
Novel Clinical Insights into Acute Myocardial Infarction

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ABSTRACT

Background and objectives: Acute myocardial infarction (Acute MI) reflects myocardial cell death due to prolonged myocardial ischaemia. At the turn of the 20th century, acute MI was a fatal condition and bed rest served as the principal management strategy. In the 1980's, pivotal early angiography studies demonstrated that patients with acute MI presenting with ST elevation on ECG were associated with an acute coronary artery occlusion in over 90% of cases. This prompted the therapeutic strategy of the 'open artery hypothesis' where re-establishing coronary patency became paramount in acute MI management. Thrombolytic therapy, percutaneous coronary intervention (PCI), and adjunctive pharmacologic strategies were all developed to re-open the occluded coronary artery and facilitate reperfusion of the myocardium. Despite these advances, acute MI remains a global issue and is associated with significant mortality and morbidity. The aim of this thesis is to examine contemporary clinical insights of acute MI, and in particular, to emphasize two novel aspects.

The first component of this thesis focuses on the identification and understanding of a clinically intriguing acute MI group. Coronary angiographic innovations have primarily focused on alleviating atherothrombotic processes that obstruct coronary blood flow, evident in most acute MI patients. However, acute MI registries report that approximately 10% of patients do not reveal obstructive coronary artery disease (CAD). The pathophysiological processes responsible for these presentations are not immediately evident at the time of angiography. These presentations are classified as "myocardial infarction with non-obstructive coronary arteries (MINOCA)", and are increasingly recognized as a clinical conundrum. In the absence of management guidelines, consequently, these patients are often discharged from hospital without secondary prevention therapies.

The specific objectives of this component are:

1. To systematically review existing literature on MINOCA (Chapter 2)
2. To evaluate contemporary clinical characteristics of MINOCA in comparison to myocardial infarction with obstructive coronary artery disease (MI-CAD) (Chapter 3)
3. To examine the risk of thrombosis in MINOCA patients (Chapter 4).

The second component of the thesis focuses on a novel management strategy for acute MI. Although timely reperfusion of the myocardium via restoration of the occluded coronary artery has evolved as the gold standard for the management of acute MI patients, reperfusion may be a double-edged sword, since the free radicals generated may also further damage myocardial tissue; a phenomenon referred to as ischaemia-reperfusion injury. Generation of reactive oxygen species (ROS) through incomplete reduction of oxygen during reperfusion has been well described and can quickly overwhelm the cell's endogenous free radical scavenging system. This, in turn, triggers additional cellular injury by reactions with intracellular components. N-acetylcysteine (NAC) has been established as a ROS scavenger, which also potentiates the vasodilator and anti-aggregatory effects of glyceryl trinitrate (GTN).

The specific objective of this component is:

4. To examine the role of NAC together with GTN in acute MI patients undergoing primary PCI (Chapter 5).

Methods: This thesis employs a number of methods to evaluate the two specific components. A comprehensive systematic review and meta-analysis were undertaken to review the literature concerning MINOCA. Contemporary clinical characteristics of MINOCA were identified via a clinical registry. Risk of thrombosis in MINOCA was assessed using thrombin generation test and thrombophilia screening. The role of NAC in acute MI patients was analysed using a randomized, double-blind, placebo-controlled clinical trial.

Summary of Major findings:

1. Chapter 2- Systematic review of the existing MINOCA literature provided the first comprehensive understanding of MINOCA and demonstrated that 6% of acute MI presentations fulfil the criteria for MINOCA. It also established that MINOCA patients are younger, more likely to be female, and have less cardiovascular risk factors compared to MI-CAD. In addition, MINOCA is associated with a guarded 12-month prognosis, and multiple aetiologies are implicated that require further evaluation.
2. Chapter 3- This is the first prospective comprehensive analysis of clinical characteristics, including chest pain features, amongst patients with MINOCA in comparison to MI-CAD. The results from this study demonstrate that MINOCA is a more common presentation (11% of acute MI) than reported from the systemic review. However consistent with the review findings, MINOCA patients were more likely to be female and present with fewer cardiovascular risk factors but the chest pain presentation is indistinguishable from MI-CAD.
3. Chapter 4- Spontaneous formation and lysis of coronary thrombosis is often hypothesised as a potential mechanism leading to MINOCA presentation. Overall thrombin generation potential, congenital thrombophilia states and acquired thrombophilia states were not different between MINOCA and MI-CAD. This suggests that despite the difference in

coronary artery anatomy of the disease progression, acute MI patients generate thrombin in a similar manner in response to local stimuli.

4. Chapter 5- In acute MI patients with an occluded coronary artery, final infarct size is determined by duration of ischaemia, area at risk, and ischaemia-reperfusion injury among others. Existing research studies indicate limiting the infarct size improves long term clinical outcomes of MI patients. Utilising a double-blind, placebo-controlled trial design, it was demonstrated that for patients with ST-elevation myocardial infarction (STEMI), early administration of NAC together with glyceryl trinitrate (GTN) reduced the final infarct size compared to placebo and GTN, as assessed by cardiac magnetic resonance imaging. NAC's intrinsic ROS scavenging properties resulting in reduced oxidative stress and its potentiation of GTN resulting in increased reperfusion may have limited the infarct size.

Conclusions: This thesis provides beneficial insights into two novel clinical aspects of acute MI in contemporary clinical practice. In regards to MINOCA, the systematic review (Chapter 2) presents the first comprehensive body of literature summarising MINOCA, especially in comparison to MI-CAD, identifying similar clinical features between these acute MI groups. Importantly, the systematic review implicates MINOCA as a working diagnosis given the role of multiple aetiologies. Subsequent to the systematic review, Chapter 3 and 4 provides contemporary clinical characteristics and mechanistic insights, in particular the risk of thrombosis, in MINOCA. Overall, this data highlights the need for optimal assessments in elucidating the underlying cause for the presentation and the requirement to generate diagnostic guidelines to inform appropriate management. In regards to MI-CAD, timely and effective myocardial reperfusion by PCI is the treatment of choice for limiting myocardial infarct size and improving clinical outcomes. However, reperfusion of the infarct artery leads to further myocardial damage via ischaemia reperfusion injury, highlighting the need for

additional pharmacological strategies. Chapter 5 presents a significant observation in that limiting infarct size is possible via the utilisation of NAC/GTN in STEMI patients. Further exploration of each of these components may enhance the diagnosis and treatment of acute MI patients and substantially improve clinical outcomes.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Last, but not least, I thank my very supportive and loving family. This thesis stands as a testament to unconditional love and encouragement from everyone mentioned here.

I dedicate this thesis to my Amma, my inspiration.

ABBREVIATIONS

AAR:	Area at risk
ACC:	American College of Cardiology
ACE:	Angiotensin converting enzyme
ACS:	Acute coronary syndrome
Ag:	Antigen
AHA:	American Heart Association
ANCOVA:	Analysis of covariance
ANOVA:	Analysis of variance
ANZCTR:	Australian New Zealand Clinical Trials Registry
APC:	Activated protein C
APCR:	Activated protein C resistance
APTT:	Activated partial thromboplastin time
ARB:	Angiotensin II receptor blocker
AT:	Antithrombin
ATP:	Adenosine triphosphate
AVOID:	Air versus oxygen in myocardial infarction
BP:	Blood pressure
CABG:	Coronary artery bypass surgery
CAD:	Coronary artery disease
CADOSA:	Coronary Angiogram Database of South Australia
CAT:	Calibrated automated thrombogram
cGMP:	Cyclic guanosine monophosphate
CI:	Confidence interval

CK-MB:	Creatine kinase - myoglobin binding
CK:	Creatine kinase
CMR:	Cardiac magnetic resonance imaging
CSANZ:	Cardiac Society of Australia and New Zealand
CTPA:	Computed tomography pulmonary angiogram
CV:	Coefficient of variation
CVD:	Cardiovascular disease
DCM:	Dilated cardiomyopathy
ECG:	Electrocardiography
EDTA:	Ethylenediaminetetraacetic acid
EDV:	End diastolic volume
EF:	Ejection fraction
ELISA:	Enzyme-linked immunosorbent assay
ESC:	European Society of Cardiology
ESV:	End systolic volume
ETP:	Endogenous thrombin potential
FIX:	Factor IX
FIXa:	Activated factor IX
FV:	Factor V
FVa:	Activated factor V
FVII:	Factor VII
FVIIa:	Activated factor VII
FVIII	Factor VIII
FVIIIa:	Activated factor VIII
FVL:	Factor V Leiden

FXI:	Factor XI
FXIa:	Activated factor XI
FXII:	Factor XII
FXIIa:	Activated factor XII
GIK:	Glucose insulin potassium
GORD:	Gastro oesophageal reflux disease
GRACE:	Global registry of acute coronary events
GTN:	Glyceryl trinitrate
H ₂ O ₂ :	Hydrogen peroxide
HCM:	Hypertrophic cardiomyopathy
HDL:	High-density lipoprotein
HOCl:	Hypochlorous acid
Hx:	History
IBS:	Irritable bowel syndrome
IQR:	Interquartile range
IV:	Intravenous
IVUS:	Intravascular ultrasound
LBBS:	Left bundle branch block
LDL:	Low-density lipoprotein
LGE:	Late gadolinium enhancement
LV:	Left ventricle
MC:	Myocarditis
MI-CAD:	Myocardial Infarction with Obstructive Coronary Artery Disease
MI:	Myocardial infarction
MINOCA:	Myocardial Infarction with Non-Obstructive Coronary Arteries

MPTP:	Mitochondrial permeability transition pore
MVO:	Microvascular obstruction
n:	Number
NAC:	N-acetylcysteine
NACB:	National Academy of Clinical Biochemistry
NACIAM:	N-acetylcysteine in ST-segment elevation myocardial infarct patients
NAD:	Diagnosis not available
NADPH:	Nicotinamide adenine dinucleotide phosphate-
NCDR:	National cardiovascular data registry
NE:	Not examined
NO:	Nitric oxide
NSTEMI:	Non ST-segment elevation myocardial infarction
OR:	Odds ratios
O ₂ :	Oxygen
O ₂ ⁻ :	Superoxide
PAD:	Peripheral artery disease
PC:	Protein C
PCI:	Percutaneous coronary intervention
PGM:	Prothrombin gene mutation
PICO:	Population, intervention, comparison, outcome
pM:	Picomolar
PS:	Protein S
PT:	Prothrombin time
RCT:	Randomised controlled trials
RISK:	Reperfusion injury salvage kinase

RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
rpm:	Revolutions per minute
SD:	Standard deviation
SPECT:	Single photon emission computed tomography
SR:	Sarcoplasmic reticulum
STEMI:	ST-segment Elevation Myocardial Infarction
t-PA:	Tissue-plasminogen activators
T2W:	T2-weighted
TF:	Tissue factor
TFPI:	Tissue factor pathway inhibitor
TM:	Thrombomodulin
TTC:	Tako-tsubo cardiomyopathy
USA:	United States of America
vWF:	Von willebrand factor
WHF:	World Heart Federation
WHO:	World Health Organization

LIST OF PUBLICATIONS

PUBLISHED MANUSCRIPTS FROM THIS THESIS

-
- i.** Systematic Review of Patients Presenting with Suspected Myocardial Infarction and Non-Obstructive Coronary Arteries (MINOCA).

