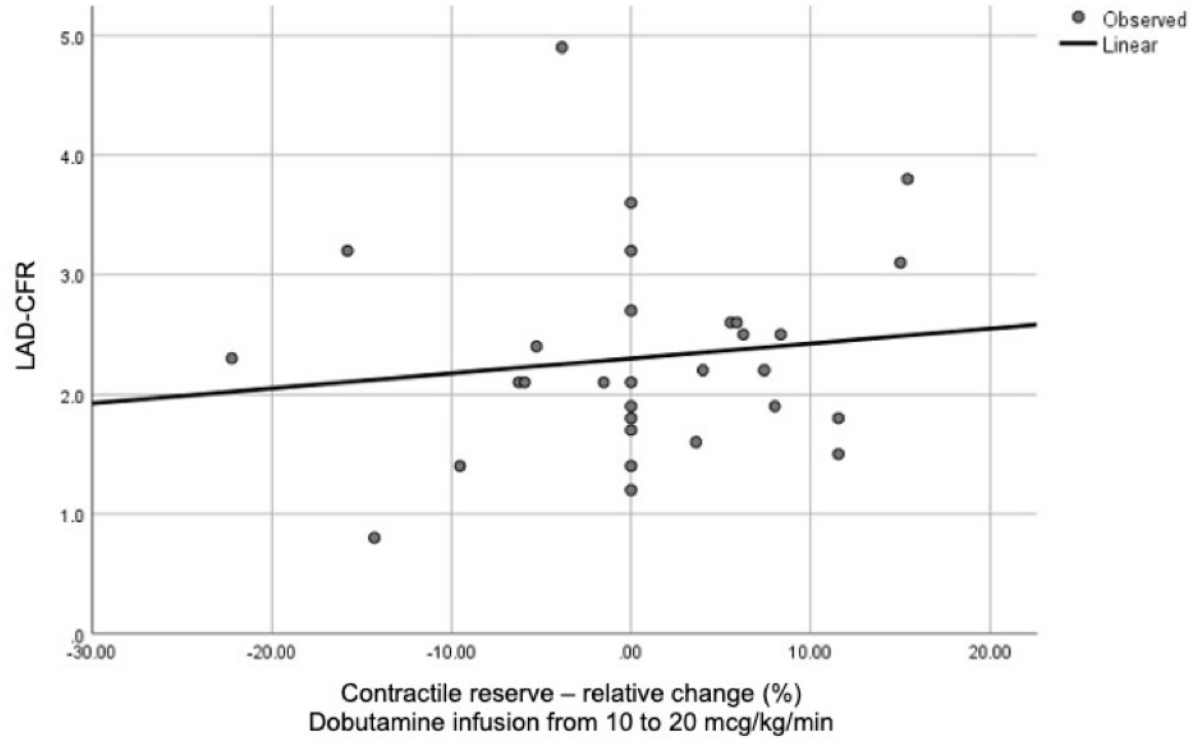


Figure 13b Contractile reserve absolute GLS change (%) vs CFR – 10 mcg/kg/min to 20 mcg/kg/min (r=0.122, p-value=0.519)

