

**Clinical Studies of Patients with Acute Coronary
Syndromes in the Absence of Obstructive Coronary
Artery Disease**

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Declaration

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Abstract

Background: Although there is extensive data on patients with obstructive coronary artery disease in relation to clinical manifestations, health outcomes and genetic predisposition, little is known about these features in patients with Non-obstructive Coronary Artery Disease (NoCAD), despite these patients representing 20-30% of patients undergoing angiography.

Objectives: This thesis examined the clinical features, health outcomes and genetic polymorphisms in patients with NoCAD. The specific objectives include (1) comparing the health outcomes of NoCAD patients who present with an acute coronary syndrome (ACS) to those who have a stable chest pain pattern; (2) examining the prevalence of acute ischaemic electrocardiographic (ECG) changes in patients with the coronary slow flow phenomenon (CSFP) admitted with an ACS; and (3) to investigate the frequency of an endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) polymorphism in patients with: (a) chest pain and NoCAD and, (b) the CSFP.

Summary of Thesis Chapters: **Chapter 1** summarises the relevant background for the studies described in this thesis. **Chapter 2** examines health outcomes, including health related quality of life (HRQoL) measures, in ACS versus stable chest pain presentations in patients with NoCAD, over 12 months follow-up. HRQoL measures were assessed using both a generic (Short-Form-36) and disease-specific (Seattle Angina Questionnaire) instrument. This study found no significant differences in health outcomes between the two clinical cohorts. **Chapter 3** assesses evidence of myocardial ischemia, utilising continuous ST/T wave monitoring to examine the frequency of ST/T wave fluctuations during an ACS in patients with the CSFP compared to healthy control subjects. This study found 92% of patients with the CSFP showed ECG evidence of myocardial ischaemia on continuous ST monitoring

during an ACS presentation, with significant ST segment and T-wave fluctuations occurring in 24% and 86% of CSFP patients; respectively. In comparison, ST/T wave fluctuations were observed in 5% of healthy control subjects. **Chapter 4** is a case-control study that investigates the frequencies of the eNOS (T-786C) and ET-1 (+138A del/ins) polymorphisms in patients with: (a) chest pain and NoCAD and (b) the CSFP. There were no significant differences in the frequency of each polymorphism associated with patients diagnosed with chest pain and NoCAD. However, the frequency of the +138 del/ins polymorphism from the ET-1 gene was significantly more prevalent in the CSFP patients compared to the control groups.

Conclusion: This thesis has demonstrated that NoCAD patients who present with an ACS have similar health outcomes to those with stable chest pain. Patients with the CSFP frequently present with an ACS and this is associated with dynamic ST/T wave fluctuations and in particular T-wave inversion. The underlying pathogenesis of the CSFP requires further study; however, endothelin appears to have an important role. Consistent with this mechanism, this study found an increased prevalence of the +138 del/ins polymorphism from the ET-1 gene in patients with the CSFP. We therefore postulate that endothelin-1 (possibly derived from inflammatory cells) produces acute microvascular vasoconstriction in patients with the CSFP resulting in myocardial ischaemia and thus an ACS presentation. Further studies are required to assess this hypothesis.

Publications and Presentations Derived from this Thesis

- **Refereed Journal Articles**

1. **Cutri N.**, Zeitz C., Kucia AM., Beltrame JF. *ST/T wave changes during Acute Coronary Syndrome Presentation in Patients with the Coronary Slow Flow Phenomenon*. International Journal of Cardiology. 2011 Feb 3;146(3):457-8.
2. Beltrame JF, Tavella R, **Cutri N.** *Quality of Life with PCI versus Medical Therapy in Stable Coronary Disease*. New England Journal of Medicine. 2008, Nov 20;359(21)2289-90.
3. Tavella, R, **Cutri N.**, Adams, R, and Beltrame JF. *Health Status of Stable Patients With Obstructive or Non-Obstructive Coronary Artery Disease Compared With Healthy Controls*. Circulation: Cardiovascular Quality and Outcomes, 2011, In Press
4. Tavella, R, **Cutri N.**, and Beltrame JF. *Health Status Outcomes in Patients With Non-Obstructive Coronary Artery Disease*. Circulation: Cardiovascular Quality and Outcomes, 2011, In Press

- **Conference Presentations**

5. **Cutri N.**, AM Kucia, Zeitz C, Beltrame JF: *ST/T wave Analysis in Patients with the Coronary Slow Flow Phenomenon* (Poster Presented at the National Heart Foundation Conference Annual Scientific Meeting in Brisbane, Australia June 2009)

6. **Cutri N**, Kucia AM, Zeitz C, Beltrame JF: *Continuous ST/T wave Monitoring During an Acute Coronary Syndrome Presentation in Patients with the Coronary Slow Flow Phenomenon* (Oral Presentation at The Queen Elizabeth Hospital Annual Research Day in Adelaide, Australia October 2008)

7. **Cutri N**, Kucia AM, Zeitz C, Beltrame JF: *ST/T wave Analysis in Patients with the Coronary Slow Flow Phenomenon* (Poster Presented at the 56th Cardiac Society of Australia and New Zealand, Annual Scientific Meeting in Adelaide, Australia August 2008)

8. **Cutri N**, Kucia AM, Zeitz C, Beltrame JF: *The Effects of Positional Changes on T-wave Amplitude in Healthy Controls* (Poster Presented at the 56th Cardiac Society of Australia and New Zealand Annual Scientific Meeting in Adelaide, Australia August 2008)

9. **Cutri N**, Tavella R, Beltrame JF: *Gene Polymorphisms in Non-Obstructive Coronary Heart Disease* (Oral Presentation Australian Society for Medical Research Annual Scientific Meeting in Adelaide, Australia June 2008)

10. **Cutri N**, Kucia AM, Beltrame JF, Adams R: *T wave Changes in patients with Normal Coronary Arteries* (Poster Presented at The Queen Elizabeth Hospital Annual Research Day in Adelaide, Australia October 2007)

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Abbreviations

A2RB	Angiotensin 2 Receptor Blocker
ACE	Angiotensin Converting Enzyme
Ach	Acetylcholine
ACS	Acute Coronary Syndrome
ADMA	Asymmetric Dimethylarginine
AP-1	Activator Protein 1
ATP	Adenosine Tri-Phosphate
bp	base pair
Ca ²⁺	Calcium Ions
CAD	Coronary Artery Disease
CaM	Calcium Calmodulin
cAMP	cyclic Adenosine Monophosphate
CCB	Calcium Channel Blocker
CCSC	Canadian Cardiovascular Society Classification
CCU	Coronary Care Unit
cDNA	complementary Deoxyribonucleic Acid
CFR	Coronary Flow Reserve
cGMP	Cyclic Guanosine Monophosphate
CHD	Coronary Heart Disease
CK	Creatine Kinase
CI	Confidence Intervals
cNOS	constitutive Nitric Oxide Synthase
COPD	Chronic Obstructive Airways Disease

CRP	C-Reactive Protein
CSFP	Coronary Slow Flow Phenomenon
CTFC	Corrected TIMI Frame Count
DNA	Deoxyribonucleic Acid
ECE	Endothelin Converting Enzyme
ECG	Electrocardiogram
EDHF	Endothelial Derived Hyperpolarising Factor
EDTA	Ethylenediaminetetraacetic Acid
eNOS	endothelial Nitric Oxide Synthase
EQ-5D	Euroqol-5D
ET-1	Endothelin-1
ETA	Endothelin Receptor A
ETB	Endothelin Receptor B
ETT	Exercise Treadmill Testing
GATA	Globin Transcription Factor
GEMS	General Electric Medical Systems
GTP	Guanosine Triphosphate
H ⁺	Hydrogen Ions
HCl	Hydrochloric Acid
HLA	Human Leukocyte Antigen
HR	Heart Rate
HT	Hypertension
IMR	Index of Myocardial Resistance
iNOS	inducible Nitric Oxide Synthase
IP ₃	Inositol triphosphate

K ⁺	Potassium Ions
KB	Kilobase
KCl	Potassium Chloride
LAD	Left Anterior Descending
L'Arg	L'arginine
LSM	Lymphocyte Separation Medium
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
MI	Myocardial Infarction
MOS	Medical Outcomes Study
MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic Acid
MSS	Mental Summary Score
MUSE	Marquette Universal System for Electrocardiology
Na ⁺	Sodium Ions
Na ₂ PO ₄	Disodium Hydrogen Orthophosphate
NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
NF-1	Nuclear Factor-1
NMR	Nuclear Magnetic Resonance
nNOS	neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NSTEMI	Non-ST-Elevation Myocardial Infarction
NYHA	New York Heart Association

OCAD	Obstructive Coronary Artery Disease
Ors	Odds Ratios
PBS	Phosphate Buffered Saline
PCR-SSP	Polymerase Chain Reaction-Sequence Specific Primer
PET	Positron Emission Tomography
PG ₂	Prostacyclin
PIP ₂	Phosphatidylinositol
PKG	Protein Kinase G
PreproET-1	Preproendothelin-1
PSS	Physical Summary Score
RNA	Ribonucleic Acid
SAQ	Seattle Angina Questionnaire
SF-36	Short-Form 36
SMC	Smooth Muscle Cell
SNP	Single Nucleotide Polymorphism
SPECT	Single Photon Emission Computed Tomography
SPSS	Statistical Package for the Social Sciences
SR	Sarcoplasmic Reticulum
STEMI	ST-Elevation Myocardial Infarction
STM	Middle of ST Segment
SV	Stroke Volume
TGF	Transforming Growth Factor
TIMI	Timi Frame Count
TNF	Tumour Necrosis Factor
WT	Wild-Type

Chapter 1. Introduction

1.1 Coronary Heart Disease Perspective

1.1.1 Epidemiology

Cardiovascular disease incorporates heart, stroke and vascular conditions, and is the leading cause of mortality and disability worldwide, responsible for a third of all deaths¹. In Australia, 3.4 million people have cardiovascular disease with 637,900 suffering from coronary heart disease. In addition approximately 260,000 Australians have self-reported angina pectoris which is a major cause of morbidity and a major clinical manifestation of ischemic heart disease. Of note, ischemic heart disease accounted for over 26,000 deaths (19.5% of all deaths) in Australia². These figures clearly indicate that this disease is a major health problem for Australians and a substantial cost burden to the health care system.

1.1.2 Definition of Coronary Heart Disease

Coronary heart disease (CHD) is a generic term used to describe a spectrum of closely related coronary disorders and syndromes. CHD can involve any part of the coronary vasculature, including the coronary arteries, arterioles, veins and/or capillaries. The most understood and common form of heart disease is obstructive coronary artery disease (CAD), which is defined as the presence of moderate to severe ($\geq 50\%$) atherosclerotic lesions which obstruct blood flow through the epicardial coronary arteries. Since the introduction of selective coronary angiography, the predominance in management of CHD has prompted a focus on epicardial coronary artery disease. Unfortunately, in the past little attention has been given to the coronary microcirculation which is responsible for the majority of coronary resistance under physiological conditions. Furthermore, the coronary microcirculation plays a fundamental role in the development of coronary microvascular dysfunction/disease.

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Coronary microvascular dysfunction can occur in the presence or absence of obstructive epicardial large vessel disease and can frequently manifest as angina pectoris, which is the cardinal symptom of CHD. In the absence of obstructive CAD, patients with heart-related chest pain can have angiographically normal or near normal coronary arteries. Unlike obstructive CAD, which is attributed to large vessel disease, patients with chest pain and non-obstructive CAD may have underlying coronary small vessel disease. Although Gould et al.³ previously demonstrated that a stenotic lesion must be $\geq 70\%$ to impede on coronary flow reserve, many studies use a 50% threshold to classify lesions as being of haemodynamic significance. In this thesis, obstructive CAD (OCAD) will be defined as patients exhibiting one or more coronary arteries with a lesion $\geq 50\%$. Those with no lesions or lesions $< 50\%$ will be referred to as non-obstructive CAD (NoCAD). The focus of this thesis will be on patients with chest pain and NoCAD, omitting cardiac entities such as heart failure and sudden cardiac death.

1.1.3 The Disease Pathway

The disease pathway model describes the health status domains involved in quantifying a patient's clinical symptoms; functional limitations and overall quality of life. In patients with chronic disease, health status refers:

to the range of manifestation of disease in a given patient including symptoms, functional limitation and quality of life, in which quality of life is the discrepancy between actual and desired function⁴.

Hence the individual patient's evaluation of health status in coronary disease is essentially important in living life as they desire.

Traditionally, clinicians mainly focused on diagnosing the disease and evaluating symptoms, whereas patients are focused on the complete range of health status. Patient-reported health

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status measures are shown to be significantly important when assessing the overall wellbeing of a patient^{5,6}. The most common framework for conceptualising health status is to independently quantify the ways in which a chronic illness affects a patient's life.

The pathway of disease in CHD depicts the path to disability where clinical symptoms caused by CHD can lead to functional limitations and an impaired quality of life (Figure 1.1)⁷. The primary symptomatic manifestation of CHD is angina pectoris, however dyspnoea, which is defined as difficult or laboured respiration, is also one of the cardinal symptoms of CHD⁸. The presence of angina pectoris in either acute or stable forms can severely impair a patient's quality of life due to increased psychological and physical morbidity⁹. Recurrent attacks of anginal pain severely restrict the ability to live and enjoy a normal life⁵. Depending on the frequency and severity of symptoms, angina can cause considerable morbidity in a patient's life.

NOTE:
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of the print copy of the thesis held in
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Figure 1.1 The Disease Pathway in Coronary Heart Disease

Modified from Spertus et al.⁶

The goal of caring for people with chronic medical conditions such as angina is not only to improve their survival rate but also to maximise their quality of life. Population studies have

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found patients with coronary disease suffer debilitating physical, emotional and social limitations or problems primarily due to symptoms of angina¹⁰. The patient with angina is limited in regard to physical exertion, leisure activity, social functioning, sexual activity and capacity for work. Furthermore, angina in patients with CHD has been correlated with subsequent cardiac events and increased mortality⁷. Therefore, enhancing daily physical functioning and wellbeing is an increasingly advocated goal in the treatment of patients with coronary heart disease.

1.1.4 Scope of Thesis

This thesis examines clinical aspects of patients presenting with chest pain who do not have evidence of OCAD on angiography. This thesis is designed to focus on a holistic approach when examining patients with chest pain and NoCAD. It particularly addresses:

- (a) their health status 12 months following angiography
- (b) if they have clinical evidence of myocardial ischaemia
- (c) genetic polymorphisms associated with this condition.

This introductory chapter will focus upon the principles underlying these investigations including:

- (a) fundamental principles in understanding the impact of disease on patients
- (b) clinical investigations for coronary heart disease
- (c) the clinical syndromes in coronary heart disease
- (d) basic pathophysiological concepts relating to coronary heart disease.

Finally the specific objectives of the thesis will be defined.

1.2 Health Outcomes in CHD

1.2.1 Definitions of Cardiovascular Health Outcomes

Cardiovascular health outcomes encompass a range of objective and subjective end-points which are used to evaluate disease progression and patients' health status. Objective outcome measures such as rates of mortality and cardiac events are generally known as *'hard'* end-points and are commonly used in clinical trials¹¹. These objective outcomes are important clinical markers; however, they are often poor indicators of a patient's physical and emotional wellbeing. Subjective outcomes consist of *'soft'* end-points such as overall quality of life, social health, pain perception, and patient satisfaction¹².

Clinicians usually focus on *'hard end-point'* measures such as mortality, myocardial infarction (MI) or other adverse cardiovascular events when examining the effectiveness of a therapy. However, by doing so they tend to underestimate a patient's disability¹³. Whilst objective measures are important outcomes for measuring physical dimensions, they do not reflect the functional limitations of the disease on a daily basis¹⁴. In the past ten years there has been an ongoing debate regarding the effectiveness of both objective and subjective outcomes. Several studies have shown that a combination between objective and subjective measures may provide a more comprehensive evaluation about the effectiveness of an intervention¹⁴⁻¹⁶.

1.2.2 Cardiovascular Events Assessment

Traditionally, mortality and complication rates such as MI and hospital re-admissions have been used as clinical end-points to assess differences in treatment strategies, healthcare needs and costs for patients with CAD in randomised clinical trials^{11,17}. In chronic conditions such

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as coronary artery disease, mortality and cardiac outcomes often need to be tracked for years before meaningful conclusions about the quality of care can be obtained¹⁸.

1.2.2.1 Cardiovascular Mortality

The number of cardiovascular deaths reported world-wide (refer to 1.1) can also be misleading due to the few autopsies performed in hospitals nowadays. Accurate reports of death caused by cardiovascular heart disease are often unreliable as they are self-reported by the patient's doctor with lack of pathological assessment. It is likely that the number of cardiovascular deaths reported is not representative of the actual number of deaths caused by coronary heart disease. Therefore there may be inconsistencies in determining a patient's actual cause of death, causing limitations in many current epidemiological and clinical studies. However, irrespective of these inconsistencies, mortality is still considered an important end-point in health outcomes research.

1.2.2.2 Myocardial Infarction

As previously mentioned, MI is often used as a major end-point in clinical and epidemiological trials. In the early 1980s the definition of MI was assessed by the measure of conventional cardiac biomarker creatine kinase and its MB isoenzyme (CK-MB). According to the universal definition published in 2007 by the European Society of Cardiology, the new definition of myocardial ischaemia is based on cardiac troponins, which are a more sensitive and specific diagnostic marker of myocardial damage compared with CK-MB¹⁹. The impact of changing myocardial diagnostic criteria in the 21st century has led to a greater proportion of patients being diagnosed with non-ST elevation MI²⁰. In addition, the new MI definition has been associated with a substantial increase in the number of coronary catherisations and cardiac hospital admissions²¹. A recent study, found that patients classified by the new

definition of MI have higher long-term mortality compared with patients that belong to the old definition²². Therefore the implications of redefining MI have had a substantial effect on patient care and health outcomes. In clinical trials the standardised and universal definition of MI is necessary for accurate comparisons and interpretations.

1.2.2.3 Hospital Re-admissions

The use of hospital admission data is frequently used in prospective and retrospective studies as a measure of outcomes in clinical studies. The purpose of analysing hospitalisation data is to improve quality of care and management strategies in patients with chronic disease. Unfortunately, hospitalisation data can be misinterpreted leading to methodological inconsistencies between studies. First, not all hospitalisations are necessarily relevant to the specific disease of interest. Second, hospitalisation can occur more frequently for some patients and not others. Third, each hospital admission may vary between patients, and the time between each admission may also vary between patients. Therefore the misinterpretation of hospitalisation data may lead to inaccurate information regarding disease burden to the patient and hospital systems²³.

1.2.3 Functional Outcome Assessment in Coronary Artery Disease

Patients with chronic conditions such as coronary artery disease are often physically limited due to the symptoms of their disease. CAD is a progressive illness relative to mortality and morbidity, primarily due to angina pectoris and MI. In patients with CAD, angina is the most common symptom causing limitations in daily functioning and wellbeing.

1.2.3.1 Canadian Cardiovascular Society Classification System

Angina severity can be assessed by the physician using the Canadian Cardiovascular Society Classification (CCSC) system, which ranges from class I (mild) to IV (severe) (*Table 1.1*)³⁰. This simple scale for angina pectoris has been used as a reliable outcome measure in studies where severity is correlated with the extent of disease²⁵. Although it is a simple clinical scale for assessing the physical limitations experienced by patients due to their angina, the CCSC has been known to have limited reproducibility and sensitivity to important clinical changes. This has been attributed to health status being measured by the physician's perspective and not self-reported by the patient²⁶⁻²⁹.

Table 1.1 Canadian Cardiovascular Society Classification Grading of Angina Pectoris
Source: Fuster et al.³⁰

<p>NOTE: This table is included on page 9 of the print copy of the thesis held in the University of Adelaide Library.</p>

1.2.3.2 Other Functional Outcome Assessments

There are other functional assessments which measure physical limitations caused by the symptoms of coronary heart disease. The Euroqol (EQ-5D) is a generic questionnaire which consists of five elements, each with three levels and a visual analogue scale which allows patients to rate their own health from 0-100 (poor to excellent health). The EQ-5D is known for its brevity; however, this questionnaire specifically assesses health status on that specific day and does not take into consideration health changes over the past month³¹. In addition, the EQ-5D is not a sensitive instrument in patients with very low levels of perceived ill-health³².

The New York Heart Association (NYHA) classification system measure was designed to assess the daily limitations caused by cardiac disease. The functional outcome is similar to the CCCC system in that it quantifies a patient's activity, symptoms and quality of life from the clinician's point of view, thus health status is not directly reported by the patient. The NYHA classification system is frequently used in clinical practice and research; however, it is known to have limited reproducibility and sensitivity to clinical change²⁶.

The Rose Angina questionnaire has been widely used in many epidemiological studies as a standardised method of measuring angina. The Rose questionnaire defines angina based on typical symptoms of chest pain which is caused by exertion. This questionnaire has a long and a current modified short version which consists of a 3-item survey. (The short version has demonstrated to be more sensitive than the long version³³. The Rose questionnaire, in both long and short forms, has been shown to independently predict morbidity and mortality in population-based studies^{34,35}. However, whilst there are studies which have tested the specificity and sensitivity of this questionnaire, at present it does not fulfil the relevant psychometric criteria needed to be properly validated³⁶.

1.2.4 Health-Related Quality of Life in Coronary Heart Disease

Health-related Quality of Life (HRQoL) represents ‘the functional effect of an illness and its consequent therapy upon a patient, as perceived by the patient’¹¹. HRQoL is assessed by the health dimensions relevant to a particular set of patients³⁷. These health dimensions can incorporate the evaluation of clinical symptoms, and bodily and emotional functions over time³⁸. For example, in patients with coronary heart disease, HRQoL assesses the patient’s perception of clinical symptoms on a physical, psychological, sexual and social scale³⁹.

The term ‘quality of life’ came into use during the 1970s as a multi-dimensional concept reflecting the overall subjective condition of the physical and mental welfare of the individual⁴⁰. Nowadays, the broad concept of HRQoL has been replaced with ‘health status’ which can be summarised as the range of manifestation of disease in a given patient including symptoms, functional limitation and quality of life, in which quality of life is the discrepancy between actual and desired function⁷.

HRQoL has recently been used as a primary outcome measure in clinical trials for determining therapeutic benefit and improving quality of care. In a randomised clinical trial, stable angina patients on long-acting anti-anginal medications experienced less symptoms, better treatment satisfaction and disease-specific quality of life compared to those on short-acting medications⁴¹. Therefore, the primary goal for treating patients with CHD is to maximise their overall quality of life and improve functional status, as this condition does not have a cure. The impact of cardiovascular disease burden is able to be reported by the patient and therefore is a more reliable outcome than mortality rates. The patient is able to describe the physical and mental disabilities caused by their symptoms, which reflects a more realistic impact of disease.

1.2.4.1 Health-Related Quality of Life Assessments

Health status evaluations can be undertaken by either the clinician or the patient. HRQoL instruments, otherwise known as questionnaires, can quantify the impact of a patient's health status and determine the extent of their symptoms, functional limitations and quality of life. An HRQoL questionnaire used in clinical trials should fulfil certain psychometric criteria. These include the properties of validity, reliability or reproducibility and responsiveness.

1. **Validity:** This measure examines whether the instrument is measuring what it is intended to measure by correlating changes in the instrument with changes in other related measures in theoretically derived predicted direction and magnitude. Additionally, the instrument should have discriminative validity in its ability to detect changes in the observed variable without provoking a 'floor' or 'ceiling' effect that reflects an inability to detect clinically significant changes.
2. **Reliability:** An instrument will be considered reliable when it measures what it truly intends to measure and reproducibility is defined by a high signal to noise ratio. If the variability in scores between patients (the signal) is much greater than the variability within patients (the noise), an instrument will be considered reliable. Reproducible results are obtained when the measure is repeatedly given to stable patients.
3. **Responsiveness:** An instrument's responsiveness is its ability to be sensitive in detecting any magnitude of clinical change^{42,43}.

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Overall, the measure employed in any clinical trial should be valid, reproducible and responsive to changes induced by treatment⁴⁴. There are two types of questionnaires that can be individually or jointly used in population-based HRQoL studies:

1. **Generic instruments** are widely used to monitor a patient's progress during an illness and give a broad assessment of that person's health status⁴⁵. Many generic questionnaires have been developed for evaluating overall quality of life in relation to any chronic disease. However, because they are general, this sometimes limits their ability to define specific limitations of that illness⁴⁶.
2. **Disease-specific instruments** are specifically targeted to a particular condition and tend to focus on areas of clinical relevance to clinicians. A disease-specific questionnaire is able to quantify the physical and emotional effects concerning a specific clinical condition⁴⁷. Disease specific tools are also more sensitive to subtle improvements in health especially in patients with co-morbid conditions^{11,46}. In clinical trials, the combination of a generic and disease-specific questionnaire provides a more comprehensive assessment of health status.

1.2.4.1.1 Short-Form 36

The Short Form-36 (SF-36) is a 36 item questionnaire constructed to assess both general physical and mental health concepts. It is the most widely used generic health status instrument in clinical trials⁴⁸. The SF-36 multi-item scales yield a profile of eight concepts, including physical and mental summary measures. The eight health concepts were selected from 40 included in the Medical Outcome Study (MOS) to represent those hypothesised to be most frequently measured in widely used health surveys and those most affected by disease

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and treatment. Furthermore, the SF-36 was constructed to have sound psychometric properties necessary for group comparisons that are not specific to any age, disease or treatment group⁴⁹.

This SF-36 was designed for use in surveys of general and specific populations, health policy evaluations, and clinical practice and research. In addition, this generic questionnaire has proved useful in comparing the relative burden of diseases and differentiates the health benefits produced by a wide range of different treatments⁵⁰⁻⁵².

The SF-36 has been shortened to the Short Form-12 (SF-12) in order to increase practicality and improve time application⁵³. The SF-12 is psychometrically comparable to the SF-36; however, there are some important differences which may influence health scores. First, the SF-36 yields more information about health status as it can be divided into eight different scale scores whereas the SF-12 gives only two summary scores. Second, the SF-36 allows room for missing data so that scores can still be calculated, whereas the SF-12 is too short and can be a limiting factor when dealing with small sample sizes⁵⁴.

1.2.4.2 Seattle Angina Questionnaire

The Seattle Angina Questionnaire (SAQ) is a 19-item self-administered questionnaire measuring five dimensions of coronary artery disease, physical limitation, angina stability, angina frequency, treatment satisfaction and disease perception. This disease-specific questionnaire has been shown to be valid, reliable, reproducible and very sensitive in detecting clinical change, making it a valuable functional health status measure³⁸. The SAQ measures how daily activities are limited by symptoms of CAD and has been specifically designed to ask questions strictly related to chest pain, chest discomfort or angina³⁸. The advantage of utilising this questionnaire in clinical practice is its ability to identify whether

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health status is improving, stable or deteriorating. In addition, health status measures obtained from the SAQ have been shown to be independently predictive for both mortality and hospitalisation in outpatients with CAD⁷. Overall, the SAQ has been shown to be a valuable instrument in cardiovascular outcomes research that measures five clinically important dimensions of health in patients with angina related coronary artery disease.

1.3 Clinical Symptoms in CHD

Patients with CHD often present with shortness of breath or less often with palpitations or pre-syncope/syncope. However, the most common presentation of CHD is chest pain or angina pectoris.

1.3.1 Angina Pectoris Definition

Angina pectoris is a clinical syndrome which is based on the presence of chest pain and other related pain originating from the heart. Angina pectoris was firstly described by historian William Heberden in 1779, who wrote,

They who are afflicted with it are seized while they are walking (more especially if it be uphill, and soon after eating) with a painful and most disagreeable sensation in the breast, which seems as if it would extinguish life, if it were to increase or continue; but the moment they stand still, all this uneasiness vanishes⁵⁵.

The symptoms of angina can be clinically classified into three types of presentations: typical angina, atypical angina and non-cardiac chest pain. The definition of typical angina is associated with retrosternal chest discomfort that may radiate to neck, jaw, epigastrium, or arms, with characteristic quality (squeezing, pressure-like, heavy), lasting two-ten minutes, worsened by physical exertion or emotional stress, and relieved by rest or nitroglycerin. Atypical chest pain refers to pain which is not characteristic of typical angina and may or may

not be due to myocardial ischaemia. Non-cardiac chest pain commonly occurs in patients with pulmonary, gastroesophageal and musculoskeletal disorders^{56,57}.

1.3.2 Angina as a Manifestation of Myocardial Ischemia

The primary manifestation of myocardial ischaemia is angina pectoris, which is caused by an imbalance between myocardial oxygen supply and myocardial oxygen demand. With respect to myocardial oxygen demand, an increase in oxygen requirements during exercise leads to subendocardial myocardial ischaemia which can manifest as a chronic form of angina. In addition, a decrease in myocardial oxygen supply due to reduced perfusion, can also lead to ischaemia which manifests as acute angina. The imbalance between myocardial oxygen supply and demand can be caused by atherosclerotic coronary artery disease, which affects coronary blood flow and metabolism.

In the presence of transient epicardial coronary artery occlusion and/or vasospasm, the onset of myocardial ischaemia causes various hemodynamic events and electrocardiogram (ECG) changes which form part of the 'Ischemic Cascade'. The ischemic sequence of events which occur over time usually begins with metabolic and biochemical changes; followed by impaired left ventricular diastolic function; then impaired left ventricular systolic function; next is the development of ECG abnormalities with ST/T wave changes; and finally angina is the clinical consequence. The cascade of ischemic events can progress from start to finish within 30 seconds from the onset of ischaemia⁵⁸⁻⁶⁰. However, not all episodes of ischaemia progress to the final symptomatic angina stage in patients with CAD, as demonstrated in patients with silent or asymptomatic ischaemia. Therefore, the use of non-invasive testing is important in detecting the presence of myocardial ischaemia at different stages of the cascade.

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As a separate clinical entity, patients with angina and angiographically NoCAD may also have microvascular ischaemia which has been attributed to coronary microvascular dysfunction⁶¹. The coronary etiology of angina with normal coronary arteries is supported by the frequent but not universal evidence of ischaemia during exercise stress testing. Although the exact underlying mechanism(s) of microvascular dysfunction in these patients remains uncertain, these patients may exhibit a variety of subjective and objective symptoms which indicate the presence of myocardial ischaemia.

1.3.2.1 Myocardial Ischemia Definition

Myocardial lactate production is considered to be the gold standard technique for detecting myocardial ischaemia, although studies have shown this technique lacks sensitivity^{62,63}. Therefore, metabolic evidence of myocardial ischaemia may not always present in patients with angina, and clinicians must rely on other measures to determine whether ischaemia is truly present. Consequently, as a result of the latter finding, the definition of myocardial ischaemia remains somewhat ambiguous with varying opinions of what is considered to be myocardial ischaemia⁶⁴. One researcher has stated that the limitation of coronary blood flow can produce both physiological and/or biochemical consequences which can cause angina symptoms⁶⁵. However, as previously mentioned, angina does not always equate to objective evidence of myocardial ischaemia in every instance and biochemical consequences do not always manifest in ischemic myocardial tissue, which makes it difficult to determine whether classic symptoms of angina have an underlying ischemic substrate. Although the close relationship between angina and ischaemia is universally recognised, the mechanism responsible for cardiac pain is not entirely understood^{66,67}.

1.3.2.2 Determinants of Myocardial Ischemia

As discussed above, myocardial ischaemia is characterised by an imbalance between oxygen supply and demand. There are several factors which can influence the control of oxygen delivery and consumption by the heart. The supply of oxygen to the myocardium is determined by the magnitude of coronary blood flow and the oxygen-carrying capacity of the red blood cells. The regulation of coronary blood flow is the most important determinant of oxygen supply and is modulated by several factors—metabolic control, autoregulation, extravascular compressive forces, duration of diastole, humoral factors and neural control⁶⁸. All of the above factors regulate coronary blood flow, by altering coronary resistance, via different complex mechanisms which are beyond the scope of this thesis.

The three main determinants of myocardial oxygen demand are factors primarily involved in the regulation of myocardial metabolism. These include heart rate, contractility and systolic wall tension⁶⁹. Heart rate is the most important determinant of myocardial oxygen demand, as when heart rate increases so does the amount of oxygen uptake. Myocardial contractility is influenced primarily by the sympathetic nervous system. An increase in contractility leads to an increase in myocardial oxygen demand, causing a proportional increase in coronary blood flow. Left systolic wall tension is directly correlated with myocardial oxygen demand. In addition, wall tension is proportional to left ventricular systolic pressure, left ventricular diameter and inversely proportional to ventricular wall thickness. The contribution of each determinant in the regulation of coronary blood flow is important in maintaining adequate cardiac metabolism and perfusion, especially during an increased cardiac work-load⁷⁰.

Consequently, in the presence of myocardial ischaemia coronary blood flow is decreased due to an increase in coronary artery resistance, which is altered by specific regulatory influences.

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Furthermore, the determinants influencing myocardial oxygen supply and demand appear to affect certain parts of the myocardium more than others during disease states. The area of myocardium most susceptible to ischaemia is the sub-endocardium, due to the influence of mechanical and metabolic forces⁶⁸.