Pasupathy S, Air T, Dreyer R, Tavella R, Beltrame JF.

Circulation 01/2015; 131(10).

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- ii.** The What, When, Who, Why, How and Where of Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA).

Pasupathy S, Tavella R, Beltrame JF.

Circ J. 2016;80(1):11-6.

-
- iii.** Myocardial Infarction with Non-Obstructive Coronary Arteries – Diagnosis and Management.

Pasupathy S, Tavella R, McRae S, Beltrame JF.

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SUBMITTED MANUSCRIPTS FROM THIS THESIS

- i. The early use of N-acetylcysteine (NAC) with Glyceryl Trinitrate (GTN) in ST-segment Elevation Myocardial Infarction patients undergoing primary percutaneous coronary intervention (NACIAM Trial).

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The Lancet.

- ii. Risk of thrombosis in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA).

Pasupathy S, Rodgers S, Tavella R, McRae S, Beltrame JF.

Coronary Artery Disease

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- i.** The early use of N-acetylcysteine (NAC) with Glyceryl Trinitrate (GTN) in ST-segment Elevation Myocardial Infarction patients undergoing primary percutaneous coronary intervention (NACIAM Trial).

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Late breaking clinical trial presentation

Annual meeting of European Society of Cardiology congress, Rome, Italy.

-
- ii.** Risk of thrombosis in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA). 2016

Pasupathy S, Rodgers S, Pope S, Tavella R, McRae S, Beltrame JF.

Poster Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand, Adelaide, Australia

Heart, Lung and Circulation , Volume 25 , S64

-
- iii.** Electrocardiographic-assessed myocardial area at risk in patients with Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA).

Pasupathy S, Leow K, Wu S, Lee A, Du Y, Tavella R, Beltrame JF. 2016

Poster Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand, Adelaide, Australia.

Heart, Lung and Circulation , Volume 25 , S44

- iv.** Diagnostic utility of cardiac magnetic resonance imaging (CMR) in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA) Patients. 2016
Pasupathy S, Tavella R, Potamianos R, Arstall M, Chew D, Worthley M, Zeitz C, Beltrame JF.

Mini oral Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand, Adelaide, Australia.

Heart, Lung and Circulation , Volume 25 , S41

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- v.** Chest pain characteristics of myocardial infarction with non-obstructive coronary arteries (MINOCA) in comparison to myocardial infarction with coronary artery disease (MI-CAD). 2016

Pasupathy S, Tavella R, Arstall M, Chew D, Worthley M , Zeitz C, Beltrame JF.

Poster presentation

Annual meeting of American Heart Association, Quality of Care and Outcomes Research, Florida, USA.

Circ Cardiovasc Qual Outcomes. 2016;9:A129.

-
- vi.** Myocardial Infarction with Non-Obstructive Coronary Artery Disease (MINOCA): Prevalence, Clinical Features and Outcomes. 2015

Pasupathy S, Tavella R, Arstall M, Chew D, Worthley M , Zeitz C, Beltrame JF.

Poster Presentation

Annual meeting of American Heart Association, Quality of Care and Outcomes Research, Florida, USA.

Circ Cardiovasc Qual Outcomes. 2015;8:A273

- vii.** Clinical profile of acute myocardial infarction patients in the absence of significant coronary artery disease. 2015

Pasupathy S, Tavella R, Arstall M, Chew D, Worthley M, Zeitz C, Beltrame JF.

Poster Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand, Melbourne, Australia.

Heart, lung and circulation 01/2015; 24:S142.

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- viii.** Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA): A Systematic Review and Meta-analysis. 2014

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Poster Presentation

World Congress of Cardiology: Melbourne, Australia.

Global Heart, Volume 9, Issue 1, e274.

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- ix.** Measurement of Area at Risk by Cardiac Magnetic Resonance Imaging: Comparison of T2-Weighted Imaging with the Endocardial Surface Area Method.

Pasupathy S, Neil C, Beltrame JF.

Poster Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand (CSANZ): Brisbane, Australia.

Heart, Lung and Circulation 12/2012; 21:S256-S257

PRESENTATIONS AT INTERNATIONAL/LOCAL MEETINGS

- i.** The early use of N-acetylcysteine (NAC) with Glyceryl Trinitrate (GTN) in ST-segment Elevation Myocardial Infarction patients undergoing primary percutaneous coronary intervention (NACIAM Trial).

Pasupathy S, Tavella R, Raman B, Grover S, Mahadavan G, Zeitz C, Arstall M, Selvanayagam J, Horowitz J, Beltrame JF.

Late breaking clinical trial Presentation

Annual meeting of European Society of Cardiology congress, Rome, Italy. 2016

-
- ii.** Myocardial Infarction with Non-Obstructive Coronary Artery Disease (MINOCA): Prevalence, Clinical Features and Outcomes. 2015

Pasupathy S, Tavella R, Arstall M, Chew D, Worthley M, Zeitz C, Beltrame JF.

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- iii.** Diagnostic utility of cardiac magnetic resonance imaging (CMR) in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA) Patients. 2016

Pasupathy S, Tavella R, Potamianos R, Arstall M, Chew D, Worthley M, Zeitz C, Beltrame JF.

Mini oral Presentation

Annual meeting of the Cardiac Society of Australia & New Zealand, Adelaide, Australia. 2016

AWARDS AND RECOGNITION

2015 Research Day (Basil hetzel institute for translational health research)

Ivan De La Lande Award

2015 School of Medicine Travel Grant (The University of Adelaide)

The University of Adelaide

2013 Research Day (Basil hetzel institute for translational health research)

Best Clinical Presentation Award

2012 Higher Degree by Research Scholarship (The University of Adelaide)

Australian Postgraduate Award (APA)

CHAPTER 1

1 INTRODUCTION

1.1 ACUTE MYOCARDIAL INFARCTION (MI)

Cardiovascular disease (CVD) is one of the most important causes of death worldwide. The World Health Organisation (WHO) estimated that in 2008, 17.3 million (30%) of all deaths were due to CVD¹ and by the year 2030, more than 23 million people will die annually as a result of CVD². In 2012, CVD accounted for 523,805 hospitalisations in Australia and was reported as the leading cause of death among Australians, with 43,946 deaths recorded (almost 30% of all deaths in Australia)³.

CVD is a collective term used for heart and blood vessel related diseases. This includes coronary heart disease, stroke, and peripheral vascular disease⁴. Coronary heart disease, the most prevalent form of CVD, comprises of coronary vasculature disorders and frequently manifests as coronary artery disease (CAD). Acute coronary syndrome (ACS) and stable angina account for two major forms of coronary heart disease⁴. The term ACS refers to a group of clinical symptoms caused by active myocardial ischaemia, typically a result of underlying atherosclerotic plaque rupture and/or thrombus within the coronary artery, which includes acute myocardial infarction (acute MI) and unstable angina pectoris⁵. Patients exhibiting clinical symptoms of ischaemia, but with no evidence of myocardial necrosis are considered to have unstable angina⁶ whereas myocardial cell necrosis (as clinically evident by an elevated cardiac biomarker such as troponin) is indicative of acute MI⁷.

The WHO estimated that for the year 2008, 7.3 million (42%) of all cardiovascular deaths were due to acute MI¹. The National Heart Foundation of Australia estimate around 550,000 Australians experience acute MI annually, which is equal to one acute MI every 10 minutes. It claimed the lives of 9000 Australians in 2012, or 26 each day on average³. The past two decades have seen considerable advances in the understanding and management of acute MI. Upon completion of this chapter, the clinical, pathological, pathophysiological, and management strategies of acute MI will be discussed.

1.2 DEFINITION OF ACUTE MI

1.2.1 HISTORICAL EVOLUTION OF DEFINING ACUTE MI

The clinical definition of acute MI has considerably advanced in the last fifty years. The first clinical description of acute MI was provided by James B. Herrick at the 1912 meeting of the Association of American Physicians, titled "Clinical Features of Sudden Obstruction of the Coronary Arteries"⁸. With Herrick's insights, the electrocardiographic (ECG) changes associated with coronary artery ligation in dogs were documented by Fred Smith⁹ leading to the description of ECG changes associated with acute MI in humans¹⁰. In the early 1950s, Karmen et al¹¹ reported the release of aspartate aminotransferase during myocardial necrosis and demonstrated the diagnostic use of serum markers during acute MI. However, creatine kinase (CK) evolved as "the cardiac marker" in serum to identify myocardial necrosis with higher specificity compared to aspartate aminotransferase in late 1960's¹².

The progression of the clinical definition of acute MI over the last five decades is summarised in Figure 1. The first clinical guideline to define acute MI was published by the WHO in 1959¹³ in an attempt to standardize the definition of acute MI, with a revision following in 1979¹⁴. The

definition provided by the WHO was based on (i) clinical history, (ii) ECG findings and (iii) serum biomarkers including CK-myoglobin binding (CK-MB).

CK-MB⁷, an isoform of CK relatively specific to heart in the absence of skeletal muscle damage, first evolved as the gold standard for detection of myocardial necrosis in the early 1970's¹⁵ and subsequently the WHO officially incorporated cardiac biomarker findings in the definition of acute MI. However, acute MI was diagnosed if at least one of the three criteria was present. Consequently, this definition was often nonspecific and allowed for considerable interpretation bias.

The significant changes in the clinical definition of acute MI occurred following the recognition of the importance of cardiac biomarkers, in particular cardiac troponin. The cardiac biomarker was added to the clinical definition as a key criterion. Although CK-MB has been the hallmark for the diagnosis of acute MI for several decades and fundamental in measuring the extent of damage (infarct size), CK-MB is not entirely cardiac specific¹⁶. In addition, CK-MB released during myocyte damage has a rapid release and clearance rate which consequently limited the diagnosis of acute MI in late presentations¹⁷. The need for a biomarker specific for myocardial injury remained a challenge until the introduction of cardiac troponin. Following years of experiments, a double antibody, two-step enzyme linked immunoassay for detecting cardiac troponin in serum was developed¹⁸, thus began the cardiac troponin era.

Following the identification of cardiac troponin, it became clear that patients previously labelled as “unstable angina” patients, i.e., those with normal CK-MB, had an increased cardiac risk if cardiac troponin was elevated¹⁹. The majority of cardiac troponin is bound to myofilaments and a very small proportion found in cytosol in comparison to CK-MB²⁰.

Cardiac troponin has no baseline value, has a slow clearance rate and has minimal cross reactivity with skeletal muscle²¹. Due to its advantages over CK-MB, cardiac troponin has replaced CK and CK-MB as the preferred MI marker²². Considering this, the National Academy of Clinical Biochemistry (NACB) issued, in 1999, the first guidelines for the use of cardiac markers in ACS that included cardiac troponin²³.