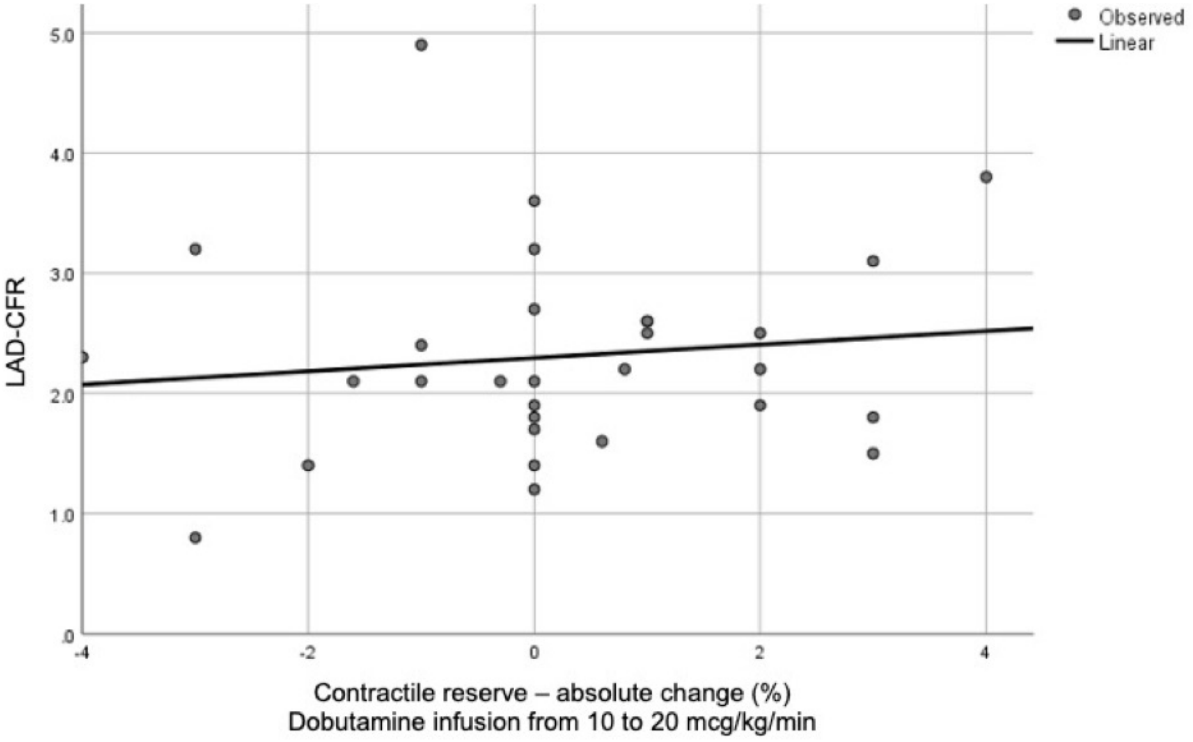
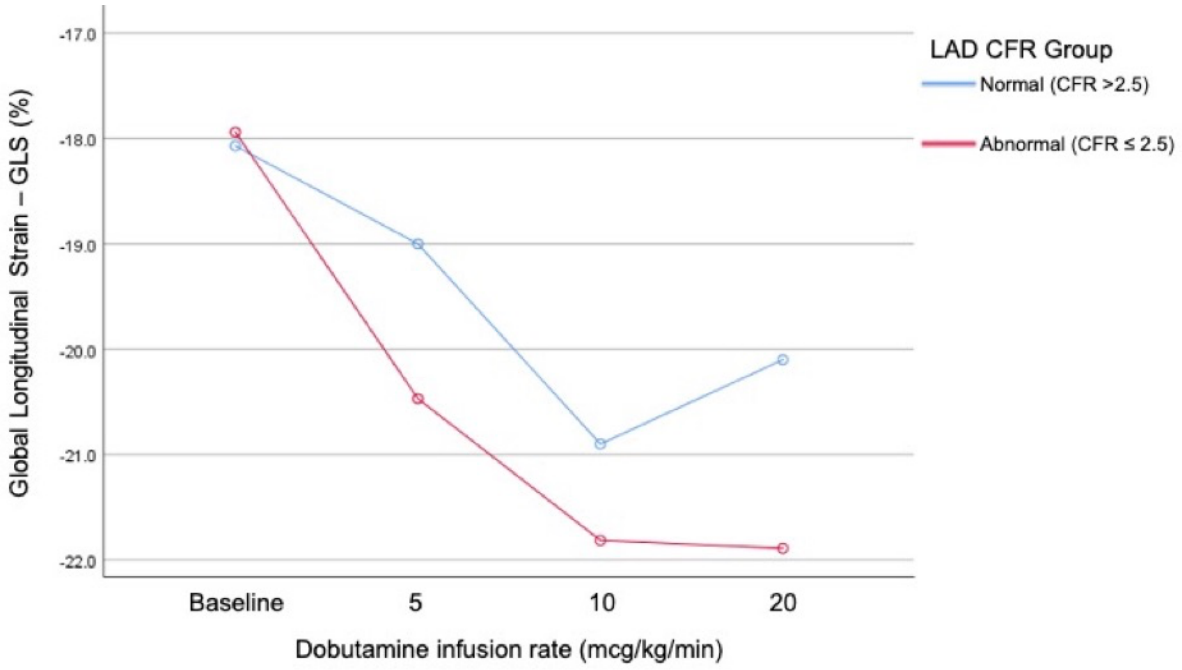