1.3.2.3 Ischemia Spectrum

Clinically, angina can be described as exertional, rest or mixed-pattern angina. These reflect different underlying pathophysiological mechanisms. Exertional angina occurs during exercise when an increase in oxygen requirements produces myocardial ischaemia. At the other end of the spectrum is rest angina, which occurs at rest or during low levels of activity and is not preceded by an increase in myocardial demand but is associated with a transient impairment in coronary blood flow. A study conducted by Maseri et al.⁷¹ found that a majority of patients in clinical practice were more likely to have both types of angina. This clinical finding was termed ‘mixed-pattern angina’, caused by both an increase in oxygen demand and transient decrease in oxygen supply. Mixed-pattern angina can occur from either a fixed reduction of coronary flow reserve or, a coronary flow reserve which is variable at different times of the day. The underlying pathogenic mechanisms of this angina syndrome may include; (a) flow-limiting epicardial stenoses and (b) epicardial and/or microvascular vasoconstriction⁷².

1.3.2.4 Assessment of Myocardial Ischemia

In current clinical practice myocardial ischaemia can be objectively assessed by pathological changes in electrical, cellular and biochemical functions. Various non-invasive functional tests can be performed to assess the presence and extent of coronary myocardial ischaemia and thus can be used to determine myocardial viability.

1.3.2.4.1 12-Lead Electrocardiogram

The clinical gold-standard measure for recording ischaemia is the 12-lead ECG, with ST segment and T wave changes being well described markers of ischaemia. The 12-lead ECG can also determine heart rate and rhythm, and identify structural and electrically abnormalities, chamber enlargement and conduction defects⁷². Therefore, it is not surprising that the ECG is considered to be one of the most important diagnostic tools in clinical practice and cardiology research.

The ST segment and T-wave changes

It is widely recognised that ST elevation is considered an indicator of transmural ischaemia and ST depression is a marker of subendocardial ischaemia. However, transient ST segment elevation and depression can also occur in the absence of angiographically epicardial coronary artery disease, suggesting ST elevation may be secondary to microvascular ischaemia (particularly when chest pain is present). The T wave is another ischemic marker on the ECG, which also yields important diagnostic information. In addition, the T wave has been shown to be prognostically just as important as ST segment changes⁷³.

1.3.2.4.2 Exercise and Treadmill Testing

Exercise treadmill testing (ETT) is another non-invasive diagnostic test used to predict the presence or absence of CAD. During the testing the patient's ECG, heart rate and blood pressure are continuously monitored to determine myocardial functional capacity and estimate prognosis. In the presence of myocardial ischaemia, exercise can produce both systolic and diastolic ventricular abnormalities, as well as ECG changes and chest pain⁷⁴. ETT is useful in clinical decision-making when determining whether a patient has a high or low probability of coronary disease. The likelihood of coronary disease can be calculated using Bayes Theorem,

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which takes into account the pre-test results to determine the post-test outcome. Bayes Theorem is clinically useful in separating subjects with and without CAD as demonstrated by coronary angiography⁷⁵. However, controversy surrounds the validity of this theory in predicting the probability of coronary disease, as a normal angiogram does not exclude a person from having small vessel disease.

Nevertheless, the mean sensitivity and specificity of exercise ECG for detecting obstructive CAD is approximately 70%⁷⁶. However, false positive tests may occur as a result of both coronary and non-coronary causes. In the absence of obstructive large vessel disease, an objectively positive ETT is described as a ‘false-positive’, which is not accurate as the absence of rate-limiting stenoses does not preclude the presence of ischaemia. In addition, a positive ETT in patients without large vessel disease may reflect the presence of microvascular ischaemia, as can occur in cardiological syndrome X⁷⁷.

1.3.2.4.3 Single Photon Emission Computed Tomography

Myocardial scintigraphy involves a number of nuclear medicine imaging techniques which are used to detect abnormalities in myocardial cellular function or metabolism. Single Photon Emission Computed Tomography (SPECT) uses gamma rays to provide cross-sectional images of functional organs in a 3D representation. SPECT imaging is used to detect abnormalities in myocardial cellular function or metabolism. Myocardial perfusion is evaluated by the uptake of radioactive labelled perfusion tracers such as thallium-201 and technetium-99m. These tracers can be used to quantitatively assess viable myocardium⁷⁸.

1.3.2.4.4 Positron Emission Tomography

Positron Emission Tomography (PET) is a non-invasive assessment of regional blood flow and function using positron-emitting radioactive tracers to detect abnormalities in myocardial perfusion and viability. PET is one of the most accurate diagnostic measurements of myocardial ischaemia in both large vessel and microvascular coronary disease⁷⁹.

1.3.2.4.5 Biochemical Studies

Trans-myocardial lactate production is considered to be the gold-standard technique for determining the presence of myocardial ischaemia. Myocardial lactate is the metabolic by-product produced by anaerobic metabolism resulting from hypoxia or anoxia⁶². Myocardial lactate testing has demonstrated to be highly specific but appears to be an insensitive measure for detecting microvascular ischaemia⁸⁰. Other biochemical studies include trans-myocardial measurement of pH⁸¹ and coronary sinus oxygen saturation which are also used to assess the presence of ischaemia. Coronary sinus oxygen abnormalities and pH changes caused by decreased myocardial oxygenation are both invasive procedures and may be subject to sampling error^{82,83}.

1.3.2.4.6 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-invasive method used to detect the presence of altered high energy phosphates released by the myocardium during ischaemia. NMR can directly identify the metabolic effects of ischaemia in patients with coronary artery stenoses. In addition, the presence of ischaemia has also been detected by NMR in individuals with chest pain and no significant coronary artery stenoses, demonstrating NMR is a sensitive method of detecting ischaemia⁶⁶.

1.3.2.4.7 Left Ventricular Function Assessment

The assessment of left ventricular (LV) function is important in assessing the presence and amount of myocardial ischaemia in patients with CHD. LV function can be determined by measuring LV ejection fraction (LVEF) at rest and during exercise. In healthy individuals, LVEF as well as stroke volume (SV) usually increase during exercise; however, in patients with impaired LV function, LVEF and SV remain constant or decrease⁸⁴⁻⁸⁶.

Stress Echocardiography

Stress echocardiography is a popular technique used to assess LV function in patients with chronic angina⁸⁷. This test involves either a physical, pharmacological or provocative stimulus to assess the LV function of coronary circulation by comparing the results at rest and immediately following exercise^{88,89}. The presence of inducible wall motion abnormalities has been used in predicting prognosis in patients undergoing exercise or pharmacological stress echocardiography⁹⁰.

1.3.2.4.8 Haemodynamic Functional Assessments

The most common haemodynamic functional assessments used to measure coronary blood flow are (a) Coronary Flow Reserve (CFR) and, (b) Index of Myocardial Resistance (IMR). CFR is defined as the ratio of hyperaemic to resting myocardial blood flow. The assessment of coronary blood flow can be measured by coronary sinus thermodilution or oxygen saturation techniques, coronary doppler or PET techniques. An impaired coronary flow reserve can be due to obstructive epicardial CAD or microvascular dysfunction. Thus in patients with normal angiography, it typically reflects microvascular dysfunction.

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The recently developed IMR technique assesses the status of the coronary microcirculation by measuring pressure and estimating coronary flow simultaneously, using a pressure-temperature sensor- tipped coronary wire⁹¹. IMR is able to measure coronary blood flow in both the epicardial and the microvascular coronary arteries, whereas the measure of CFR only assesses blood flow through the epicardial coronary arteries.

1.3.3 Angina Syndromes

The symptom of angina encompasses a heterogeneous spectrum of conditions which are classed together irrespective of the underlying cause of the condition. *“The word syndrome identifies a collection of symptoms and signs but does not identify the precise cause of illness”*⁹². The CHD syndromes that manifest as angina can be either acute or chronic in nature.

1.3.3.1 Acute Coronary Syndrome

The term acute coronary syndrome (ACS) is used to classify a heterogeneous spectrum of clinical conditions including unstable angina, acute ST elevation myocardial infarction (STEMI) and acute Non-ST elevation myocardial infarction (NSTEMI). These clinical conditions are classified as syndromes and we are able to identify them by a similar pattern of signs and symptoms. Acute coronary syndromes typically present with prolonged rest angina and may result in cardiac events including MI or sudden cardiac death. The exact aetiology of an ACS presentation is unknown, although it involves the interplay between atherothrombosis, inflammation and vasomotor reactivity.

1.3.3.1.1 Unstable Angina

The syndrome unstable angina covers a broad spectrum of patients including those with and without coronary disease. This heterogeneous disorder is defined as chest pain that occurs at rest or with progressively lesser degrees of exertion; and can also be accompanied with ST/T changes on the resting ECG. The diagnosis of unstable angina is determined by clinical evaluation of symptoms and the presence of ST changes, which can be reversible once chest pain has subsided. Unstable angina is differentiated from acute MI by the absence of a significant troponin rise suggesting that major myocardial necrosis has not occurred. However this condition warrants aggressive management as unstable angina may progress onto acute MI.

The Braunwald classification of unstable angina assesses the risk of death or MI in one year by differentiating the severity and clinical circumstances surrounding the presentation of unstable angina, considering the presence of ECG changes and intensity of medical therapy⁹³.

1.3.3.2 Acute Myocardial Infarction

Acute myocardial infarction is the term used to describe blood supply to the heart being reduced and causing myocardial necrosis manifest by the leakage of troponin from the myocardium into the circulation. Acute MI is diagnosed on the presence of two of the following three features—clinical symptoms, elevated cardiac markers (eg Troponin) in the blood and ECG changes such as ST/T wave changes which may include the development of Q waves and/or persistent T wave inversion. Further evaluation of the ECG allows a distinction to be made between ST elevation MI (STEMI) and non-ST elevation MI (Non-STEMI). The hallmark of STEMI is ST segment elevation, which may also be associated with T wave abnormalities and later the development of Q waves⁹⁴. NSTEMI is characterised by the

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absence of ST elevation and may include ST depression and T wave changes. STEMI is often associated with transmural myocardial damage which indicates injury to the entire thickness of the myocardial wall and warrants urgent reperfusion therapy. NSTEMI often reflects subendocardial infarction which reflects injury to the layers just beneath the myocardium. Often a coronary angiogram is performed in patients with an acute myocardial infarct in order to visualise narrowings or obstructions of the coronary arteries and determine if revascularisation of the infarct-related artery is feasible. The manifestation of STEMI and NSTEMI is commonly attributed to epicardial coronary artery disease, although this is not always the case. Less than 5% of patients who undergo coronary emergency angiograms for suspected MI are diagnosed with normal coronary arteries^{95,96}.

1.3.4 Chronic Stable Angina

A chronic coronary syndrome usually presents in the form of stable angina, manifesting as typical exertional angina. These may limit the patient's physical capacity and thus quality of life, but have a lower risk of cardiac events when compared to patients with acute coronary syndromes. The exertional angina may result from obstructive large vessel disease (chronic stable angina) or microvascular dysfunction (Syndrome X). The disability incurred by this symptom can be categorised by the CCSC. This utilises a grading scale from I-IV with class I not limiting the patient's physical activity to class IV where any activity produces chest pain (Table 1.1). Considering the exertional nature of this type of angina, an exercise stress test is a useful assessment. With this provocation, myocardial ischaemia may be assessed by ischaemic ST-segment changes, perfusion changes on myocardial scintigraphy or MRI contrast-perfusion, or ischaemic left ventricular dysfunction on stress echocardiography or MRI³⁰.

1.3.5 Chest Pain and Non-Obstructive Coronary Artery Disease

The angina syndromes described in section 1.3.3 are predominately a manifestation of coronary artery disease. As previously mentioned, in the diagnosis of coronary artery disease, chest pain and dyspnoea are the two most important features. The underlying cause of acute and stable angina manifestations in large vessel disease has been attributed to atherosclerosis. However, the post-mortem studies undertaken by Tuzcu et al.⁹⁷ found that atherosclerotic lesions are commonly present in asymptomatic individuals, suggesting that coronary atherosclerosis does not always cause chest pain. Clinically the term angina pectoris is used to describe chest pain strictly related to coronary disease however this term can be inaccurately applied, as coronary disease can occur without pain, and alternatively chest pain can occur without coronary disease. Hence the importance of utilising functional assessments to detect myocardial ischaemia and exclude non-cardiac causes of chest pain such as reflux, musculoskeletal and lung disease.

1.3.5.1 Atherosclerosis

The disease process underlying obstructive CAD is atherosclerosis, which commences in childhood and clinically manifests itself in adulthood at any age. Coronary atherosclerosis is a disease of the large and medium-sized arteries of the heart and is characterised by the formation of atheromatous plaques, which represent a build-up of lipids, cholesterol, calcium, and macrophages within the intima of the epicardial arterial wall. Growth of the fibrous plaque results in Glagov remodelling (compensatory enlargement of coronary arteries)⁹⁸, luminal obstruction, abnormalities of blood flow and compromised oxygen supply to the affected artery⁷⁰. In addition, fibrous plaques can undergo calcification, ulceration, thrombus formation and aneurismal dilation. These conditions compromise arterial blood flow and weaken affected arteries which may cause symptoms of angina or dyspnoea.

1.3.5.2 Coronary Artery Disease and Ischaemia

In a normal heart, coronary blood flow correspondingly increases as oxygen demands rise. In patients with atherosclerotic CAD, coronary flow reserve is limited and blood flow cannot increase proportionally to deliver adequate oxygen when demand is increased. Although obstruction may not be enough to produce myocardial ischaemia at rest, an increase in myocardial oxygen demand during exertion can precipitate myocardial ischaemia causing stable angina. The typical underlying cause of stable angina is a fixed coronary stenosis, which causes compromised blood flow. The underlying pathophysiology of unstable angina usually involves plaque rupture with secondary platelet aggregation, coronary artery spasm or thrombosis. Furthermore, the manifestation of unstable angina and/or MI can be caused by atheromatous plaque rupture, which obstructs a blood vessel causing coronary insufficiency or microembolisation⁹⁹. Gould et al.³ previously demonstrated that a stenotic lesion must be $\geq 70\%$ to impede on coronary flow reserve during exertion causing stable angina. A stenotic lesion of $>90\%$ decreases coronary blood flow dramatically and may produce rest angina¹⁰⁰.

1.3.5.3 Assessment of Coronary Artery Disease

Coronary imaging has become important in assessing coronary anatomy and function in patients with CAD. There are several diagnostic invasive and non-invasive techniques that can be used to detect myocardial ischaemia caused by CHD. In addition, the assessment of myocardial cellular function or metabolism can be examined using a variety of radioactive tracers to detect the presence of ischaemia.

1.3.5.3.1 Coronary Large Vessel Disease

Invasive selective coronary angiography is the routine, clinical, gold-standard assessment method to determine the presence and severity of CAD. It is most commonly utilised to

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evaluate patients with significant chest pain for the presence of CAD but may also be used to exclude significant CAD in patients with acute pulmonary oedema or malignant arrhythmias. The procedure involves the insertion of a cannula into a peripheral artery (femoral radial or brachial artery) to access the arterial system and then advancing catheters through the cannula (referred to as a sheath) to the heart. The preformed catheters are selectively placed in the left or right coronary ostia under x-ray guidance. Radio-opaque contrast is then injected into the coronary artery enabling the coronary arteries to be visualised radiographically. The severity of a coronary stenosis can then be qualitatively assessed by the angiographer, with functionally obstructive stenoses considered to be those encroaching on the lumen $\geq 70\%$ ³. Lesions that are 50-70% are often considered intermediate as they could potentially be obstructive and may warrant further investigation. Those $<50\%$ are generally considered not to be responsible for causing angina due to myocardial ischaemia.

Coronary stenoses may also be assessed by quantitative angiography. This involves digitisation of lesion assessment to provide a more accurate and unbiased assessment of absolute and relative coronary artery dimensions during angiography. However this technique may be inconclusive in the borderline lesions (40% to 60% diameter obstruction)¹⁰¹.

The rate of passage of contrast through the coronary circulation during coronary angiography may be used as a surrogate for coronary blood flow. Qualitative and semi-quantitative measures have been developed by the Thrombolytic In Myocardial Infarction (TIMI) group and include (a) TIMI flow grade, (b) TIMI frame count and (c) TIMI perfusion grade. It should be pointed out that these measures do not directly assess volumetric blood flow but at best reflect blood flow velocity¹⁰².

1.3.5.3.2 TIMI Flow Grade

The TIMI flow grading system is a widely used qualitative measure which classifies successful reperfusion after thrombolysis by assessing distal epicardial coronary flow. This coronary angiographic method utilises four different descriptive categories based upon the penetration of radiographic contrast beyond the lesion of interest—grade 0 (no flow), grade 1 (penetration without perfusion), grade 2 (partial perfusion) or grade 3 (complete perfusion)^{102,103}. The TIMI flow grade has been used to assess the efficacy of thrombolytic agents and also to predict clinical outcomes in angiographic trials. However, there are limitations associated with this angiographic end-point method, including its subjective nature which allows for data variability to occur between angiographic clinical trials. In addition, the TIMI flow-grade system categorically measures blood flow and this can lead to inconsistent assessments.

1.3.5.3.3 TIMI Frame Count

The TIMI Frame Count is another index measure used to assess epicardial coronary blood flow during angiography. This more quantitative method known as the Corrected TIMI Frame Count (CTFC) utilises defined coronary arteriographic landmarks and measures the number of cine frame counts to reach these vascular landmarks. A correction factor to compensate for the longer length of the left anterior descending artery (LAD) compared with the circumflex and right coronary arteries is made and referred to as the CTFC; the number of frames required for dye to traverse the LAD is divided by 1.7)^{103,104}. A normal frame count was initially calculated to be 21 ± 3 , although this value can vary between studies^{103,105,106}.

In addition to being more quantitative, the CTFC is a continuous rather than a categorical variable, and is sensitive to flow changes¹⁰³. Furthermore, the CTFC method is reproducible

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and provides a more objective, and continuous index of coronary flow^{103,104,107}. Of note, the CTFC method has been reported to be highly useful in predicting clinical outcomes and has been prospectively validated as providing independent risk stratification in MI studies^{107,108}.

1.3.5.3.4 TIMI Myocardial Perfusion

The TIMI myocardial perfusion (TMP) grading is a recently developed qualitative angiographic method used to assess the degree of myocardial perfusion. This method involves assessing the filling and clearance of contrast dye in the myocardium and is measured by the presence or absence of myocardial blush in the myocardium¹⁰⁴. This perfusion-grading system has been shown to predict mortality in patients with impaired perfusion undergoing thrombolytic therapy, although this system is subjectively measured. A limitation of this system is that its unable to measure epicardial coronary blood flow^{108,109}.

During coronary angiography, additional functional tests of the coronary circulation may be undertaken such as:

- (a) the assessment of coronary artery spasm via provocative testing with ergonovine, acetylcholine or other stimuli
- (b) determination of the coronary flow reserve (CFR) with intracoronary Doppler wire
- (c) estimation of the functional significance of a coronary stenosis with Fractional Flow Reserve evaluation using a pressure wire
- (d) further assessment of coronary atheroma using intracoronary vascular ultrasound.

1.3.5.4 Coronary Computerised Axial Tomography Angiography

Computerised Axial Tomography (CT) angiography is a non-invasive technique to image the coronary arteries. It is undertaken with ultra-fast CT equipment and requires the patient to be

in sinus rhythm with a slowed heart beat with the use of beta-blockers. The technique enables coronary vessels that are >1.5mm in diameter to be imaged and assessment of lesions is only qualitative. Its clinical utility is best used for excluding obstructive coronary artery disease¹¹⁰.

1.3.5.5 Coronary Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive high resolution imaging technique that uses a powerful magnetic field to accurately evaluate wall-motion, myocardial tissue morphology, left ventricular structure and systolic function. Cardiac MRI imaging can provide a greater contrast of soft tissues without the use of ionising radiation¹¹¹. Furthermore, in patients with chest pain and normal coronary arteries, MRI can evaluate both myocardial perfusion and assess myocardial flow reserve¹¹². Although it continues to be developed for coronary artery imaging, currently it is not routinely used for this purpose as coronary CT angiography provides a better alternative.

1.3.5.6 Functional Assessments

Myocardial ischaemia can be assessed by a number of functional assessments which are described in section 3.2.4 of this chapter. These tests involve a provocation to elicit ischaemia and assess the functional response produced by exercise or pharmacologic intervention. As previously mentioned, coronary angiography is considered to be the gold standard procedure for assessing the presence of CAD; however, this technique is a measure of anatomy and not function and therefore may not always be accurate in diagnosing coronary vascular dysfunction.

1.4 Chest Pain and Non-Obstructive Coronary Artery Disease

The syndrome of chest pain with non-obstructive CAD (NoCAD) was first published in 1910 by Professor William Osler who described a patient who had lived for many years with recurrent angina, only to find the presence of normal coronary arteries during autopsy¹¹³. Since the arrival of coronary angiography, we have seen patients with classic anginal symptoms and normal coronary arteries¹¹⁴. Recent studies show approximately 10-30% of patients undergoing coronary angiography for the investigation of chest pain, have normal or near-normal coronary arteries¹¹⁵⁻¹¹⁹. Angiography is undertaken in these patients as they often have clinical symptoms that are indistinguishable from those with obstructive CAD^{120,121}. The rate of normal angiography is also the result of excessive investigation of benign atypical chest wall pain and dyspepsia (often driven by financial incentives).

Chest pain presentation in patients with NoCAD is often reported by clinicians to be atypical in nature, with both stable and unstable symptoms of angina. However, in a population study of women undergoing coronary angiograms, the data found typical versus atypical angina does not discriminate between obstructive and NoCAD¹²⁰. Patients with angina and angiographically normal coronary arteries have been reported to have ST/T wave changes, and this number ranges between 15-30%^{117,121,122}. Due to the limited literature regarding acute presentations in non-obstructive angina patients, the overall consensus is that they were less likely to present with an acute coronary syndrome. An investigative coronary angiogram is performed based on angina symptoms and to exclude the presence of obstructive CAD¹²³. However, the exclusion of visible stenotic plaques does not rule out the possibility that angina may be caused by other cardiac or coronary causes. Similarly, patients with coronary atherosclerotic CAD may have chest pain which may in fact not be heart-related.

1.4.1 Causes of Chest Pain in Non-Obstructive CAD

Chest pain and NoCAD has many causes which may or may not be cardiac in origin. These patients have been described as heterogeneous and there are difficulties in confidently sub-phenotyping many patients beyond their clinical presentation. Patients with chest pain and NoCAD can have either non-cardiac or cardiac aetiologies. In addition, in the absence of obstructive disease, angina can also be due to coronary large vessel dysfunction and/or small vessel dysfunction (Figure 1.2).

1.4.1.1 Non-Cardiac Causes

Non-cardiac causes of recurrent chest pain and normal angiography have been associated with various aetiologies including: gastro oesophageal reflux disease, musculoskeletal disorders, respiratory disorders, psychiatric disorders^{124,125} and increased sensitivity to pain¹²⁶.

1.4.1.2 Non-Coronary Causes

Non-coronary causes associated with myocardial disease include several cardiomyopathies such as hypertrophic cardiomyopathy, tako-tsubo syndrome, dilated cardiomyopathy, myocarditis, amyloid heart disease and Anderson-Fabry disease¹²⁷. Additionally, pericarditis and valvular heart disease have been associated with chest pain and normal or near-normal angiography.

1.4.1.3 Coronary Large Vessel Disease

Chest pain with NoCAD can be attributed to dynamic, functional disease of the epicardial coronary arteries. Variant angina, first described by Prinzmetal et al.¹²⁸ in 1959, reported 12 patients with chest pain not caused by exertion. In 1975, Maseri et al.¹²⁹ conducted a study

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demonstrating coronary vasospasm as a cause of myocardial ischaemia. In 1976 the underlying pathophysiology of Prinzmetal angina was proven to be coronary spasm,¹³⁰ which can be directly visualised during catheterisation by provocation testing using acetylcholine to induce epicardial coronary artery spasm allowing diagnostic angiographic, ECG and clinical responses to be observed. The prognosis for patients with isolated coronary artery spasm and normal or near normal coronary arteries is good, as calcium channel blockers diminish vasospasm. The clinical presentation associated with this disorder includes transient ST elevation in association with transient acute myocardial ischaemia, which is initially indistinguishable from transmural myocardial ischaemia¹²⁹. The incidence of variant angina occurs more frequently in the Japanese population compared to Caucasian populations¹³¹. In a white population study, only 2-3% of patients with chest pain undergoing coronary angiography were diagnosed with variant angina¹³².

1.4.2 Coronary Small Vessel Disease

Over 30 years ago Cannon and Epstein suggested that the underlying mechanism of chest pain with normal coronaries is microvascular ischaemia caused by the heart experiencing abnormalities in the small blood vessels¹¹⁵. Coronary small vessel disease involves functional and structural alterations of small arteries, arterioles and resistance vessels. Structural changes may involve the presence of occult atherosclerosis undetectable by coronary angiography, further complicated by plaque rupture which can lead to peripheral coronary microembolization⁹⁷. Functional disorders which have been associated with microvascular dysfunction include cardiac Syndrome X, Microvascular Angina and the Coronary Slow Flow Phenomenon (Figure 1.2).

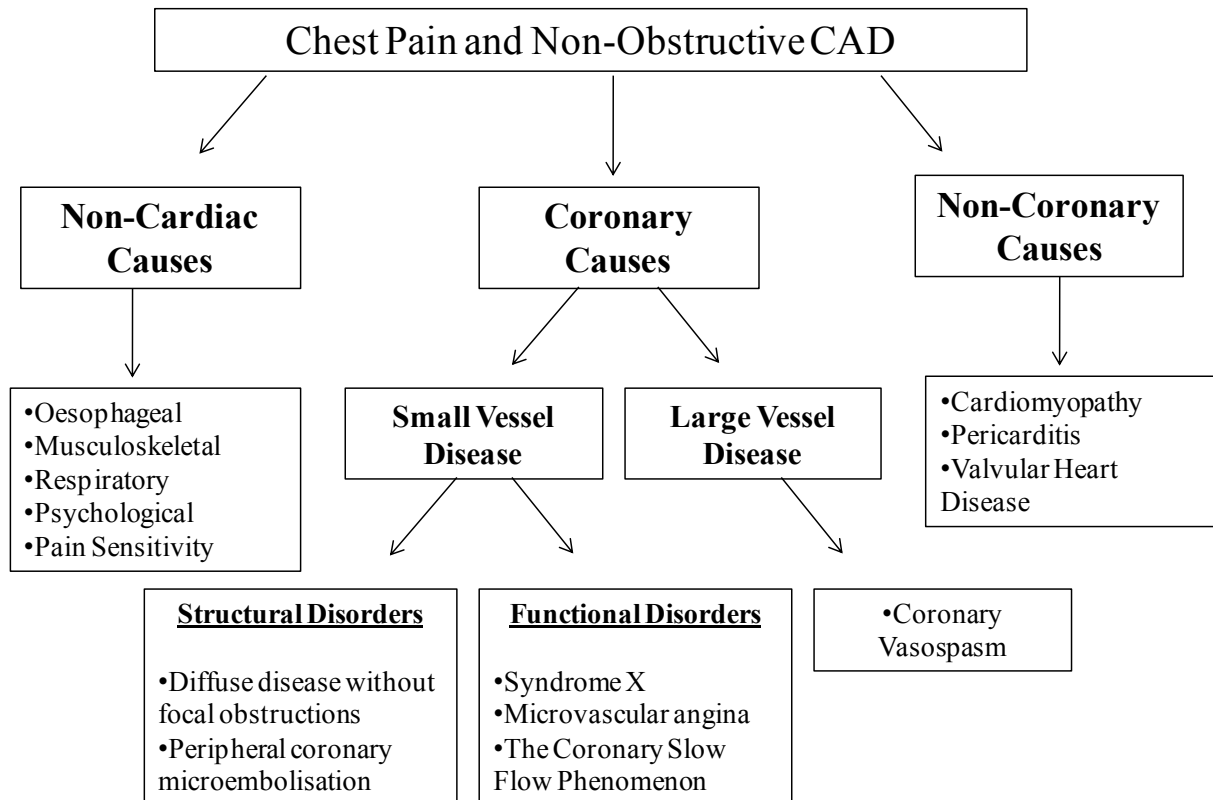


Figure 1.2 Causes of Chest pain in Non-Obstructive CAD
 Patients with chest pain and non-obstructive CAD can have a variety of non-cardiac, coronary and non-coronary causes.

1.4.2.1 Difficulties of Assessing Microvascular Dysfunction

Several investigators have researched the concept of chest pain caused by small vessel disease. However, the area of coronary microcirculatory dysfunction has been quite difficult to study because the anatomic microvasculature cannot be easily visualised; therefore we rely on functional studies to diagnose myocardial ischaemia. Additionally a quantitative assessment of responses often relies upon indirect measures such as coronary blood flow, coronary flow reserve or markers of myocardial ischaemia. Unfortunately, many studies have failed to identify the presence of ischaemia using lactate studies^{80,133}, although the lack of lactate production does not exclude the possibility of myocardial ischaemia, especially when clinical symptoms and signs are present. Recent evidence suggests that an ischemic mechanism may play a pathogenic role as a result of studies demonstrating coronary

microvascular endothelial dysfunction¹³⁴⁻¹³⁷ and exercise-induced myocardial ischaemia¹³⁸.

Clearly, the idea of coronary small vessel disease causing chest pain needs to be further explored in order to understand the underlying pathophysiology of this condition.

1.4.3 Microvascular Clinical Syndromes

The manifestation of clinical syndromes associated with chest pain and angiographically normal coronary arteries remains elusive. Irrespective of the underlying cause, chronic and acute clinical manifestations as evidenced in patients with microvascular spasm or dysfunction, in the absence of obstructive CAD. As previously mentioned in section 1.4.2, the clinical syndromes in patients with microvascular dysfunction are Syndrome X, Microvascular Angina and the Coronary Slow Flow Phenomenon. The features of these three clinical microvascular syndromes are summarised in Table 1.2 on page 44.

1.4.3.1 Cardiological Syndrome X

In a landmark study over 30 years ago, Arbogast and Bourassa¹³⁹ performed atrial pacing in 11 patients with obstructive CAD (group C) and ten with angina but normal angiography (group X). Interestingly both the control and the experimental group had ECG and transmyocardial lactate evidence of myocardial ischaemia. In the accompanying editorial by Kemp, Group X was referred to as syndrome X, and is now frequently used as a diagnostic label for patients with:

- (a) stable exertional angina
- (b) positive response to stress testing
- (c) angiographically normal coronary arteries, and

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- (d) no other cardiac cause of chest pain such as coronary spasm, left ventricular hypertrophy, cardiomyopathy, valvular heart disease, as these cardiac disorders have been associated with microvascular dysfunction and chest pain.

Clinical Characteristics

A longitudinal study by Kaski et al.⁷⁷ found patients with syndrome X had distinct clinical characteristics which differed from other coronary disorders and these include:

- (a) prolonged episodes of typical angina pain during rest and exercise
- (b) 80% of patients were often females with 61% being post-menopausal, and
- (c) continuous ECG monitoring demonstrated horizontal and down sloped ST depression in both silent and exertional episodes of chest pain.

Syndrome X patients have a low risk of cardiac events¹⁴⁰, however persistent chest pain and associated psychosocial effects of this disorder often impair a patient's quality of life severely¹⁴¹⁻¹⁴³.

Pathophysiology

There are ischemic and non-ischemic mechanisms which have been identified in the underlying pathophysiology of syndrome X. Microvascular ischaemia has been postulated as an underlying pathogenic mechanism found by abnormal perfusion scans on MRI, decreased oxygen saturation during chest pain, and variable transmural lactate production during stress testing. However, myocardial ischaemia has been objectively documented in approximately 20-25% of patients, which suggests other pathogenic mechanisms may be in play^{77,144}.

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Chest pain of non-ischemic origin has been evidenced in patients with syndrome X. These include:

- (a) altered pain perception to cardiac stimuli linked to a defect in the nociceptive pathways responsible for angina pectoris¹²⁶
- (b) impaired coronary endothelium-dependent vasodilatory responses¹⁴⁵
- (c) insulin resistance causing impaired endothelial function¹⁴⁶
- (d) oestrogen deficiency, as hormone therapy has been found to improve chest pain and endothelial function in post-menopausal female patients¹⁴⁷.

The treatment for syndrome X remains a clinical challenge as controversy surrounds the pathogenesis of syndrome X. It is evident that this syndrome encompasses a variety of pathogenic sub-groups. It is therefore not surprising that conventional anti-anginal therapies are somewhat limited in relieving symptoms. Subsequently, treatment depends on the underlying cause of chest pain.

1.4.3.2 Microvascular Angina

Operk et al.¹⁴⁸ first reported reduced coronary flow reserve in patients with angina and normal coronary angiograms. Cannon and Epstein¹⁴⁹ described patients with an inappropriate coronary blood flow response to vasomotor stimuli, despite normal coronary arteries. These patients were identified as having ‘microvascular angina’ and were clinically distinct from syndrome X, as they did not usually have ischemic ST segment changes on stress testing. However, microvascular angina and syndrome X have been reported as co-existing with each other⁶¹.

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Clinical Characteristics

Microvascular angina is a dynamic coronary microvascular disorder defined as reduced vasodilatory response to provocative vasomotor stimuli, in the absence of epicardial artery spasm. Other clinical characteristics of this disorder include:

- (a) chest pain that usually occurs at rest¹⁴⁹
- (b) patients seldom have ischemic ST changes on stress testing, with positive stress testing in only 10% of patients¹⁵⁰
- (c) patients with this disorder can have abnormal resting left ventricular ejection fraction. A sub-group of patients with microvascular angina and left bundle branch block has been identified, with left ventricular ejection fraction deteriorating over time¹⁵¹
- (d) generalised smooth muscle dysfunction has been linked with microvascular angina, including impaired forearm vasodilator reserve¹⁵², bronchial hyperreactivity,¹⁴⁹ and abnormal esophageal motility¹⁵³.