In 2000, the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC) Committee redefined the diagnostic criteria of acute MI and provided the first universal definition. Given its superior sensitivity and specificity, cardiac troponin was recommended as the biomarker of choice when a significant rise and/or fall is identified in serially measured concentrations and at least one value is above the 99th percentile. In addition, the presence of either ischaemic symptoms, ischaemic ECG changes, or coronary artery intervention was required²⁰. Subsequently, the ESC/ACC Committee was expanded as ‘The Task Force’ and provided the second universal definition of acute MI with several subtypes of acute MI (Type 1 to Type 5)⁷. The advances in high sensitivity cardiac troponin assays in the early 2010s²⁴ resulted in a shift from unstable angina to acute MI in the setting of ischemic imbalance (Type 2 acute MI) which lead to the latest third universal definition of acute MI²⁵.

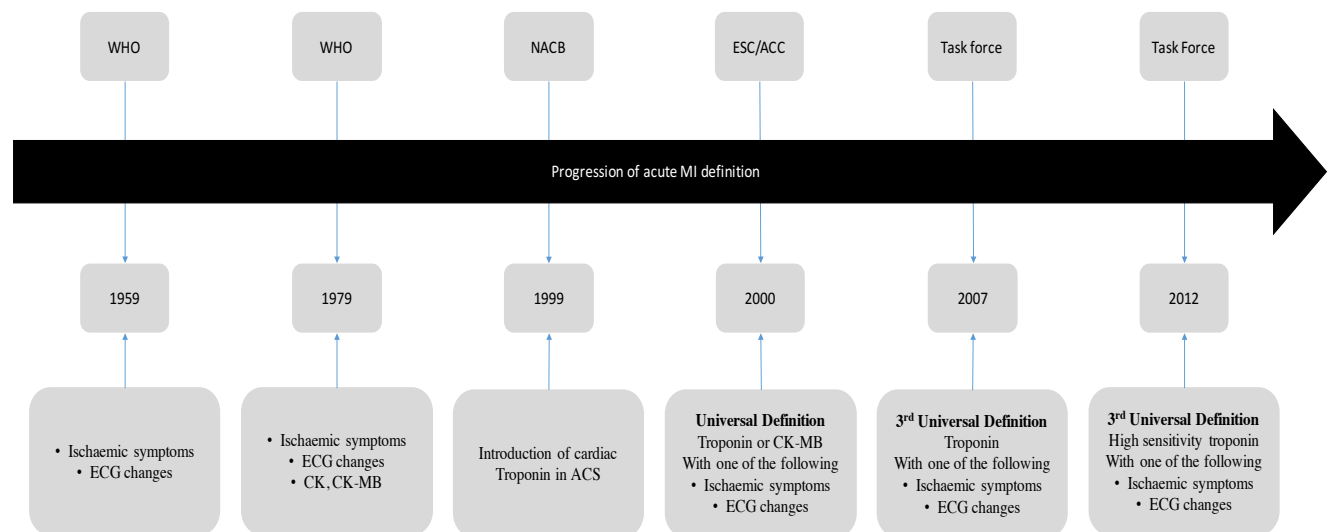


FIGURE 1: PROGRESSION OF ACUTE MI DEFINITION.

ACC, American College of Cardiology; ACS, Acute coronary syndrome CK, Creatine kinase; CK-MB, CK-myoglobin binding; ECG, Electrocardiography; ESC, European Society of Cardiology; NACB, National Academy of Clinical Biochemistry; WHO, World Health Organisation.

1.2.2 UNIVERSAL DEFINITION OF ACUTE MI

In 2012, The Task Force presented an updated and third universal definition of acute MI by integrating the latest clinical research insights, in particular high sensitivity cardiac troponin biomarkers, based on which, the following criteria is required for the diagnosis of acute MI. The components described in detail in sections 1.1.2.1 -1.1.2.4.

- Detection of rise and/or fall of cardiac biomarker values (preferably cardiac Troponin with at least one value above the 99th percentile upper reference limit with at least one of the following:
 - Clinical symptoms of ischaemia.
 - Significant ST-Segment-T wave (ST-T) changes, new left bundle branch block (LBBB) or development of pathological Q waves on ECG.
 - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

1.2.3 BIOCHEMICAL MARKERS

As the clinical definition of acute MI progressed, the detection of myocardial cell necrosis using cardiac biomarkers also has undergone major advances. The biomarkers utilized in the detection of infarction included aspartate aminotransferase, lactate dehydrogenase²⁶, CK, and CK-MB²⁷, all of which failed to demonstrate the nearly absolute specificity as well as high clinical sensitivity when compared to cardiac troponin²⁸. Cardiac troponins, made up of three subunits; C, T and I, are regulatory proteins that control the myocardial contractile function. Troponin C is expressed by cells in both cardiac and skeletal muscle. In contrast, the amino acid sequences of troponins I and T are unique to cardiac muscle. This difference has allowed the development of rapid, quantitative assays to detect elevations of cardiac troponins in the serum. The plasma troponin level of I and T in healthy subjects is hypothesised to be 0.1–0.2 ng/L, due to the continuous microscopic loss of cardiomyocytes during normal life²⁹.

Although elevated values reflect myocardial damage, it does not reflect the mechanism. Therefore, an elevated value in the absence of clinical indications may imitate other clinical conditions such as myocarditis²⁰. The third universal definition highlighted instances where elevated cardiac troponin may indicate conditions other than acute MI and elaborated that cardiac troponin is an indication of myocardial necrosis/injury regardless of the underlying pathophysiology.

1.2.4 ISCHAEMIC SYMPTOMS

Symptoms suggestive of acute MI can be identified from the patient's history at clinical presentation. Most reported ischaemic symptoms include chest pain and/or shortness of breath, which last at least 20 minutes. The presentation may include additional symptoms of nausea, diaphoresis or syncope. Atypical symptoms include rapid palpitations, or cardiac arrest, and it is also possible for myocardial necrosis to occur without any of these symptoms. In some instances, the ischaemic symptoms can be misdiagnosed and attributed to gastrointestinal, neurological, pulmonary or musculoskeletal disorders²⁵.

1.2.5 ECG FINDINGS

ECG plays a crucial role in the identification and management of acute MI patients. Changes in ST, T wave, and QRS components may indicate signs of myocardial ischaemia. The temporal changes in ST segment morphology during myocardial ischaemia was first described by Pardee et al³⁰ indicating current flow changes during ischaemia. Injury currents flowing from the depolarized ischaemic regions to normal regions result in the appearance of ST segment elevation or depression, depending upon whether the ischaemic region is transmural or subendocardial³¹. A working definition of acute MI can be anticipated in the presence of clinical ischaemic symptoms described with either (i) ST-segment elevation or

(ii) without ST-segment elevation, i.e. ST-segment depression or T wave abnormalities³². ST-segment elevation myocardial infarction (STEMI), refers to MI with raised cardiac biomarker and ST segment elevation, and is often identified as the more severe subtype of acute MI. Non ST-segment elevation myocardial infarction (NSTEMI), refers to MI with raised biomarker and ischaemic symptoms but without ST segment elevation³³.

1.2.6 IMAGING INVESTIGATIONS

Imaging investigations are used when the diagnosis of suspected acute MI is not evident by standard means in order to rule out or confirm the presence of ischaemia and identify the non-ischaemic causes of ischaemic symptoms. Echocardiography, single photon emission computed tomography (SPECT) and cardiac magnetic resonance Imaging (CMR) are commonly used imaging investigations²⁵.

1.3 PATHOPHYSIOLOGY OF ACUTE MI

Acute MI is myocardial tissue damage due to prolonged myocardial ischaemia as a result of obstructed blood supply to the myocardium. The mechanism behind myocardial ischaemia will be discussed in section 1.4. The pathophysiology of acute MI reflects the causes of occlusion of the coronary arteries. Coronary artery occlusion from rupture or erosion of atherosclerotic plaques is the most common cause of acute MI, accounting for at least 70% of fatal events^{34, 35}. Other causes of MI include coronary spasm, coronary embolism, and thrombosis in non-atherosclerotic normal vessels³⁶. In short, a combination of atherosclerosis, thrombosis and coronary artery spasm, underpin the pathophysiological basis of acute MI. Subsequent sections of this chapter will discuss each of these components.

1.3.1 ATHEROSCLEROSIS

Coronary atherosclerosis refers to the plaque formation in the intima of large and medium sized coronary arteries. Progression of coronary atherosclerosis takes many years and is accelerated by the presence of risk factors such as hypertension, hyperlipidaemia, smoking, diabetes and a family history of premature CAD^{35, 37}. The formation of the response-to-injury hypothesis proposed that endothelial dysfunction is the first step in atherosclerosis³⁸. The influence of risk factors such as elevated and modified low-density lipoprotein (LDL), presence of free radicals via smoking, hypertension, diabetes mellitus, genetic components, and specific infectious conditions may damage the endothelium, which leads to endothelial dysfunction in coronary arteries³⁸. When the endothelium is damaged, it leads to an accumulation of inflammatory cells in the subendothelium, and through differentiation processes, they form macrophages which subsequently develop fatty streaks. The amount of macrophages in smooth muscle cells plays a crucial role in plaque vulnerability and propensity for the rupture³⁹. Although atherosclerosis can result in ACS, the progression rate

of atherosclerosis is nonlinear, unpredictable and clinically silent in most cases^{40, 41}. An atherosclerotic plaque may partially or totally obstruct the blood supply. A ruptured plaque or formation of thrombus on the plaque's surface, or a combination of both, could lead to prolonged ischemia manifesting as acute MI. In many acute MI cases, a sudden morphologic change in atherosclerotic plaque leads to plaque disruption⁴².

1.3.2 THROMBOSIS

Endothelial dysfunction also leads to increased thrombogenicity of the blood through a series of events^{39, 43}. The lipid and tissue factor content of the plaque, severity of the plaque rupture, the amount of inflammation at the site, and the antithrombotic-prothrombotic balance are each important factors in regards to thrombus formation and the progression into an acute ischaemic event⁴⁴⁻⁴⁶. Thrombus can be formed from an exposed thrombogenic lipid core as a result of the fibrous cap detachment from the plaque or in a defective endothelial layer as a result of plaque erosion⁴². Extrinsic coagulation pathway activation also leads to thrombus formation⁴⁷. The formed thrombus either lyses spontaneously or remains incorporated in the artery, progressing to a total or near total coronary artery obstruction⁴⁸. Plaque haemorrhage resulting from blood vessel rupture leading to the deposition of blood in plaques, in turn, can enlarge the plaque, with lipid and inflammatory content⁴⁹ and subsequently obstruct the blood flow.

1.3.3 CORONARY ARTERY SPASM

Coronary artery spasm refers to abnormal vasomotor reactivity, in particular vasoconstriction of coronary arteries that leads to total or subtotal coronary artery occlusion and consequently myocardial ischaemia. Prinzmetal et al⁵⁰, for the first time, proposed a new form of angina pectoris referred to as variant angina and postulated this occurs as a result of coronary artery spasm. Following the introduction of coronary angiography, Sones et al⁵¹ documented coronary spasm angiographically during a variant angina attack. Coronary artery spasm has been reported in many variant angina cases, particularly in Japan^{52, 53}. It is now known that prolonged occlusive coronary spasm results in acute MI⁵⁴.