Figure 14: The relationship between GLS at different dobutamine infusion rate (baseline, 5, 10 and 20mcg/kg/min) with LAD CFR. Abnormal LAD CFR was defined as ≤ 2.5. (p=0.393)



Chapter 7

Final Discussions and Future Directions

7.1 Final discussions

It is increasingly recognized that patients can experience angina despite having no obstructive coronary artery disease, an entity, termed as ischaemia with no obstructive coronary artery disease (INOCA), with emerging standardised diagnostic criteria using invasive coronary physiology study. However, there is a relative lack of confirmatory non-invasive diagnostic techniques available at present to assess INOCA. INOCA patients may not necessarily be associated with significant increase in cardiac mortality, but suffer from impaired quality of life[37] and have substantial financial burden on welfare and health services together with limited effective therapeutic options.[3,5,6]

OSA is an important public health concern, with its increasing prevalence, and is significantly associated with coronary artery disease, independent of traditional cardiovascular risk factors. The potential mechanisms of OSA affecting vascular function leading to adverse cardiovascular outcomes are complex and likely related to changes on a haemodynamic, autonomic, and/or neuronal basis, linked to hypoxic-related sympathetic surge, oxidative stress, inflammation and/or endothelial dysfunction.

The findings made during this doctoral thesis have provided comprehensive review of INOCA and the second chapter of this thesis relates to understanding of the association between OSA and CAD. The third and fourth chapters revealed the significant relationship with OSA as an associative risk factor, utilizing retrospective data from the large, established CADOSA registry and prospectively with an invasive coronary assessment as a pilot study. Chapter three of this thesis provides information on the prevalence and clinical predictors of OSA in patients with anginal symptoms who have undergone coronary angiogram in South

Australia. In addition to established risk factors for OSA, this study found INOCA to be independent predictor of OSA; especially in those presenting with a stable angina presentation. This suggests that coronary vasomotor disorders may be associated with OSA. Chapter 4 examined the relationship of OSA in INOCA, by invasive coronary physiology study as a pilot study. This study has provided novel evidence of the important observations of a high prevalence of abnormal coronary haemodynamics in patients with INOCA and the presence of OSA was a significant independent predictor of abnormal coronary microvascular function.

The following two chapters concerns the ability to evaluate INOCA non-invasively with advanced echocardiographic technique, given the invasiveness of current diagnostic criteria requiring instrumentations of the apparently normal coronary arteries. Chapter 5 provided the insight of myocardial ischaemia detection which has come a long way since the clinical symptom was scientifically described several centuries ago and matched with pathophysiological correlation. Establishing the extent of ischaemia is critical in the diagnosis, management and prognostication of coronary artery disease. Stress echocardiography is a modality that is easily available, cost effective and radiation free, in establishing ischaemia. Myocardial ischaemia prediction, utilising non-invasive speckle tracking derived GLS, providing a novel prospect.[13,14] Chapter 6 investigates the possibility of low dose dobutamine stress echocardiogram speckle tracking derived contractile reserve in predicting the invasive coronary microvascular measures, specifically CFR and HMR, in patients with INOCA. This study found that there were more GLS derived contractile reserve at 5mcg/kg/min of dobutamine infusion between normal and abnormal coronary haemodynamic indices which suggest hibernating myocardium contractility recruitment during the infusion. This was not seen on the higher dose of dobutamine. Unfortunately, this pilot study has small numbers of patient samples. Therefore, limited conclusions can be made from these data beyond this.

OSA patients not only suffers from poor sleep quality, reduced quality of life and cardiovascular comorbidities, but is also independently associated with CAD. The pathogenesis of atherosclerosis remains complex and poorly understood. CPAP therapy has been linked with reduction in major adverse cardiovascular events, however, recent pivotal randomised controlled trials failed to demonstrate its significance. INOCA is a relatively new entity with novel evidence of haemodynamic association with OSA but the prognostic impact of OSA treatment on both this endotype and obstructive CAD remains unresolved.

7.2 Future directions

INOCA has often been dismissed as ‘false positive’ in the current ‘stenotic centric’ era in managing coronary artery disease. There are no large prevalence studies to date, but international registry collaborations have been established, namely by the COVADIS study group. Hopefully, a unifying diagnostic criterion established by this group will pave the way to a better understanding towards the nature of this condition, namely pathophysiology, clinical determinants and effective targeted therapy.