There has been evidence of pain sensitivity and panic disorders associated with microvascular angina.

Pathophysiology

Patients with microvascular angina have evidence of dynamic coronary microvascular dysfunction, as coronary flow reserve is reduced in response to vasomotor stimuli causing abnormal vasodilator reserve or vasoconstriction during atrial pacing¹⁵⁴. In addition, investigators have previously demonstrated a reduced coronary flow response to dipyridamole, adenosine and ergonovine. Unfortunately the absence of trans-myocardial lactate ischemic changes observed during atrial pacing has caused speculation about whether an ischemic origin for this disorder truly exists. However, it has been suggested that the

techniques used to assess microvascular function are inadequate since the affected vessels are too small to investigate in vivo with standard tests¹²².

1.4.3.3 The Coronary Slow Flow Phenomenon

In 1972, Tambe et al.^{154,155} described the angiographic slow progression of contrast through the epicardial coronary arteries in a group of patients with angina pectoris. Since then the CSFP has been defined as an angiographic observation characterised by delayed opacification of distal vasculature in the absence of obstructive CAD¹⁵⁵. The diagnosis of coronary slow flow is determined based on the extent of opacification as assessed during angiography. While there is some variation between studies the most widely used measures of diagnosis are a TIMI frame count of >22 frames or >3 cardiac beats to fill the vessel¹⁵⁶.

Clinical Characteristics

Until recently the clinical characteristics of this disorder were not fully investigated. In 2002, Beltrame et al.¹⁵⁵ undertook the first case-controlled and observational study in patients with the CSFP and concluded the following about their clinical characteristics:

- (a) This finding is prevalent in males with a tendency to affect the middle aged.
- (b) This angiographic disorder presents as a form of unstable angina that usually occurs at rest.
- (c) Patients often present with an acute coronary syndrome, prompting emergent angiography, accounting for 4% of unstable angina admissions.
- (d) Spontaneous ST segment and T wave changes are commonly observed during the initial presentation, despite no angiographic evidence of epicardial spasm.
- (e) The incidence of metabolic syndrome X has been shown to be prevalent in patients with the CSFP compared to non-CSFPs.

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Clinically more than half have objective evidence of myocardial ischaemia in the absence of epicardial coronary spasm suggesting the presence of microvascular dysfunction⁸⁰. Although an underlying ischemic substrate has been suggested as a cause, only 27% have abnormal serial ECG changes with less than 20% exhibiting an abnormal exercise stress test. In addition, myocardial scintigraphy abnormalities are only present in about a third of patients. Furthermore, there is considerable morbidity associated with this disorder, where 84% of patients suffer recurrent chest pain and 27% require re-admission into the coronary care unit with severe chest pain¹⁵⁵.

Preliminary epidemiological studies in patients with the CSFP have found that cardiac death and MI is rare but continued chest pain remains a problem, as described above. Indeed, Voelker et al.¹⁵⁷ observed 16 patients with the CSFP and found these patients were more likely to develop recurrent pain than those without this phenomenon. Unfortunately, conventional anti-anginal therapy is of limited benefit in the chronic management of these patients, as coronary microvessels function differently to large coronary vessels⁸⁰.

Pathophysiology

It has been suggested that CSFP may be an acute and recurrent perturbation of microvascular function. Patients with the CSFP often present with an acute coronary syndrome which suggests an underlying ischemic basis for this disorder^{122,139}. Coronary hemodynamic studies have found delayed opacification seen during angiography is associated with an increased resting coronary vascular resistance. These have been attributed to microvascular abnormalities both structural and functional.

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Structural Disease

In 1986, Mosseri et al.¹⁵⁸ obtained endomyocardial biopsy specimens from the right ventricle and reported hypertrophy of the myofibres and marked thickening of the intramural coronary arteries, all which suggest the presence of small vessel disease. In 1996, Mangieri et al.¹⁵⁹ obtained left ventricular endomyocardial biopsies and observed thickened vessel walls with a reduction in the lumen diameter. The presence of structural abnormalities suggests an atherosclerotic aetiology of occlusive small vessel disease.

Functional Abnormalities

It has been suggested that structural disease alone cannot explain the acute presentation of these patients. Therefore a dynamic microvascular component has been suggested and is supported by the fact that small vessel and not large vessel vasodilators reverse the angiographic phenomenon^{105,159}. Interestingly, the presence of ischemic ST elevation with chest pain in the absence of large vessel spasm has been observed in one patient with the CSFP¹⁵⁵. Also, endothelin-1 (ET-1) and neuropeptide Y have been implicated as potential mediators of the microvascular constrictor response, as intracoronary infusion of these microvascular vasoconstrictors have been able to mimic this angiographic phenomenon in dogs^{160,161} and humans¹⁶². Furthermore, endothelial function has been shown to be impaired in slow flow patients as demonstrated by reduced flow mediated dilatation in the brachial artery¹⁰⁶. In addition, nitric oxide and ET-1 bioactivity¹⁶³, oxidative stress¹⁶⁴ and diastolic dysfunction¹⁶⁵ have all been associated with this disorder.

Table 1.2 Clinical Syndromes in Patients with Chest Pain and Normal Angiography

Source: Beltrame et al.¹⁵⁶

Feature	Cardiological Syndrome X	Microvascular Angina	Coronary Slow Flow Phenomenon
Diagnostic Investigation	ST depression on Stress Testing	Impaired coronary flow reserve	Delayed contrast opacification
Gender Distribution	Predominantly females	Predominantly females	Predominantly males
Angina Characteristic	Typically exertional	Usually rest pain	Often rest pain
Ischemic ST changes ¹ on Stress Testing	ST depression in all (by definition)	Infrequent (<30% of patients)	Infrequent (<20% of patients)
Ischemic lactate changes ² with Rapid Atrial Pacing	Variable	Seldom	Seldom

1.4.4 Gender and Non-Obstructive CAD Studies

Cardiac research studies undertaken in patients with OCAD are usually focused on men while research in NoCAD is almost entirely focused on women. This has led to a disturbing trend where a large number of women are left undiagnosed and continue to experience persistent and disabling chest pain¹⁶⁶. Middle-aged post-menopausal women with syndrome X symptoms are the subjects most often used in studies on NoCAD. Therefore the true impact of chest pain with NoCAD in a non-biased population is seldom observed due to the exclusion of men and also women who do not fit the syndrome X criteria.

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Several investigators have found patients with chest pain and normal or non-obstructive coronary arteries are two to five times more likely to be women than men¹⁶⁷⁻¹⁷¹. More than half of women with chest pain undergoing coronary angiography are found to have normal or near normal coronary arteries. In addition, the incidence of ‘false positive’ ETT is reported to be more common in women than in men. The high ‘false positive rate’ for clinically significant coronary artery stenoses among symptomatic women was first reported approximately 30 years ago and the rate remains the same today¹⁷¹⁻¹⁷⁵. Perhaps we may need to consider the possibility that objectively false-positive ETT results may in fact be due to microvascular ischaemia.

For many years research in coronary artery disease has been focused on men, yet this chronic disease is also the major cause of disability in women. In the past women without OCAD were considered a low risk population^{171,173-175}. However, recent data suggests that women with chest pain and NoCAD are at increased risk of major cardiovascular adverse events and even death¹²⁰.

The syndrome of chest pain with NoCAD clearly has important clinical implications for females, although this disorder can be equally debilitating in males. In a study by Sullivan et al.¹⁷¹ 65% of males had continued chest pain after follow-up time of 2.4 years and 28% continued to take anti-anginal medications. Surprisingly, the latter findings are similarly comparable with the female cohort in the same study. Irrespective of gender, the majority of patients with NoCAD continue to have chest pain, take anti-anginal medication and require hospital re-admissions, which suggests this may not be a gender specific disorder.

1.5 Disease Mechanisms in Coronary Heart Disease

CHD is primarily associated with hemodynamic changes that affect the regulation of coronary blood flow causing angina symptoms. The presence of structural and/or functional myocardial abnormalities may cause changes in coronary arterial resistance which leads to changes in the regulation of the coronary circulation. Several studies have also shown the clinical importance and influence of dynamic alterations in coronary resistance occurring in either large or small vessels¹⁷⁶. The following section will focus upon the basic pathophysiological concepts related to CHD.

1.5.1 Functional Anatomy

The coronary vasculature is a fundamental circulatory system within the body, consisting of an arterial and venous network, as well as a microcirculation component. Anatomically there are structural and functional differences between the arteries, veins and capillaries. However, functionally, the coronary vessels work simultaneously to supply oxygen and nutrients to the myocardial cells and remove waste products.

1.5.1.1 Conduit Vessels

The left and right coronary arteries and their branches lie on the surface of the heart and are therefore known as epicardial or conduit vessels. These conduit vessels transport large quantities of blood to the myocardium and due to their size have minimal resistance to flow. Conduit arteries have a diameter ranging from 500 μm -5mm and are visible during coronary angiography. These large vessels subsequently branch off into smaller arteries which act as resistance vessels.

1.5.1.2 The Microvasculature

The microvasculature is an intrinsic network of vessels that are generally less than 500µm in diameter which include the arterioles, pre-arterioles, venules and capillaries. In the coronary arterial microvasculature, the pre-arterioles (100 to~500µm in diameter) maintain arterial pressure when coronary flow changes and the arterioles (< 100 µm in diameter) are involved in metabolic regulation of blood flow. The arterioles give rise to a densely packed capillary network (approximately 7-9 µm in diameter) which is important for the regulated exchange of oxygen and other metabolites. The capillaries have a thin wall to allow the exchange of oxygen and waste products from the myocardial cells¹⁷⁷.

1.5.1.3 Veins

The veins are vessels that receive blood from the capillaries after the exchange of oxygen and carbon dioxide, and transport deoxygenated blood to the heart and lungs. They are similar to arteries in that they constrict and dilate; however, they have no role in regulating physiological blood pressure and blood flow.

1.5.2 Vascular Histology

The arteries, veins and capillaries have individual functions when transporting blood throughout the body. However, there are fundamental histological similarities in the structural composition of all blood vessels. The wall of the blood vessel is composed of three tunicae which are defined as the intima, media and adventitia.

The intima is located on the interior surface of the blood vessel and is made up of a thin tightly packed layer of endothelial cells that are separated from the media by the internal elastic lamina⁷⁰. The endothelial cells are crucial in maintaining vascular homeostasis by

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influencing the activity of vascular smooth muscle by releasing various vasoactive substances¹⁷⁸.

The media is the middle and thickest layer of a blood vessel, consisting of vascular smooth muscle cells arranged in a circular and spiral manner connected together by collagen and gap junctions. Vascular smooth muscle cells are abundant in the arterial wall (particularly in the resistance vessels) and are the primary mechanism responsible for the regulation of vascular tone in blood vessels. The media is separated from the adventitia by a dense elastic membrane called the external elastic lamina⁷⁰.

The adventitia is the outermost layer of the vessel and consists primarily of loose connective tissue, nutrient vessels and autonomic nerves. In medium-sized to large-sized arteries, the adventitia contains small capillaries which supply nutrients to tissues in the outer one-half of the wall of the blood vessel. The adventitia is controlled by autonomous perivascular innervations which are involved in the regulation of smooth muscle compliance¹⁷⁹.

1.5.2.1 Endothelium

The endothelium is an endocrine organ that releases a number of substances to influence the contractile activity of vascular smooth muscle. Vascular smooth muscle cells modulate vascular tone through contraction and dilatation by either direct autonomic signals or by local hormonal responses. In a landmark study by Furchgott and Zawadski¹⁸⁰, it was first demonstrated that acetylcholine (Ach), which normally elicits vasodilation in vivo, produces coronary vasoconstriction in the absence of endothelial cells in the rabbit aorta. This resulted in the concept of endothelial dysfunction, which can influence vasomotor responses.

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The endothelium releases both relaxing and constricting factors which can influence the underlying vascular smooth muscle. Endothelium-dependent relaxation of blood vessels is produced by endothelial-derived vasodilating factors; these include endothelium-derived hyperpolarising factor (EDHF)¹⁸⁰ and nitric oxide (NO)¹⁸¹. Endothelium-derived constricting substances include ET-1¹⁸² and prostacyclin (PG₂)¹⁸³. To date, NO and ET-1 are the most researched and published autocooids involved in modulation of vascular tone, and are particularly relevant to this thesis.

1.5.2.2 Nitric Oxide

NO is one of the most important vasodilators released by the endothelium. NO generated by the vascular endothelial cells regulates coronary blood flow, by acting on vascular smooth muscle cells through endothelium dependent-dilatation under normal physiological conditions¹⁸⁴. NO can also indirectly influence vascular tone through interaction with the autonomic central nervous system by decreasing sympathetic activity in animals and humans^{185,186}. In addition, other physiological functions of NO include inhibiting vascular remodelling and proliferation¹⁸⁷ and also preventing angiogenesis^{188,189}. Impaired NO release is associated with vascular disorders and risk factors including atherosclerosis, hypertension, diabetes, hypercholesterolaemia and cigarette smoking to mention a few.

1.5.2.3 Endothelin-1

ET-1 is a 21 amino acid peptide that is released by endothelial cells¹⁹⁰ or vascular smooth muscle cells¹⁹¹ to elicit potent vasoconstriction. ET-1 maintains normal regulation of vasomotor tone and also plays a major role in the control of peripheral blood resistance in humans. The production of ET-1 is stimulated by hormonal, metabolic and/or physical factors, including angiotensin-II, catecholamines, vasopressin, thrombin, growth factors,

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hypoxia, insulin, oxidised-low density lipoprotein and sheer stress. The formation of nitric oxide and PG₂ interact with endothelin receptors to inhibit the production of ET-1¹⁹². Furthermore, elevated plasma ET-1 levels have been reported to be associated with several cardiovascular pathologies, including atherosclerosis, heart failure, cardiogenic shock and diabetes⁹⁹.

1.5.2.4 Humoral Mediators

There are several humoral mediators that can alter vascular function in the regulation of coronary blood flow. These include circulating catecholamines, bradykinin¹⁹³, histamine, serotonin, vasopressin¹⁹⁴, thromboxane A₂¹⁹⁵, angiotensin II, atrial natriuretic peptide, kinins, leukotrienes, and various recreational drugs.

1.5.2.5 Neurological Mechanisms

Coronary resistance can be controlled through the autonomic nervous system by sympathetic and parasympathetic cholinergic nerve activation. The sympathetic and parasympathetic adrenergic nerves innervate the myocardium and stimulate vascular smooth muscle cells to release local metabolites to modulate vascular tone⁷⁴. The role of neural activation in controlling vasomotor tone involves a variety of autocooids and also a number of adrenoceptors which are beyond the scope of this thesis.

1.6 Coronary Physiology

Changes in coronary resistance and perfusion pressure are the primary mechanisms by which coronary blood flow is regulated. The major determinant of resistance is vessel radius as blood flow can be dramatically regulated by influencing vascular tone.

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Ohm's Law

The most fundamental haemodynamic law of blood flow is derived from Ohm's Law. Flow through any circuit, in analogy to Ohm's law, is proportional to the pressure gradient and inversely proportional to resistance. Hence blood flow and oxygen supply is increased by an increase in pressure difference across the coronary arteries, and decreased by an increased coronary resistance, as occurs in coronary atherosclerosis¹⁹⁶.

Flow (Q) is defined as the total volume of fluid moving through a tube per unit time and is directly proportional to the pressure gradient (P) across the tube. The constant (k) describes the relationship between Q and P, which reflects the ease with which fluid will flow through the tube. By convention the resistance to flow in a tube should be considered, thus the reciprocal of the constant (1/k) reflects resistance (R). Mathematically this relationship may be summarised as follows:

$$Q = \frac{P}{R}$$

Expansion of this equation can be derived from Poiseuille's equation assuming that flow (Q) is laminar. Poiseuille's equation states:

$$Q = \frac{P \times r^4}{\eta \times l \times 8}$$

where P= the pressure difference across the tube

r= the radius of the vessel

n= the viscosity of the fluid

l=the length of the tube

1.7 Regulation of Coronary Blood Flow

Anatomically, the coronary arterial system is comprised of a three compartmental system—the proximal, intermediate and distal compartments. Functionally the arterial system can be categorised into conductive or resistance blood vessels. There are, however, physiological differences between each compartment¹⁷⁷. The proximal compartment is represented by the large epicardial coronary arteries, which have a capacitance function and contribute <10% in resistance to coronary blood flow. The conductive epicardial arteries are able to mediate their vascular responses through flow-dependent dilatation¹⁹⁷. Coronary resistance can also be controlled by sheer stress responses which can cause endothelium-dependent dilatation. This in turn triggers flow mediated dilatation in epicardial arteries.

The resistance vessels are found in the intermediate compartment of the arterial coronary system, where the pre-arterioles reside. The pre-arterioles are characterised by a measureable drop in pressure along their length and contribute 45-50% of the total coronary resistance, with the remainder residing in the arterioles¹⁹⁸. The pre-arterioles are not under direct vasomotor control by diffusible myocardial metabolites due to their extramyocardial position and arterial wall thickness. Instead blood flow is thought to be controlled by auto-regulatory mechanisms, where a change in resistance throughout the overall network leads to myogenic dilatation of pre-arterioles¹⁷⁷.

The distal compartment is represented by arterioles, also known as resistance vessels and they have the largest pressure drop along their length. Coronary arterioles have a high resting tone and can regulate blood flow in response to diffusible myocardial metabolites which mediate vasomotor tone¹⁹⁹. Furthermore, the capillaries and veins primarily function as conduit and capacitance vessels, and therefore have minimal involvement in total coronary resistance. The

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prime function of the coronary circulation is to match myocardial blood supply and myocardial oxygen consumption by regulating changes in resistance across all three compartments of the coronary arterial system²⁰⁰.

1.8 Aims of Thesis

In the preceding components of this chapter, the morphological and physiological aspects of the coronary circulation and the clinical disorders associated with the small and large coronary arteries have been described. Patients with chest pain and NoCAD are regarded as a heterogeneous group due to the varied underlying aetiologies associated with this syndrome. The acute clinical syndromes that are associated with NoCAD are poorly understood and are especially difficult to medically treat, hence further research is required. *This thesis aims to define the various clinical, prognostic and genetic characteristics of patients with chest pain and NoCAD.* In addition, patients with the CSFP will also be analysed as an independent cohort, given this is a homogenous population of patients.

Chapter 2 of this thesis involves an epidemiological clinical study which examines health-related quality of life outcomes in patients with NoCAD, particularly focusing on those presenting with acute chest pain and determining if they differ clinically and prognostically to those with stable chest pain presentations.

Chapter 3 entails a case-controlled clinical investigation which will examine the electrocardiographic changes in patients presenting with an ACS and NoCAD, during continuous 12-lead ST/T monitoring. Continuous 12-lead ST/T wave monitoring is a useful and non-invasive tool for monitoring cardiac ischaemia in patients suffering from chest pain. In patients with the CSFP, those presenting with recent onset rest pain have had ischemic ECG changes prompting urgent admission to the coronary care unit and coronary angiography. Therefore, our aim is to investigate whether patients with the CSFP have transient ischaemic ECG changes which occur during the acute phase of this disorder.

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Chapter 4 is a laboratory-based study and it examines the frequencies of genetic polymorphisms in patients with non-obstructive CAD and compares these frequencies with both an obstructive and healthy control group. The aim of this study is to determine whether a genetic background influences the pathogenesis of NoCAD; and in particular the CSFP, which may involve microvascular endothelial dysfunction. In the literature there are several genetic single nucleotide polymorphisms (SNPs) which have been associated with cardiovascular endothelial dysfunction and/or increased risk of coronary spasm. After an extensive literature search we chose to examine the prevalence of the following genetic polymorphisms in our study cohorts:

1. ET-1 +138 Deletion/Insertion SNP
2. eNOS T-786C SNP.

Finally, **Chapter 5** summarises the important findings of the various chapters and of what has been explored in this thesis, and links together the clinical and quality of life outcomes. It also comments on the possible genetic characteristics of patients who present with chest pain and NoCAD.

Chapter 2. Health Outcomes in Patients with Acute Chest Pain and Non-Obstructive Coronary Artery Disease

2.1 Introduction

Approximately 10-30% of patients undergoing coronary angiography have angiographically normal coronary arteries; as a result of this finding, patients are commonly classified as having non-cardiac chest pain^{117,166,201}. This diagnosis can be misleading as the etiology of this syndrome remains unclear due to the heterogeneous nature of this disorder. Irrespective of the underlying cause, the symptom of chest pain with non-obstructive coronary artery disease (NoCAD) is a well-recognised clinical entity. The occurrence of this chest pain syndrome is reportedly more commonly in women than in men and their symptoms are often indistinguishable from those with obstructive CAD¹³⁴. At present, treatment remains elusive; however, management strategies have focused on: (a) improving a patient's quality of life, and (b) decreasing the financial burden imposed on the health care system due to recurrent hospital admissions for on-going chest pain.

The overall consensus in the literature is that patients with chest pain and NoCAD have a good prognosis with several studies reporting excellent long term survival and low risk of myocardial infarction^{77,121,140,175,202}. However, many patients continue to have persistent chest pain¹⁷⁰ some are re-hospitalised^{170,203} and approximately 30% undergo repeat angiography²⁰⁴. Chambers and Bass²⁰⁵ reported that the incidence of myocardial infarction and death is low among patients with chest pain and NoCAD. In addition, patients who had continuing cardiac symptoms were more likely to have frequent hospitalisations, medication use and job disability, which are consistent with other studies^{166,170}.

The impact of chest pain and NoCAD on mortality and morbidity rates in the short-term has recently been explored in an acute clinical setting. Bugiardini et al.¹³⁴ reported that the prognosis of this cohort is not as favourable as once thought, as this syndrome is associated

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with a 2% risk of myocardial infarction or death over, after 30 days post-angiography, when in the context of unstable angina. It is well established that anginal status in obstructive coronary artery disease is associated with ACS hospitalisations and increased mortality²⁰⁶. Furthermore, self-reported patient health status measures in acute coronary artery disease syndromes have demonstrated a lower quality of life both at baseline and after 12 months follow-up¹⁷⁰. In the specific context of acute angina and NoCAD, little is known about the long-term risk of mortality and morbidity. Unfortunately, the majority of prognostic studies in the literature have assessed long-term functional disabilities using non-specific questionnaires in patients with chronic angina and NoCAD^{170,207-209}, thus quality of life indices in patients with ACS-CAD has never been compared using valid disease-specific health-related quality of life (HRQoL) instruments.

Patient-centered health status involves the assessment of a patient's symptoms, function, and quality of life using valid, reliable and responsive HRQoL instruments⁶. The evaluation of HRQoL as perceived by the patient focuses on treatment strategies which not only prolong life, but also relieve symptoms and improve function²¹⁰. The overall spectrum of health outcomes in patients with chest pain and NoCAD patients is poorly characterised and thus requires further investigation. The overall aim of this study is therefore to examine the HRQoL measures in patients with acute chest pain presentation and NoCAD, as self-reported by the patient using both a generic and a specific-disease HRQoL instrument.

2.2 Study Objective

The primary objective of this study is to compare 12-month health outcomes in NoCAD patients who present with either an ACS or stable angina. Thus the null hypothesis to be tested in this study is:

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- (1) There is no difference in health outcomes between acute and stable NoCAD patients at baseline and then at 12 months follow-up.

2.3 Materials and Methods

This is a prospectively designed, observational, longitudinal study conducted to evaluate health outcomes over a period of 12 months in patients with NoCAD. Patients were recruited at The Queen Elizabeth Hospital and The Lyell McEwin Hospital, Adelaide South Australia, between April 2003 and May 2007. The study was approved by the Central Northern Adelaide Health Service Ethics of Human Research Committee.

2.3.1 Study Patients

Patients undergoing invasive coronary angiography for suspected ischemic heart disease were approached to participate in this study. Those with insignificant CAD (<50% stenosis) were included in this study. Patients with NoCAD were subsequently angiographically categorised into either (a) elective (stable) or (b) acute (unstable) clinical presentations. Patients with a chronic stable presentation were referred for angiography from outpatients, whereas acute cases were admitted via the Emergency Department.

Inclusion Criteria

- (a) Elective (stable) angina patients were defined as having angina with the following features:
 - (i) NoCAD
 - (ii) Elective angiography for stable chest pain symptoms (ie no hospital admission for chest pain within the previous two weeks).

- (b) Acute (unstable) angina patients were defined as experiencing angina with all the following features:
 - (i) NoCAD

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- (ii) Acute coronary syndrome presentation (unstable angina, non-ST elevation myocardial infarction or acute ST elevation myocardial infarction) resulting in immediate hospital admission (within 48 hours of pain onset) and urgent angiography (i.e. during admission or within two weeks).

Exclusion Criteria

Patients with evidence of (a) obstructive coronary artery disease (>50% stenosis), (b) valvular heart disease, (c) pericarditis and (d) cardiomyopathy were excluded from this study.

2.3.2 Study Protocol

All patients recruited in this study were invited to participate with informed consent obtained after hospital admission in preparation for their procedure. Prior to angiography, patients were administered the Short Form-36 (SF-36) and Seattle Angina Questionnaire (SAQ) questionnaires to complete on their own as part of their baseline health assessment. Patient history, risk factor, clinical symptoms, blood chemistry, ECG, and other clinical variables were obtained from the patient's case notes. Longitudinal data was obtained by mailing out the SF-36 and SAQ questionnaires to all patients at 1, 6 and 12 months post-angiography, as part of their self-reported health assessment. Consenting patients completed the questionnaires and mailed them back to us. Follow up phone calls were also made as part of assessing the patient's clinical progress. If no response was obtained from mailed surveys, attempts were made to contact the non-respondents by letter or phone call within four weeks of the last mail out.

2.3.3 Parameters Assessed

Clinical characteristic data such as demographics and cardiovascular risk factors were obtained via patient interview, clinical examination or from the case notes. Longitudinal follow-up data post-angiography was reported by the patient during telephone interview, where patients were asked if they had any chest pain, cardiac related re-admissions or procedures. Enquiries were also made about medication changes and other cardiac and non-cardiac events. Any documented fatalities were obtained via a hospital administrative health database.

2.3.4 Health-Related Quality of Life Assessment

HRQoL was assessed using both a generic and disease specific instrument, which provides a more comprehensive measure of disease impact^{50,211}. The SF-36 is a well-established generic instrument, which has been shown to be valid, reliable and responsive both in patients with CAD^{50,211} and in reference norm populations²¹². The questionnaire is comprised of 36 items and grouped into eight scales: physical functioning, social functioning, physical impairment, emotional impairment, emotions, vitality, pain and global health. These 8 scales are coded into two component summary scales known as the physical and mental summary scores. The scoring of the SF-36 ranges from 0 (poor) to 100 (high), which quantifies a patient's level of functioning and overall quality of life⁴⁷. The summary scales are standardised such that the mean score for the general population is set at 50 with a standard deviation of 10. In addition, a difference of ≥ 5 points is considered to be clinically and socially relevant²¹³.

The SAQ is a disease specific functional status measure which measures the physical and emotional effects of CAD³⁸. It is comprised of 19 questions that quantify five clinically relevant dimensions related to angina which include: angina frequency, angina stability,

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physical limitation, treatment satisfaction and quality of life. The SAQ has well established psychometric properties and is a highly specific measure in capturing physical limitations related to CAD^{39,50}.

The Canadian Cardiovascular Society Class (CCSC) of angina pectoris scale, which corresponds well with the SAQ⁴⁷, was also used by the clinician to measure a patient's functional level associated with chest pain at baseline and then at 12 months follow up.

2.3.5 Study Endpoints

The primary end point of the study is to evaluate the change in SF-36 Physical Summary Score (PSS) at 12 months compared to the baseline. The secondary end-points are to evaluate the change in SF-36 Mental Summary Score (MSS), SAQ domain scores, chest pain-free status at 12 months and mortality and myocardial infarction at 12 months. The definition of 'no chest pain' at 12 months was defined as no episodes of chest pain in the preceding four weeks as recorded by either the angina frequency domain of the SAQ or the self-reported patient assessment during the 12 month follow up telephone call.

2.3.6 Power Calculation

There is currently no published SF-36 data on NoCAD patients in the literature; therefore we could not calculate a sample size prior to the commencement of this study. As a result, we chose to recruit as many patients as we could for the entire duration of this study.

2.3.7 Statistical Analyses

For all estimates and analyses, the complete data set was used with no imputation for missing values. Logistic regression was used to compare demographics, risk factors and clinical characteristics at 12 months. The longitudinal changes in SF-36 and SAQ scores over 12 months between ACS and stable angina patients were compared using linear regression models. Frequency data and means (\pm SD) were expressed relative to the number of patients available for each clinical and demographic variable. The Odds ratios (Ors) with 95% confidence intervals (CIs) and p values were reported. A p value of <0.05 was considered statistically significant. Also, a cross-sectional comparison of clinical characteristics between patients who completed the 12 month follow up (respondents) and those who dropped out (non-respondents) was undertaken. Characteristics of non-respondents were compared using logistic or linear regression (age adjusted). All analyses were performed with STATA (version 10, StataCorp, Texas, USA).

2.4 Results

From April 2003 until May 2007, 1249 patients who underwent coronary angiography for the investigation of chest pain were recruited into the study. Of this total number, 819 patients had obstructive CAD and 105 were excluded from the study cohort due to valvular heart disease, pericarditis and cardiomyopathy. Quality of life follow up data at 12 months was available in 173 (53%) patients.

2.4.1 Patients Characteristics

Demographics, cardiac and non-cardiac characteristics of the study population are listed in Table 2.1. The mean age of the study population was statistically different between ACS and stable NoCAD patients, with ACS patients being younger. The majority of cardiovascular risk factors were matched except for hypercholesterolemia which was greater in the acute patients. In terms of cardiac history, the ACS patients were more likely to have a previous acute chest pain admission and prior myocardial infarction. Additionally, patients presenting to the emergency department with ACS are more likely to be prescribed anti-platelets, anti-coagulants, nitrates and calcium channel blockers. Non-cardiac co-morbidities were matched between the study groups.

To gauge the extent of the ischaemic insult in the acute cohort, a breakdown between ACS, STEMI, NSTEMI and the CSFP was determined. A total of 43 (60%) unstable angina, 2 (3%) STEMI, 4 (6%) NSTEMI and 23 (32%) CSFP patients made up the acutely presenting group.

Table 2.1 Clinical Characteristics of Stable-NoCAD and ACS Non-CAD Patients at Baseline Angiography

	<i>n</i> *	Stable <i>n</i> = 253 Mean ± SD/%	<i>n</i> *	ACS <i>n</i> = 72 Mean ± SD/%	<i>p</i> Age Adjusted
Demographics & Risk Factors					
Age (years)	253	58±12**	72	52±14	<0.01
Female	253	57%	72	56%	0.555
Hypertension	243	58%	71	59%	0.16
Hypercholesterolemia	236	57%	68	71%	0.026*
Diabetes Mellitus	253	19%	70	14%	0.679
Current	243	16%	72	28%	0.359
Ex-Smoker	239	34%	71	27%	0.336
Family History	239	63%	67	58%	0.13
Past Cardiac History					
Previous chest pain history	253	44%	72	32%	0.141
Previous ACS admission	253	8%	72	26%	<0.001**
Myocardial Infarction	253	4%	72	13%	0.008*
Stress Test performed	253	44%	72	36%	0.308
Scintigraphy performed	253	19%	70	11%	0.169
Echocardiography performed	253	26%	72	25%	0.921
Previous Angiogram	253	12%	71	10%	0.936
Pre-angiogram Medications					
Anti-platelet	252	65%	72	76%	0.029*
Anti-Coagulants	251	10%	72	36%	<0.001**
Nitrates	252	31%	72	63%	<0.001**
Calcium Channel Blocker	251	41%	72	68%	<0.001**
Beta-blockers	252	25%	72	17%	0.293
Other anti-anginals #	251	3%	72	3%	0.97
Statins	250	40%	71	56%	0.03*
ACE-inhibitor	250	24%	71	30%	0.155
Angiotensin Receptor Blocker	251	12%	71	9%	0.71
Co morbidities					
Cerebrovascular disease	244	9%	67	6%	0.706
Obstructive airways disease	244	24%	67	16%	0.15
Chronic heart failure	244	0%	67	0%	-
Peripheral arterial disease	244	1%	67	2%	0.86
Gastroesophageal disorders	244	30%	68	34%	0.109
Musculo-skeletal disorders	244	20%	67	27%	0.074
Psychiatric disorders	242	13%	72	16%	0.387

*n** number of patients with available data for parameter assessed. **p*<0.05, ***p*<0.01

#other anti-anginals includes nicorandil and perhexiline

Abbreviation: ACS; acute coronary syndrome, ACE; angiotensin converting enzyme

2.4.2 Cardiac Outcomes

Several cardiac outcomes were evaluated over the 12 month period including emergency department chest pain presentation, chest pain re-admission, MI, repeat angiography, mortality and the combined end-point of MI and mortality. Whilst the majority of these end-points were similar between the two groups, the mortality rate and the combined end-point of MI/mortality rate is three times higher in the ACS patients, compared to the stable chest pain patients. This trend was not statistically significant ($p = 0.08$, Table 2.2).