The pathophysiologic basis of coronary artery spasm is multifactorial. The autonomic nervous system, inflammation, endothelial dysfunction, oxidative stress, and genetic mutations have been found to be associated with coronary artery spasm⁵⁵. The differences in the incidence of coronary artery spasm in different countries is also well documented. The Japanese population appear to be at high risk of developing coronary artery spasm compared to western populations⁵⁶. Although the specific reasons for these racial differences are unknown, it has been postulated that clinical and pathophysiological differences between Japanese and Caucasian patients are a result of low prevalence of fixed coronary stenoses and diffuse coronary hyperreactivity^{56, 57}.

It is important to recognize that the diagnosis of coronary artery spasm depends on coronary angiography and provocation test, for which a universal method is not yet established. Therefore, although a racial difference exists in coronary vasoconstrictor response, the prevalence for different populations is yet to be determined⁵⁶.

1.4 PATHOLOGY OF ACUTE MI: MYOCARDIAL ISCHAEMIA

As stated in section 1.3, acute MI refers to myocardial tissue damage due to prolonged myocardial ischaemia as a result of obstructed blood supply to the myocardium. Pathological evidence of atherosclerotic plaques and thrombotic occlusion during acute MI is demonstrated in autopsy studies and early angiographic studies. Davies et al⁵⁸ demonstrated 95% of autopsy studies of acute MI patients had thrombotic occlusion of a ruptured or eroded atherosclerotic plaque. Pioneering coronary angiographic studies by DeWood and colleagues³³ showed that 87% of acute MI patients presenting with onset of symptoms within four hours had complete thrombotic occlusion of the infarct related artery. However, the incidence was reduced to 65% at 12-24 hours after symptom onset, due to either spontaneous lysis of the thrombus, relaxation of spasm, or both. Gould et al⁵⁹ demonstrated the relationship between coronary artery stenosis and ischaemia using myocardial blood flow measurements in which the myocardial blood flow is not compromised at stenosis less than 50%. A lesion must be at least 70% to impair coronary flow but more severe lesions of at least 90% could result in myocardial ischaemia at rest.

Coronary circulation is critical for the normal function of the heart as it delivers oxygen to myocytes. The loss of blood supply during coronary occlusion diminishes the oxygen supply to the myocytes with subsequent damage and loss of function²⁵ via a series of biochemical and metabolic changes in the myocardium as illustrated in Figure 2. During ischaemia, reduced availability of molecular oxygen and metabolic substrates results in a deficit of adenosine triphosphate (ATP). Ca^{2+} uptake mechanisms via the sarcoplasmic reticulum (SR) are impaired leading to intracellular Ca^{2+} accumulation. This results in hypercontractility, ATP depletion, structural damage to mitochondria and myocardial stunning⁶⁰⁻⁶². Anaerobic metabolism is associated with intracellular accumulation of inorganic phosphate, lactate, and

H^+ . Activation of the Na-H exchanger by intracellular acidosis leads to accumulation of intracellular Na^+ . This Na^+ overload is exacerbated by inhibition of the sodium pump due to ATP depletion. Increasing intracellular concentrations of solutes resulting in osmotic swelling may cause sarcolemmal fragility or disruption. Activation of Ca^{2+} - dependent proteases and phospholipases may further accelerate the cellular level myocardial damage. The acidic conditions during ischaemia prevent the opening of the mitochondrial permeability transition pore (MPTP)^{63, 64}. The process of irreversible injury is time-dependent, and in unrelieved ischaemia, will result in the pathological features of necrosis.

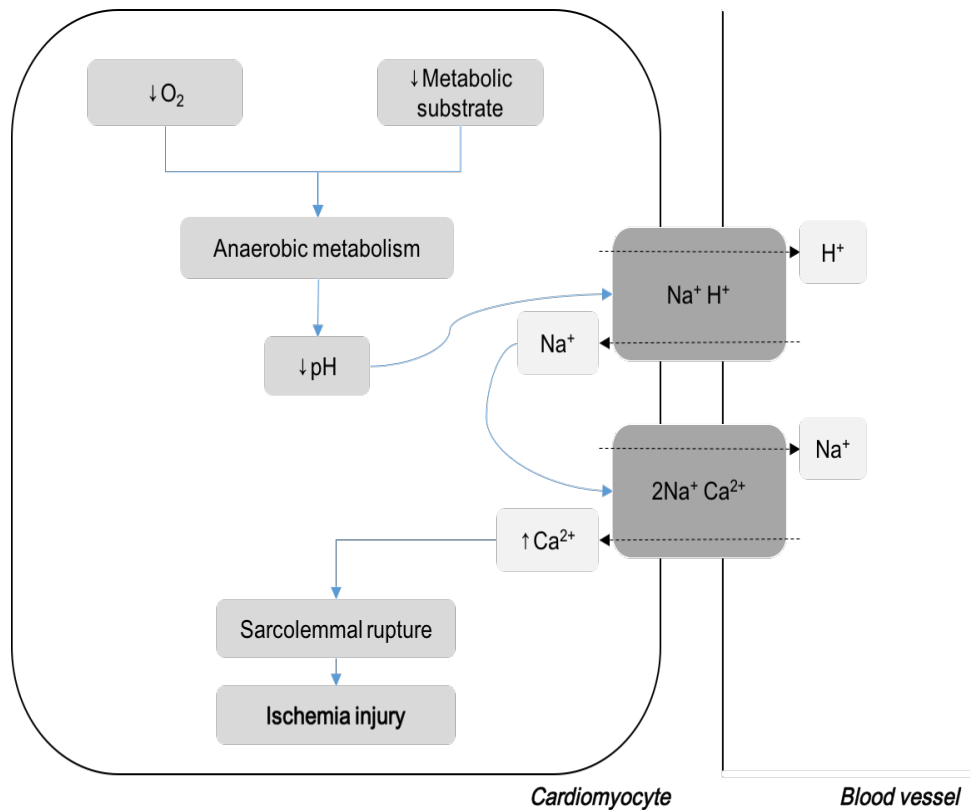


FIGURE 2: PROCESS OF MYOCARDIAL ISCHAEMIA

Myocardial cell death does not begin immediately after the onset of myocardial ischaemia but takes a period of time to develop, and requires hours to be evident on microscopic/macrosopic post mortem examinations²⁰. The complete process of myocardial cell death requires at least two hours and up to six hours due to a number of factors, including collateral circulation to ischaemic zone, persistent or intermittent coronary occlusion, sensitivity of myocytes to ischaemia, pre-conditioning and/or individual demand for oxygen and nutrients⁶⁵.

The morphological patterns in myocardial infarct areas reflect the progression of myocardial ischaemia with a gradient of perfusion deficit from the centre to the peripheral infarct or ischaemia zone. In an established myocardial infarct area, the central infarct zone contains necrotic myocytes with relaxed myofibrils, whereas the peripheral infarct zone exhibits necrotic myocytes with contraction bands and calcium deposits. Other myocytes in the outermost periphery of the infarcts show features of less severe injury, including lipid droplet accumulation^{66,67}.

An acute coronary artery occlusion lasting for greater than 20 minutes initiates myocardial necrosis. The developing myocardial cell death spreads from the centre to the edge of the occluded vessel territory, and the endocardial layer are subject to severe ischaemia⁶⁸. The evolution of infarct within the ischaemic zone is defined as a wave front phenomenon and was first described by Reimer et al⁶⁹. The morphology of the infarct zone represents a sharp delineation between ischaemic and non-ischaemic myocardium at the lateral margins of the myocardial bed-at risk. The onset of irreversible injury begins after about 20 to 30 minutes in the ischaemic sub endocardium, where the perfusion deficit is most severe, compared with the sub epicardium, which receives some collateral blood flow. Irreversible myocardial injury

then progresses in a wave front movement from the sub endocardium into the sub epicardium.

1.5 DIAGNOSIS OF ACUTE MI: INITIAL ASSESSMENTS

As established in section 1.4, although factors such as collateral circulation attempt to limit the myocardial cell death following the onset of prolonged myocardial ischaemia, it only takes a few hours to result in irreversible injury. Therefore, it is essential that the diagnosis of acute MI is established as accurately and as early as possible to begin appropriate management.

1.5.1 ECG

As established in the clinical definition of acute MI, the ECG is a clinically important tool for the evaluation of patients presenting with suspected acute MI. The 12-lead ECG is the most immediately accessible and widely used diagnostic tool to identify proximal blockages of coronary arteries, which result in large acute MIs, it adds significant information for risk stratification and aids the decision to begin immediate treatment strategies.

1.5.2 STEMI

Patients presenting with predominant ST segment elevation on ECG in the presence of cardiac biomarkers are classified as STEMI. The underlying aetiology is transmural ischaemia/infarction generally caused by thrombus occluding the infarct related coronary artery, except in cases of coronary artery spasm^{70, 71}. The acute ECG of STEMI patients provides information about the infarct related artery, location of infarct, size of infarct, and area at risk, hence facilitating appropriate management^{71, 72}. Delayed diagnosis and management, in particular delayed reperfusion of coronary arteries, results in increased

mortality in STEMI patients⁷³. Reperfusion of the infarct related artery resolves the ST segment elevation⁷⁴.

1.5.3 NSTEMI

Patients presenting with no predominant ST elevation in the presence of cardiac biomarkers are classified as NSTEMI⁷¹. The absence of ECG changes does not exclude the diagnosis of NSTEMI, as approximately 5% of patients with ischaemic symptoms such as chest pain and no ECG changes are found to have NSTEMI⁷⁵. ST segment depression is a frequent finding in acute MI; reflecting ischaemia confined to the sub-endocardium⁷¹.

1.5.4 CARDIAC BIOMARKERS

For patients presenting with suspected acute MI, cardiac biomarkers such as cardiac troponin (T or I) or CK-MB are usually performed to confirm the myocardial injury²⁵. Cardiac troponin is now considered the ‘gold standard’ method for the diagnosis of myocardial infarction^{76, 77}. It rises approximately four to six hours after the onset of myocardial injury and peaks at approximately 24 hours. Recent advances have led to the development of high sensitivity troponin assays which are more accurate than previous assays and result in successful diagnosis of acute MI within three hours of the onset of symptoms^{78, 79}. Although cardiac biomarkers are important to confirm the myocardial injury, they do not affect the initial or crucial management of acute MI patients due to the time delay in the rise and/or fall. In addition, patients with elevated cardiac troponin may be associated with causes other than MI such as myocarditis, congestive heart failure, renal insufficiency, renal failure, hypertension with left ventricular hypertrophy, aortic valve disease, hypertrophic obstructive cardiomyopathy with significant left ventricular hypertrophy, strenuous exercise, pulmonary embolism, amyloidosis, sepsis, systemic inflammatory response syndrome, severe burns,

hypotension, and others⁸⁰. However, cardiac troponin serves as a class I indication for risk stratification in ACS patients. Increased cardiac troponin values correlate with severity and presence of CAD^{81, 82}. Furthermore, cardiac troponin has well documented prognostic implications. Patients presenting with increased troponin values are associated with worse outcomes^{78, 83, 84}.