However, there is limited uptake of provocation testing prevails in most busy cardiovascular intervention suites exacerbated by a lack of physician awareness of the prevalence and diagnostic, therapeutic and prognostic significance of INOCA. The lack of demand for provocation testing has resulted in, limited technology advancement in this area. Disappointingly, ComboWire XT wire manufacturing was discontinued in 2020 and currently the only available pressure wire for this purpose is the PressureWire X.

The evolution and long-term prognostic implication of INOCA remains unknown, while emerging evidence suggest that this may not be as uncommon or benign as we originally thought. Without accurate data on the life trajectory of INOCA or evidence to support the importance of this condition in the lexicon of cardiac diseases, it remains challenging to increase awareness within the cardiology, general medical and pharmaceutical community.

The observations made during the course of this thesis have provided important insights into INOCA; with its evolving terminology, and OSA association, and novel prospect in non-invasive assessment of myocardial ischaemia, potentially in microvascular angina endotype of INOCA. However, many questions remain unanswered, some of which are discussed below.

This thesis provides the evidence in a South Australian cohort of patients undergoing coronary angiography for the investigation of suspected ischaemic chest pain, OSA was observed in 11% and associated with previously described OSA risk factors (male gender, hypertension, dyslipidaemia, diabetes, depression), as well as the novel finding of the presence of INOCA was predictive (particularly in those with a stable angina pattern) of OSA.

Our pilot study to investigate the association of OSA in INOCA patients, by invasive coronary physiology study, has demonstrated important observations of a high prevalence of abnormal coronary haemodynamics in patients with INOCA, and the presence of OSA was a significant independent predictor of abnormal coronary vasomotor disorder. Consequently, OSA may play a significant pathophysiological role in the coronary vasomotor mechanism of angina in this subgroup and screening of OSA should be considered. However, this study has several limitations. Firstly, about a third of patients' diagnosis of OSA relied upon a previous

diagnosis. Secondly, the study does not provide any insights into the mechanism underlying this observed phenomenon. Thirdly, the number of patients with pure epicardial spasm was too small to draw a meaningful conclusion between OSA and epicardial spasm. Finally, previous studies have demonstrated in the general population an association between OSA and traditional cardiovascular risk factors including male sex, hypertension and diabetes.[7-11] With no healthy control in our study cohort, we were unable to demonstrate a meaningful difference to reflect this in our study cohort being limited without a healthy control cohort.

OSA is an important public health concern, with its increasing prevalence, and is significantly associated with coronary artery disease, independent of traditional cardiovascular risk factors. The pathogenesis of OSA affecting vascular function leading to adverse cardiovascular outcomes are complex and poorly understood. INOCA is a relatively new entity with novel evidence of haemodynamic association with OSA but the prognostic impact of OSA treatment on both this endotype and obstructive CAD remains unresolved. The benefit of treatment of OSA in this subset remains unknown but these results suggest a randomised controlled trial of nocturnal CPAP for this cohort is worth pursuing. Generally, patients with INOCA are resistant to conventional angina management, so alternative treatment options are urgently required. Also, further mechanistic studies evaluating the pathophysiological relationship in this sub-group of patients is important to understand the likely complex relationship between these two conditions.

Non-invasive diagnostic techniques are either not easily accessible due to cost (CMR) or has ionising radiation exposure (PET). Echocardiography with emerging speckle tracking technique may be a potential non-invasive means of diagnosing MVA but lack large validation studies. Stress echocardiography is a modality that is easily available, cost effective and

radiation free, in establishing ischaemia but remain at the mercy of image quality. Finally, our pilot study that investigated the utility of speckle tracking derived contractile reserve during low dose dobutamine stress echocardiogram in predicting the invasive coronary microvascular measures provided the novel prospect of speckle tracking technique in assessment angina with no obstructive coronary artery disease with microvascular angina endotype. Unfortunately, this study had limited number of patients limiting substantive conclusions. Further evaluation of such techniques, including the utilisation of myocardial work to reduce the confounding effect of afterload in a larger number of patients would warrant further investigation and be essential for our understanding of this novel investigation technique.

Chapter 8

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