Table 2.2 Clinical Progress Over 12 Months Post Angiography

Changes over 12 months	Stable n = 253		ACS n = 72		P Age Adjusted
	n*	%	n*	%	
Cardiac Endpoints					
ED Chest Pain Presentation	182	4%	50	10%	0.165
Chest pain re-admission	180	3%	50	10%	0.146
Myocardial infarction	178	1%	50	0%	1.0
Repeat angiography	178	6%	50	0%	1.0
Mortality	253	0%	72	3%	0.08
MI/Mortality	253	0.4%	72	3%	0.082
Medication Changes					
Any Changes	179	50%	49	45%	0.699
Started Any Anti-anginal	180	26%	49	25%	0.801
Started Nitrates	180	9%	49	10%	0.763
Started CCB	180	11%	49	12%	0.772
Started Beta Blocker	180	10%	49	4%	0.301
Started other anti-anginal#	180	2%	49	4%	0.286

n*number of patients with available data for parameter assessed

#other anti-anginals includes nicorandil and perhexiline

Abbreviations: ED; emergency department, MI; myocardial infarction, CCB; calcium channel blocker

2.4.3 Quality of Life Assessment

The presence of angina in relation to HRQoL was assessed using the SF-36 scores, SAQ domains and CCSC at baseline (Table 2.3) and then at 12 months (Table 2.4). At baseline,

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there were no differences in the SF-36 physical and mental component scores between stable and ACS patients; although the SF-36 MSS confirmed the presence of depressive symptoms in both stable and ACS NoCAD patients. Baseline scores showed a substantial impact from chest pain with scores around one standard deviation below population mean. In addition, the SAQ domains were similar between the two groups, although the change in acute patients had a significantly lower SF-36 bodily pain score. In addition, there were no significant differences in angina grade as assessed by the CCSC at baseline and 12 months.

At 12 months, there were no significant differences in the SF-36 physical and mental summary scores, although the change in SF-36 MSS was five points lower in the ACS patient groups; which is clinically relevant and confirms the presence of depressive symptoms. The quality of life domain on the SAQ was notably lower in the ACS patients compared to the stable patients and this was close to reaching statistical significance ($p=0.056$) (Table 2.4). In relation to the longitudinal changes, logistic regression analyses demonstrated no changes in both SF-36 and SAQ scores over the 12 month follow-up (Table 2.5)(Figures 2.1 and 2.2). In relation to self-reported chest pain, stable angina patients reported more on-going pain at one month post-angiography than the acute patients (Figure 2.3).

Table 2.3 HRQoL at Baseline Angiography for Stable-NoCAD and ACS Non-CAD Patients

	Stable n =253		ACS n =72		P
	n*	Mean ± SD	n*	Mean ± SD	Age Adjusted
Short-Form 36					
Physical Functioning	247	55±28	70	63 ±26	0.235
Role Limitations-Physical	244	36±42	69	33 ±42	0.221
Bodily Pain	234	54±22	67	45 ±23	0.003
General Health	249	56±23	70	57 ±22	0.831
Vitality	244	43±23	70	44 ±22	0.808
Social Functioning	246	64±27	70	60 ±26	0.383
Role Limitations-Emotional	241	48±45	67	54 ±42	0.372
Mental Health	243	64±20	70	61 ±20	0.574
Physical Summary Score	229	38±11	65	38 ±11	0.505
Mental Summary Score	229	41±11	65	40 ±11	0.752
Seattle Angina Questionnaire					
Physical Limitation	232	59±25	64	64 ±24	0.593
Angina Stability	219	44±30	64	41 ±33	0.377
Angina Frequency	228	65±24	66	63 ±25	0.483
Treatment Satisfaction	221	89±15	67	91 ±13	0.13
Quality of Life	224	22±45	68	40 ±20	0.124
CCS Class					
I	101	46%	31	50%	0.817
II-IV	117	54%	31	50%	

*n**number of patients with available data for parameter assessed

Table 2.4 HRQoL at 12 Months Angiography for Stable-NoCAD and ACS-NoCAD Patients

	Stable n =253		ACS n =72		P
	n*	Mean ± SD	n*	Mean ± SD	Age Adjusted
Short-Form 36					
Physical Functioning	115	63±27	26	57 ± 24	0.102
Role Limitations-Physical	115	55±42	25	54 ±47	0.459
Bodily Pain	114	63±22	26	61 ±27	0.566
General Health	115	56±21	26	50 ±18	0.179
Vitality	114	51±21	25	49 ±20	0.535
Social Functioning	115	76±24	26	69 ±23	0.188
Role Limitations-Emotional	115	67± 42	26	59 ±42	0.278
Mental Health	114	73±18	25	65 ±20	0.089
Physical Summary Score	113	40±11	24	40 ±11	0.3
Mental Summary Score	113	46±10	24	43 ±11	0.322
Seattle Angina Questionnaire					
Physical Limitation	107	70±23	24	69 ±21	0.182
Angina Stability	103	73±27	25	67 ±27	0.363
Angina Frequency	108	88±17	25	80 ±23	0.079
Treatment Satisfaction	103	89±15	24	89 ±12	0.786
Quality of Life	105	71±22	25	59 ±24	0.056
CCS Class					
I	72	80%	23	79%	0.73
II-IV	18	20%	6	21%	

n*number of patients with available data for parameter assessed

Table 2.5 HRQoL Scores from 1-12 Months Compared to Baseline between Stable-NoCAD and ACS NoCAD patients

Seattle Angina Questionnaire	<i>n</i>*	Stable Mean ± SD	<i>n</i>*	ACS Mean ± SD
Physical Limitation				
Baseline	232	59 ± 25	64	64 ± 24
1 Month	142	65 ± 23	39	68 ± 25
6 Month	113	70 ± 22	30	74 ± 22
12 Month	107	70 ± 24	24	69 ± 21
Angina Stability				
Baseline	219	44 ± 30	64	41 ± 33
1 Month	140	74 ± 29	37	68 ± 35
6 Month	110	74 ± 25	31	78 ± 29
12 Month	103	73 ± 27	25	67 ± 27
Angina Frequency				
Baseline	228	65 ± 24	66	63 ± 25
1 Month	146	79 ± 21	39	78 ± 25
6 Month	116	83 ± 23	31	84 ± 18
12 Month	108	88 ± 17	25	80 ± 23
Treatment Satisfaction				
Baseline	221	89 ± 15	67	91 ± 13
1 Month	145	86 ± 17	39	88 ± 17
6 Month	112	86 ± 18	30	92 ± 12
12 Month	103	89 ± 15	24	89 ± 12
Quality of Life				
Baseline	224	45 ± 22	68	40 ± 20
1 Month	142	64 ± 24	39	60 ± 24
6 Month	114	70 ± 21	31	58 ± 27
12 Month	105	71 ± 22	24	59 ± 24
Short Form-36	<i>n</i>*	Stable Mean ± SD	<i>n</i>*	Acute Mean ± SD
Physical Summary Score				
Baseline	229	38 ± 11	65	38 ± 11
1 Month	148	38 ± 11	36	42 ± 10
6 Month	123	41 ± 11	27	46 ± 11
12 Month	113	41 ± 11	24	41 ± 11
Mental Summary Score				
Baseline	229	41 ± 11	65	40 ± 11
1 Month	148	44 ± 11	36	41 ± 13
6 Month	123	45 ± 11	27	43 ± 12
12 Month	113	46 ± 10	24	43 ± 11

*n**number of patients with available data for parameter assessed

2.4.4 Missing Follow-up Data

The baseline clinical and socio-demographic characteristics of respondents versus non-respondents were compared (Table 2.6). Socio-economic status was defined by the Australian Bureau of Statistics' socio-Economic Indexes for Areas scores²¹⁴. Non-respondents were younger and more likely to be smokers and of lower social class. The scores on the SF-36 demonstrated poorer physical and social functioning, as well as more bodily pain in the non-respondents. In addition, the SF-36 PSS was lower in the non-respondents compared to the respondents ($p=0.06$). Furthermore, patients who dropped out scored lower in the SAQ quality of life domain compared to patients who completed the study ($p=0.053$) (Table 2.7).

Table 2.6 Patient Characteristics of Respondents versus Non-Respondents

	Respondents n = 143		Non-Respondents n = 182		P
	n*	Mean±SD/%	n*	Mean±SD/%	Age Adjusted
Demographics & Risk Factors					
Age (years)	143	60 ± 12	182	54 ± 39	<0.001**
Female	143	57%	182	57%	0.306
Current smoker	137	10%	178	25%	0.014*
Hypertension	139	58%	175	58%	0.174
Diabetes Mellitus	141	16%	178	19%	0.181
Hypercholesterolemia	132	57%	172	63%	0.198
Low Socio-economic status	133	17%	167	30%	0.053
Cardiac History					
Stable angina	143	41%	182	41%	0.667
Unstable angina	143	14%	181	11%	0.457
Previous MI	143	8%	182	4%	0.188
Previous angiogram	143	12%	181	11%	0.786
Co-morbidities					
Stroke	140	4%	171	4%	0.574
Airways disease	140	21%	171	23%	0.42
Renal disease	140	6%	171	4%	0.504
Liver disease	140	4%	171	0.6%	0.08
Cancer	139	22%	171	18%	0.746
Gastroesophageal disease	140	36%	172	27%	0.124
Musculo-skeletal disease	140	20%	171	23%	0.201
Psychiatric disorders	139	22%	171	18%	0.232

n*number of patients with available data for parameter assessed, *p<0.05, **p<0.01.

other anti-anginals includes nicorandil and perhexiline.

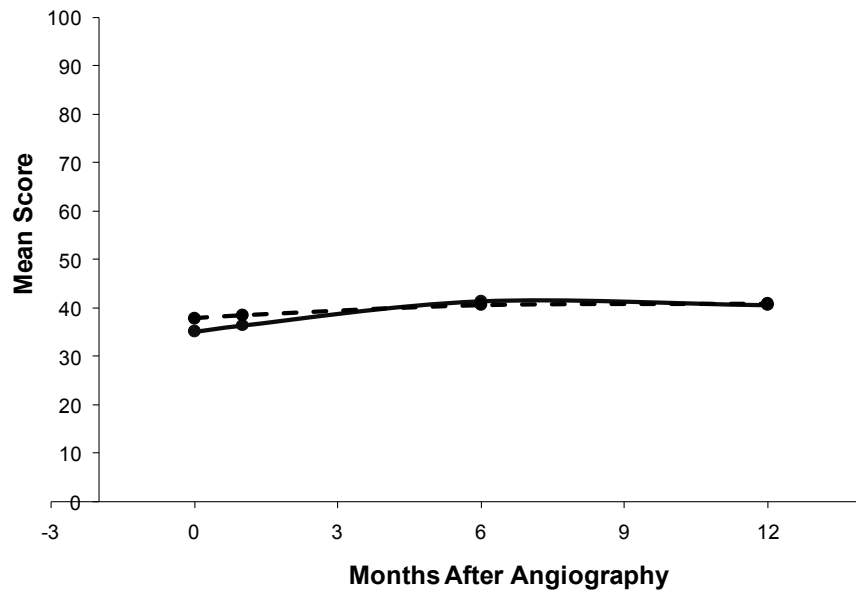
Abbreviations: MI; Myocardial Infarction

Table 2.7 Health-Related Quality of Life at Baseline for Respondents and Non-Respondents

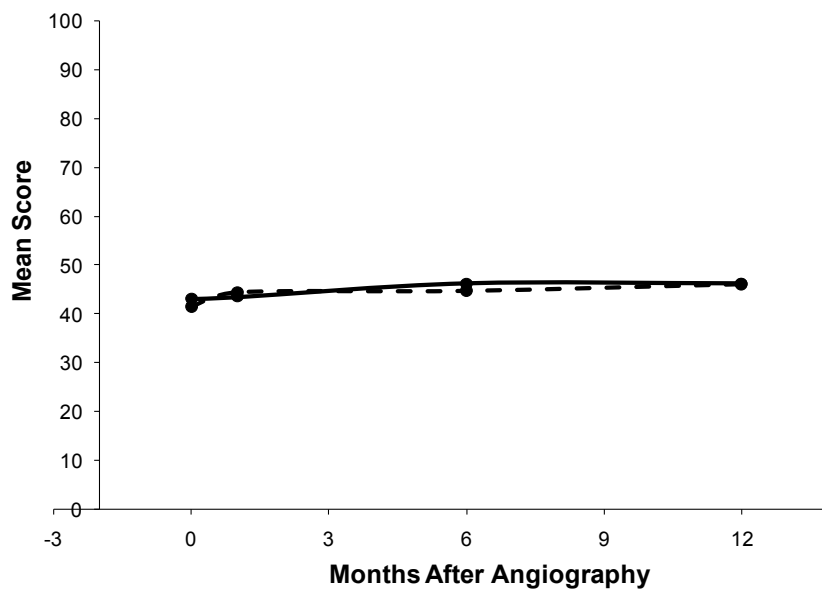
	Respondents n = 143		Non-Respondents n = 182		P
	n*	Mean ± SD	n*	Mean ± SD	Age Adjusted
Short-Form 36					
Physical Functioning	141	59 ± 26	176	54 ± 29	0.006**
Role Limitations-Physical	140	35 ± 42	173	36 ± 41	0.457
Bodily Pain	136	55 ± 22	165	50 ± 23	0.03*
General Health	141	57 ± 21	178	55 ± 24	0.507
Vitality	139	45 ± 22	175	42 ± 24	0.2
Social Functioning	140	66 ± 26	176	61 ± 27	0.093
Role Limitations-Emotional	136	52 ± 44	172	48 ± 44	0.32
Mental Health	139	65 ± 20	174	62 ± 21	0.513
Physical Summary Score	133	38 ± 11	161	38 ± 11	0.066
Mental Summary Score	133	42 ± 11	161	40 ± 12	0.572
Seattle Angina Questionnaire					
Physical Limitation	131	61 ± 23	165	60 ± 26	0.121
Angina Stability	120	43 ± 31	163	44 ± 31	0.913
Angina Frequency	128	66 ± 23	166	63 ± 25	0.155
Treatment Satisfaction	124	90 ± 14	164	88 ± 15	0.315
Quality of Life	130	47 ± 22	162	41 ± 21	0.017*

n* number of patients with available data for parameter assessed, *p<0.05, **p<0.01.
other anti-anginals includes nicorandil and perhexiline

A. Physical Summary Score



B. Mental Summary Score



—●— Stable -●- Acute

Figure 2.1 SF-36 Physical and Mental Summary Scores over 12 months

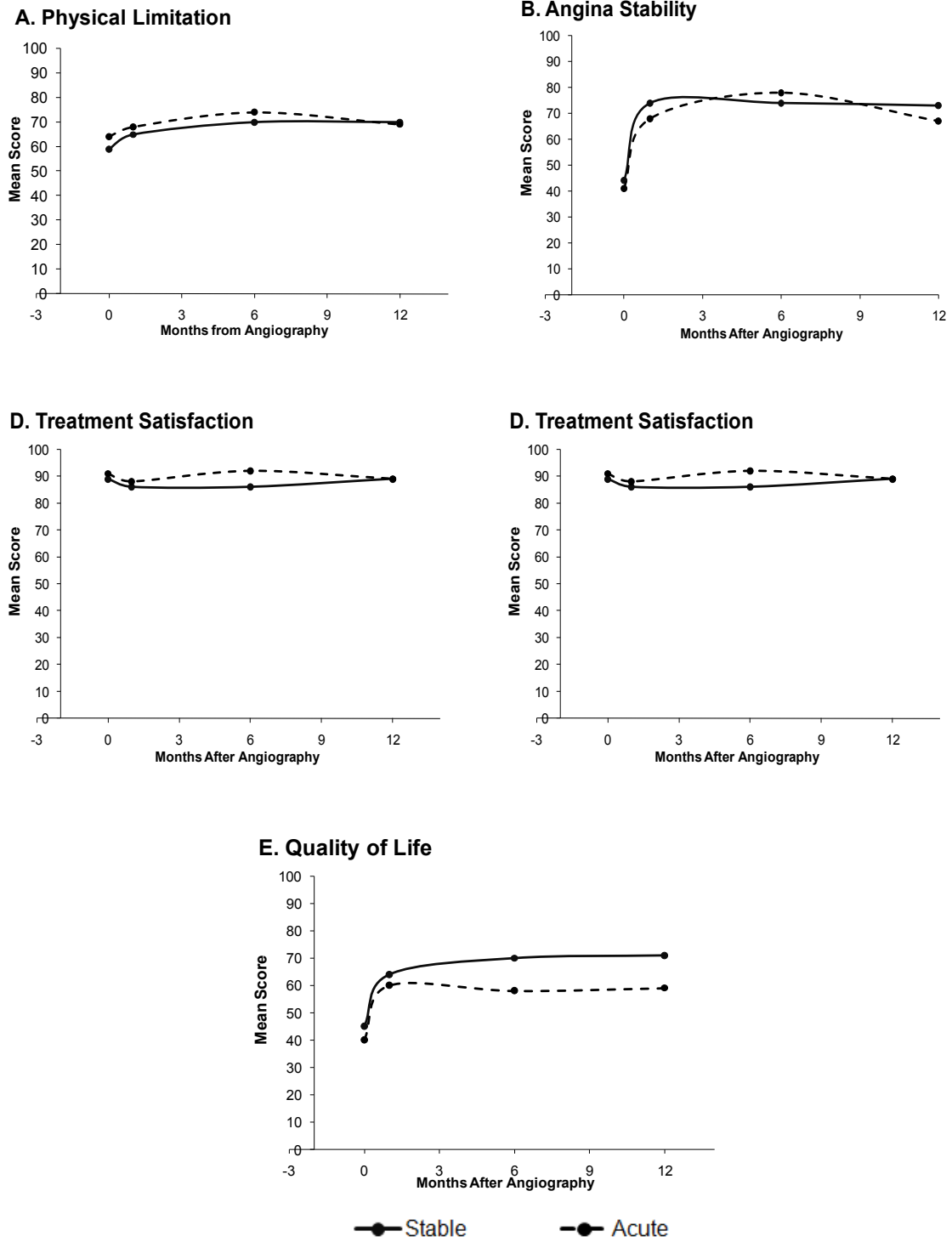


Figure 2.2 SAQ domain scores over 12 months

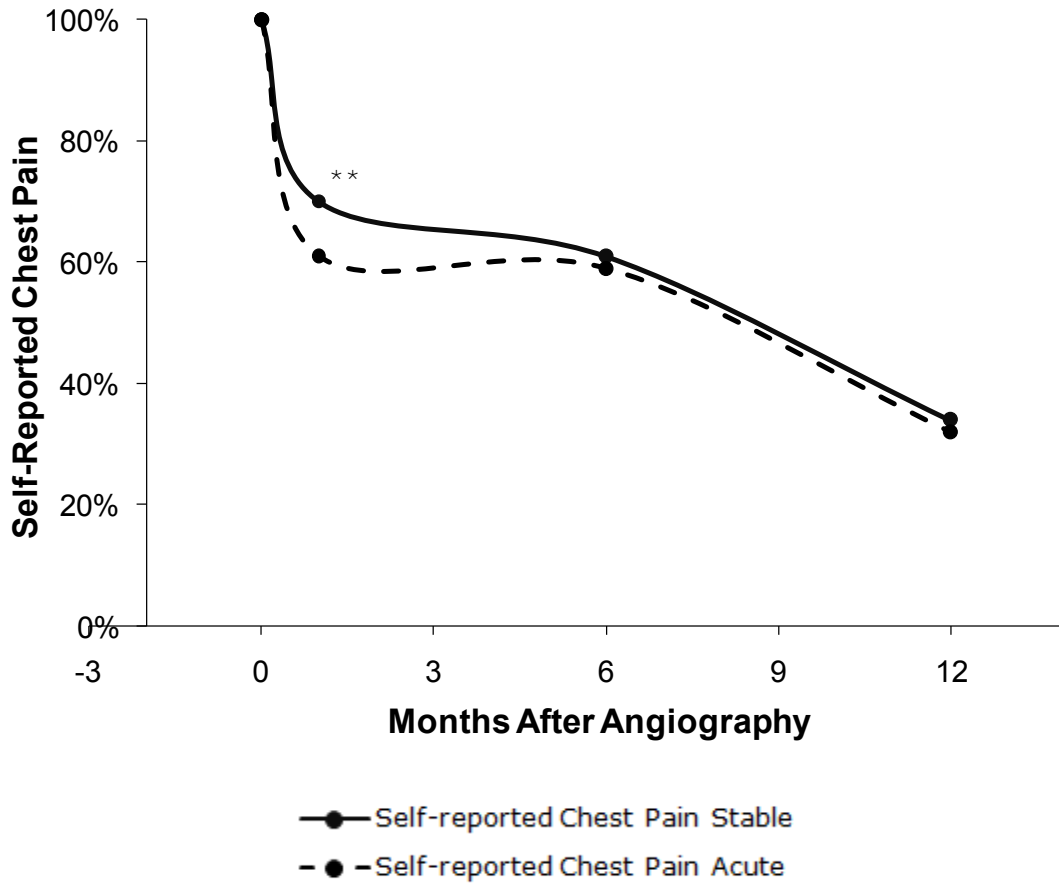


Figure 2.3 Self-Reported Chest Pain for Stable and Acute Chest Pain in NoCAD Patients

* $p < 0.05$, ** $p < 0.01$ for Stable Chest Pain versus Acute Chest Pain
P values obtained from logistic regression

2.5 Discussion

To date, no previous study has quantitatively evaluated health outcomes in patients diagnosed with an ACS and NoCAD. The four key findings of this study include:

- (1) no significant changes in HRQoL after one year in ACS-NoCAD patients
- (2) no statistical differences in HRQoL between ACS-NoCAD and stable-NoCAD patients over the 12 month follow up, although both SF-36 PSS and MSS scores were very low in all patients with NoCAD
- (3) no significant differences in the SAQ scores between the two groups, however ACS-NoCAD patients reported a poorer ‘quality of life’; indicating a worsening of symptoms and health status (Table 3)
- (4) the prevalence of MI/mortality is three times higher after one year in the ACS-NoCAD patients compared with the stable-NoCAD patients; however, this was not statistically significant as the study was underpowered for this endpoint.

In addition, self-reported chest pain was greater in the stable-NoCAD patients at one month post-angiography compared to patients with an acute chest pain presentation; although the frequency of chest pain was similar at the 12 month follow up.

HRQoL is a multi-dimensional concept which involves the physical, mental and social aspects of health. The principal components of measuring HRQoL are related to a patient’s physical and mental well-being⁴⁸. The three scales which correlate most highly with the SF-36 physical component summary and contribute most to the scoring of the physical summary score, are:

- (a) physical functioning
- (b) role-physical
- (c) bodily pain.

Although the SF-36 PSS remained unchanged between the two groups, the bodily pain score in the ACS-NoCAD patients was significantly worse at baseline compared to the stable-NoCAD patients. This finding is not surprising as patients who present to the emergency department with acute chest pain have had a recent onset of symptoms as a result of a traumatic event, whereas patients with stable angina have had chest pain for a longer period.

2.5.1 Acute Chest Pain Studies

In this study, ACS-NoCAD patients tended to be younger than those with stable-NoCAD, although they are essentially indistinguishable from the latter in respect of gender, risk factor (except cholesterol status) or co-morbid conditions, and are more often prescribed anti-platelet agents and nitrates at the time of angiography in keeping with their ACS presentation. In the subsequent 12 months following angiography, we found that the SF-36 PSS and MSS scores did not improve in the ACS-NoCAD patients. Although we found that the HRQoL scores in both ACS-NoCAD and stable-NoCAD were lower than those observed in a normal Australian population²¹² and also similar to those with significant illness such as cancer^{212,215}, Chronic Obstructive Pulmonary Disease (COPD)²¹⁶ and inflammatory arthritis²¹⁷. This is quite an alarming finding, as it demonstrates a significant level of impaired physical and mental functioning due to poor health status. Other authors have also found no differences in HRQoL outcomes after one year in patients with obstructive CAD. Beck et al.²¹⁸ found that quality of life in patients diagnosed with an acute MI was low and did not improve after one year; attributing this finding to increasing age and psychosocial factors such as depression.

Over 12 months, the ACS-NoCAD patients scored very low on the SF-36 MSS and this reflects a diminished functioning in mental health, as a score ≤ 45 is considered to be the threshold for categorising cardiac patients with depressive symptoms. Patients with stable-

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NoCAD also scored low on the SF-36 MSS prior to angiography however their score improved by five points after 12 months, which is a clinically relevant improvement. A difference of five points in mean HRQoL scores is a small change but is considered to be clinically and socially relevant with regard to daily functioning²¹³. The SF-36 MSS assesses the impact of health on cognitive and social/emotional functions, and psychosocial characteristics at baseline are known to affect HQRoL in the long term²¹⁸.

A statistical trend was observed in ACS-NoCAD patients who reported a poorer quality of life, as assessed by the SAQ, compared with their stable-NoCAD counterparts. The 'quality of life' domain measures the overall impact of a patient's condition and takes into account social, as well as, psychological aspects that can affect everyday living³⁸. Rumsfeld et al.⁴ reported that patients with ACS were more physically impaired than MI patients; this has been attributed to underlying pathophysiological differences between the two syndromes, because patients with ACS are more likely to experience recurrent chest pain and have higher cardiac events rates²¹⁹.

2.5.2 Stable Chest Pain Studies

In this study, patients with stable NoCAD reported a poor HQRoL at angiography, however, the SF-36 MSS but not the PSS score improved over the 12 month follow-up. The physical impairment reported by the patient at 12 months is likely due to on-going chest pain. Boini et al.²²⁰ reported that persistent chest pain can have a negative effect on HRQoL for many years. In addition, over 50% of patients with stable chest pain presentations, mostly women, experience persistent disabling chest pain which is resistant to anti-anginal medication¹²⁰. Furthermore, over 75% of patients with stable chest pain presentations and NoCAD continue to see their doctor for pain management²²¹, and this may be one of the reasons why patients

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with stable-NoCAD reported more chest pain at one month post-angiography compared to unstable angina patients. Also, Johnson et al¹²⁰ reported that women with stable persistent chest pain and NoCAD are twice as likely to experience a cardiac event within five years compared to patients whose pain resolves within 12 months of their initial diagnosis. In contrast to the previous study, our study reported a low cardiac event rate (0.4%) for patients with stable-NoCAD; however the end-point was assessed over a shorter period of time. Therefore the prognosis of patients with stable persistent chest pain and NoCAD may change over five years; thus cardiac event rates will need to be tracked for several years to adequately evaluate long-term prognostic outcomes in this study cohort.

2.5.3 Previous HRQoL Studies in Patients with Chest Pain and NoCAD

Assessment of health status in CHD requires further studies both to document its extent and determine the benefit of particular therapies. To date, the treatment of CHD has focused on prevention of cardiac events³⁷. The assessment of HRQoL is an important outcome in the management and care of patients with angina, as cardiac related chest pain decreases a patient's quality of life and is associated with increased risk of ACS and death^{7,206,222}. Unfortunately there is relatively little literature that examines the current use of health status in patients with NoCAD; however, there are some studies which have assessed HRQoL in patients with acute and stable angina without evidence of CAD. Perers at al.²²³ found that patients with unstable angina presenting with an acute coronary syndrome, with no ECG signs of ischaemia and/or elevated blood cardiac markers, were more likely to have a poorer quality of life than patients with clinical evidence of myocardial infarction. Brink et al.²²⁴ found patients presenting with an ACS and a 'non-confirmed MI' scored lower on the mental health component summary of the SF-36 compared to a normal population group after a one-week hospital admission and also 12 months post-discharge. This is not surprising as depression has

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been found to be one of the strongest predictors of overall physical and mental health status in patients with CAD^{4,225,226}.

In this study, the SF-36 MSS confirmed the presence of depressive symptoms in the total NoCAD cohort. Tavella et al.²²⁷ defined a threshold score on the SF-36 MSS of ≤ 45 which indicates the presence of depression in a cardiac population that consisted of patients undergoing angiography for the investigation of chest pain. The identification of depressive symptoms in this cohort of NoCAD patients raises theoretical questions such as: does depression precede the pain? Or is pain a consequence of the depression itself? The answers to these questions are unknown and we can only speculate that depression may not be the cause of pain but rather a consequence of persistent chest pain, as has been previously reported in these patients. In addition, the SF-36 MSS scores tended to improve in the stable angina patients, suggesting that despite the persistence of pain, mental health functioning possibly improved due to improved coping mechanisms; although patients who had been admitted for the first time with an ACS have been shown to assess their illness as less threatening, and may use different strategies for coping compared to those with long-term cardiac illness²²⁸. In light of these conflicting findings, the factors related to the improvement of mental health functioning need further exploration.

2.5.4 Death and Cardiac Events in Non-Obstructive CAD

The current consensus in the literature indicates that patients with chest pain and NoCAD have a favourable prognosis in terms of mortality of cardiac events. Long term studies have reported myocardial infarction and cardiac mortality rates are $\leq 1\%$ after a 10 year follow-up²⁰⁵. Furthermore, the risk of cardiac events after five years is doubled in patients with moderate (30-50%) compared to minor coronary lesions ($<30\%$)²²⁹.

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More recently, Bugiardini and Merz¹³⁴ reported a 2% risk of death/MI after a 30 day follow up in patients presenting with an acute coronary syndrome, which suggests the risk of cardiac events and death appears to be dependent upon the clinical presentation. Our findings are comparable to the study by Bugiardini and Merz¹³⁴, as the combined end point of death/MI is three times more common among patients presenting with an ACS compared to the stable angina group after one year.

The results of our study suggest that the risk of MI/mortality appears to be greater in patients presenting with an ACS and no significant coronary stenoses. The prognostic difference between our study and other non-obstructive studies is likely to relate to the characterisation of study populations; as angina syndromes are rarely defined in patient groups. Furthermore, it is difficult to compare the present work with that of other studies, as HRQoL outcomes in this study were assessed between stable and acute angina patients in the context of NoCAD. Unfortunately the cause of death in the patients who presented with ACS was unable to be determined; as the patients died outside of hospital therefore only time of death and not the cause was notifiable.

The findings of our study are novel as the incidence of MI and/or death in patients with acute coronary syndromes and non-obstructive disease has never been investigated in a prospective trial setting. As a result of these preliminary findings, further consideration should be given to patients presenting with ACS and NoCAD. Ideally a larger sample size is required to adequately investigate health outcomes in patients with ACS and NoCAD.

2.5.5 Etiology of Chest Pain in Patients with Non-obstructive CAD

The etiology of patients with chest pain and NoCAD is considered to be heterogeneous and various cardiac, coronary and non-cardiac or coronary causes have been previously stipulated (See chapter 1, section 1.4.1). Cardiologists are sometimes unable to distinguish whether chest pain is of cardiac origin and therefore patients with this syndrome are usually referred for coronary angiography in order to dismiss the presence of CAD.

In this study several known cardiac etiologies that cause chest pain in patients with NoCAD were excluded. These include valvular heart disease, pericarditis, recurrent pulmonary emboli, severe pulmonary hypertension and cardiomyopathy. Clinical entities such as variant angina, syndrome X, microvascular angina and the coronary slow flow phenomenon can also cause chest pain, however these disorders, were not routinely investigated in our cohort of patients.

Non-cardiac etiologies such as gastrointestinal disease, musculoskeletal disease and psychiatric disorders can also cause chest pain in patients without significant coronary stenosis. Although we did not specifically assess non-cardiac causes of chest pain in NoCAD patients presenting with an ACS, we found 27% had a history of musculo-skeletal disorders, 34% had gastroesophageal disorders and 16% had psychiatric disorders (Table 2.1), thus eliminating potential confounders caused by co-morbidities. It is important to note that this study was not designed to determine the etiology of chest pain but to assess to HRQoL in patients presenting with an ACS and NoCAD.

2.5.6 Study Limitations

The strengths of this study include the prospective recruitment of patients, the clinical and angiographic categorisation of patients and the use of both a generic (SF-36) and a disease-

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specific (SAQ) instrument, which provides a more comprehensive assessment of symptoms and HRQoL.

There were several potential limitations which should be addressed in this study. The main limitation was the modest survey response rate (53%) which resulted in a small sample size. Post Hoc analyses confirmed that in order to detect a three point difference in SF-36 PSS score an additional 210 patients must be recruited in each group to power the study. The factors underlying drop-out were related to health and socio-economic differences. In particular, patients from the ACS cohort were less likely to complete their questionnaire compared to patients with a stable presentation. A possible explanation for this discrepancy may be related to the longstanding problems associated with chronic disease, which may motivate these patients to get followed up. Whereas the symptoms associated with an acute chest pain presentation may resolve and thus patients from this cohort are less interested in long term follow up.

Patients who did not return their questionnaires had a poorer quality of life and were of lower socio-economic status than respondents. Bosworth et al.²³⁰ found that patients who had low educational attainment and lacked social support were more likely to have a significantly poorer quality of life than those with better education and higher levels of support. Therefore, we cannot rule out the possibility that HRQoL may have been underestimated at the 12 month follow up, and that missing follow up data has potentially introduced bias into our study. The non-respondents were also more likely to be cigarette smokers, which is associated with low socio-economic status and may influence disease severity leading to refusal to participate.