1.5.5 CORONARY ANGIOGRAPHY

A new era in cardiovascular medicine was introduced following the application of coronary angiography. As a reliable in-vivo tool for the visualization of coronary arteries, the coronary angiogram not only provided the evidence to support the clinical diagnosis but led to significantly improved understanding of CAD and function of the myocardium, and contributed to the discoveries that are important in the management of coronary heart disease, including acute MI.

1.5.6 ACUTE MI WITH CAD (MI-CAD)

Early acute MI coronary angiography studies undertaken by DeWood and colleagues demonstrated the role of obstructive CAD in acute MI. These pioneering studies demonstrated that in patients presenting with STEMI, almost 90% had an occluded coronary artery provided that angiography was undertaken within four hours of chest pain onset³³. In contrast, in NSTEMI patients, only 26% had an occluded coronary artery when angiography was performed within 24 hours of symptom onset⁸⁵. In both of these landmark studies^{33, 85} greater than 90% of the acute MI patients had angiographic evidence of obstructive CAD which underscored the importance of the atherosclerotic process in the pathogenesis of acute MI.

1.5.7 ACUTE MI WITHOUT SIGNIFICANT CAD

Although DeWood's studies highlighted the importance of CAD in acute MI, it is fascinating that a small proportion of the patients investigated had no significant CAD on coronary angiography. This finding is confirmed in several large acute MI registries where 1-11% of acute MI's occurred in the absence of obstructive CAD⁸⁶⁻⁸⁹. These acute MI presentations constitute an intriguing subgroup now being referred to as Myocardial Infarction with Non-Obstructive Coronary Arteries or MINOCA⁹⁰. The inconsistency in the literature regarding this syndrome underestimates its importance. In addition, the fundamental conundrum confronting researchers and clinicians regarding MINOCA is 'are they clinically the same as a patient with MI-CAD and therefore should be managed the same?', and 'what are the mechanisms behind the clinical presentation?'. In chapters 2 to 4 of this thesis, novel aspects of MINOCA will be discussed in detail.

1.6 MANAGEMENT OF ACUTE MI

Following the initial assessments described in section 1.5, acute MI management decisions are dependent upon on the ECG findings and coronary angiogram findings. This section provides an overview of these management steps.

1.6.1 INITIAL MANAGEMENT

The initial management of acute MI consists of restoring the balance between oxygen supply and demand to prevent further myocardial ischaemia, pain relief and prevention of treatment complications. Oxygen has been used in acute MI treatment for over 100 years and was first advocated by Steele⁹¹. The justification for which is that it increases the oxygen delivery to ischaemic myocardium thereby reducing the size of MI and improving clinical outcomes. The routine use of supplemental oxygen for treatment of acute MI is recommended by international clinical guidelines⁷⁴. However, a Cochrane review in 2013 demonstrated no advantage of oxygen over room air for patients with suspected MI⁹². Subsequently, the Air Versus in Myocardial Infarction (AVOID) trial confirmed this⁹³. Therefore, more recent guideline suggests that oxygen should be administered when blood oxygen saturation is less than 90% or if the patient is in respiratory distress⁹⁴.

Pain relief approaches are important for patient comfort and also since pain is associated with sympathetic activation, it could increase the cardiac workload. Therefore, intravenous morphine, intravenous beta-blockers, and nitrates are considered for MI patients when there are no contraindications⁹⁵.

1.6.2 MANAGEMENT OF MI-CAD: ‘THE OPEN ARTERY HYPOTHESIS’

Since Herrick et al's⁹⁶ landmark description of coronary artery features in acute MI in 1912, clinical and research insights transformed acute MI from a medical curiosity to a treatable disease. Following Reimer et al⁹⁷, and De Wood et al's³³ findings, the pathophysiological understanding of acute MI was exemplified and the focus shifted towards limiting the spread of ischaemia (infarct size). Over a number of years, it became well-established that timely reperfusion salvaged severely ischaemic myocardium⁹⁸ and restoring the patency of infarct related artery, in any manner, declined the mortality rate⁹⁹. These findings supported the ‘open artery hypothesis’ first described by Braunwald et al¹⁰⁰. Improvements in myocardial reperfusion are largely due to (i) the development of efficient thrombolytic methods for lysis of thrombi; and (ii) the advances in mechanical interventions in restoring the coronary artery patency. The ‘door to needle time’ and ‘door to balloon time’ were established as markers to assess the efficacy of thrombolysis and mechanical intervention¹⁰¹.

1.6.3 THROMBOLYTIC THERAPY

Although Tillett et al's¹⁰² fortuitous research on thrombolytic agents laid the ground work for the use of thrombolytic therapy using streptokinase in early 1930s, the intracoronary infusion of streptokinase was initiated only in late 1970s following DeWood et al's³³ angiographic study. The first randomized multicentre trial, Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI), in 1986¹⁰³, validated streptokinase as an effective therapeutic method and established a fixed protocol for its use in acute MI, and thus the thrombolytic era began. Following streptokinase, tissue-plasminogen activators (t-PA) evolved as an optimal method. The Global Utilization of Streptokinase and Tissue plasminogen activator for Occluded coronary arteries (GUSTO-1), a landmark trial, demonstrated optimal vessel patency at 90 minutes after the administration of intravenous t-

PA administration, which resulted in 15% reduction in mortality rate in comparison to streptokinase¹⁰⁴.

1.6.4 PERCUTANEOUS CORONARY INTERVENTION (PCI)

In 1977, Gruentzig et al^{105, 106} introduced one of the most important therapeutic advances of 20th century medicine by the re-opening of severely stenosed coronary artery in humans using balloon angioplasty. Adoption of this technique was widespread, and a number of technological advances have evolved the procedure to what is now referred to as percutaneous coronary intervention (PCI), a collective term used for coronary angioplasty, thrombus extraction and stenting. While PCI is intensive and more difficult to undertake than administration of thrombolysis, it offers better clinical outcomes. A meta-analysis of 23 randomized trials with 7,739 acute MI patients demonstrated PCI resulted in reduced mortality rate, non-fatal re-infarction, and stroke compared to thrombolysis¹⁰⁷. However, the benefit of PCI is only evident when patients are treated early after the onset of symptoms¹⁰⁸. Efficient and effective clinical systems that are able to deliver timely and consistent reperfusion allow for the advantage of primary PCI.

1.6.5 REPERFUSION STRATEGIES FOR STEMI PATIENTS

As patients presenting with suspected STEMI are presumed to have an occlusive thrombosis in the epicardial coronary artery, immediate reperfusion is the principal focus. It includes either (i) primary PCI or (ii) thrombolytic therapy, and if the PCI and thrombolytic therapies are unsuccessful or not amendable, (iii) coronary artery bypass surgery is considered⁹⁵. Intracoronary stents are widely used in current clinical practice as the recurrent stenosis rates are less compared to balloon angioplasty¹⁰⁹.

In the event that the PCI procedure cannot be performed within 90 minutes after initial medical contact with the patient⁷⁴, pharmacological methods of reperfusion, such as thrombolytic therapy, are considered to restore blood flow. The effectiveness of which is highest in the first two hours⁹⁵. Although restoring patency with intravenous thrombolytic therapy results in a reduced infarct size, improved preservation of left ventricular (LV) function and thus improved cardiac mortality following STEMI, it is limited by restoration of infarct-related artery patency in only 50% of patients, and some risk of associated cerebral haemorrhage. However, rates of nonfatal re-infarction and stroke are significantly reduced in PCI compared to thrombolysis¹⁰⁷. As a consequence, PCI has evolved as the optimal reperfusion therapy⁹⁵. Although PCI is the preferred treatment for STEMI presentation, diagnostic angiography also identifies patients unsuitable for this procedure during the acute phase⁷⁴ and thus CABG may be used as the primary reperfusion modality. Stone et al¹¹⁰ reported that 11% of STEMI patients required CABG during hospital admission and the outcomes were similar to those who received PCI. However, the incidence of patients requiring emergency CABG after PCI has decreased during the last few decades¹¹¹, especially, since the introduction of adjunctive treatments such as heparin, clopidogrel, and glycoprotein IIb/IIIa inhibitors¹¹².

1.6.6 REPERFUSION FOR NSTEMI PATIENTS

Unlike STEMI, early thrombolytic trials in patients with NSTEMI demonstrated harm rather than benefit, consistent with the angiographic observations of DeWood et al⁸⁵ that patients with NSTEMI typically had non-occluded vessels. The acute management of NSTEMI consists of several goals, including relief of ischaemic symptoms, assessment of hemodynamic status and correction of abnormalities, and estimation of risk for prevention of adverse ischaemic events¹¹³. An early invasive strategy requires a routine cardiac

catheterization within days of admission followed by revascularisation with either PCI or CABG depending on the extent of CAD. In contrast, some patients receive initial medical management and revascularization only if the patient experiences ischaemia recurrently despite vigorous medical management. Non-invasive management is applied generally in patients presenting with lower Global Registry of Acute Coronary Events (GRACE) score and low risk features¹¹⁴.

1.6.7 ADJUNCTIVE THERAPY

Following reperfusion and stabilization of acute MI patients, medical management aims to prevent long term complications, modify risk factors, restore normal function and control symptoms, and includes antiplatelet therapy, anticoagulant therapy, statin therapy, beta blockers, calcium channel blockers, and nitrates⁹⁴.

1.7 MYOCARDIAL ISCHAEMIC REPERFUSION INJURY IN MI-CAD

Although the reperfusion of the myocardium represented a major innovation in the treatment of acute MI, it is not flawless. While it reduces the spread of ischaemic cell death, it also increases the risk of myocardial injury. Jennings et al¹¹⁵ demonstrated harmful reperfusion after the release of temporary coronary artery occlusion. The study was based on experiments with canine hearts subject to coronary ligation in which reperfusion appeared to increase the development of necrosis. They showed that histological staining changes following only 30-60 minutes of ischaemia-reperfusion were comparable to the degree of necrosis normally seen after 24 hours of permanent coronary occlusion¹¹⁵. This was further elaborated by Kloner et al¹¹⁶ who showed that reperfusion caused further microvascular damage with swelling of capillary endothelial cells and of myocytes. As a result, myocardial reperfusion may be considered a 'double edged sword' with the resulting damage known as myocardial ischaemia reperfusion injury¹¹⁷.

The processes leading to myocardial ischaemia-reperfusion injury are shown in Figure 3. The restoration of coronary artery patency introduces the sudden re-introduction of molecular oxygen, causing re-energization of mitochondria and reactivation of the electron transport chain with production of reactive oxygen species (ROS). This may stimulate further ROS production (ROS induced ROS release) and generation of reactive nitrogen species (RNS) in the presence of nitric oxide (NO). ROS/RNS cause oxidative and nitrosative damage to cellular structures including the SR leading to Ca^{2+} release. Also, under conditions of restored ATP production, the activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is restored, leading to the extrusion of Na^+ in exchange for Ca^{2+} , and SR Ca^{2+} release is further accentuated by restoration of ATP, leading to cytosolic Ca^{2+} overload. ROS also mediates the opening MPTP, acting as a neutrophil chemo-attractant, and mediating dysfunction of the SR. The combined effects of

Ca^{2+} accumulation in the mitochondrial matrix, ROS/RNS, and increasing intracellular pH due to H^+ washout, favour the formation/opening of the MPTP, leading to cardiomyocyte contracture. The mechanisms leading to activation of the apoptotic program are yet to be understood but may be related to either mitochondrial or extracellular death signals.

Clinically, ischaemia-reperfusion injury may be attributed to four different types of cardiac dysfunction: myocardial stunning (persistent mechanical dysfunction despite restored blood flow); the no-reflow phenomenon after opening of an infarcted coronary artery; reperfusion arrhythmia; and lethal, irreversible injury of the myocardium¹¹⁸.

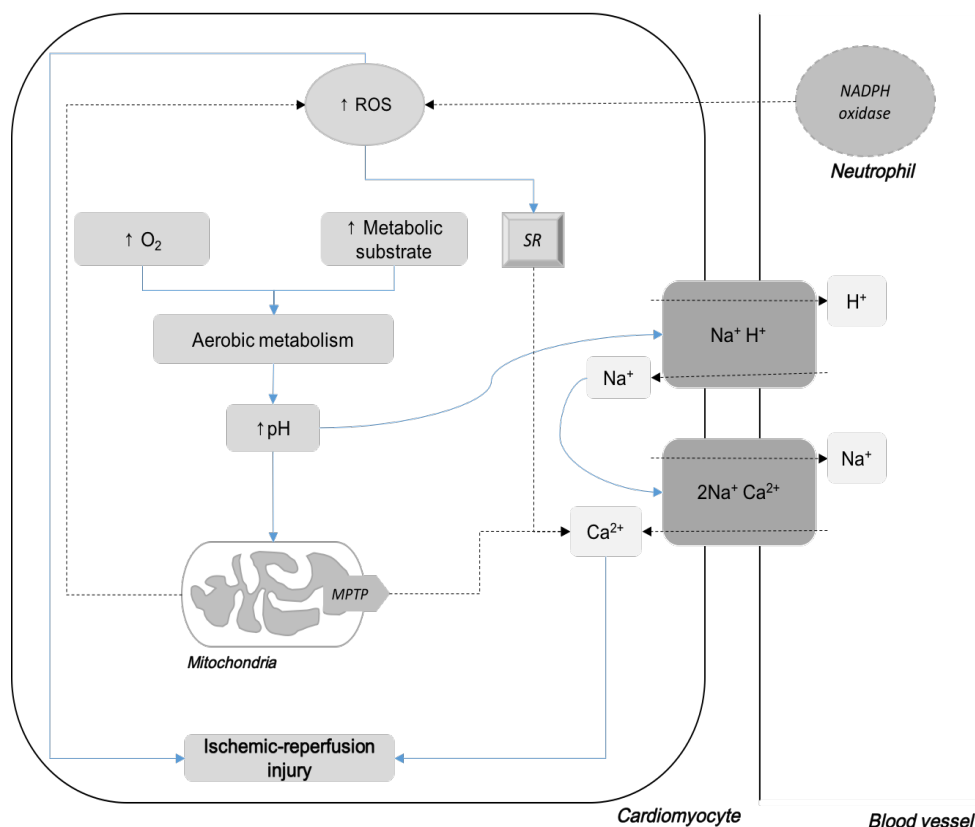


FIGURE 3: MYOCARDIAL ISCHAEMIA REPERFUSION INJURY.
MPTP, Mitochondrial permeability transition pore; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; ROS, Reactive oxygen species; SR, sarcoplasmic reticulum

1.8 DETERMINANTS OF ACUTE MI SIZE

Myocardial infarct size is one of the important predictors of clinical outcomes of acute MI patients, in particular for those presenting with STEMI. As the result of infarction, ventricular remodelling occurs in the normal myocardium in attempt to normalize the increased stress on the left ventricle. It involves hypertrophy and apoptosis of cardiomyocytes, formation of new cardiomyocytes, and connective tissue changes^{67, 69, 119}. Although controlled remodelling can lead to wall stress normalization, excessive wall stress can lead to fixed structural dilation of the ventricle and subsequent heart failure¹²⁰. Patients with a larger myocardial infarct size are directly associated with increased heart failure incidence and death^{121, 122}. Myocardial infarct size can be divided into two categories: myocardial damage arising from myocardial ischaemia; and myocardial damage arising from reperfusion.

In regards to myocardial ischaemia related damage, the major determinants of final infarct size are the duration and severity of ischaemia, the size of the myocardial bed-at-risk, and the amount of collateral blood flow available shortly after coronary occlusion^{67, 69}. In regards to reperfusion related damage, as established in section 1.7, ischaemia-reperfusion injury following restoration of myocardial perfusion results in significant damage. In addition, restoration of blood flow also results in structural damage to the microvasculature. This concept is referred to as the no-reflow phenomenon and plays a significant role in final infarct size¹²³. In patients receiving mechanical reperfusion, stent deployment may precipitate distal embolization, a form of microvascular injury referring to distal arterial occlusion.

Reperfusion strategies have evolved to limit the infarct size arising from myocardial ischaemia, but additional pharmacological interventions have also been examined to limit the damage arising from reperfusion. The success of some agents has been limited to

experimental models of ischaemia and reperfusion. It is important to distinguish therapeutic strategies for ischaemia versus reperfusion and it is possible that a combination of agents is required to elicit the maximum clinical benefits. Clinical trials employing a combination of pre-ischaemic and pre-reperfusion strategies are currently in progress to identify the optimal pharmacological approach to limit reperfusion injury. The final chapter of this thesis aims to investigate a novel therapeutic intervention in reducing infarct size.

1.9 PROGNOSIS OF ACUTE MI

Following an acute MI, patients are at risk of further adverse cardiovascular events such as recurrent infarct, death, stroke and heart failure. These outcomes vary depending on the clinical profile and comorbidities; thus risk stratification models should be applied in predicting prognosis. Many reports have shown that short term (in-hospital and one month) and long term (greater than six months) mortality rates following acute MI have been decreasing over the last three decades in developed countries. These improvements have been attributed to the increasingly widespread use of revascularization procedures, effective acute treatment, and long-term secondary prevention^{99, 124-127}.

Registries examining rates and trends in the mortality rates of STEMI have reported a decreasing mortality over the last 30 years however most of these studies are presented with data collected prior to 2005 – prior to implementation of current management and secondary prevention strategies¹²⁸⁻¹³². More contemporary patient registries also continue to report decreasing mortality in STEMI patients^{124, 133, 134}. The Registry of Information and Knowledge about Swedish Heart Intensive Care, Sweden, reported that in-hospital mortality decreased from 11.8% to 5.1 % and one-month mortality reduced from 14.2% to 6.3% from 1996 to 2007¹³⁴. In contrast, a North California based health database reported from that 1999 to 2008 there was no significant reduction in mortality rate (odds ratio 0.93; 95% confidence interval 0.71 to 1.20)¹³³. This may be attributed to the differences in clinical practices between countries¹³⁵. Short-term mortality is lower in patients with NSTEMI (2% to 4%) compared to patients with STEMI (3% to 8%) treated with primary PCI within two hours of hospital arrival^{101, 136-140}. Better short-term outcomes for patients with NSTEMI have also been noted in other studies (e.g., in-hospital mortality 5% to 7% compared with 7% to 9.3% with STEMI in the GRACE and European Heart registries)^{138, 140, 141}.

1.10 THESIS OBJECTIVES

Although considerable advances have been made in the understanding and management of acute MI in the past 50 years, there are significant areas that require further evaluation. This thesis will provide novel data in two of these areas. Firstly, in relation to progressing the understanding of MINOCA, and secondly concerning adjunct therapy in the management of acute STEMI.

Concerning MINOCA, the specific objectives are:

1.10.1 CHAPTER 2: SYSTEMATIC REVIEW AND META-ANALYSIS OF MINOCA

This study aims to detail the clinical attributes of these patients by systematically evaluating the published literature in regards to (i) the prevalence, clinical features, and 12-month prognosis of MINOCA patients, and (ii) the major underlying pathophysiological mechanisms responsible for this disorder.

1.10.2 CHAPTER 3: CLINICAL CHARACTERISTICS OF MINOCA

This study aims to compare the clinical profile, particularly the characteristics of chest pain in relation to location, quality, precipitating factors, associated symptoms, and duration between MINOCA and MI-CAD patients. In addition, it also provides an evaluation of cardiovascular risk factors, GRACE risk assessment at presentation, discharge medications, and in-hospital outcomes between the two groups.

1.10.3 CHAPTER 4: RISK OF THROMBOSIS IN MINOCA

This study aims to compare the pro-thrombotic tendency of patients with MINOCA compared to MI-CAD by evaluating congenital and acquired thrombophilic conditions, markers of coagulation activation, and global coagulation via the thrombin generation assay.

In relation to adjunct therapy in the management of acute STEMI, this thesis also presents the findings of the NACIAM (N-AcetylCysteine In Acute Myocardial infarction) study:

1.10.4 CHAPTER 5: THE ROLE OF N-ACETYLCYSTEINE AND GLYCERYL TRINITRATE IN STEMI

The objective of the NACIAM trial is to evaluate the efficacy of the addition of intravenous high dose N-acetylcysteine (NAC) to low dose glyceryl trinitrate (GTN) for reduction of infarct size in STEMI patients.

CHAPTER 2

2 SYSTEMATIC REVIEW AND META-ANALYSIS OF MINOCA

This chapter includes an outline and the manuscript in the form as it appears in the manuscript, “Systematic Review of patients presenting with suspected Myocardial Infarction and non-obstructive coronary arteries” authored by Sivabaskari Pasupathy, Tracy Air, Rachel Dreyer, Rosanna Tavella, and John F. Beltrame, and published in *Circulation*, 2015.

In keeping with this style of this thesis, the abstract has been removed, the table and figures re-numbered, the references incorporated into the thesis’s master reference list and the manuscript repaginated.

2.1 STATEMENT OF AUTHORSHIP

Systematic Review of Patients Presenting with Suspected Myocardial Infarction and Non-obstructive Coronary Arteries

Publication Status	Published
Publication Details	Circulation. 2015; 131:861-870
Principal Author	Sivabaskari Pasupathy
Contribution	Acquisition of data; Analysis and interpretation of data; Drafting of manuscript, Critical revision of manuscript
Overall percentage (%)	80%

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature and Date 14/08/2016

Co-Author Contributions

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

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

Chapter 2

Name	Tracy Air
Contribution	Analysis and interpretation of data; Critical revision
Signature and Date	12/08/2016

Name	Rachel Dreyer
Contribution	Critical revision
Signature and Date	11/08/2016

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Contribution	Critical revision
Signature and Date	12/08/2016

Name	John F Beltrame
Contribution	Study conception and design; Critical revision; Final approval.
Signature and Date	12/08/2016

2.2 STUDY OUTLINE

As established in the introductory chapter, MINOCA refers to an intriguing subgroup of myocardial infarct patients without obstructive CAD. Contemporary narrative reviews summarizing MINOCA tend to be descriptive; do not involve a systematic search of the literature and thereby focus on a subset of patients, and although informative, often are subject to selection bias. Mulrow et al¹⁴² and Teagarden et al¹⁴³ highlighted the lack of meticulousness in the narrative reviews. The inadequacy of traditional narrative reviews and the need for systematic reviews was emphasised in the early 1990s by Lau et al¹⁴⁴ and Antman et al¹⁴⁵. This further underscored that more knowledge can be extracted by collating existing research in the same rigour undertaken for primary research or an original study.