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Patients with non-cardiac pathologies, which are capable of producing chest pain, were not excluded in this study. Unfortunately, it was very hard to delineate between cardiac and non-cardiac causes of chest pain, as the majority of patients had several non-cardiac comorbidities. However statistical analyses showed no significant differences among comorbidities between the two groups. Also, measures of ischaemia during the index admission were not routinely performed in ACS cohort; however a breakdown between anginal syndromes (outlined in section 2.4.1) demonstrated that unstable angina is the likely cause of ischaemic chest pain in acutely presenting patients. Last, mortality rates in CAD need to be tracked for years before any meaningful conclusions about the quality of healthcare can be found. Hence, a larger sample size of patients needs to be assessed over five years to adequately assess the validity of survival and cardiac event rates in this population of patients.

As a result of the above limitations, any conclusions drawn from this study must be cautiously interpreted. However, these are the first preliminary results derived from a NoCAD cohort of patient which has shown these patients to be functionally impaired. Clearly further investigations are warranted in order to properly assess these novel findings.

Previous prognostic studies have reported up to 80% of patients with NoCAD have persistent chest pain which can cause significant morbidity in a patient's life. It is therefore not surprising that HRQoL scores in patients with chest pain and NoCAD were akin to those with chronic debilitating illnesses, indicating the presence of significant physical and mental disability. Furthermore, the treatment for chest pain in patients with NoCAD is limited, as many pharmacologic medications offer some but not complete relief of symptoms. Patients with ACS-NoCAD are clinically distinct in that they appear to be at increased risk of cardiac events compared to stable-NoCAD patients; and thus may require additional cardioprotective therapy such as statins and aspirin to decrease their risk of MI or death. Whilst anti-anginal

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measures can help alleviate cardiac symptoms, the assessment of HRQoL can be used to identify patients with a poor quality of life and assess the effects of treatment strategies. HRQoL measures are an important research tool used to assess the impact of disease as self-perceived by the patient and should be utilised in clinical practice for better patient management and decision-making.

2.5.7 Conclusion

This study concludes that although no differences exist between ACS and stable NoCAD patients, both groups have a very poor HRQoL which does not improve over a 12 month follow-up. Furthermore, despite a modest follow-up of patients, this study reported an increased risk of cardiac event rates associated with ACS-NoCAD over the 12 months following angiography. In particular patients with ACS-NoCAD should not be dismissed as having a benign prognosis and careful consideration should be employed to minimise the risk of MI or death. These preliminary findings have given us insight into important clinical and prognostic outcomes of patients with chest pain and NoCAD. Future studies should assess HRQoL in a larger patient population and further explore the factors related to health outcomes in both acute and stable chest pain presentations with NoCAD.

2.5.8 Clinical Significance

A poor HRQoL is associated with work absenteeism, premature retirement or disability pension, and also increased use of healthcare services. In this study, patients with chest pain and NoCAD have been shown to have significant functional and psychological impairment as demonstrated by low HRQoL scores, which indicates that health care needs are not being met. In addition, patients with ACS-NoCAD appear to be more psychologically impaired than their stable chest pain counterparts and thus may require tricyclic antidepressants to improve their

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mental health component of HRQoL. The presence of a psychiatric condition is a potential non-cardiac cause of chest pain, although cardiac-related chest pain can also co-exist with psychiatric illness; either way the presence of depressive symptoms can further reduce a patient's quality of life, hence the importance of assessing mental health status. Furthermore, clinicians should consider aggressive medical management to decrease the risk of cardiac events after 12 months in patients with ACS and NoCAD, which may also improve their overall quality of life. In clinical practice the primary goal of therapy in patients with chest pain and NoCAD is to decrease the severity of symptoms and improve a patient's overall quality of life. This can be achieved by optimising quality of health care through the assessment of HRQoL as perceived by the patient.

Chapter 3. ST/T Wave Fluctuations During Acute Coronary Syndrome Presentation in Patients with the Coronary Slow Flow Phenomenon.

3.1 Introduction

The coronary slow flow phenomenon (CSFP) was first described by Tambe and colleagues in 1972¹⁵⁴ when it was attributed to microvascular dysfunction but until recently has attracted little attention. The clinical characteristics of this disorder are intriguing as patients frequently present with an ACS presentation, clinically indistinguishable from acute myocardial infarction thereby warranting admission to a coronary care unit¹⁵⁹. Despite their ACS clinical presentation there is limited evidence of myocardial ischaemia when these patients have been assessed on an elective, non-acute basis. Previously we have performed rapid atrial pacing, cold pressor testing and intracoronary acetylcholine infusion during the chronic phase of their condition and failed to identify transmyocardial lactate production with any of these provocative manoeuvres. Furthermore, one patient experienced chest pain and ST segment elevation during acetylcholine provocation in the absence of transmyocardial lactate production, questioning the sensitivity of the later methodology. Moreover, coronary haemodynamic studies^{80,231,232} have demonstrated normal coronary and myocardial perfusion flow reserve in response to pacing and pharmacologic stimuli during the chronic phase of this disorder. However, consistently there has been evidence of increased resting coronary microvascular resistance in these patients^{80,231}.

The lack of objective evidence of myocardial ischaemia in these studies may not only reflect methodological limitations but also the timing of the studies considering the dynamic nature of the condition. Hence if the assessment of myocardial ischaemia were to be undertaken when the condition was clinically profound (as during an ACS presentation) then the findings may differ. Undertaking the above invasive techniques during an ACS presentation is logistically difficult and so ECG monitoring is a practical alternative.

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Continuous 12-lead ST/T wave monitoring is an established clinical method for detecting myocardial ischaemia²³³ that has been clinically-validated against contrast echocardiography^{234,235} and shown to predict cardiac outcomes in patients with ACS²³⁶⁻²³⁹. It avoids the technical difficulties encountered with serial ECG recordings and thus may increase the possibility of detecting transient ischaemic ECG fluctuations during an ACS presentation. Thus considering the clinical utility of continuous ST/T wave monitoring, the objective of this study was to determine the frequency of significant ST/T wave fluctuations during an ACS presentation in patients with the CSFP.

3.2 Background

3.2.1 Electrocardiogram

The ECG is one of the most important tools in cardiology as it is imperative in the diagnosis of myocardial ischaemia and infarction. In addition the ECG can determine heart rate and rhythm, and identify structural and electrical abnormalities, chamber enlargement and conduction defects⁷². The 12-lead ECG records the electrical activity of the heart via the skin surface. The views of the heart obtained using this system are often referred to as ‘ECG leads’. These electrodes are attached to leads and consist of 3 bipolar (or limbs) leads and 9 unipolar (or precordial) leads which are attached on the chest wall. The location of change, morphology and number of affected leads on the admission ECG can yield both diagnostic and prognostic information²⁴⁰.

3.2.2 ECG Waveforms

In 1887 Waller and colleagues, recorded the first electrical currents generated from the human heart using a Lippman’s capillary electrometer. Electrical potentials were measured by

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altering the surface tension of the mercury-sulphuric acid mixture in the capillary column²⁴¹. A decade later, Einthoven invented the string galvanometer which recorded and measured electrical currents generated from the beating heart on film. He mathematically calibrated and corrected the waveform to allow for inertia and friction in the capillary tube of the refined Lippman's capillary electrometer. The latter was an important breakthrough in electrocardiology as 4 deflections were identified and labelled using the letters ABCD. Later on Einthoven mathematically corrected the waveform to increase the speed of electrical currents being transmitted on film and thus labelled the new deflections as PQRST to allow for adaptation for future waves²⁴² (Figure 3.1).

NOTE:
This figure is included on page 92
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 3.1: The QRS complex. Source: Scheidt, S.²⁴³

The P wave represents atrial depolarisation that commences in the sino atrial node and travels through the atria. The QRS complex represents depolarisation of the ventricles. The ST segment represents the end of ventricular depolarisation and the beginning of ventricular repolarisation. At this stage of the cardiac cycle, the ventricular action potential is in the plateau stage and normally is isoelectric. The T-wave is the final major wave on the ECG and

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represents repolarisation of the ventricles. The T-wave is the final major wave shown on the ECG during a cardiac cycle, representing repolarisation of the ventricles⁷². The characteristics and variation of normal T-waves is well established. Normal T-waves are asymmetric, rounded and have the same polarity as the terminal QRS complex²⁴⁴. The U wave was identified last by Einthoven and represents repolarisation of the His Purkinje fibres²⁴⁵.

3.2.3 The ST Segment

ST segment changes are well recognized on the ECG as a sign of severe myocardial ischaemia or infarction²⁴⁶. ST changes can be described as elevated, depressed or isoelectric. In most instances ST elevation reflects transient total occlusion of a major epicardial coronary artery; however acute episodes or variant angina can also cause ST elevation. ST elevation is associated with several electrical and structural abnormalities including early repolarisation and pericarditis. However, it is more commonly identified as a marker of transmural injury which affects the entire thickness of the heart and leads to necrosis of heart tissue. Downward deviations of the ST segment also known as ST depression may represent either a reduction of flow or an increase in myocardial oxygen demand^{247,248}. ST depression can be a sign of subendocardial ischaemia or infarction. Subendocardial ischaemia or infarction is less severe than transmural ischaemia, as it does not involve the entire thickness of the myocardial wall.

3.2.4 The T-Wave

T-wave changes are based on variations in amplitude and morphology on the ECG. Variations in T-wave amplitude are described as either hyperacute or inverted and variations in morphology are described as biphasic or non biphasic. A peaked T-wave may be present in the early stages of acute MI or can be caused by sudden narrowing or total occlusion of a coronary artery.

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Regional ischaemia is generally confined to the ECG zone of ischaemia and may be characterized by the appearance of tall peaked T-waves caused by sudden narrowing or obstruction of an epicardial artery²⁴⁹. Another pattern of T-wave change in ischaemia is that which accompanies ST segment depression and inverted T-waves, in the absence of tachycardia may reflect an acute reduction in regional coronary flow. If this change occurs with tachycardia it reflects an increase in myocardial demand²⁵⁰. After an ischaemic event the T-wave may undergo progressive normalization. Following an ischaemic episode of acute transmural ischaemia if the supply/demand balance of myocardial oxygen supply is restored, the T-wave in the affected leads will become inverted. If the T-wave does not invert there is some implication that the restoration of myocardial oxygen supply is not complete. In some cases, inverted T-waves may undergo pseudonormalisation during a recurrent ischaemic event²⁵¹. Tall peaked T-waves are usually a sign of sudden narrowing or obstruction of an epicardial artery, and can be one of the earliest signs of infarction. Deep symmetrical T-wave changes may represent a sign of injury and are usually associated with subendocardial ischaemia. Terminal T-wave inversion may be associated with a previous infarct and can persist for months or remain as a permanent sign of injury.

3.2.5 QT in Ischaemia

The QRS complex on the ECG is produced by activation of both ventricles. The QT interval is a time measure between the start of the Q wave and end of the T-wave. The QT interval is dependent on heart rate and therefore has to be adjusted to aid in interpretation. The standard clinical correction is to use *Bazett's formula*²⁵² which is:

$$QTc = \sqrt{\frac{QT}{RR}}$$

QT complex (QTc) is the QT interval corrected for heart rate, and RR is the interval from the onset of one QRS complex to the onset of the next QRS complex, (*measured in milliseconds*) often derived from the heart rate (HR) as 60/HR. The evolution of T-wave inversion following an ischemic event is correlated with increased ventricular action potential and is associated with QT prolongation.

3.2.6 Electrocardiographic Indicators of Acute Ischaemia and Infarction

The ST segment is clinically important if elevated or depressed, as it can be a sign of ischaemia or infarction. Significant ST segment elevation (STE) will occur at the epicardial level by reducing local myocardial flow below 50% compared to control values. Reducing myocardial flow further, consequently increases the amplitude of STE in proportion to the reduction in myocardial flow²⁵³. However the most common cause of ST segment changes is the result of altering the transmembrane potential in the ischaemic region compared with the adjacent non ischaemic region. Inadequate blood flow to the heart causes electrical and mechanical disturbances. The basic molecular and electrical cellular mechanisms underlying acute ST segment changes on the electrocardiogram involve (a) changes in the transmembrane action potential and (b) alteration in cell to cell coupling²⁴⁶.

Mechanistically, ischaemia leads to an increase in extracellular K⁺ with membrane depolarisation, depletion of intracellular ATP and action potential shortening. Together, these cellular events lead to electrical disturbances and generate injury currents between ischaemic and adjacent non-ischaemic cells which shift the ST segment in the ECG. The change in the transmural action potential involves the loss of intracellular K⁺ ions from ischaemic cells, which consequently changes the resting membrane potential because of increasing extracellular K⁺. These ionic changes during ischaemia, as well as action potential shortening,

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are believed to be the result of the activation of ATP- sensitive K^+ channels as a primary mechanism. A recent study by Li et al.²⁵⁴ directly linked the activation of sarcolemmal kATP channels to ischaemic ST elevation in knock-out mice. It has also been hypothesized that ionic changes may be caused by the inhibition of energy-dependent K^+/Na^+ pump and it has suggested that cellular K^+ redistributes due to lactate build up.

The change in electrical cell-to-cell uncoupling develops shortly after coronary occlusion and is completed after within 30-40 minutes. The exact cause(s) of cell-to-cell uncoupling is unknown however it has been postulated that an increase in intracellular Ca^{2+} initiates the onset of cell-to-cell uncoupling. Other metabolic changes that occur during ischaemia are the accumulation of lipid metabolites and acidification which have been suggested to play a role in cell-to-cell uncoupling. During myocardial ischaemia there is a dissociation between the very early stages in the transmembrane action potentials and the delayed electrical cell-to-cell uncoupling. It is this dissociation that allows the flow of injury current and the generation of the early ST-segment changes in the ECG to occur. The changes in transmembrane potential build up the driving force for injury current flow, the current flow itself requires low resistance pathways between the ischaemic and non-ischaemic region²⁴⁶. ST segment deviation caused by ischaemia is subject to both spatial and non spatial factors that influence its magnitude²⁵⁵.

The ST segment and T-wave represent different electrophysiological events, although they are still commonly referred to as ‘ST-T wave changes’ irrespective of whether both changes are present. The exact cause of T-wave changes is unknown however, it is believed electrical and mechanical disturbances, similarly like the ST segment, have been speculated to be involved. Nevertheless, abnormalities of the ST segment and T-wave frequently co exist and they are

well known markers on the electrocardiogram that are associated with acute myocardial ischaemia.

3.2.7 Pathological Factors that can Cause ST/T Wave Changes

A variety of pathological factors can alter ventricular repolarisation and cause ST/T wave changes that can impede the diagnosis of myocardial ischaemia and infarction. These factors include metabolic and electrolyte variations, structural and conduction abnormalities, cardiac sympathetic dysfunction and drug effects (Table 3.1). The aforementioned pathological factors can affect the ECG similarly to coronary ischemic syndromes. More recently, ischemic ST/T wave changes have been observed in various cardiac abnormalities such as Takosubo syndrome²⁵⁶, microembolization²⁵⁷, and also ischemic stroke²⁵⁸.

Table 3.1 Pathological Factors that cause ST/T Wave Changes

Metabolic and Electrolyte	Structural and Conduction	Sympathetic	Drug and Treatment Effects
Hyperkalemia	Ventricular aneurysm	Subarachnoid haemorrhage	Digitalis
Hypokalemia	Pericarditis	Hypothermia	Quinidine
Hypocalcaemia	Myocarditis	Takosubo syndrome	Tricyclic Antidepressants
	Microembolism	Ischemic Stroke	Procainamide
	Early Repolarisation		Phenothiazines
	LVH		Pericardiocentesis
	LBBB		

3.2.8 Non-specific ST Segment and T-waves

Non-diagnostic ST segment and T-waves were first described by Burch et al.²⁵⁹ in the 1950's and were suggested to reflect early signs of coronary artery disease. Non-specific or non-diagnostic T-waves are often described in the literature as common changes that do not quite meet the criteria for a significant change²⁶⁰. However, non-specific ECG changes have been observed in 4% of patients subsequently diagnosed with an acute MI²⁶¹. In 1961 Friedberg and Zager assessed the association between non-specific ST segment and T-wave changes and found that they were markers for subendocardial and transmural ischaemia²⁶². Kannel et al.²⁶⁰ proposed that non-specific ST segment and T-wave changes may be a manifestation of asymptomatic silent coronary heart disease. The interpretation of non-specific ST segment and T-wave fluctuations can be hazardous in regards to treating patients with acute MI because non-specific ECG changes in patients who are asymptomatic or have chest pain are at high risk of developing heart disease²⁶¹. In addition, patients who initially present to emergency with non-specific ECG changes are heterogeneous with regard to diagnosis and prognosis therefore accurate clinical evaluation is imperative to determine a positive outcome²⁶³.

3.3 Study Objectives and Hypotheses

The objective of this study was to determine the frequency of significant ST segment and T wave fluctuations during an ACS presentation in patients with the CSFP. The current study was conducted to test the following null hypotheses:

- (1) There will be no difference in the frequency of ischaemic ST Segment and T-wave fluctuations between patients with the CSFP and control patients.
- (2) There will be no difference in the ST segment and T-wave amplitude measurements between the CSFP and control patients.

3.4 Materials and Methods

Utilising an observational case-control study design, we compared CSFP patients admitted with an ACS with healthy control subjects. The study protocol was approved by The Central Northern Adelaide Health Service Ethics Committee.

3.4.1 Study Population

CSFP was defined as significant delay contrast opacification on selective coronary angiography in the absence of obstructive (<50% stenosis) coronary artery disease, where significant delay was considered as 3 or more cardiac cycles to opacify distal vessels. Patients were included if they had; (a) angiographic documentation of the CSFP, and (b) an admission to the coronary care unit with an ACS where at least 4 hours of continuous ST/T wave monitoring was undertaken.

Healthy subjects were recruited via local media advertisements for volunteers to undergo 4 hours of continuous ECG monitoring for ST/T wave analysis. These patients were selected for inclusion on the basis (a) age ≥ 35 years, and (b) no history of cardiac disease or chest pain.

Exclusion Criteria

Patients with the following were excluded from the study: (a) a clinical history of cardiomyopathy, pericarditis, or myocarditis, (b) angiographic evidence of obstructive CAD in any vessel, or (c) ECG evidence of a previous myocardial infarction, bundle branch block, left ventricular hypertrophy, sustained atrial or ventricular arrhythmias.

3.4.2 Study Procedure

Baseline clinical characteristics were collected on all study participants including coronary risk factors, cardiac history and current medications. A resting 12-lead ECG was performed and evaluated for the presence of left ventricular hypertrophy using both the Cornell and Sokolow-Lyon voltage criterion²⁶⁴. The characteristics of the 12-lead ECG recorded when the patients were pain-free, were evaluated in both CSFPs and healthy control patients. In the CSFP patients, this was recorded pre-discharge, when their chest pain symptoms had resolved. In addition to the above ECG data, echocardiographic findings were retrieved from the clinical case record for those CSFP patients in whom it was clinically undertaken. The echocardiographic studies were reviewed for evidence of left ventricular hypertrophy.

Patients with the CSFP underwent continuous ST/T wave monitoring as part of the clinical assessment of their ACS presentation. They were monitored for up to a period of 22 hours however only the first four hours of monitoring was evaluated in this study. These patients received routine therapy for their ACS that included anti-platelet agents, intravenous heparin and nitrates. In contrast, healthy controls underwent elective continuous ST/T wave monitoring for a 4-hour period.

Continuous ST/T wave monitoring involved automated acquisition and digitization of serial 12-lead ECGs using the General Electric Medical System (GEMS) ST-Guard monitoring system. This ECG data was archived on the Marquette Universal System for Electrocardiography (MUSE) Management System allowing subsequent off-line analysis²⁶⁵. This system obtains ST segment and T-wave amplitude measurements from the bedside monitor every 60 seconds and plots these fluctuations over time to form linear trends (Figure

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3.2). Throughout the duration of the monitoring period, the baseline ECG is continuously compared to the instantaneous ECG.

For the purpose of this study, significant ST segment fluctuation was defined as ST segment deviation of $\geq 1\text{mm}$ in ≥ 2 contiguous leads on ≥ 2 consecutive QRST complexes. The reference point for assessing the ST segment deviation was the middle of the ST-segment (STM), which was calculated by measuring the R-R interval between each waveform (Figure 3.3). The most commonly used parameter for measurement of ST-segment amplitude is 60 milliseconds post J-point. The disadvantage of having a set point of measurement is that variations in heart rate can result in the ST segment being measured at a different point in the QRS waveform. Therefore, the measurement of the R-R interval to calculate the middle of the ST segment is able to compensate for variations in heart rate and offers a more accurate measurement of amplitude change²⁶⁶. A significant T-wave fluctuation was defined as T-wave fluctuation of $\geq 1\text{mm}$ deviation in ≥ 2 contiguous leads on ≥ 2 consecutive ECG complexes.

Both qualitative and quantitative analyses of the continuous ST/T wave monitoring data were undertaken. Qualitative analyses were performed by an independent blinded observer who assessed each of the 4 hour monitoring periods, reviewing the ECG recorded every 60 seconds during this period. The observer categorised each patient/subject as having no or significant ST/T wave fluctuations. Quantitative analysis of the ST/T wave monitoring data involved obtaining the maximal ST/T amplitude fluctuation (μm) at 60-second intervals using the admission ECG as the baseline reference as calculated by the GEMS system. This process was undertaken throughout the entire monitoring period in order to determine the maximal amplitude fluctuation for both the ST segment and T-wave.

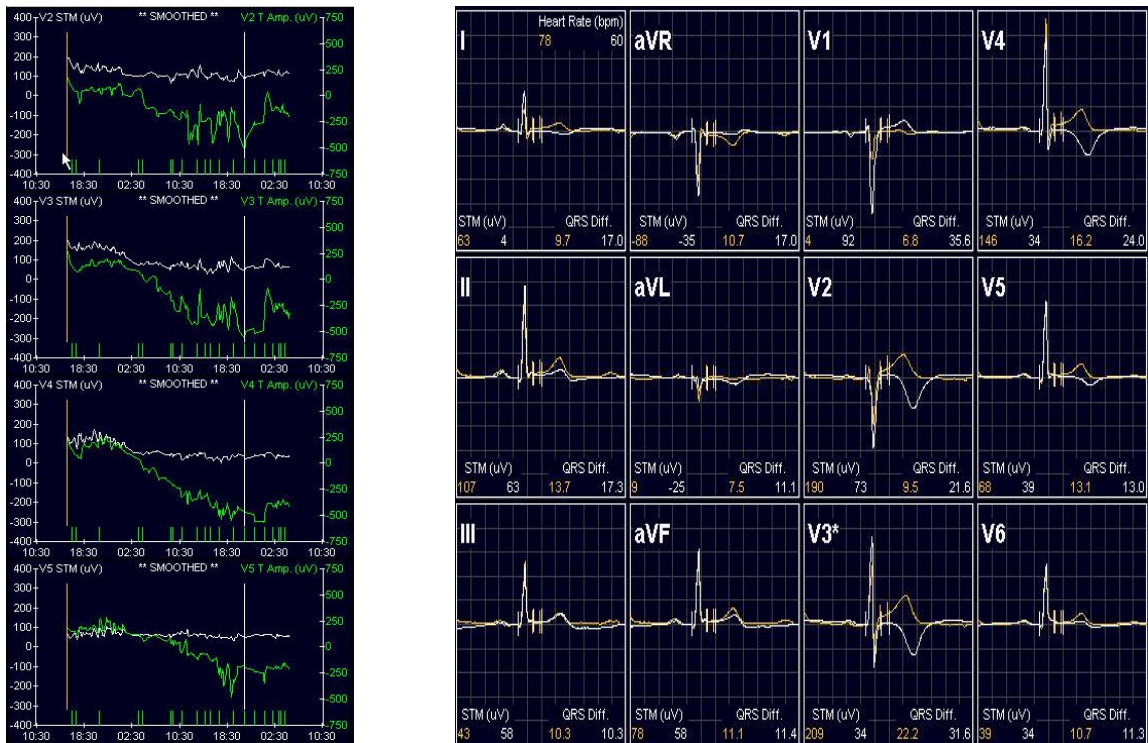


Figure 3.2 Screen capture of 12 Medians (GEMS ST Guard)

Figure 3.2 illustrates the ST-segment (white) and T-wave (green) trends on the left hand panel. On the right hand panel, the gold medians represent the baseline ECG recorded at CCU admission (corresponding to the gold cursor in the panel on the left). The white medians represent the ECG 9 hours later (corresponding to the white cursor on the left hand panel). An additional feature of the GE ST guard is that ST and T wave trends can be individually or simultaneously displayed in 4 chosen leads and changes can be analysed at the same point in time. The ST segment and T wave amplitude measurements for each of the 12 leads are then plotted against time to form a linear trend.

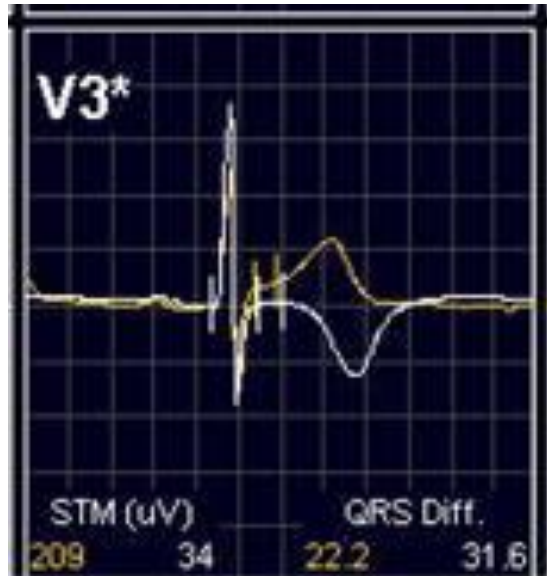


Figure 3.3 Middle of ST segment (STM) measurements (GEMS ST GUARD)

Figure 3.3 shows 2 median complexes in Lead V₃. The first complex shows no ST or T wave abnormalities. The overlaying complex shows 1.5 mm of T wave inversion. The first marker denotes the beginning of the QRS complex. The second marker denotes the end of the QRS or “J” point. The third marker shows the calculated STM.

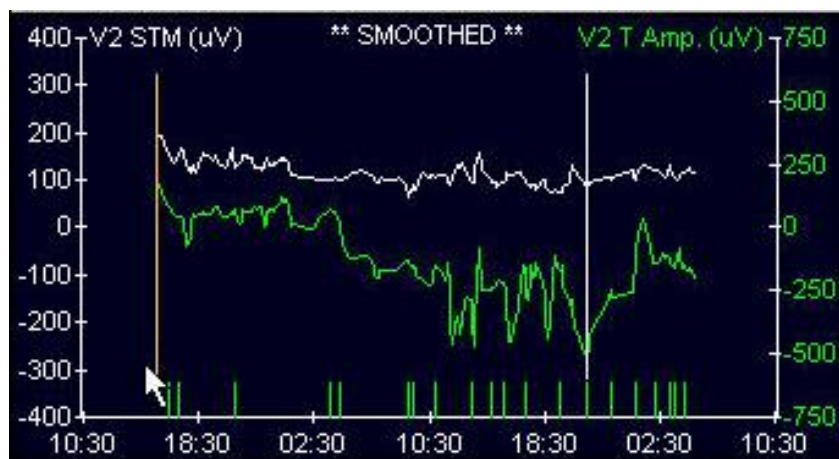


Figure 3.4 ST-segment trend (GEMS ST Guard)

Figure 3.4 demonstrates a trend of ST-segment amplitude over time for a single lead (Lead V₂) Amplitude (in microvolts) is plotted on the y-axis and time (in hours) on the x-axis.

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The GEMS ST Guard offers the option of viewing changes to the QRS complex, ST segment and T wave in a “median” format, which allows direct comparison of the baseline complex with a subsequently acquired ECG median complex for each of the 12 leads. (Figure 3.4) T wave amplitude changes and heart rate change are also able to be documented.

3.4.3 Data Analysis and Statistics

Descriptive statistics were expressed as frequencies for the ordinal data and mean \pm SD for the continuous parametric data. Comparisons between groups were conducted using a Fisher’s Exact Test or Chi-Squared Test for the non-parametric ordinal data and unpaired t-tests for the parametric data. Analyses were performed using Statistical Package for Social Science (SPSS Version 16, Chicago).

3.4.4 Sample Size Calculations

Sample size calculations were based upon previous study findings where 34% of CSFP patients had ST/T wave fluctuations on serial ECG¹⁵⁹. Assuming that 1% of healthy controls have ST/T wave fluctuations on continuous ST monitoring, than 20 patients/group are required to detect a difference in the frequency of ST/T wave fluctuations between the CSFP and healthy control patients for 80% power at the alpha 0.05 level.

3.5 Results

The study population comprised of 37 CSFP patients admitted with an ACS and 20 healthy controls, each of whom had undertaken at least 4 hours of continuous ST/T wave monitoring. Of note, 4 (11%) of the CSFP patients had elevated cardiac markers consistent with an acute myocardial infarct during their admission. In relation to baseline clinical characteristics, the CSFP patients were younger, more likely to be male and had more coronary risk factors including smoking, hypertension and hypercholesterolaemia compared with the healthy controls (Table 3.2). Also compared with the healthy controls, the CSFP patients were more likely to have resting ST changes on their ECG when pain-free (ST elevation in 11 patients and ST depression in 2 patients). However there was no difference between groups in resting rhythm, heart rate, abnormal T-wave fluctuations or QTc interval (Table 3.2). Furthermore, neither group had ECG evidence of left ventricular hypertrophy (as per study criteria); nor was there echocardiographic evidence of hypertrophy or left ventricular dysfunction in the 17 CSFP patients who underwent this investigation as part of their routine diagnostic work-up.

In contrast to the pain-free ECG's, the CSFP patient's acute admission ECG's had faster heart rate (65 ± 10 vs. 73 ± 17 bpm, respectively $p = 0.01$) and shorter QTc interval (426 ± 33 vs. 407 ± 21 msec, respectively, $p = 0.009$). Furthermore amongst the CSFP patients, 10 (30%) showed ST elevation, 4 (12%) ST depression, and 3 (9%) had T-wave abnormalities on the admission ECG.

Table 3.2 Baseline Characteristics in CSFP and Healthy Controls

	CSFP (n=37)	Controls (n=20)
	Mean ± SD/ (n)%	Mean ± SD/ (n)%
Demographics & Risk Factors		
Age (years)	49±14.6* years	54.8±13.5 years
Males	(27) 73%*	(9) 45%
Smoking History	(19) 51%	(5) 25%
Hypertension	(19) 51%**	(0) 0%
Hypercholesterolemia	(20) 54%*	(6) 30%
Diabetes Mellitus	(9) 24%	(1) 5%
Pain-free Resting ECG		
Sinus Rhythm	(33) 100%	(20) 100%
Heart Rate	65±10 bpm	60±7 bpm
Resting ST fluctuations	(13) 39%**	(0) 0%
Inferior ST elevation	(9) 27%*	(0) 0%
Anterior ST elevation	(1) (3%)	(0) 0%
Resting T- wave fluctuation	(5) 15%	(1) 5%
Inferior T-wave fluctuation	(2) 6%	(1) 3%
Anterior T-wave fluctuation	(2) 6%	(0) 0%
QTc Interval	426±33 msec	410±10 msec

*p<0.05 or **p<0.01; significant difference between CSFPs & healthy controls

Abbreviations: bpm; beats per minute, LVH; left ventricular hypertrophy, STE; ST elevation, Tw; T wave, msec; millisecond; CSFP, Coronary Slow Flow Phenomenon.

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During continuous ST/T wave monitoring patients admitted with an ACS were more likely to be prescribed aspirin, nitrates and calcium channel blockers, and this is irrespective of the angiographic diagnosis. In addition, patients with the CSFP were more likely to be prescribed statins compared to ACS/NoCAD (Table 3.3).

Table 3.3 Medications Administered During Continuous ST/T-wave Monitoring

Medication	CSFP (n=39)	Controls (n=20)
Asprin	(36) 92% **	(0) 0%
Nitrates	(38) 97% **	(0) 0%
Beta blockers	(3) 8%	(0) 0%
Calcium channel blockers	(33) 84% **	(0) 0%
Anti-platelet	(0) 0%	(0) 0%
Statins	(18) 46% **	(0) 0%
Ace-inhibitor	(7) 18%	(0) 0%
A2RB	(4) 10%	(0) 0%
Anti-depressants	(5) 13%	(2) 10%

* $p < 0.05$ or ** $p < 0.01$; significant difference between CSFPs & healthy controls
Abbreviation: A2RB; Angiotensin 2 Receptor Blocker; CSFP Coronary Slow Flow Phenomenon

Qualitative assessment of the continuous ST/T wave monitoring data demonstrated a non-significant trend in ST segment changes in the CSFP patients; however T-wave fluctuations were substantially more common amongst the CSFP patients (Table 3.4). Quantitative assessment of the total continuous ST/T wave data demonstrates greater maximal ST segment and T-wave fluctuations in the CSFP patients as compared with controls (Table 3.4). In the CSFP patients, the average fluctuation in maximal ST segment deviation from baseline was

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almost 1 mV (95% CI: 0, 3mV, Table 3.4). In contrast, the average maximal T-wave deviation from baseline was almost 2.5mV in the CSFP patients (95% CI = 0, 5mV) as compared with almost 1.5mV in the healthy controls (95% CI= 0, 2mV, Table 3.4).