Systematic reviews have evolved as a tool to summarise the results of available original studies and provide evidence on the effectiveness of healthcare interventions¹⁴⁶. They can provide a comprehensive approach to evaluate a number of primary studies with disparate findings and substantial uncertainty. As the name implies, systematic reviews typically involve a detailed and comprehensive plan and search strategy derived priori, with the goal of reducing bias by identifying, appraising, and synthesizing all relevant studies on a particular topic. In addition, systematic reviews can include a meta-analysis component, which involves using statistical techniques to synthesize the data from several studies into a single quantitative estimate or summary effect size. Hence, a systematic review and meta-analysis approach is an extremely efficient method to obtain a clinical understanding of MINOCA.

As proposed by Hulley et al¹⁴⁷, the formulation of a research question should consider feasibility, interest, and relevance, and provide an opportunity to fill gaps in existing knowledge. In that view, the objectives to be addressed in this systematic review were clearly defined and structured prior to the review work. Objective 1 aims to identify the clinical profile of MINOCA in comparison to patients with MI and coronary artery disease (MI-CAD). Objective 2 aims to identify the mechanisms of MINOCA. The research topic was “Systematic Review of patients presenting with suspected Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA)

A systematic review involves a well-planned, rigorous attempt to identify all relevant primary research publications of the chosen topic in order to provide unbiased output. Identifying as many relevant research publications as possible is a key step in this research approach. PubMed, the largest free medical journal search engine maintained by the United States National library of Medicine, and Embase, a significantly large database produced by Elsevier indexing many journals that are not covered by PubMed, were utilized for the publication identification. To identify the publications, the Population, Intervention, Comparison, Outcome (PICO) logic grid method¹⁴⁸ was applied to the search terms. The three main concepts used in the logic grid were: Myocardial Infarction, Non Obstructive Coronaries and Coronary Angiogram. The next step was to identify synonyms, alternative terms, singular/plurals etc. for each of those three main concept terms. MeSH database was used to find at least one MeSH for the concepts. The search for the same terms in titles and abstracts of citations was found using [tiab] command. Standard logical operators OR, AND, NOT were used in between the search terms.

Using the PICO logic grid, the following search criteria were applied in the search engines to identify publications:

(Myocardial infarction [MH] OR myocardial infarct* [tiab] OR cardiac infarct* [tiab] OR heart attack* [tiab] OR myocardium infarct* [tiab] OR subendocardial infarct* [tiab] OR transmural infarct* [tiab] OR ventricle infarct* [tiab] OR ventricular infarct* [tiab])
AND (Non-Obstructive [tiab] OR nonobstructive [tiab] OR unobstruct* [tiab] OR unobstructive [tiab] OR normal [tiab] OR non significant [tiab] OR nonsignificant [tiab] OR No significant [tiab] OR “not significant” [tiab])
AND (angiogr* [tiab] OR coronary [tiab] OR stenosis* [tiab] OR cardioangiogra* [tiab] OR arteriogra*[tiab] OR Arteriosclerosis [tiab] OR CAD [tiab] OR artery [tiab] OR arteries [tiab] OR arterial [tiab] OR arteriography [tiab] OR vessel* [tiab])
AND (human OR Humans OR man OR men OR woman OR women)
NOT case reports [pt]
NOT Review [pt] NOT (Animals [MH] NOT Humans [MH])

Similar to PubMed, the Embase search was also built on the basis of a logic grid and the three main concepts were connected using the AND rule and synonyms/alternate terms were built using the OR rule. In addition, the Cochrane database of systematic reviews and meta-analyses was searched to confirm that there was no systematic review on this topic.

To be included in the systematic review, the following inclusion criteria were considered: Language: English; Original research studies; Evidence of AMI defined by Thygesen et al²⁵; and coronary angiogram performed in the context of acute MI. Studies were excluded if any of the following criteria were met: Language: Non English; Review articles; Case reports; coronary angiography performed in the context other than AMI and reproduced data from a former study.

An independent observer reviewed the titles and abstracts by screening the title and/or abstract and rejected many of the articles that did not fulfil the inclusion criteria. A second observer screened the identified articles for eligibility according to the inclusion criteria. Any disputes were discussed with a third observer. All relevant information from each of the included studies was extracted, which included details of the study, patient characteristics, relevant interventions and results.

The collected data was analysed using two methods in this systematic review. The primary objective of this study focused on the epidemiology/prevalence of patient characteristics. Therefore, data were pooled and analysed using a random effects meta-analysis model¹⁴⁹. This approach assumes that individual studies are estimating different treatment effects. Heterogeneity of the studies was assessed using the I^2 statistic with high values indicating larger heterogeneity between studies. Studies comparing two groups were assessed by summary of odds ratios (ORs) or mean difference and exact 95% confidence intervals (CI). Studies addressing the second objective focusing on mechanisms of MINOCA were analysed by pooling of frequency data and expressed as a qualitative assessment.

2.3 MANUSCRIPT: SYSTEMATIC REVIEW OF PATIENTS PRESENTED WITH SUSPECTED MINOCA

Introduction

Contemporary management strategies of acute ST-elevation myocardial infarction (STEMI) are based upon the pioneering early angiographic studies of DeWood and colleagues³³, who demonstrated an occluded coronary artery in almost 90% of these patients. Accordingly, the ‘open artery’ management strategy was employed, initially with the use of thrombolytic therapy and subsequently with percutaneous coronary interventions. In contrast, early angiography in patients with non-ST elevation myocardial infarction (NSTEMI) showed an occluded vessel in less than a third of these patients⁸⁵ so that strategies focusing on maintaining arterial patency were developed. However both of these acute myocardial infarction (MI) angiographic studies^{33, 85} demonstrated the presence of significant obstructive coronary artery disease (CAD) in more than 97% of these MI patients, thus underscoring the importance of obstructive coronary atherosclerotic disease in this condition.

With the widespread use of coronary angiography in the early clinical management of MI, multicentre MI registries have evolved and reported that as many as 10% of MI patients have no evidence of obstructive coronary artery disease⁸⁶. These patients with MINOCA (Myocardial Infarction with Non-Obstructive Coronary Arteries)⁹⁰ represent a conundrum as the underlying cause of their MI is not immediately apparent. Furthermore, whether they have similar clinical features and outcomes as patients with MI-CAD (Myocardial Infarction with obstructive Coronary Artery Disease) is unclear. Ascertaining whether MINOCA is a distinct clinical entity with specific clinical features, outcomes and pathophysiological mechanisms is paramount to determining the appropriate management strategy for these patients, yet to-date there is no systematic review of the published literature concerning these

patients. Furthermore, given the limited investigation of these patients, it is not surprising that there are no professional guidelines on the management of MINOCA.

Accordingly, the primary objectives of this systematic review are to detail the clinical attributes of these patients by systematically evaluating the published literature in regards to (A) the prevalence, clinical features, and 12-month prognosis of MINOCA patients, and (B) the major underlying pathophysiological mechanisms responsible for this disorder.

Methods

This study utilized a comprehensive structured systematic approach that included a methodical literature search, well-defined inclusion criteria for MINOCA, extraction of available raw data and pooling of the data to determine the frequency of each of the pre-determined study endpoints.

Published Literature Search

An unrestricted literature search was conducted using PubMed and Embase. The search terms in each of these databases and the subsequent evaluation process are summarized in Figure 4. In brief, searches were conducted in both databases focusing on the terms ‘myocardial infarction’, ‘non-obstructive’ and ‘angiography’. Only original human clinical research studies published in English were considered. However, the references in recent key review papers were also crosschecked with the database searches to ensure a comprehensive source of original papers. A search of the Cochrane database revealed no relevant systematic reviews on this topic.

Systematic Assessment of the Available Literature

Of the original human myocardial infarction research studies (1,033 publications) between 1966 and 2013 (inclusive), reference to non-obstructive coronary artery disease was evident in 237 publications (Figure 4). These manuscripts were reviewed for the following pre-specified inclusion and exclusion criteria by two of the investigators (SP, RT).

Inclusion Criteria. For consideration in this meta-analysis, it was essential for the following criteria to be documented in the protocol of the published study:

1. Evidence of an MI²⁵ as defined by (a) significant elevation of a cardiac biomarker and (b) at least one of the following – ischaemic symptoms, new ST/T changes or new left bundle branch block.
2. Qualitative coronary angiography findings to allow determination of the presence/absence of obstructive coronary artery disease.

MINOCA was defined as the presence of an MI (as per the above criteria) in the absence of obstructive coronary artery disease (i.e. no epicardial vessel with a stenosis $\geq 50\%$ on angiography). Those MI patients with significant obstructive coronary artery disease (at least one stenosis $\geq 50\%$) were designated as MI-CAD. The decision to utilize a $<50\%$ lesion threshold to delineate non-obstructive CAD from the obstructive CAD is based upon the following rationale: (a) well-established criteria in clinical guidelines¹⁵⁰, accordingly (b) it is the most frequently used definition in published angiographic studies, (c) considering the limitations of angiography, the presence of angiographic smooth vessels does not exclude the presence of significant atherosclerosis, (d) attention should be focused on why myocardial infarction/injury has occurred in the absence of a functionally obstructive lesion, and (e) the more inclusive definition allows future prognostic studies to determine if there is clinical utility in delineating those with angiographically smooth vessels from those with minor CAD.

Exclusion Criteria. Publications were excluded from further consideration if:

1. coronary angiography was not performed in the context of an MI admission,
2. tako-tsubo cardiomyopathy or myocarditis were the primary focus of the paper,
3. there was no original data or the data was reproduced from a former study, and
4. isolated case report format.

Utilizing these inclusion and exclusion criteria, 152 original MI publications had sufficient data to clearly identify those patients with MINOCA. Further analysis was dependent upon the specific objectives of this study, namely (A) determining the clinical (primary objective) or (B) pathophysiologic (secondary objective) attributes of MINOCA (Figure 4). The studies utilized in the analyses are listed in Supplemental Table 1 of the Data Supplement.

As the primary objective requires a representative sample to quantitatively assess the clinical attributes of MINOCA, only publications that recruited (i) at least 100 patients with MI, and (ii) consecutive MI patients, were included in the analysis. The specific definitions used in these studies for the various cardiovascular risk factors are listed in Supplemental Table 2 of the Data Supplement.