Table 3.4 Qualitative and Quantitative Data in CSFP Patients and Healthy Controls

Medication	CSFP (n=39)	Controls (n=20)	P Value
<i>Qualitative</i>			
ST-segment Fluctuations	(9) 24%	0%	P=0.09
T-wave Fluctuations	(32) 86%	5%*	P<0.001
<i>Quantitative</i>			
Max ST-segment deviation (mV)	0.97±0.07	0.39±0.22*	P<0.001
Max T-wave deviation (mV)	2.46±0.163	1.45±0.59*	P<0.001

*p<0.05 or **p<0.01; significant difference between CSFPs & healthy controls
Abbreviation: CSFP; Coronary Slow Flow Phenomenon

To exclude the influence of resting ST/T wave changes on the dynamic ST/T wave fluctuations observed during continuous ST monitoring, a sensitivity analysis was conducted. The 20 CSFP patients who had both normal ST segments and T-waves on the resting pain-free ECG were compared with healthy controls. Whereas only one (5%) healthy control subject had ST/T wave abnormalities on continuous ST/T wave monitoring, 2 (10%) of CSFP patients with normal resting ST segments had new ST fluctuations and 16 (80%) had new T-wave fluctuations on continuous ST/T wave monitoring (p<0.01).

3.6 Discussion

This study demonstrates that 92% of patients with the CSFP exhibit ECG evidence of myocardial ischaemia on continuous ST monitoring during an ACS presentation, with significant ST segment fluctuations occurring in 24% and T-wave fluctuations in 86%. This compares with healthy control subjects where only 1 of 20 patients (5%) showed any ST/T wave fluctuations. These findings support an ischaemic basis to the ACS presentation in patients with the CSFP and suggest that T-wave fluctuations may be a marker of microvascular dysfunction.

3.6.1 Myocardial Ischaemia and the CSFP

The CSFP is angiographically defined by the delayed passage of contrast in the epicardial vessels implicating increased downstream resistance. Although the diagnosis is usually made during an ACS presentation¹⁵⁵, angiography is typically undertaken when the patient is pain-free and has implicated an underlying increased resting coronary resistance. This has been confirmed in coronary haemodynamic studies^{80,231} and is consistent with biopsies that have reported pathological changes in the microvasculature. However, unlike many other coronary microvascular disorders, the ability of the microvasculature to respond to hyperaemic stimuli does not appear to be impaired in the CSFP^{80,231}.

Considering that the microvasculature in patients with the CSFP can appropriately respond to vasodilatory stimuli, it is not surprising that hyperaemic stimuli such as exercise and rapid atrial pacing fail to induce myocardial ischaemia. Hence exercise tests are frequently negative in patients with the CSFP¹⁵⁴ and studies assessing transmyocardial lactate production during rapid atrial pacing have failed to identify evidence of ischaemia⁷⁹. In contrast, the use of vasoconstrictor stimuli such as cold pressor testing or intracoronary acetylcholine have

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produced abnormal coronary haemodynamic responses in some patients yet still did not show metabolic evidence of lactate production⁸⁰.

The understanding of the CSFP has previously been limited by the research approach since most studies were undertaken when the patient is pain-free rather than when the symptoms are most profound as during an ACS presentation. As a metaphoric comparison, our understanding of acute myocardial infarction experienced a paradigm shift when DeWood et al.²⁶⁷ performed coronary angiography during the acute phases of infarction rather than remote from the event.

The present study is unique since it has evaluated CSFP patients during their acute presentation when the clinical manifestations are the most profound. Utilising this approach and employing the robust technique of continuous ST/T wave monitoring, we have demonstrated that most CSFP patients have ECG evidence of myocardial ischaemia on the basis of dynamic ST/T wave fluctuations.

3.6.2 Ischaemic T-wave Fluctuations

Although 27% of the patients with CSFP exhibited ischaemic ST segment fluctuations, the majority only demonstrated T-wave fluctuations. The importance of isolated T-wave fluctuations as a marker of ischaemia has not received as much attention as ST segment fluctuations although there has been some interest in the context of ACS presentations. Jacobson et al.²⁶⁸ demonstrated that T-wave amplitude and the number of leads with significant T-wave abnormalities were predictive of 30-day cardiovascular events including death, myocardial infarction and refractory angina. Although they reported that ST depression was a better marker for cardiovascular events, this finding was uncommon and the

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T-wave abnormalities were predictive in the absence of ST changes²⁶⁸. In a broader population, Beckerman et al.⁷³ reported that combined ST segment and T-wave abnormalities were the greatest predictor of cardiovascular mortality but that major T-wave abnormalities (Minnesota Code 5-1 and 5-2) were more predictive than severe ST depression.

In the present study, T-wave fluctuations were associated with ST segment fluctuations in 22% of CSFP patients. T-wave inversion occurred in 68% and peaked T-waves in 32%. There was no relationship between the extent of T-wave fluctuation and the TIMI frame count assessed on index angiography. Surprisingly, 4 (11%) of CSFP patients with ST/T wave changes on their admission ECG had elevated troponin levels which is consistent with a myocardial infarction. Acute MI with non-obstructive CAD is a recognized clinical entity but it is generally an uncommon occurrence. The aetiology of MI with normal coronaries remains unknown; however epicardial coronary spasm, microembolism and platelet dysfunction have been postulated as underlying causes²⁶⁹. Unfortunately the patients in this study did not undergo (a) provocative testing for vasospasm, (b) platelet studies or (c) intravascular ultrasound to exclude the aforementioned causes of MI in the 4 CSFP patients. Therefore, the underlying cause of MI in patients with the CSFP remains speculative.

3.6.3 Definition of an Abnormal T-wave Definition

The prognostic significance of the T-wave in cardiology is somewhat controversial as there are several studies which have demonstrated no adverse outcomes associated with an abnormal T-wave change^{270,271}. The reason for this discrepancy is sought out in the definition of the T-wave abnormality and what constitutes an abnormal T-wave change in the literature. For example, a significant T-wave change, in the context of a MI, has quantitatively been described as T-wave inversion in ≥ 1 mm of change in ≥ 2 lead(s)^{201,272} or ≥ 2 mm of change in

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≥ 1 lead(s)^{263,270}. An abnormal T-wave has also been described by visually identifying them as either inverted, negative, positive or hyperacute T-waves²⁷³⁻²⁷⁵. The study by Jacobson et al.²⁶⁸ classified a T-wave abnormality in the 12 lead ECG using a quantitative approach correcting for gender and age, electrocardiographic lead and QRS axis. In the context of STEMI, a recent study defined an abnormal T-wave as positive or negative if it was ≥ 0.5 mm above or below the iso-electric line, or alternatively measured more than 120 ms after the J point²⁷⁶. In addition, there are several studies that do not even specify how they define a significant T-wave change and simply describe changes as ST or T ~~abnormalities~~^{122,239,277}. Unfortunately, the definition of an abnormal T-wave change has not yet been validated in context of acute coronary syndrome and therefore a standard definition in the literature does not exist.

Unlike the T-wave, the definition of a significant ST change in the context of an ACS is globally defined. The Global Use of Strategies to Open Occluded Arteries in Acute Coronary Syndromes IIb trial was one of the first publications to define and validate the criteria for significant ECG fluctuation in acute coronary syndromes and provide quantitative data to classify patients into categories on the basis of the ECG findings on presentation²³⁷. Therefore the definition of a significant ST fluctuation in the context of an ACS is globally defined as ≥ 1 mm change in ≥ 2 mm contiguous leads.

3.6.4 The T-wave Amplitude

The T-wave amplitude is known to vary with age, gender²⁷⁴ and position²⁷⁸, although age and gender specific T-wave analysis in healthy subjects has not thoroughly been investigated. In this study we found CSFP patients had significantly larger amplitude T-wave changes compared to controls suggesting that amplitude changes of ≥ 2 mm may be an

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electrocardiographic marker of transient coronary microvascular ischaemia. In 1961, Friedberg and Zager first identified that a T-wave change of ≥ 2 mm was associated with recent cardiac chest pain in a group of patients with a heterogenous diagnosis²⁶². In addition, the clinical significance of T-wave amplitude changes post infarction was investigated first by Haines et al.⁷² describing ≥ 2 mm of T-wave inversion was predictive of coronary artery stenosis and a poor outcome even when treated medically²⁶⁰. Clearly, our patient cohort is not comparable to patients with obstructive CAD however the clinical significance of ≥ 2 mm appears to be equally important in both obstructive and non-obstructive CAD patients.

3.6.5 Study Limitations

There are several study limitations in this study. First, there may be a selection bias as consecutive CSFP patients admitted to CCU with an ACS were recruited in the study. Thus the more severe end of the CSFP spectrum may have been included in the study and thus more likely to have ischemic ECG fluctuations.

Second, although no patient had ECG evidence of left ventricular hypertrophy, only a limited number of patients underwent echocardiography to exclude this diagnosis. Thus potentially some patients may have left ventricular hypertrophy to account for their ECG fluctuations although it was not observed in any of the 17 CSFP patients who did undergo echocardiography.

Third, the control patients did not undergo cardiac imaging to exclude coronary heart disease. However there was no clinical history of coronary heart disease. Furthermore, the study groups were unfortunately not age or gender matched, both of which may influence T-wave fluctuations.

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Lastly, the medications administered during continuous ST/T wave monitoring could be considered potential confounders. One can argue that the highly prescribed statins may increase the frequency of ST/T wave fluctuations in the CSFP patients; however this would be unlikely as this type of drug serves to improve myocardial coronary perfusion²⁸⁵ and theoretically should decrease ischemic episodes of chest pain.

3.6.6 Conclusion

This study demonstrates that most patients with the CSFP exhibit ST/T wave fluctuations during an ACS presentation. The strong association with T-wave fluctuations in patients with this condition raises the possibility that the T-wave may be a marker of microvascular dysfunction. In addition, a T-wave amplitude change of $\geq 2\text{mm}$ may be a significant quantitative marker of microvascular ischaemia on the ECG. These novel findings require confirmation in a larger study population. Furthermore, continuous ST/T wave monitoring should be considered as a useful investigative technique to detect episodes of dynamic ischaemia in patients with acute chest pain in the absence of significant CAD.

3.6.7 Clinical Significance

In the past, evidence of myocardial ischaemia was seldom observed in patients with the CSFP, although the presence of clinical symptoms associated with their ACS presentation suggested otherwise. The findings of this study are clinically relevant as patients with the CSFP have transient ST/T wave changes consistent with myocardial ischaemia during an ACS presentation. This suggests that the current functional assessments used to detect myocardial ischaemia, at the microvascular level, are not sensitive enough and thus any negative results need to be cautiously reviewed. The presence of ST/T wave fluctuations observed during continuous monitoring, strongly suggests an underlying ischaemic substrate

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is involved in the pathogenesis of the the CSFP. Of course, future studies are needed to investigate the cause of microvascular ischaemia.

Chapter 4. Genetic Polymorphisms

4.1 Introduction

Coronary artery disease (CAD) has been described as a complex genetic disease which is caused by a combination of environmental and genetic (inherited) factors²⁷⁹. The precise molecular mechanisms that lead to CAD are not understood; however, it has been postulated that neither the environment alone nor a single gene can cause disease²⁸⁰. There are genetic variations in causative genes that form the basis of molecular mechanisms and, in conjunction with environmental factors, can lead to CAD and determine its clinical manifestation. Over the past ten years there has been a number of genetic association studies designed to identify gene variations associated with the increased risk for obstructive CAD or its related phenotype, myocardial infarction (MI)²⁸¹⁻²⁸³. However, relatively little is known about the genetic risk factors which are associated with NoCAD and its related phenotype, the Coronary Slow Flow Phenomenon (CSFP). Patients with the CSFP have recurrent episodes of chest pain at rest which can be the result of myocardial ischaemia caused by microvascular endothelial dysfunction. The imbalance of Endothelin-1 (ET-1) and endothelial nitric oxide (NO) are thought to contribute to the development of coronary endothelial dysfunction. Therefore, the association between cardiovascular disease and the imbalance of endothelial mediators makes these autocooids and their systems a favourable target for genetic association studies. As yet no studies have addressed the contribution of endothelial nitric oxide synthase (eNOS) and ET-1 genetic polymorphisms in an Australian Caucasian population with chest pain and NoCAD.

4.2 Background

4.2.1 Genetics

The genome contains several million individual DNA sequence variants (or alleles) which encode the functional differences underlying protein variants. The process of identifying DNA variations that may increase susceptibility to disease is able to be explored through mapping of single nucleotide polymorphisms (SNPs), throughout the genome²⁷⁹. The identification of SNPs that associate an underlying genotype with a disease phenotype allows us to develop a better understanding of how genes contribute to CAD. A SNP can influence the disease phenotype by altering a specific biological function that consequently leads to the development of disease²⁸⁴. In the context of this study, a genetic variation can cause the up-regulation or down-regulation of important vascular mediators such as ET-1 or NO, which are believed to be involved in the underlying disease process of microvascular dysfunction.

4.2.2 Nitric Oxide

Vascular NO is one of the most important cardioprotective autocooids described due in part to its potent vasodilatory actions. In addition to regulating smooth muscle, NO can inhibit cell-to-cell migration and proliferation²⁸⁵, as well as platelet adhesion and aggregation^{187,286}. NO is constitutively synthesised by eNOS from the amino acid L-arginine and molecular oxygen with the by-products of this reaction being citrilline and water²⁸⁷. Once released from the endothelium, NO acts on the underlying smooth muscle cells to induce vasodilatation by activating soluble guanylyl cyclase. This activation leads to a cascade of events commencing with an increase in intracellular concentrations of cyclic guanosine monophosphate (cGMP), followed by the activation of the G kinase which, in turn, reduces the intracellular concentration of calcium leading to smooth muscle relaxation^{288,289} (Figure 4.1). NO can also signal through a cGMP-independent pathway via n-nitrosylation, which involves protein

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modification related to cell signaling²⁹⁰. In endothelial cells, constitutive NO production is up-regulated by physiological stimuli such as ET-1, acetylcholine, thrombin, adenosine disphosphate, estrogens, sphingosine 1-phosphate, serotonin, bradykinin, histamine or sheer stress^{291,292}. The release of NO can be down-regulated by hypoxia inducible factors 1 and 2, oxidised low density lipoproteins, ageing and smoking²⁹³.

NOTE:

This figure is included on page 119 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4.1 Relaxation of vascular smooth muscle cells by endothelial derived nitric oxide and other vasodilators. Source: Vanhoutte et al²⁹⁴.

Endogenous agonists such as Ach and bradykinin activate NO synthesis, which leads to an increase of intracellular calcium (Ca^{2+}) released from the sarcoplasmic reticulum (SR) and into the cytoplasm where it binds to calmodulin (CaM) and activates eNOS, resulting in NO synthesis from L'arginine (L'arg). NO diffuses into the smooth muscle cell (SMC) where it

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activates soluble guanylate cyclase and increases cGMP synthesis from guanosine triphosphate (GTP). The cGMP binds and activates protein kinase G (PKG) resulting in a decrease in intracellular Ca^{2+} , which inhibits Ca^{2+} dependent muscle contraction. Also released from endothelial cells is Prostacyclin (PGI_2), which is catalysed from arachidonic acid from cyclooxygenases. Once diffused from the endothelial cell, PGI_2 activates adenylate cyclase, leading to increased production of cyclic AMP (cAMP) and cell muscle relaxation. Endothelium-derived hyperpolarising factor (EDHF) causes Ca^{2+} -dependent K^+ channels in vascular SMCs to open, leading to their hyperpolarisation. *Abbreviations: R, receptor; AA, arachidonic acid; NOS, NO synthase; L-Arg, L-arginine; R, membrane receptor; SR, sarcoplasmic reticulum; X, unknown precursor.*

4.2.3 Nitric Oxide and Endothelial Dysfunction

The classic definition of endothelial dysfunction is characterised by an impairment of endothelium-dependent vasorelaxation caused by loss of NO bioactivity²⁹⁵. However, circulating increased levels of ET-1 in conjunction with decreased NO are thought to play a key role in the development of endothelial dysfunction²⁹⁶. Traditional risk factors for atherosclerosis; such as age, smoking, hypertension, hypercholesterolemia and diabetes, can predispose individuals to endothelial dysfunction. In addition impaired endothelial dependent-vasodilation is associated with several adverse cardiovascular events including ACS and death^{297,298} (Figure 4.2).

In the coronary vasculature, NO deficiency can be caused by either reduced activity of eNOS or by decreased bioavailability of NO⁹⁹. The underlying mechanisms associated with NO deficiency include injury by oxidative stress, inflammation, and endogenous inhibitors of eNOS. The process of oxidative stress can cause NO to chemically degrade by combining

with reactive oxygen species that decrease the effectiveness of NO as a signaling molecule²⁹⁵. Inflammatory mediators such as c-reactive protein (CRP) and high levels of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of eNOS have also been shown to cause NO deficiency^{299,300}. Moreover, a decrease in endothelial NO production can increase synthesis of potent vasoconstrictors such as ET-1 and angiotension II, thereby leading to increased vascular reactivity and contributing to pathophysiological states such as coronary spasm¹⁹². Lastly, NO production can also be influenced by gene variations which are associated with endothelial dysfunction. Thus, NO production may also be genetically determined.

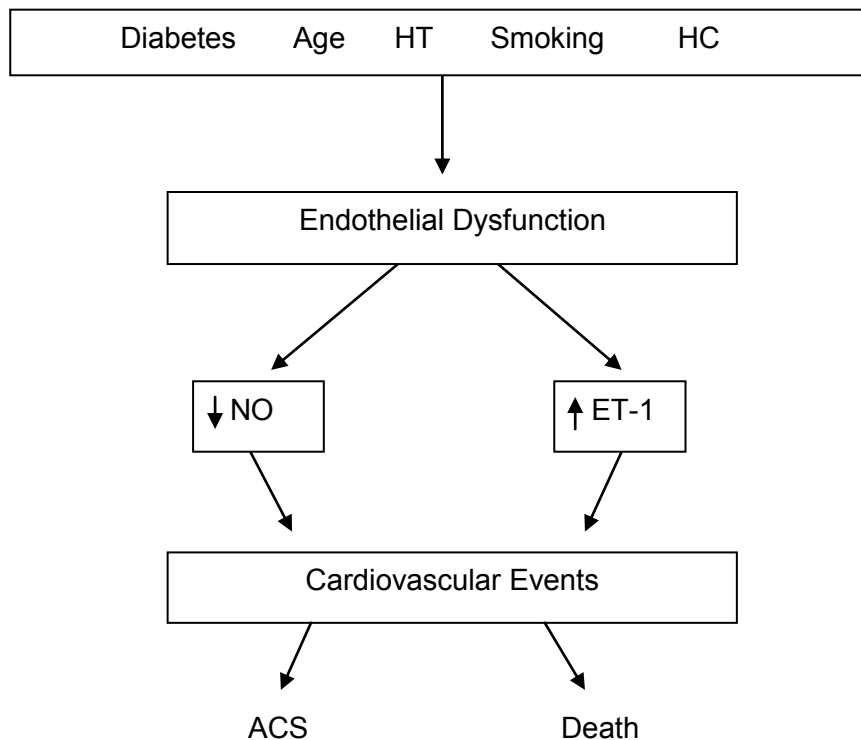


Figure 4.2 Diagram of Risk Factors, Mechanisms and Cardiovascular Events associated with Endothelial Dysfunction

Abbreviations: HT, hypertension; HC, hypercholesterolemia; NO, nitric oxide, ET-1, Endothelin-1; ACS, acute coronary syndrome; MI, myocardial infarction.

4.2.4 Nitric Oxide Synthases

There are three nitric oxide synthase enzymes including constitutive nitric oxide synthase (cNOS), inducible nitric oxide synthase (iNOS) and neuronal NOS (nNOS). The cNOS involves both neuronal and endothelial NOS which are constitutively expressed and are Ca^{2+} /calmodulin dependent enzymes^{301,302,303}. nNOS has been found to be localised in cerebral and peripheral neuron cells^{304,305}. This isoform has been shown to play a role in the regulation of the central nervous system where NO acts as a neurotransmitter associated with memory and learning functions^{301,306,307}.

The eNOS enzyme was first cloned from bovine and human endothelial cells and has been identified as a membrane bound homodimer with a molecular weight of 135kDa^{308,309}. eNOS is localised to the caveolae, specialised invaginated cytoplasmic vesicles that contain caveolin-1. The activity of eNOS is Ca^{2+} /calmodulin dependent and this isoform is induced by the release of calcium ions from subsarcolemmal storage sites via flow-dependent or receptor-stimulated NO formation.

iNOS is a Ca^{2+} independent protein which is only expressed during immune responses and is transcriptionally regulated by cytokines and lipopolysaccharides³¹⁰. iNOS can be activated by various inflammatory cells which secrete large amounts of NO which act as a "killer" molecule causing oxidative injury to the targeted cell³¹¹. This cytosolic enzyme is not compartmentalised and when iNOS is expressed, it produces a significantly greater amount of NO compared to the cNOS enzymes³¹².

4.2.5 Endothelial Nitric Oxide Synthase Gene

The eNOS gene is expressionally and functionally regulated through multiple regulatory steps^{313,314}. The structural organisation of the eNOS gene has been determined by genomic clones. This homodimeric enzyme is located on chromosome 7q35-36 with 1203 amino acids. The eNOS gene consists of 26 exons spanning 21 kilobases of genomic DNA, encoding a messenger RNA of 4052 nucleotides, and is present as a single copy in the haploid human genome. The ET-1 gene does not contain a TATA box however contains a number of cis-regulatory DNA sequences located near the transcription start site. These include specificity protein-1 (Sp1), globin transcription factor-1 (GATA), activating protein-1 (AP-1), nuclear factor-1 (NF-1), shear-stress response elements, and sterol-regulatory elements³⁰⁹. The promoter also contains a number of other putative binding domains that may be regulated by a number of transcription mediated signals. There are a number of physical and exogenous stimuli that can alter up-regulate eNOS gene expression at the mRNA, transcription or post-translation levels. At a molecular level the eNOS gene can be up-regulated by shear stress, transforming growth factor beta (TGF-beta), vascular endothelial growth factor, and hypoxia inducible factors. The down-regulation of eNOS is induced by inflammatory mediators such as tumour necrosis factor alpha (TNF-alpha), hypoxia, low density lipoproteins and proliferation²⁹³. In humans, hundreds of genetic variants of the eNOS human gene have been detected; however, only few variants have been reportedly associated with cardiovascular pathologies³¹⁵.

4.2.6 T-786C (eNOS) Polymorphism

The T-786C mutation is a functional variant located in the 5'- flanking region of the eNOS gene and is associated with a thymine to cytosine mutation at nucleotide position -786 (Figure 4.3). The detailed genetic properties of this allelic variation are referenced in the

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Geneworks National Centre for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/pubmed). In the literature, the T-786C polymorphism has been associated with several cardiovascular disease phenotypes, which include hypertension³¹⁶, ischemic heart disease³¹⁷, diabetes³¹⁸, acute coronary syndrome³¹⁹ and coronary artery spasm³²⁰. Nakayama et al.³²¹ found this polymorphism to be an independent risk factor for patients diagnosed with myocardial infarction and angiographically normal coronary arteries, suggesting this variant may be a marker of coronary spasm.

NOTE:
This figure is included on page 126
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Figure 4.3 eNOS Genome Structure Source: Zanchi et al.³²²

The T-786C polymorphism and other polymorphisms (intron 4a, G894T and intron 13) of the eNOS gene are indicated by arrows. *Abbreviations: Kb; kilobase, and bp; base pair.*

4.2.6.1 Functional Significance

This polymorphism is located in the promoter region of the eNOS gene, which is the region that modulates transcription of that particular gene. Miyamoto et al.³²³ used human umbilical

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vein endothelial cells from individuals carrying the T-786C polymorphism to investigate the regulation of eNOS gene activity. Reporter gene studies found the T-786C polymorphism modified gene expression by reducing the promoter activity of the eNOS gene. The molecular mechanism for suppressed eNOS gene transcription was determined by purifying a protein, RPA1 that is identical to a DNA binding protein, A1, which is found in vivo. A1 is a negative regulatory factor which binds the T-786C mutated sequence and consequently acts as a repressor protein to decrease gene transcription. Therefore individuals carrying the T-786C polymorphism may have a defective NO production pathway caused by altered eNOS gene expression³²³.

4.2.7 The Endothelins

The endothelins belong to a family of potent vasoconstrictor peptides that regulate vascular tone and blood pressure³²⁴. Three endothelin genes have been identified encoding three closely related peptides, Endothelin-1, Endothelin-2 and Endothelin-3³²⁵. ET-1 is known as the most potent endogenous vasoconstricting agent in the human coronary vasculature¹⁸². In addition to its long-lasting vascular effects, ET-1 plays a role in inhibiting cell proliferation, fibrosis and inflammation through its mitogenic activity³²⁶. ET-2 has also been reported to be constitutively expressed in the several cell lines such as the kidney and digestive system, however the precise role of this isoform remains unclear³²⁷. ET-3 is not synthesised by vascular endothelial or SMCs, but has been found in plasma and other tissues such as the heart, brain and adrenal gland³²⁸.

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Figure 4.4 Mechanisms of Signalling Induced by Endothelin-1 through the Endothelin-A Receptor. Source: Levin et al.³²⁷

ET-1 binds to the ET-A receptor on the vascular SMC and activates phospholipase C through a G-linked protein, causing the formation of diacylglycerol and inositol 1,4,5-triphosphate (IP₃) from phosphatidylinositol (PIP₂). The IP₃ directly stimulates the release of Ca²⁺ from the sarcoplasmic reticulum. The formation of diacylglycerol from PIP₂ activates protein kinase C, and these events lead to an increase in intracellular Ca²⁺ which results in vasoconstriction and cell proliferation. Alterations in ion channels also occur via activation of Na⁺-H⁺ anti-porter and deactivation of K⁺ channels. *Abbreviations: ETA, Endothelin-A Receptor; Na⁺, Sodium ions, K⁺, Potassium ions; H⁺, Hydrogen ions.*

4.2.8 Endothelin-1

ET-1 is a 21 amino acid peptide, originally isolated from culture supernatant of porcine aortic endothelial cells³²⁹. This vasoactive protein is initially synthesised by the vascular endothelium in the form of an inactive 212 amino acid precursor, preproendothelin-1

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(preproET-1), which is cleaved by a furin enzyme to form a 38 amino acid called Big ET-1³³⁰. The conversion of Big ET-1 to the biologically active ET-1 is catalysed by endothelin converting enzyme (ECE)³³¹, which cleaves the Tryptophan²¹-Valine²² bond to form the active ET-1³³². ET-1 is continuously released, mostly from endothelial cells, by a constitutive and regulated pathway³³³ for the regulation of vascular smooth muscle tone³³⁴. The constitutive pathway involves ET-1 being continuously released from secretory vesicles in the endothelium by a cAMP independent pathway. The regulated pathway involves ET-1 being stored in Weibel-Palade bodies and released when there is an influx of calcium or cAMP¹⁹⁰. Vascular smooth muscle contraction is stimulated by ET-1 via the IP₃ signalling pathway which regulates the opening of voltage-gated (L-type) Ca²⁺ channels, causing an increase in the concentration of intracellular Ca²⁺ (Figure 4.4).

The production of ET-1 is regulated at the gene level by various stimuli such as angiotensin II, TGF-beta, thrombin, interleukin-1, TNF alpha, lipoproteins, insulin, hypoxia, vascular endothelial growth factor and low shear stress. These physical and chemical stimuli can directly up-regulate ET-1 mRNA expression during inflammatory responses, by infiltrating cells such as neutrophils and lymphocytes³³⁵. Its production is inhibited by endothelium-derived NO, atrial natriuretic peptides, prostacyclin, prostaglandins and ET-3^{336,337,338,339}.

4.2.8.1 Endothelin-1 Receptors

ET-1 functions in an autocrine or paracrine manner through two G-protein-coupled subtypes of receptor isolated and cloned from mammalian tissues^{340,341}. The Endothelin-1 type A (ET_A) receptor is primarily located on vascular SMCs and interacts with ET-1 to produce constriction. The Endothelin-1 type B (ET_B) receptor is found on both endothelial cells and SMCs, where its role is to exert vasodilatory and vasoconstrictor responses, respectively^{342,343}.

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In the coronary vasculature, ET-1 released from the endothelial cells diffuses into to plasma membrane to activate both ET_A and ET_B receptors on vascular SMCs and mediate vasoconstriction.

4.2.8.2 Endothelin-1 and Endothelial Dysfunction

Considerable evidence suggests that ET-1 plays a key role in the development of endothelial dysfunction through binding to both ET_A and ET_B receptor types. The up-regulation of ET-1 in picomolar concentrations induces a long lasting and dose-dependent vasoconstriction¹⁸² and therefore it is not surprising that the altered production of this autocooid has been linked to several cardiovascular pathologies. For example, the underlying pathophysiology process of endothelial dysfunction appears to precede the onset of atherosclerosis where vasoconstriction can be seen at the site of plaque formation³⁴⁴. At present, the role of ET-1 in contributing to endothelial dysfunction in atherosclerotic large arteries is well documented⁹⁹; however, less is known about the functional effects of this protein in the microvasculature. In vivo evidence in mice has shown that over expression of ET-1 is associated with impaired NO-dependent vasodilatation in resistance vessels, thus suggesting ET-1 may play a role in microvascular endothelial dysfunction³⁴⁵.

4.2.8.3 Endothelin-1 Gene

In 1989, the full length of the human preproET-1 gene was cloned, as well as the corresponding cDNA to determine the complete nucleotide sequence. The human preproET-1 mRNA consists of 2026 nucleotides, excluding the poly(A) tail, which is encoded by the human 6836 base pair ET-1 gene³²⁵. The ET-1 gene is localised on human chromosome 6 and is composed of five exons and four intervening sequences³⁴⁶. The first Exon 1 contains the whole 5'-untranslated region and the coding sequence for the first 22 amino acid residues of

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proendothelin-1. The second exon includes sequences corresponding to the 21 amino acids of endothelin. The third exon encodes the endothelin-like peptide and the fourth exon contains sequences for preproET-1 residues 131-178. The fifth exon contains the AATAAA sequence of preproET-1 near the 3' un-translated region. The five exons span approximately 5.5kb and consist of a 636 nucleotide open reading frame encoding preproET-1, which is flanked by an approximately 250 nucleotide 5' un-translated region and a 1127-nucleotide 3' un-translated region (Figure 4.5). At the 5' end of the gene is a TATAAA sequence, commonly known as the TATA box, which is involved in RNA polymerase II binding of DNA. The promoter region of this gene is believed to be located 65 base pairs 5' from the TATAAA sequence which codes a CAAT sequence. Other regulatory elements upstream from the promoter region include GATA binding protein-2³⁴⁷ and AP1³⁴⁸ protein binding sites which can induce gene transcription³⁴⁹. At the 3' end there is an AATAAA sequence which represents the sequence that codes for the poly A tail signal on the mRNA. Furthermore, in the 3' end of the first exon there is a ten base pair sequence which confers with TGF- β and may induce transcription of the ET-1 gene^{346,350}. DNA sequencing studies have identified two single main genetic variants of the ET-1 gene, +138 deletion/insertion (+138 del/ins) and Lys198Asn, which are both associated with cardiovascular disease³¹⁵.

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Figure 4.5 Genome structure of human endothelin-1. Modified from Banno et al.³⁵¹

The arrows indicate the direction of the sequencing, and the shaded sections in exons 1 and 2 represent the sequences that code for the mature ET-1 protein. The +138 del/ins variant is located in exon 2 which codes the preproET-1 gene. The Lys198Asn polymorphism is another common variant located in exon 5 of the ET-1 gene.

4.2.8.4 +138 Deletion/insertion polymorphism

A genetic variant located 138bp downstream from the transcription site in the 5' un-translated region from the preproET-1 gene in exon 1 is known as the +138 del/ins (-3A/-4A) polymorphism. This mutated sequence involves the insertion of an extra adenine nucleotide in the DNA sequence, therefore changing the nucleotide sequence from AAA' to AAAA'. A literature search found this polymorphism to be associated with hypertension^{351,352}. In addition, Banno et al.³⁵¹ found elevated ET-1 levels in hypertensive patients who were carriers of the +138 del/ins variant, indicating the presence of impaired endothelial function. Furthermore, impaired endothelium-dependent vasodilatation has been successfully demonstrated in hypertensive patients³⁵³, which suggests the polymorphism may play a role in mediating the resultant phenotype of this disease. More recently, a study by Lee et al.³⁵⁴ found the 4a haplotype is associated with variant angina in Caucasians, whilst the 3a haplotype is protective against the disease. These findings suggest this polymorphism may be a marker of impaired coronary vasomotor reactivity.

4.2.8.5 Functional Significance

Popowski et al.³⁵⁵ used human vein umbilical cord cells, from homozygotes with the 4a allele and heterozygotes carrying the 3a allele, to observe the gene expression. The cells containing the +138 del/ins variant (4a allele) showed changes in the production of Big ET-1, which subsequently lead to increased expression of the active ET-1 protein. The authors of this study concluded that the increased ET-1 expression found in homozygotes with the 4a allele was due to enhanced preproET-1 mRNA stability. In absence of the polymorphism, the half-life of preproET-1 mRNA is short and degrades within seven seconds. Reporter gene studies found that the half-life of preproET-1 mRNA from cells containing the polymorphism was increased by 20 seconds (total stability of 27 seconds before being degraded)³⁵⁵. Therefore if the

stability of the mRNA protein is increased, the transcription of the protein is also increased, resulting in more ET-1 production. Furthermore, a study by Tanaka et al.³⁵² found that gene expression of Big ET-1 was increased in the 4a allele (polymorphic) carriers compared to the 3a (wild-type) allele carriers in hypertensive patients, indicating gene expression is modified by this mutation.

4.2.9 Other Gene Polymorphisms from the ET-1 and eNOS Genes

Several SNPs of the ET-1 gene have been identified and associated with cardiovascular and non-cardiovascular disease phenotypes. However, most association studies lack biological plausibility, which makes it difficult to directly relate SNPs with phenotypes. In regard to cardiovascular phenotypes, the most frequent SNP investigated from the ET-1 gene is Lys198Asn located on exon 5 near the carboxyl terminal region (Figure 4.5). The Lys198Asn SNP has been associated with high systolic blood pressure in overweight individuals^{356,357}. Unfortunately, most of the findings related to the Lys198Asn SNP are isolated and have not been independently replicated. In addition, there is no data which demonstrates any functional relevance of the Lys198Asn, and therefore the interpretation of these studies remains unclear. It is for these reasons we chose to exclude the Lys198Asn polymorphism from our study selection. Furthermore there are several polymorphisms from the Endothelin Converting Enzyme (ECE) gene and the ET_A and ET_B receptor genes which have been genotyped in frequency studies³⁵⁸. However, most of these SNPs have been associated with non-cardiovascular phenotypes and there is also a lack of association studies, which makes these gene polymorphisms unsuitable candidates for studies in vascular disorders.

Similar to ET-1, there are two polymorphisms from the eNOS gene which are commonly associated with cardiovascular disease. These include the T-786C polymorphism and the

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G298T missense mutation (Figure 4.3). The G298T mutation is located in exon 7 and encodes an amino acid change from glutamate to aspartate. Like the T-786C polymorphism, the G298T polymorphism from the eNOS gene is another variant which has been genotyped in patients with the CSFP. Caglayan et al.³⁵⁹ recently showed a lack of association between the G298T polymorphism and the CSFP, although ethnic variation of the population may account for the above finding. Nevertheless the negative results of one study should not set the precedence for future studies, as conflicting results in the literature commonly exist. Furthermore, this variant has been speculated to modify eNOS gene activity by increasing the risk of proteolytic cleavage³⁶⁰. The exact underlying molecular mechanism however has yet to be demonstrated and thus its biological plausibility is yet to be established.

Another mutation from the eNOS gene is the 4ab variant which is a 27 bp tandem repeat in intron 4 which has been associated with a smoking dependent risk of CAD³⁶¹. The eNOS4ab polymorphism has been shown to affect NO synthase promoter activity³⁶² and may also alter plasma concentration of NO metabolites³⁶³. However, the intron 4ab polymorphism is reported to modulate the functional effects of the T-786C variant in linkage disequilibrium,³⁶² hence the intron 4ab variant mimics the functional effects of the T-786C polymorphism and influences the promoter function per se (Figure 4.3).

4.2.10 Endothelial Dysfunction and The Coronary Slow Flow Phenomenon

To date only a limited number of studies have focused on the etiology of the CSFP; however, endothelial dysfunction has been implicated in the underlying pathogenesis. A study by Hirata et al.¹⁶⁰ investigated the role of ET-1 on the coronary vasculature in rabbits and found that ET-1 produced no evident constriction in the large epicardial arteries, but demonstrated delayed filling of contrast medium into the distal coronary artery. This suggests that ET-1

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may induce a long lasting contraction and diffuse constriction of small coronary arteries. Turner et al.³⁶⁴ assessed endothelial reactivity in human subcutaneous microvessels and discovered an increased sensitivity to ET-1 in the CSFP patients compared with controls. This was not evident with other agonists such as serotonin, noradrenalin and thromboxane. These findings suggest vascular function in CSFP patients can be altered by the effects of ET-1, which can potentially lead to microvascular dysfunction. Clinical studies conducted by Sezgin et al.¹⁰⁶ found reduced plasma NO levels, in conjunction with impaired flow-mediated dilatation, in patients with the CSFP compared to controls. Camsari et al.¹⁶³ also showed increased ET-1 production and decreased NO plasma levels in 25 CSFP patients compared with healthy subjects. Beltrame et al.³⁶⁵ reported increased systemic levels of ET-1 in CSFP patients and a positive association between higher ET-1 levels with increasing angina frequency, suggesting a possible correlation between ET-1 and angina severity in CSFP patients.

4.2.11 Study Objectives

The primary objective of this study is to evaluate the frequencies of the (eNOS) T-786C and (ET-1) +138 del/ins single nucleotide polymorphisms in patients with:

- (a) non-obstructive CAD and
- (b) the CSFP.

The secondary objective is to examine the cardiovascular risk associated with these gene polymorphisms in both NoCAD and the CSFP. The current study was conducted to test the following null hypotheses:

- (2) There will be no difference in the gene frequencies of the (eNOS) T-786C and ET-1 +138 del/ins SNPs between cases (NoCAD and CSFP patients) and the control groups.

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There will be no increase in disease risk associated with each SNP.

4.3 Methods

4.3.1 Study Design

This case-control study design used a targeted gene approach to determine if there is an association between the SNP and the mutation. A case-control study is advantageous as it enables a larger number of controls to be recruited to minimise the effects of population stratification. Most importantly, case control studies possess a greater power to identify genetic variants with modest effect when compared with linkage studies³⁶⁶.

4.3.2 Selection of Candidate SNP's

In selecting the T-786C and +138 del/ins polymorphisms from the eNOS and ET-1 genes respectively, we first searched the literature and found evidence of endothelial dysfunction associated with these SNPs. The next step was to discover whether the SNP had biological plausibility by again reviewing the literature. The target SNPs were selected on the basis of the potential biological mechanism. Biological plausibility is the most important criterion for SNP selection, which describes how the expression of the gene product is altered by the presence of the chosen SNPs. Both the T-786C and +138 del/ins SNPs were found to functionally alter the expression of each gene—these functional studies are previously described in this chapter (see sections 4.2.6.1 and 4.2.8.5). The genomic sequence containing the SNP was needed for oligonucleotide primer design. The chosen SNPs were listed on the Entrez SNP database (<http://www.ncbi.nlm.gov/SNP>).

4.3.3 Sample Size Estimation

Table 4.1 summarises the reported frequency for the study SNPs and the estimated sample size based upon a 2:1 prevalence of the polymorphism in the NoCAD cohort compared with controls, at 80% power with an alpha of 0.05. As the genotype distribution may vary between ethnic groups preference was given to studies performed on Australian or Caucasian populations. Genotype frequency data from previous studies gave us an indication of the probability of exposure among controls and cases. SNPs were considered as candidates if a sample size of up to 550 patients was calculated, as this number was a feasible target given the clinical resources and recruitment time available. Hence the sample size for ET-1 +138 del/ins polymorphism was too large and therefore not a suitable candidate for genotyping. However, we still chose to investigate the frequency of the ET-1 +138 del/ins SNP in our cases and report the results as a pilot study.

Table 4.1 Sample size estimations for the +138 del/ins and T-786C polymorphisms based on genotype frequency data in a Caucasian population

Polymorphism	Frequency (%)	Ratio	Sample (80% power at 0.05)	Reference
+138 del/ins	8.8	2:1	549	Charron et al. ³⁶⁷
+138 del/ins	7	2.1	708	Popowski et al. ³⁵⁵
T-786C	14.9	2.1	294	Granath et al. ³⁶⁸
T-786C	19.2	2:1	213	Rossi et al. ³⁶⁹

4.3.4 Patient Recruitment

Participants were selected from an epidemiological angiographic study at The Queen Elizabeth Hospital, which consisted of a cohort study of 1249 men and women aged 21-80 years and from South Australia. A total of 1144 patients were selected from the angiographic

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study who were subsequently diagnosed with either obstructive or non-obstructive CAD. From August 2007 to November 2008, these patients were invited to participate in the present study and venesection performed for genotyping following completion of written informed consent.

4.3.3.1 Study Population

The categorisation of patient groups was determined by the angiographic data and clinical information. Based on a patient's angiographic finding and clinical presentation, subjects were categorised into three groups:

- 1) Obstructive CAD:
 - chest pain
 - $\geq 50\%$ stenosis in one or more epicardial vessel
- 2) NoCAD:
 - chest pain
 - $< 50\%$ stenosis in one or more epicardial vessel
- 3) The CSFP:
 - ≥ 3 beats for contrast to opacify an epicardial vessel (TIMI II flow)
 - $< 50\%$ stenosis in one or more vessels.

Patients with the CSFP were identified during angiography and for accuracy, the angiographic data was re-reviewed by a blinded observer. A case report including demographic information, risk factors, current medications prescribed and medical history was filled out for all patients.

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Exclusion Criteria

Patients with evidence of valvular heart disease, pericarditis, cardiomyopathy and diminished mental capacity were excluded in this study.

4.3.3.2 Recruitment of Healthy Control Subjects

Healthy subjects from another study³⁷⁰ involving cerebral vascular disease were recruited for this study. The names and contact details of controls were obtained from four hospitals in metropolitan Adelaide, South Australia: The Queen Elizabeth Hospital, The Lyell McEwin Health Service, Flinders Medical Centre and The Royal Adelaide Hospital.

Inclusion Criteria were:

- (a) age range between 18-75 years
- (b) no psychological disability
- (c) no pregnancy or breast feeding patients
- (d) no history of hypertension, heart disease or cerebral vascular disease.

Healthy control subjects were contacted via mail to participate in this study and were given a choice to consent to the study by sending a letter back to the researcher. Selected controls were contacted by telephone by a professional interviewer who initially screened them for the study and then upon verbal agreement an appointment was organised for them to be seen in the outpatient department at The Queen Elizabeth Hospital.

4.3.5 Genotyping Procedure

4.3.3.3 DNA extraction

DNA from 10ml of whole blood and was either extracted immediately or stored at room temperature overnight before extraction. Extracting DNA involves a two-step process:

1. separation of lymphocytes and
2. precipitation of DNA.

The following reagent mixture was prepared in advance and used in the first stage of extracting DNA.

Dulbecco's Phosphate Buffered Saline (PBS)

NaCl (sodium chloride) 8.00g

KCl (Potassium chloride) 0.20g

Na₂HPO₄ (Disodium hydrogen orthophosphate) 1.15g

KH₂PO₄ (Potassium dihydrogen orthophosphate) 0.20g

Dissolved in double distilled water to 1.00L and adjust pH to 7.4

a) Separation of Lymphocytes

- 1) Genomic DNA was isolated from circulating lymphocytes firstly by separating the red blood cells on lymphocyte separation medium (LSM) (M.P. Biomedicals).
- 2) 2ml of whole blood was added to a conical end tube and 5ml PBS solution was added. The tube was mixed by inversion.
- 3) 2ml of LSM was inserted by a pipette under the blood/PBS mixture to create two distinct layers.
- 4) The tube was centrifuged at 1230rpm for 20 minutes allowing red blood cells to pass into the LSM layer, leaving the mononuclear cells as a buffy layer at the interface between the blood and LSM.

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- 5) The buffy layer was removed by aspiration and transferred to a 10ml tube. 10ml of PBS was added and the tube was centrifuged at 1230rpm for ten minutes to wash the mononuclear cells.
- 6) The PBS was decanted and the white cell pellet was re-solubilised in the remaining fluid.

b) Precipitation of DNA

1. 100 µl pelleted cells were added to 1mL DNazol (Molecular Research Centre Inc, USA) in a 2ml microtube (Scientific Specialities Inc, Ca, USA).
2. DNA is precipitated by adding 500µl of 100% ethanol and mixing thoroughly by inverting the tube and then centrifuging for two minutes. The DNA is insoluble in the alcohol and will come out of solution, and the alcohol serves as a wash to remove DNazol and any cellular or histone protein bound to the DNA.
3. After centrifugation the ethanol was poured off and the DNA pellet was washed again with 100µl of 75% alcohol and centrifuged for two minutes. This step was repeated once more.
4. All excess alcohol was removed using a fine tip 100µl pipette and 200µl of 8mM NaOH was added to each microtube to re-suspend the DNA pellet.

The DNA solution was stored at 4°C until required for genotyping.

4.3.3.4 Genotyping Methods

A polymerase chain reaction- sequence specific primer (PCR-SSP) method was used to detect the presence or absence of an SNP. Sequence-specific DNA primers are designed to anneal at the 3'-nucleotide end to an SNP present in the allele of interest, allowing amplification if the

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polymorphism is present. A consensus primer is designed to bind to a non-polymorphic region of the opposite strand of DNA, within 300-400 base pairs of the SNP. Taq polymerase (Taq 1000, Applied Biosystems) inhibits the repair of 3'-terminal primer nucleotide, so that only a primer that is annealed well at the 3' end can be amplified. PCR-SSP requires a positive control added to each reaction tube, to detect failed versus negative PCR reactions. Two additional oligonucleotide primers were used in multiplex reactions that generated a 600bp amplicon from the HLA-DRB3 gene and this was therefore used as the positive control. PCR-SSP was performed in 96-well PCR plates (Corbett Research) using a Peltier PTC-200 thermal cycler.

4.3.3.5 Oligonucleotide Primer Design

Oligonucleotide primers were designed for SNPs using sequences obtained from the Entrez Human Genome public database (www.ncbi.nlm.nih.gov/SNP) and were manufactured locally (Geneworks Pty Ltd, Adelaide, South Australia). An SNP dependent nucleotide was placed on the 3'-terminal nucleotide of the primer in the eNOS SNP.

For the ET-1 mutation, we designed a wildtype (WT) primer which ended in 3A + G. The insert primer ended with 4As and G, because we could not identify the 'hotspot' by the same mechanisms as eNOS (i.e. an altered terminal nucleotide). The oligonucleotide primer sequences, genbank accession codes and PCR product sizes are listed in Table 4.2. A BLAST search was performed on all oligonucleotide primer sequences (www.ncbi.nlm.nih.gov/BLAST) and checked for minimal cross-reactivity with other DNA regions and also for lack of self-adherence.

Table 4.2 Oligonucleotide Primer sequences, Genebank Accession numbers and PCR Product sizes

SNP	Primer Sequence	Genebank Accession No.	Position (5'3')	Primer size	Product size
+138 del/ins					
D allele	CTT TTT CTC CCC GTT AAA G	AY434104	2002-2021	19	305
I allele	CCT TTT TCT CCC GTT AAA AG		2002-2021	20	
Consensus	ACG GGG AAA AAT AGA AGC AAG		2306-2286	21	
T-786C					
T allele	CAT CAA GCT CTT CCC TGG CT	AF519768	1113-1132	20	335
C allele	CAT CAA GCT CTT CCC TGG CC		1113-1132	20	
Consensus	TGC AGG TTC TCT CCT TCA CC		1447-1428	20	

4.3.6 PCR-SSP Method

4.3.3.6 Oligonucleotide Primer Preparation

The final working concentrations and manufacturers of the reagents for a control primer stock containing the HLA-DRB3 positive control primers are listed below in Table 4.3. SNP-specific primers were added the HLA-DRB3 positive control primers. A primer optimisation was initially performed to determine the SNP-specific primer concentration that achieved amplification of the polymorphic DNA sequence. The final primer concentrations are listed in Table 4.4.

Table 4.3 HLA-DRB3 Positive Control Primer Stock

Cresol red	62µg/ml	(Fluka)
HLA-DRB3 forward primer	5µg/ml	(GeneWorks)
HLA-DRB3 reverse primer	5µg/ml	(GeneWorks)

Table 4.4 Primer Reaction Mix for each SNP

Polymorphism	Allele-Specific Primer (µg/ml)	Consensus Primer (µg/ml)
eNOS (T-786C)	20	20
Et-1 (Ins/Del)	10	10

5µl of allele specific and consensus primers was added to each well and the 8µl of DNA solution was added to each well containing 5µl of allele specific consensus primers. A pink colour change confirmed the presence of DNA in the PCR reaction mix of a 96 well PCR plate. Lastly, 10µl of paraffin oil was added to each well to minimise primer and reagent

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evaporation during PCR thermal cycling. A plastic sealing sheet was also used to cover the 96 well plate.

4.3.3.7 DNA Preparation

The following reagent mixtures were prepared in advance and used in the preparation of DNA. The final concentrations are also listed.

1. 10X PCR buffer:

- 670mM Tris Base
- pH Tris base to 8.9 with concentrated HCl
- dissolve ammonium sulphate to give a 166mM final concentration
- add 1% v/v Tween 20 (polyoxyethylenesorbitanmonolaurate—BDH Laboratory Supplies). The solution was then aliquoted and stored at -70°C.

2. TMDH Mixture

- 6ml 10x PCR buffer
- 6ml 10mM dNTP (nucleotides)—(Promega Corporation)
- 5.1 ml 25mM Magnesium Chloride—(Applied Biosystems)
- 6ml autoclaved, milli-Q water

3. The DNA solution was prepared using the following protocol:

- 100µl TMDH
- 57µl autoclaved, deionised water
- 4.25 Units AmpliTaq DNA polymerase—(Applied Biosystems)
- 3µl genomic DNA.

4.3.3.8 PCR Thermal Cycling

PCR was performed using a PTC-200 Peltier Thermal Cycler—(MJ Research).

The PCR cycling stages are stated below:

Five cycles of: 96°C for 25 seconds 70°C for 45 seconds 72°C for 45 seconds.

Twenty-one cycles of: 96°C for 25 seconds 65°C for 50 seconds 72°C for 45 seconds.

Four cycles of: 96°C for 25 seconds 55°C for 60 seconds 72°C for 125 seconds.

4.3.7 Gel Preparation

The 5X TBE (Trizma, Boric acid, EDTA) solution was made by up by first adding 600ml of H₂O to dissolve the ingredients. The final volume of the solution was adjusted to 1L using H₂O and the pH was modified to 8.3 using 10M NaOH. The 5X TBE was diluted using a ratio of 1 in 10 to make 0.5X TBE solution. The agarose gel was made by combining 4g agarose with 300ml of 0.5X TBE solution in a conical flask. The flask was heated in microwave on high for one minute, gently swirled; this step was repeated until the agarose was colourless and dissolved. The agarose solution was then cooled to 55°C in a controlled cabinet for approximately 45-60 minutes. Once the agarose solution had cooled, 40µl of Ethidium bromide was added to the flask and gently dispersed throughout the solution. The agarose gel was poured onto a plate and allowed to solidify at room temperature, with combs in place to form the sample wells in the solidified gel (approximately 30 minutes)—see below for 5X TBE solution.

5X TBE solution Reagents

53.9g Trizma base

27.1g Boric acid

1.865g EDTA

800ml Purified water

4.3.3.9 Genotype Determination

PCR-SSP products were pipetted on 1.33% agarose gel, electrophoresed for 30 minutes at 100 volts and visualised under ultra violet light³⁷¹. Gels were photographed using a high resolution digital camera (Olympus ColourPixX990) and photographs were analysed to visually determine the genotype. Figure 4.6 illustrates the genotype determination of the two SNPs in two subjects.

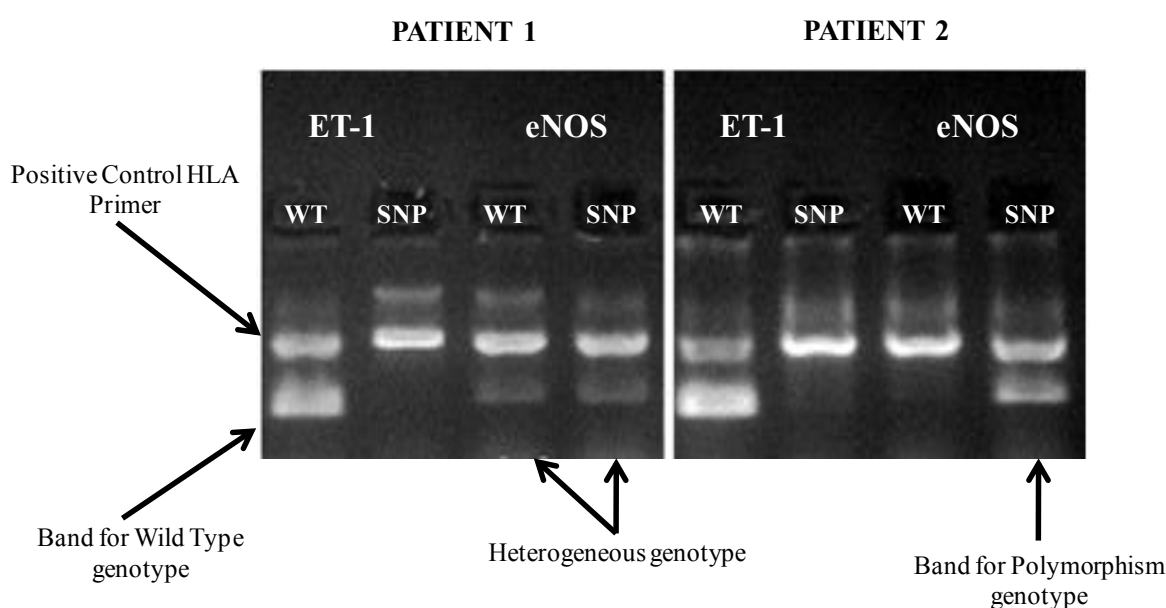


Figure 4.6 The PCR-SSP products on a gel showing genotype determination of ET-1 and eNOS genotypes in 2 subjects

The well illustrating ‘_WT’ represents the wild-type allele for either ET-1 or eNOS. The well illustrating ‘_SNP’ represents the single nucleotide polymorphism allele for each of the two genes. The larger sized positive control band indicates a successful PCR reaction. In patient 1, the presence of the WT band for ET-1, in the absence of the SNP band, indicates that the subject is homozygous WT for ET-1. The presence of both WT and SNP bands indicates that the subject is heterozygous indicating that both alleles are present in the eNOS genotype. In patient 2, the absence of the eNOS WT band indicates that this patient is homozygous for the mutant allele (SNP) for eNOS, and also a homozygous WT carrier for ET-1.

4.3.8 Quality Assurance Measure

There were two quality assurance measures performed to determine if our genotype determination using SSP-PCR was accurate:

1) Reproducibility

To test for reproducibility, SSP-PCR was repeated in 50 subjects randomly chosen for genotyping. The results obtained from the repeated genotyping were comparable and thus this method was found to be reliable and reproducible.

2) Sequence Confirmation

PCR products from both ET-1 and eNOS genes were sequenced to confirm if amplification of the DNA polymorphic sequence was specific for each gene. The PCR products of one forward primer and the consensus primer of each polymorphism were purified using ExoSAP-it (USB/Affymetrix) as per manufacturer clean up procedure. The concentration of the 'clean' product was determined using a UV NanoDrop Spectrophotometer (Thermo Scientific).

Next a Big Dye Terminator Reaction mix PCR was performed. The sample was prepared as follows:

- 0.1 μg primer
- 0.2 1 μL Big Dye Terminator dye mix
- 0.3 3.5 μL 5x Sequencing buffer
- 0.4 20-100 ng clean PCR product (determined by Nano Spectrophotometer)
- 0.5 To 2- μL with autoclaved, deionised water.

The sample was then placed into the thermal cycler for 25 cycles of 96°C of one session. After washing with 75% isopropanol, the higher molecular weight Big Dye Terminator

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reaction products were air-dried and sent to the Institute of Medical and Veterinary Science, Molecular Biosciences Laboratory, Adelaide, for gene sequencing using microcapillary electrophoresis. Product sequences for both polymorphisms were compared to the original NCBI Entrez Nucleotide sequence and were found to be accurate.

4.3.9 Statistical Methods

SPSS for windows (release 16.0; SPSS, Inc) was used for statistical analyses. Continuous variables were analysed by independent sample t-tests and categorical variables were presented as percentages and were compared using Fisher's Exact test. Odds ratio and 95% confidence intervals were determined using logistic regression analysis. Univariate analyses were adjusted for potential confounders between the groups in order to determine adjusted odds ratios for the genotype frequencies. The chi-squared test was used to compare the observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium.

4.4 Results

A total of 520 patients who had undergone coronary angiography were retrospectively asked to participate in this study. Approximately 30% of patients refused consent due to the travelling distance required for blood collection, 20% cited ethical issues with having their DNA tested and 10% cited health issues for refusal. The study population consisted of 44 non-obstructive, 18 CSFP patients, CAD patients, 131 obstructive CAD and, 45 health control subjects. The case groups were patients with:

- (a) non-obstructive CAD and
- (b) the CSFP.

The controls were patients with:

- (c) obstructive CAD and
- (d) healthy subjects.

4.4.1 Clinical Characteristics Comparisons

The characteristics and demographics of all patients are shown in Table 4.5. The NoCAD patients were likely to be females whereas the CSFP and obstructive CAD groups were predominately male dominated. The NoCAD and CSFP groups had a greater incidence of hypertension and hypercholesterolemia compared to the healthy subjects. In addition, patients with obstructive CAD had more atherogenic risk factors such as hypertension, hypercholesterolemia and diabetes mellitus in comparison to the other clinical groups, including the healthy controls. There were no statistical variations between co-morbidities and each subject group (Table 4.5).

4.4.2 Genotype Frequency Distribution

The genotype distributions and allele frequencies of the eNOS T-786C and ET-1 +138 del/ins polymorphisms are shown in Table 4.6 and Table 4.7, respectively. No deviation from the genotype distributions predicted by the Hardy-Weinberg equilibrium was observed between the cases and control subjects. The genotype distributions or allele frequencies of the eNOS T-786C polymorphism did not significantly differ between cases or each control group. On the other hand, there was a statistical difference in the homozygosity for the ET-1 +138 del/ins polymorphism between CSFP patients and healthy control patients (28% vs.2%), and CSFP patients and obstructive patients (28% vs.5%) (Table 4.7).

4.4.3 Gene Odds Ratios

The odds ratio for examining the association between the genotype frequencies in the case groups was adjusted for confounders. There were no statistical differences between the NoCAD patients and genotype frequencies in either recessive or dominant models of statistical analysis (Table 4.8). When comparing the frequency of the II' homozygotes versus the combined II and DD homozygotes (II vs. ID+DD, recessive model), the odds ratio for the CSFP group was 16.9(1.8-158.2) (Table 4.9). However, when comparing the allele frequencies of the ET-1 polymorphism between the CSFP patients and the control group, there was no statistical difference found, which implies that the double I' homozygous genotype is associated with the CSFP. In both the recessive and dominant models of statistical analysis, the eNOS genotype frequency showed no statistically significant differences between the cases and control groups.

Table 4.5 Clinical and Demographic Characteristics of the Subject Populations

	OCAD (n=131)	Healthy Subjects (n=45)	NoCAD (n=44)	CSFP (n=18)
	Mean ± SD/ (n)%	Mean ± SD/ (n)%	Mean ± SD/ (n) %	Mean ± SD/ (n)%
Demographics & Risk Factors				
Age (years)	63 ± 10*	47 ± 11	58 ± 12	60 ± 15^
Female	(34) 26%**	(26) 58%††	(31) 70%	(8) 44%
Hypertension	(85) 65%***#	(2) 4%††	(17) 39% ∞∞∞	(6) 33%^^
Hypercholesterolemia	(95) 73%*	(0) 0%††	(23) 52% ∞∞∞	(10) 56%^^
Diabetes Mellitus	(47) 36%***#	(0) 0%††	(7) 16%	(0) 0%
Current Smoker	(26) 20%	(7) 16%	(5) 11%	(2) 11%
Ex Smoker	(60) 46%	(15) 33%	(15) 34%	(10) 56%
Co-Morbidities				
Stroke	8 (6.3%)	0 (0%)	4 (9%)	1 (6%)
Airways Disease	25 (20%)	0 (0%)	10(23%)	5 (28%)
Gastrointestinal Disease	29 (23%)	0 (0%)	6 (14%)	5 (28%)
Renal Disease	9 (7%)	0 (0%)	1 (2%)	3 (17%)
Liver Disease	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Cancer	3 (2%)	0 (0%)	2 (5%)	1 (6%)
Arthritis	14 (11%)	0 (0%)	9 (21%)	5 (28%)
Psychiatric Disorders	15 (12%)	0 (0%)	5 (11%)	1 (6%)

*p <0.05, ** p <0.01; Obstructive vs. Non-obstructive CAD

†p <0.05, †† p <0.01; Obstructive vs. Healthy Subjects

#p <0.05, ## p <0.01; Obstructive vs. CSFP

^p <0.05 ^^ p <0.01; CSFP vs. Healthy Subjects

∞ ∞∞p<0.05; Non-Obstructive CAD vs. Healthy Controls

Abbreviations: OCAD; obstructive CAD, NoCAD; Non-obstructive CAD

Table 4.6 Genotype and Allele Frequency Data for eNOS

eNOS T/C genotypes	CSFP (n=18)	Healthy Controls (n=45)	OCAD (n=131)	NoCAD (n= 44)
TT	6 (33%)	12 (27%)	48 (37%)	16 (36%)
CT	9 (50%)	21 (47%)	55 (42%)	20 (45%)
CC	3 (17%)	12 (27%)	25 (19%)	8 (18%)
Allele (n%) T	58%	50%	59%	59%
Allele (n%) C	42%	50%	41%	41%

Abbreviations: CSFP; Coronary Slow Flow Phenomenon, OCAD; Obstructive CAD, NoCAD; Non-obstructive CAD; 'T' denotes the wild type allele; 'C' denotes the mutant allele

Table 4.7 Genotype and Allele Frequency Data for Endothelin-1

ET-1 D/I genotypes	CSFP (n=18)	Healthy Controls (n=45)	OCAD (n=131)	NO CAD (n= 44)
DD	8 (44%)	24 (53%)	76 (58%)	25 (57%)
DI	5 (28%)	20 (44%)	49 (37%)	14 (32%)
II	5 (28%)**	1 (2%)	6 (5%)	5 (11%)
Allele (n%) D	58%	76%	77%	73%
Allele (n%) I	42%	24%	23%	27%

*p≤0.05, **p≤0.01

Abbreviations: CSFP; Coronary Slow Flow Phenomenon, OCAD; Obstructive CAD, NoCAD; Non-obstructive CAD; 'D' denotes the wild type allele; 'I' denotes the mutant allele

Table 4.8 Adjusted Odds Ratios between Healthy Controls and the Non-Obstructive CAD patients

Genotype (eNOS)	Healthy Controls (n=45)	NoCAD (n=44)	Odds ratio (95%CI)	P
Dominant model TT vs TC/CC	21:24	28:16	0.5 (0.2-1.1)	0.08 N/S
Recessive model TT/TC vs CC	33:12	39:5	0.3 (0.1-1.1)	0.06 N/S
Genotype (Et-1)	Healthy Controls (n=45)	NoCAD (n=44)	Odds ratio (95%CI)	P
Dominant Model DD vs DI/II	24:21	19:25	1.5 (0.5-2.5)	0.2 N/S
Recessive Model DD/DI vs II	44:1	39:5	5.6(0.6-50.0)	0.09 N/S

Abbreviations: NoCAD, Non-obstructive CAD; 'T' and 'D' denotes the wild type allele; 'C' and 'I' denotes the mutant allele

Table 4.9 Adjusted Odds Ratios between Healthy Controls and the patients with the Coronary Slow Flow Phenomenon

Genotype (eNOS)	Controls n=45	CSFP n=18	Odds ratio (95%CI)	P
Dominant model TT vs TC/CC	12:33	6:12	0.7 (0.2-2.4)	0.7 N/S
Recessive model TT/TC vs CC	33:12	15:3	0.5 (0.1-2.2)	0.5 N/S
Genotype (Et-1)	Controls n=45	CSFP n=18	Odds ratio (95%CI)	P
Dominant Model DD vs DI/II	24:21	8:10	1.49 (0.5-4.3)	0.5 N/S
Recessive Model DD/DI vs II	44:1	13:5	16.9 (1.8-158.2)	0.005**

**p≤0.01. Abbreviations: CSFP, Coronary Slow Flow Phenomeonon; 'T' and 'D' denotes the wild type allele; 'C' and 'I' denotes the mutant allele

4.5 Discussion

To our knowledge, the (ET-1) +138 del/ins SNP has never been investigated in patients with NoCAD or the CSFP. The association between the (eNOS) T-786C SNP and the CSFP has previously been investigated in other ethnic groups but not in an Australian population. In the present study, we did not find an association between NoCAD and the T-786C or +138 del/ins SNPs; however, we found a strong association between the +138 del/ins polymorphism of the ET-1 gene and the CSFP. This increased risk was confined to individuals homozygous for the insertion variant. Conversely, we found no association between the T-786C SNP from the eNOS gene and the CSFP. The +138 del/ins and the T-786C genotype distributions in this study population matched the Hardy Weinberg equilibrium, indicating that our study method was appropriate.

4.5.1 +138 Deletion/insertion Polymorphism

The +138 del/ins mutation may be a putative candidate for the CSFP and may initiate the process of microvascular endothelial dysfunction by enhancing preproET-1 mRNA stability, which leads to an increased expression of the ET-1 protein. ET-1 has an important role in regulating vascular tone in both the large and small vessels of the coronary vasculature³⁷². An increase in ET-1 production could predispose patients to microvascular dysfunction or spasm, by altering endothelium-mediated vasoconstriction in the small coronary resistance arteries¹⁵⁹. There is evidence demonstrating that the ET-1 +138 del/ins polymorphism is responsible to variations in the genetic control of the plasma concentration of ET-1 in patients with endothelial dysfunction³⁵². Whilst we did not measure circulating blood ET-1 levels in this study, other investigators have reported elevated plasma ET-1 levels in patients with the CSFP^{373,374}. At present, it is not clear whether raised circulating plasma ET-1 influences local

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receptor activation or attenuates the constricting effects of other vasoactive hormones such as serotonin or noradrenalin to increase the constrictor response³⁷⁵.

Several observations have demonstrated an underlying ischemic substrate associated with coronary slow flow³⁷⁶. During ischemia, studies have found endothelium-dependent smooth muscle relaxation is more impaired in the distal part compared to the proximal part of these arteries³⁷⁷. ET-1 mediated vasoconstriction has been shown to occur at the microvascular level via ET_A and ET_B receptor-mediated responses³⁷⁸. Increased ET-1 levels can also potentiate the vasoconstriction caused by various other proteins such as norepinephrine, catecholamines and serotonin, which in turn heighten the effects of ET-1^{375,379}. In addition, the release of ET-1 in the coronary circulation has been shown to be increased during the acute phase of myocardial ischemia³⁸⁰. It would be interesting to examine whether ET-1 levels vary between the acute and quiescent stage of this microvascular disease, as this would determine whether ET-1 responses are altered during different stages of the disease process.

The importance of ET-1 in the pathophysiology of CSFP has been further highlighted by the inadequate response of conventional anti-anginal agents in these patients¹⁰⁵. In contrast, the unique combined calcium L/T channel blocker (mibefradil) has been shown to be effective. In-vitro studies on isolated microvessels have demonstrated that mibefradil is more effective in inhibiting ET-1 constrictor responses than conventional L-channel blockers in human microvessels. Furthermore, calcium T-channels were shown to be more abundant in the microvessels as compared with larger vessels.

It is thought there is an abundance of T-type calcium channels present in coronary small vessels that may be related to the increased sensitivity effects of ET-1 and contribute to the

regulation of contractile responses in the microvasculature³⁸¹. In pathological situations such as in hypertension, where endothelial dysfunction has been demonstrated, ET-1 expression is increased and the vasoconstrictor response is often enhanced in affected coronary arteries. It is possible that as a consequence of the +138 del/ins mutation, an increase in ET-1 production can predispose patients to microvascular spasm in the small vessels of the heart.

4.5.2 eNOS T-786C Gene Polymorphism

In the present study, we found that the T-786C polymorphism from the eNOS gene was not associated with the CSFP. Other investigations concerning the T-786C eNOS gene polymorphism and CSFP have given contradictory results. Nurkalem et al.³⁸² found that the T-786C polymorphism is associated with the CSFP in the Turkish population, which is discordant to our study. Three interpretations can be proposed from such diverse findings. First, there were three times as many CSFP patients (n=56) enrolled in the other study, which may indicate the possibility of a Type II error. Second, the contradictory results may reflect the diverse genetic backgrounds between two completely different population groups, one being Eastern European and the other being Australian. Third, the other study quantified coronary blood flow using the TIMI frame count method, whereas we defined CSFP based on the number of beats it takes for the contrast agent to flow through the epicardial vessel. Whilst both these methods are well known and reproducible^{80,104}, they need to be examined further and compared in larger cohorts of different ethnic groups.

4.5.3 Study Limitations

In the present study there are several limitations. First, plasma ET-1 and NO metabolite levels were not measured in the CSFP patients, therefore we were unable to provide evidence of a mechanistic link between the ET-1 polymorphism and this clinical disorder. However, as

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previously mentioned plasma ET-1 levels have been measured in other studies and found to be elevated in CSFP patients. Second, the study cohort was not age or risk factor matched. The CSFP patients were slightly younger and had a greater incidence of hypertension and hypercholesterolaemia, as compared with the healthy controls. The prevalence of hypertension in the CSFP is a potential confounder as hypertension has been associated with the +138 del/ins polymorphism whereas cholesterol and ageing has not. Among the OCAD patients, the number of patients with hypertension was doubled compared to the CSFP patients (65% vs. 33%), however the frequency of the homozygous insertion (II) variant is significantly less in the obstructive group compared to the CSFP group (5% vs. 28%). One can therefore assume that the probability of hypertension influencing the frequency of the +138 del/ins polymorphism in the CSFP group is unlikely; and univariate analyses determined the +138 del/ins polymorphism was the sole independent risk factor for developing the CSFP. Third, the study group sample was small, introducing the possibility of a Type 1 error for the ET-1 +138 del/ins SNP, or a Type II error in regards to the eNOS T-786C SNP, thus a larger sample population of CSFP patients should be examined to confirm the relationship between each gene polymorphism and the CSFP.

In regards to the NoCAD patients, according to the frequencies obtained from the study by Rossi et al.³⁶⁹ we had sufficient power to detect a difference in the T-786C SNP. Furthermore, a Post Hoc analysis was conducted to determine if there was a Type II error between the T-786C SNP and the NoCAD patients. The results of this statistical test showed that an additional 18,000 patients are required to detect a difference using a genotype frequency of 3%. Clearly the recruitment of 18,000 patients is not feasible and therefore it is likely that the T-786C SNP may not be associated with NoCAD in an Australian population. In addition, there was no association between the +138 del/ins SNP of the ET-1 gene and

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NoCAD, although this null finding is may reflect a Type II error, as this investigation was underpowered and this was acknowledged at the commencement of the study. A larger sample size is therefore warranted to adequately investigate the relationship between the +138 del/ins SNP and NoCAD.

4.5.4 Conclusion

In summary, this study reported no association between the T-786C and +138 del/ins SNPs in a heterogeneous group of patients with NoCAD. However the frequency of the +138 del/ins polymorphism of the ET-1 gene was more prevalent in the CSFP, suggesting an underlying genetic background exists amongst this population. The preliminary findings of this study have identified the +138 del/ins polymorphism from the ET-1 gene as a potential genetic risk factor for the CSFP, thus patients who carry this mutant variant may be genetically predisposed to developing this debilitating microvascular condition. Further confirmation of these findings is required in a larger study cohort and also in other ethnic populations to strengthen the genetic association between the CSFP and the +138 del/ins SNP.

4.5.5 Clinical Significance

The importance of establishing the +138 del/ins SNP as a prognostic marker would be of clinical interest, especially in the long-term assessment of chest pain. This ET-1 polymorphism may potentially be used to predict poor functional outcomes in patients with on-going chest pain, as occurs with the CSFP. Unfortunately, we could not assess the association between the ET-1 polymorphism and on-going chest pain, as there was not enough epidemiological data to detect a clinically significant change in this patient cohort. Therefore, future studies are needed to investigate the role of this polymorphism as a prognostic marker in patients with the CSFP. Furthermore, the over production of ET-1 due to

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a genetic mutation is an attractive drug target system, as pharmacological treatment strategies can focus on opposing the actions of ET-1 in the microcirculation; and this can be facilitated by ET-1 antagonists, ETA selective antagonists and ECE inhibitors that are specific for small vessel disease. The regulation of ET-1 levels, via the activity of pharmacological agents, may potentially decrease episodes of microvascular spasm/dysfunction and alleviate re-current painful episodes of chest pain in CSFP patients. Although, this research is still in the early stages, the potential discovery of a genetic risk factor is clinically valuable and can lead to new treatments. Further studies are needed to investigate the exact role of the +138 del/ins polymorphism in the genetic predisposition of the CSFP.

Chapter 5. Conclusions

5.1 Overview of Studies

The syndrome of chest pain with non-obstructive coronary artery disease (NoCAD) is poorly understood; even less is known about acute clinical presentations associated with NoCAD. This thesis has addressed the various clinical, prognostic and genetic characteristics of patients with chest pain in the absence of obstructive CAD. The three studies undertaken for this thesis were diverse and particularly assessed:

- (1) the health status in patients with ACS and NoCAD over 12 months (Chapter 1)
- (2) the presence of myocardial ischaemia in CSFP patients presenting with ACS (Chapter 2)
- (3) genetic associations related to NoCAD and its related biological phenotype, the CSFP (Chapter 3).

The first study, detailed in **Chapter 2**, measured health outcomes in patients with chest pain and NoCAD over a 12-month follow-up. Health-Related Quality of Life (HRQoL), as self-reported by the patient, was assessed using both a generic (SF-36) and disease-specific (SAQ) HRQoL instrument. The objective of this study was to identify the clinical and prognostic differences in patients with ACS-NoCAD as compared with patients with stable-NoCAD. This investigation found no significant differences in HRQoL scores at baseline and at the 12 months follow-up between the two study groups. A major noteworthy observation was that the SF-36 Physical Summary (PSS) and Mental Summary (MSS) scores were very low and akin to patients with obstructive CAD and other chronic debilitating diseases. In addition, patients with ACS-NoCAD suffered depressive symptoms at angiography and their mental health status did not improve over 12 months of follow-up. Furthermore, an increased risk of cardiac events in ACS-NoCAD was reported in patients within 12 months following angiography. The latter prognostic outcome needs further exploration, however, as no formal

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sample size was calculated in this study—although a statistical trend in relation to cardiac event rates indicates this finding may be underpowered.

The second study, detailed in **Chapter 3**, focused on patients with ACS and NoCAD, in particular those with the CSFP. This case-control study investigated the frequency of ST segment and T-wave fluctuations during continuous ST/T wave monitoring in patients with the CSFP compared to healthy control subjects. This clinical investigation found that 92% of patients with ACS and the CSFP had evidence of ischaemic ST/T wave changes, with a third having ST segment fluctuations and over 80% having T-wave fluctuations. In addition, patients with the CSFP had significantly greater T-wave amplitude changes compared to healthy controls, which suggests that T-wave amplitude change of ≥ 2 mm may be an electrocardiographic marker of coronary microvascular ischaemia. The utility and established technique of continuous ST/T wave monitoring has demonstrated ECG evidence of myocardial ischemia during the acute phase of this disorder. Future studies are needed to confirm the prevalence of ST/T wave changes in a larger cohort of patients.

The final study, detailed in **Chapter 4**, assessed the frequencies of two genetic polymorphisms in patients with NoCAD, and its related phenotype the CSFP. This investigation was undertaken to determine whether a genetic background influences the pathogenesis of NoCAD, especially the CSFP, which is associated with microvascular endothelial dysfunction. This lab-based pilot study found the frequency of the ET-1 polymorphism was more prevalent in the CSFP patients compared to patients with NoCAD, obstructive CAD and healthy controls. A statistical difference was found between the +138 del/ins SNP of the ET-1 gene and the CSFP; although a larger sample size is required to eliminate the potential of a Type 1 error. In addition, the lack of genetic association between

the CSFP and the T-786C SNP of the eNOS gene may be due to a Type II error—reinforcing the importance of investigating these frequencies in a larger patient cohort.

5.2 Advances in Understanding Acute Chest Pain with Non-obstructive CAD

Chest pain in the absence of obstructive CAD is a heterogeneous disorder which has many causes that may or may not be cardiac in origin. In this thesis the possible cardiac and non-cardiac causes of chest pain in NoCAD have been described, but we have specifically focused on understanding acute chest pain associated with coronary microvascular dysfunction, as occurs with the CSFP. Typically patients with the CSFP initially present with an ACS which has been previously shown to be associated with recurrent chest pain at rest and an impaired quality of life at baseline¹⁵⁵. This thesis extends on the previous research by demonstrating that patients with acute chest pain and NoCAD are more likely to have higher psychological morbidity after 12 months compared to those with stable chest pain, however, no clinical differences were reported between the two clinical presentations. Patients presenting with the CSFP were not identified in our epidemiological study, and therefore could not be compared as an independent cohort. Future studies are needed to evaluate whether clinical differences exist between the CSFP and stable angina syndromes with NoCAD.

The pathogenesis of chest pain with NoCAD is multifactorial; however, there appears to be an underlying genetic determinant that may contribute to the predisposition of microvascular dysfunction, as occurs with the CSFP. The +138 del/ins SNP from the ET-1 gene is independently associated with the CSFP, suggesting that ET-1 may be genetically up-regulated and contribute to the development of this condition. On the basis of previous observations by Hirata et al.¹⁶⁰ ET-1 can induce the CSFP without evidence of epicardial

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constriction, suggesting a pathogenic role may exist in the development of microvascular dysfunction. In addition, the effects of ET-1 on the human microvasculature have been reported by Turner et al.³⁸³ who observed an increased microvascular sensitivity to ET-1 in the CSFP patients compared to controls; thus ET-1 might contribute to increased microvascular tone, leading to microvascular ischemia, which ultimately leads to the manifestation of chest pain. The aforementioned studies provide evidence which suggests ET-1 is involved in the pathogenesis of the CSFP. Furthermore, the +138 del/ins SNP from the ET-1 gene is a potential mechanism that may possibly play a role in the development of microvascular endothelial dysfunction and predispose carriers of this variant to the CSFP.

The acute chest pain presentation associated with the CSFP has been attributed to acute episodes of coronary microvascular dysfunction which can cause ischaemia. The notion of microvascular ischaemia as a cause of chest pain in NoCAD has been discussed for over two decades; however, the relationship between ischemia and microvascular dysfunction is poorly understood. The observation of frequent ST/T wave changes in patients with the CSFP is an important and novel finding which supports the concept that those presenting with an ACS have underlying microvascular ischaemia.

At present, the underlying pathophysiological processes which lead to microvascular ischemia are speculative, but could be explained by the effects of inflammatory responses and endothelial dysfunction. The presence of increased plasma CRP, an acute phase inflammatory response protein, has reportedly been observed in patients with the CSFP³⁸⁴. The presence of inflammation, indicated by an elevated CRP level, may be associated with increased endothelin-1 released from mast cells and macrophages. In addition, genetic variants such as the +138 del/ins SNP can increase ET-1 expression and thus may increase circulating levels

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of ET-1 and contribute to the development of myocardial ischemia. One can therefore speculate that ET-1 acts as a potent microvascular constricting peptide, which can lead to the development of microvascular ischaemia and can be severe enough to cause a microspastic form of angina.

Furthermore, during an acute event circulating plasma ET-1 levels are increased and this may be a potential pathophysiological mechanism which can trigger adverse cardiovascular outcomes³⁸⁵. Therefore, an ET-1 receptor antagonist could be a useful therapeutic agent for lowering cardiac events by blocking the effects of ET-1. A randomised, double-blind, placebo-controlled clinical trial using Bosentan, a non-selective ET-1 receptor antagonist, in patients with the CSFP is currently being undertaken at The Queen Elizabeth Hospital, Adelaide, South Australia. This trial aims to investigate whether Bosentan alleviates the debilitating clinical symptoms associated with this condition. Bosentan works by competing with ET-1, thus binding to the ET_A and ET_B receptors which are responsible for eliciting long lasting constriction that leads to impaired vascular tone³⁸⁶. Theoretically, Bosentan should decrease episodes of chest pain by improving endothelial function, resulting in a reduction of cardiovascular events and attenuation of the disease process. The potential therapeutic benefits of this oral drug would be significant as health outcomes in patients with chest pain and NoCAD would be improved.

Although patients with ACS-NoCAD may have a worse prognostic outcome compared to patients with stable chest pain and NoCAD, the effects of on-going symptoms in either clinical group causes significant physical and psychological morbidity that does not improve after 12 months. Therefore, the long term negative effect of chest pain in NoCAD patients

represents an unrequited need for effective treatment in order to reduce the frequency and severity of symptoms and increase quality of life.

In clinical practice, chest pain with NoCAD remains a frequent problem as there is no standard therapeutic strategy for patients with this condition. Moreover, effective treatment is required in patients with ACS-NoCAD given the elevated risk of adverse cardiac outcomes reported in this thesis; however, these retrospective findings require confirmation in a larger prospective clinical trial. Furthermore, there is currently no available treatment in patients suffering from the debilitating effects of the CSFP, although previous therapeutic studies have been undertaken in these patients. Mibefradil, a unique calcium T-channel blocker, has been shown to significantly improve angiographic coronary blood flow and reduce spontaneous anginal episodes in a randomised, double-blind, placebo-controlled, cross-over study in patients with the CSFP¹⁰⁵. Unfortunately, Mibefradil has been withdrawn from the market and is not longer available due to harmful interactions with other drugs. Dipyridamole, a vasodilator, has been investigated in an open-label observational study of patients with the CSFP and was found to be of benefit, although the study was not randomised. Further controlled studies are therefore needed to determine the long-term effect of this therapy³⁸⁷. There are various other agents such as statins and ACE inhibitors which have shown to be of benefit in patients with coronary microvascular dysfunction, however, randomised, controlled, clinical trials are warranted to assess the true benefits of these drugs. Future research should be directed at developing new therapies to treat both the acute and chronic management of patients with the CSFP. In addition, a better understanding of the underlying pathophysiology of coronary microvascular dysfunction will continue to give us more insight into the acute clinical presentation associated with this condition.

5.3 Summary

The diverse investigations undertaken for this thesis are novel and have offered new insights into the clinical, prognostic and genetic factors related to patients with acute chest pain and NoCAD. In particular this thesis has expanded our current understanding of health outcomes in patients with ACS-NoCAD and revealed new clinical and genetic findings, associated with the CSFP. It is now imperative that further research is undertaken to: (a) improve the quality of life of all patients with NoCAD (b) further understand the underlying pathophysiological mechanisms, associated with microvascular ischaemia, that lead to the manifestation of an ACS in CSFP patients and, (c) clarify the role of ET-1 in the underlying pathophysiology of the CSFP. Although valuable progress has been made, further research is warranted to enable the development of new therapies which can potentially reduce both the health burden and economic costs associated with the debilitating and on-going clinical symptoms of NoCAD

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Appendices

Appendices: 'MAC Project' and 'Seattle Angina Questionnaire' are included the print copy of the thesis held in the University of Adelaide Library.

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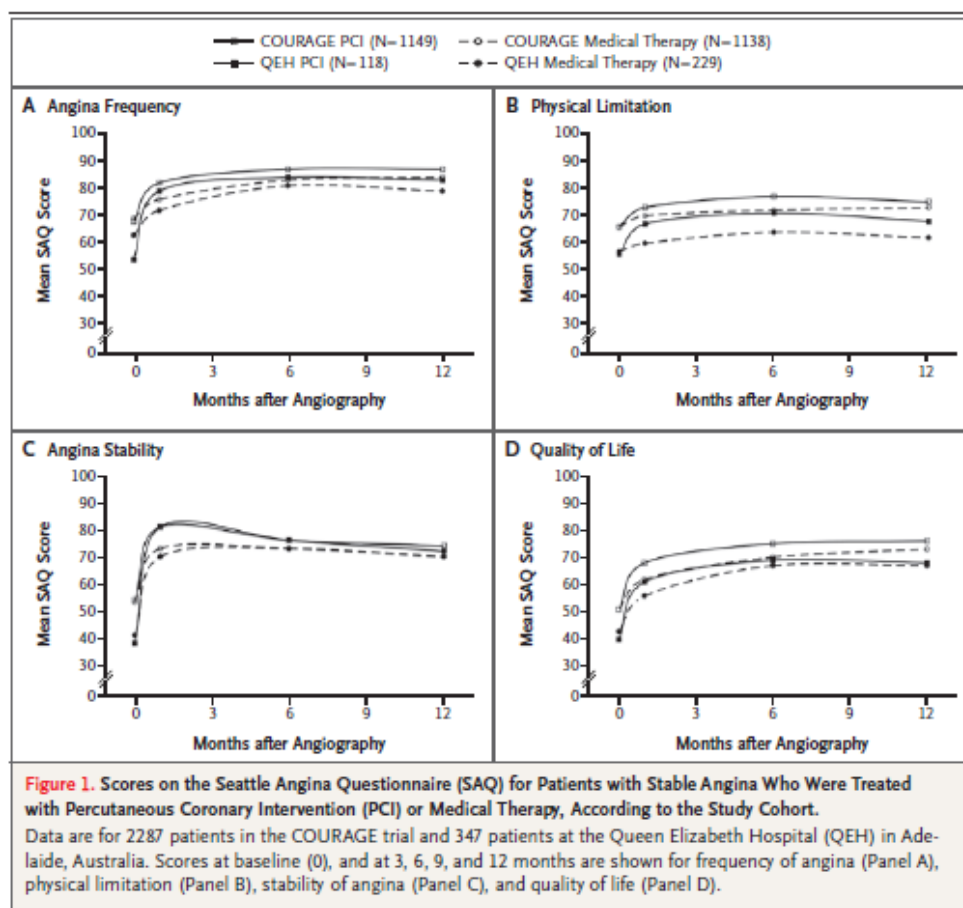
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Quality of Life with PCI versus Medical Therapy in Stable Coronary Disease

TO THE EDITOR: Weintraub et al. (Aug. 14 issue)¹ report the results of the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial, which examined the effect of percutaneous coronary intervention (PCI), as compared with medical therapy alone, on the quality of life in patients with stable coronary disease. The Seattle Angina Questionnaire (SAQ), as well as other measures, was used to assess the effect of treatment on health status. The authors

conclude that patients who received PCI had small but significant incremental benefits that disappeared by 36 months.

Concern has been expressed about the generalizability of the results of this study because of the highly selected nature of the cohort (of 35,539 patients who were screened, only 2287 were enrolled), the underrepresentation of women, and the possibility that the marked improvement in the condition of patients who received medical



therapy might have reflected the free supply of drugs. To address these concerns, we analyzed serial SAQ scores of 347 consecutive patients undergoing elective angiography for stable angina at the Queen Elizabeth Hospital in Adelaide, Australia, who were treated with PCI or medical therapy, as recorded in an observational registry.

In our study, the PCI group and the medical-therapy group had similar risk factors and baseline SAQ scores, except for more frequent angina in the PCI-treated patients. In this observational, "real-world" cohort of patients, 28% of whom were women and all of whom were treated in an Australian health care system with government-subsidized medicines, the trends were similar to those in the COURAGE cohort, despite poorer scores at baseline in our cohort (Fig. 1). In particular, patients in the medical-therapy group had early improvement in SAQ indexes, with the results approaching those in the PCI group, except for scores on the physical-limitation scale. These findings strongly support the quality-of-life data in the COURAGE study and underscore the study's real-world implications for patients with stable angina.

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1. Weintraub WS, Spertus JA, Kolm P, et al. Effect of PCI on quality of life in patients with stable coronary disease. *N Engl J Med* 2008;359:677-87.

TO THE EDITOR: In the COURAGE trial, Weintraub et al. determined that patients with stable angina who were treated with either optimal medical therapy alone or PCI derived similar benefits at 3 years. The rate of success of PCI in this trial was considerably lower than that in contemporary PCI studies. In-hospital PCI clinical success was achieved in only 89% of patients.¹ In the Argentine Randomized Study of Coronary Angioplasty with Stenting versus Coronary Bypass Surgery in Patients with Multiple Vessel Disease (ERACI) and the Arterial Revascularization Therapies Study (ARTS),²⁻⁴ initial clinical success with PCI was greater than 95% in patients with significantly more complex baseline characteristics than those of patients in the COURAGE trial. A meaningful comparison of the two treatment strategies in the

COURAGE trial is difficult because of the high rate of unsuccessful PCI (11%), the use of bare-metal stents in patients with stable angina (such stents were used in >97% of the patients in the PCI group), and the fact that complete data were available for only one third of the patients after 36 months.

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TO THE EDITOR: Weintraub et al. report that patients with stable coronary artery disease who underwent PCI had transiently fewer anginal symptoms than did those who received optimal medical treatment alone. Of the patients who had symptoms at baseline, fewer than one third in the PCI group had relief from angina within 1 month after the intervention. By contrast, PCI has been shown to be immediately and highly efficacious in relieving symptoms that are triggered by the stenosis that is the target of treatment.^{1,2} This finding suggests that the symptoms of many patients in the COURAGE trial were not caused by ischemia or that revascularization was incomplete despite PCI. We conclude from these data that classic, ischemia-driven angina may not have caused the symptoms of a majority of patients in the COURAGE trial. Alternatively, patients may have received optimal medical therapy but not optimal interventional treatment. Therefore, the conclusions drawn from the original report of the COURAGE trial³ may not apply to patients with

angina who undergo complete revascularization by means of PCI.

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TO THE EDITOR: Coronary stenoses are like chocolate — they are very difficult to leave alone. Stenting a stenosis provides a very visible and visceral gratification for the interventionalist and the patient. Although many point the finger at the interventionalist, our patients are just as subject to the temptations of the angiogram as we are.

For this reason, one of the most important implications of the COURAGE trial is to discourage proposals for computed tomographic angiography or other noninvasive imaging of coronary arteries in asymptomatic adults. Such approaches risk deluging catheterization laboratories with patients who have coronary stenoses that, although visually tempting, do not require PCI.

On the other hand, the COURAGE trial suggests that PCI provides more rapid resolution of symptoms in patients with frequent stable angina, as confirmed by functional tests. It would be a travesty if these patients were denied early relief of symptoms because of the overall results of the COURAGE trial or inadequate surveillance for medical failure.

Finally, the COURAGE trial does not address myocardial infarction or unstable angina, two conditions that together account for two thirds of PCIs.¹ Early revascularization offers greater benefits in patients with these conditions.^{2,3}

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TO THE EDITOR: The editorial by Peterson and Rumsfeld¹ that accompanies the article on the COURAGE trial states that in a patient with stable angina, a trial of medical therapy is a “reasonable” initial approach. However, the 21% crossover rate at 3 months that is noted in the editorial is an underestimation. In fact, by the end of the original COURAGE study, 32.6% of patients in the medical-therapy group crossed over to undergo PCI because of symptoms or ischemia. The large number of crossovers and the small number of drug-eluting stents that were used mute the initial benefit in health status observed in the PCI group. The editorial concludes, “Thus, a very reasonable ‘take-home’ message from the COURAGE trial is to pursue optimal medical therapy initially and if this is ineffective, turn to PCI.” This statement neglects to mention that all the patients in the COURAGE trial underwent coronary angiography before enrollment. As written, the take-home message can easily be misinterpreted to indicate that patients with angina do not need coronary angiography, which was clearly not the strategy in the COURAGE trial.

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1. Peterson ED, Rumsfeld JS. Finding the courage to reconsider medical therapy for stable angina. *N Engl J Med* 2008;359:751-3.

TO THE EDITOR: In their editorial, Peterson and Rumsfeld caution against the use of a “PCI-first” strategy for stable coronary artery disease, claiming that PCI would result in approximately 2 deaths for every 1000 patients treated. Yet in the original COURAGE trial, in which the primary outcome was a composite of death from any cause and nonfatal myocardial infarction, there was no significant difference in outcome between the PCI group and the medical-therapy group. It is possible that periprocedural mortality in the PCI group was offset by lower long-term mortality, but whatever the cause, it is faulty logic to imply that a PCI-first strategy will result in 2 extra deaths per

1000 patients treated, when mortality has been proven to be equivalent for patients treated with PCI and those treated medically.

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THE AUTHORS REPLY: We congratulate Beltrame et al. for their study of the SAQ scores, which shows the similarity and generalizability of our results to that of a “real-life” population.

Rodríguez et al. speculate that our trial suffered from substandard PCI, the use of bare-metal stents, and missing data on the quality of life. A detailed study of angiographic outcomes in our trial is forthcoming and should allay their first concern. Although stenting technology has evolved since the launch of our trial, we believe it is necessary to demonstrate that survival and quality of life are superior with the use of drug-eluting stents, as compared with optimal medical therapy or the use of bare-metal stents, rather than presume so in the absence of empirical data. Although missing data are always problematic, less than one third of data were missing at 3 years and less than that at earlier periods. Extensive analyses revealed no evidence of bias, making it unlikely that missing data substantially affected the results.

Although Reppel et al. suggest that many patients in our trial may not have had angina, we believe this to be unlikely, given our careful screening and entry criteria. They further question the completeness of revascularization and postulate that incomplete procedures were responsible for suboptimal angina relief in the PCI group. Not only will forthcoming angiographic data be reassuring concerning the quality and completeness of revascularization, but the 66% rate of freedom from angina (as defined by Canadian Cardiovascular Society criteria class) at 1 year in our original trial exactly matched that for patients undergoing complete revascularization in the Coronary Artery Surgery Study.¹ It is also unclear whether PCI in major trials always offers immediate and highly efficacious angina relief. Quality of life was first assessed at 6 months in the Medicine, Angioplasty, or Surgery Study (MASS II) and at 3 months in the Randomized Intervention Treatment of Angina 2 (RITA-2) study.^{2,3} Among patients undergoing PCI, the results on the 36-

item Medical Outcomes Study Short-Form General Health Survey (SF-36) from both MASS II and RITA-2 are almost identical to those of the RAND 36-item health survey used in our trial. Most important, patients in both study groups in our trial had rapid improvement in health status, as measured by the SAQ, a well-validated tool for the assessment of angina.

We agree with Kinlay that patients with severe angina may benefit from early rather than deferred PCI and that our findings do not apply to patients with acute coronary syndromes. We hope that our trial provides an evidence base that physicians can use to inform patients that the primary benefit of PCI in chronic coronary disease is to alleviate symptoms and improve the quality of life, ideally after medical therapy alone has failed to do so.

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THE EDITORIALISTS REPLY: Brown et al. raise several points regarding our editorial about the results of the COURAGE trial. First, they note that 21% of patients in the medical-therapy group underwent revascularization within 3 months and that 32.6% of patients underwent such therapy by the end of the trial. Our editorial also discussed treatment crossovers, emphasizing that the COURAGE study was a strategy trial rather than a head-to-head comparison. With that, the inverse must also be considered — namely, that two thirds of patients who received medical therapy alone did not require revascularization during follow-up. In contrast, all the patients in the PCI group underwent up-front intervention, and 21.1% of these patients had to undergo more than one procedure.

Second, Brown et al. ask whether the findings might have been different if more drug-eluting stents had been used. Although coated stents reduce the need for repeat revascularization, they do not alter rates of death or myocardial infarction, as compared with the rates for bare-metal stents, and their effect on the quality of life of patients is marginal on the basis of available studies (a gain in quality-adjusted life-years that ranges from 0 to 0.08).^{1,2}

Third, Brown et al. point out that patients in the COURAGE trial underwent cardiac catheterization and that the small proportion of patients whose anatomy was not suitable for PCI (i.e., those with left main coronary artery disease) were excluded from the study. Many of these high-risk patients could be identified and selected for catheterization on the basis of noninvasive imaging studies.^{3,4} That said, it is true that the COURAGE trial specifically addressed treatment strategy after coronary anatomy was defined. Yet it is uncertain whether a “look but don’t touch” strategy can be executed in clinical practice, given the lack of incentives for the provision of optimal medical therapy in the current health care system.

Finally, Smith expresses concern that we overemphasized the procedural risks of PCI, which was not our intent. We noted that long-term rates of death and myocardial infarction were similar for the two strategies. However, we would also note that most patients discount future events. A strategy that avoids present-day risks may be preferred, even if long-term outcomes are similar.

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Malaria Prevention in Short-Term Travelers

TO THE EDITOR: In his Clinical Practice article on malaria prevention in short-term travelers, Freedman (Aug. 7 issue)¹ offers clinical advice for the family of three described in the vignette. For the 29-year-old wife, who is 15 weeks pregnant and has won a trip to Zambia in a corporate sales competition, it might be best to provide a medical letter that she could give to corporate sales, advising postponement of her trip for a year. In addition, Table 3 of the article, which lists prophylactic drug regimens, does not mention the latest artemisinin-based combination therapy.²

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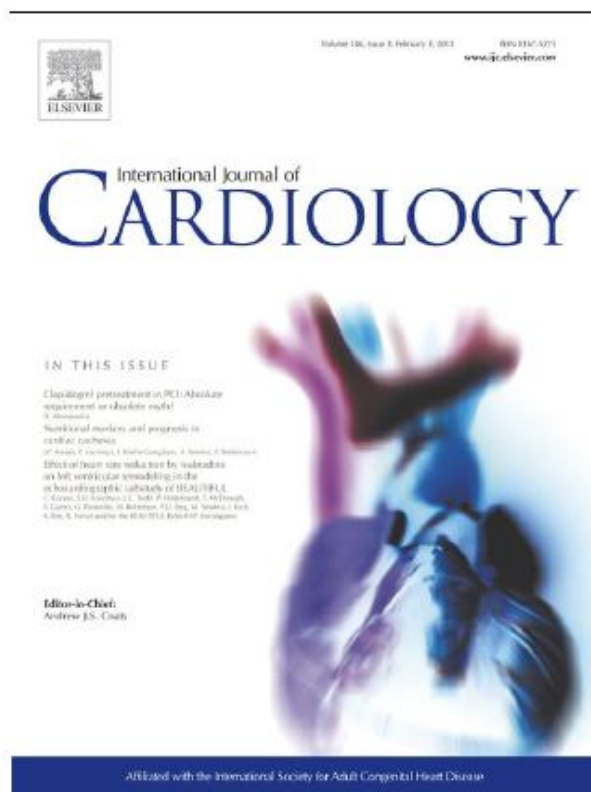
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THE AUTHOR REPLIES: Artemisinin derivatives have revolutionized malaria treatment programs worldwide and are the drugs of choice for falciparum malaria in most countries where it is endemic. No artemisinin compound is currently licensed in the United States. Intravenous artesunate is available from the Centers for Disease Control and Prevention (CDC) for emergency use for severe and complicated falciparum malaria (www.cdc.gov/malaria; telephone, 770-488-7788) under an investigational-new-drug (IND) application. An oral combination drug containing artemether and lumefantrine for the treatment of uncomplicated falciparum malaria is currently under expedited review by the Food and Drug Administration. Unfortunately, the extremely short half-life of the artemisinin drugs precludes their use as chemoprophylactic agents.

The advice of the CDC, the World Health Organization, and myself that pregnant women not

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