For the second objective, original studies fulfilling the above inclusion/exclusion criteria were included if they performed systematic diagnostic evaluations on a group of MINOCA patients with the intention of exploring the underlying pathophysiologic mechanism/s responsible for the MI. These included myocardial imaging studies such as cardiac magnetic resonance (CMR) imaging and functional studies such as provocative spasm testing and thrombophilia screening (Figure 4). For consistency, the total frequency of each abnormal pathophysiologic investigation was documented although it is acknowledged that the findings may be time-dependent. Accordingly, the results of early investigations (i.e. within 6 weeks of MI) are also described.

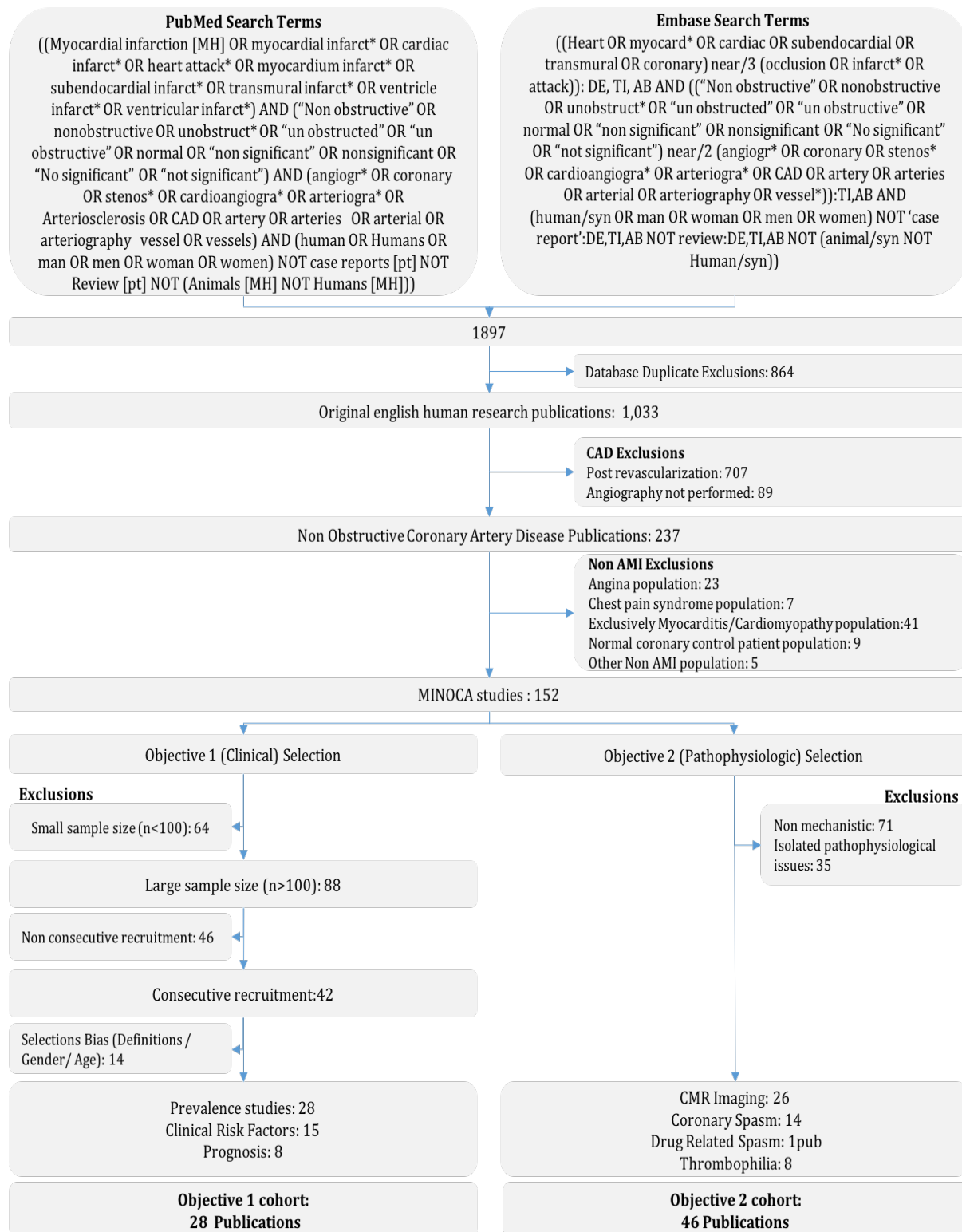


FIGURE 4: FLOW DIAGRAM OF STUDY SELECTION PROCESS.
 CAD, Coronary artery disease; CMR, Cardiac magnetic resonance imaging;

Data Extraction and Analysis

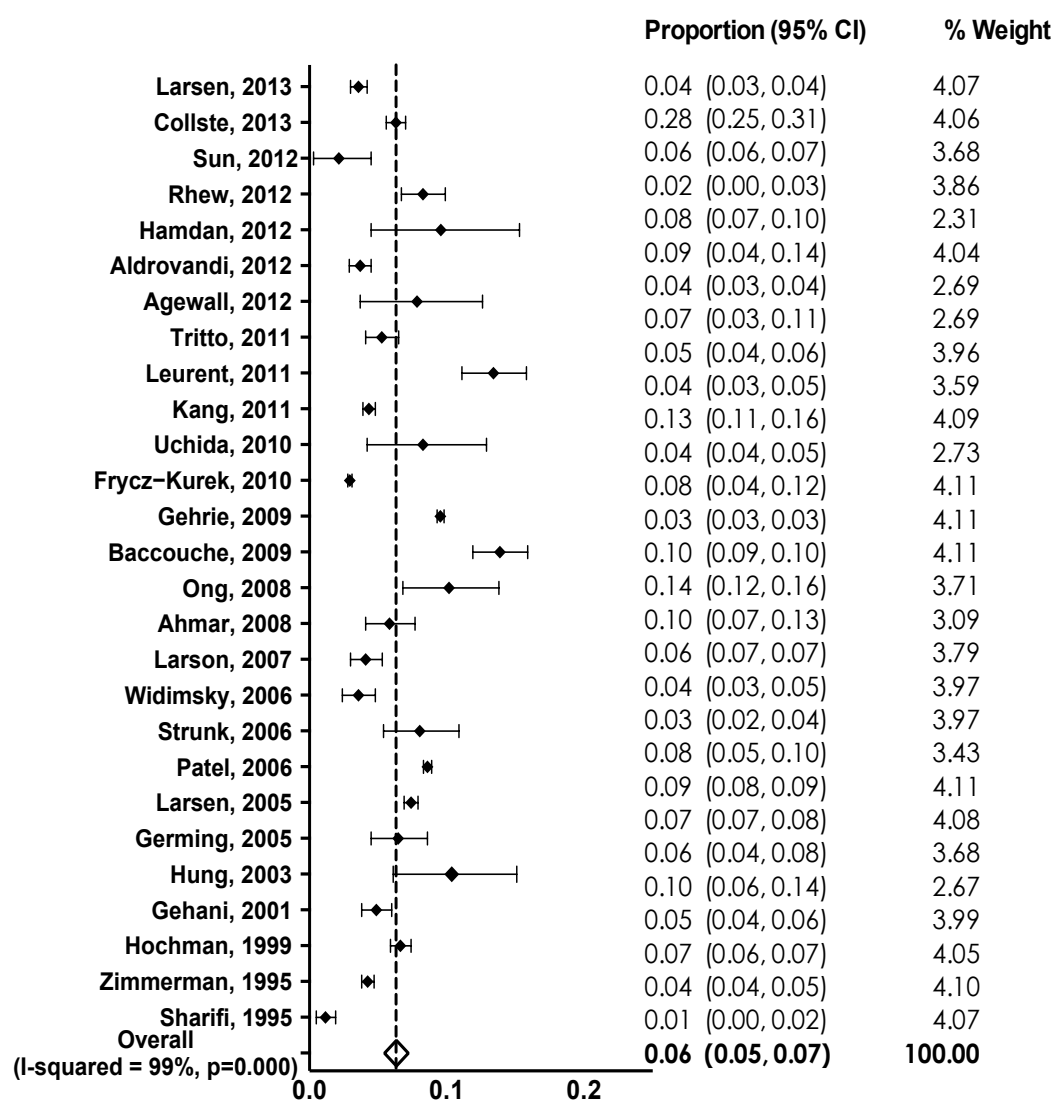
The endpoints evaluated in the primary objective included (a) prevalence of MINOCA, (b) clinical features including age, gender, MI type (STEMI or NSTEMI), cardiovascular risk factors, and (c) prognosis (including in-hospital and 12-month all-cause mortality). Data for these endpoints were pooled and analyzed using random effects meta-analysis models¹⁴⁹. This conservative approach assumes that individual studies are estimating different treatment effects. Heterogeneity in the study estimates were assessed using I-squared statistics¹⁵¹ with larger values indicating increasing heterogeneity between studies. In addition, for studies including both MINOCA and MI-CAD patients, the summary odds ratios (OR's) or mean difference and exact 95% confidence intervals (95% CI) were calculated. Data from the pathophysiologic mechanistic publications was more limited so that qualitative assessment could only be undertaken. This involved pooling of frequency data from studies with similar endpoints. All analyses were performed using STATA (version 12, College Station, TX, USA).

Results

From the 152 MINOCA publications identified on PubMed and Embase, we embarked upon (A) quantitative assessment of 28 studies to evaluate the clinical attributes of the condition and (B) qualitative evaluation of 46 studies that focused on its pathophysiologic attributes (Figure 4 & Data Supplement: Supplemental Table-1). These clinical and pathophysiologic attributes of MINOCA are detailed below.

Prevalence

The prevalence of MINOCA was determined from 27 large clinical trials/registries involving 176,502 consecutive MI patients who had coronary angiography performed. These studies reported a prevalence of MINOCA ranging from 1-14% with an overall prevalence calculated at 6% (95%CI: 5, 7%), based upon random effects analysis (Figure 5). The I-squared statistic was estimated to be 99%.



Note: Weights are from random effects analysis

FIGURE 5: PREVALENCE OF MINOCA.

Forest plot of published studies examining the prevalence of MINOCA using random effects meta-analysis. Data presented as percentage (%) and 95% confidence intervals (CI; %).

Clinical Features

In 15 publications there was sufficient detail to evaluate gender, age, cardiovascular risk factors, STEMI presentation, and angiographic characteristics of MINOCA patients. Some of these studies provided the opportunity to compare these features with those from patients with MI-CAD.

Gender. In the 15 publications reporting gender (n=11,334), pooled analyses revealed that only 40% (95%CI: 33, 46%) of MINOCA patients were women. However, pooled analysis of 10 studies that recruited both MINOCA (n=5,322) and MI-CAD (n=70,253) patients, revealed an over-representation of women with MINOCA (43%; 95%CI: 35, 51%) relative to that observed with MI-CAD (24%; 95%CI: 19, 30%; Table 1).

Age. Sufficient data was available in 13 studies (n=9,986) to determine the pooled mean age of MINOCA patients. This was calculated to be approximately 55 years (95%CI: 51, 59 years) with no significant heterogeneity between studies as estimated by the I-squared statistic. In 6 publications, data on age was available for both MINOCA (n=3,927) and MI-CAD (n=48,082) patients, with the respective pooled mean ages between 58.8 (95%CI: 51.6, 66.1 years) and 61.2 years (95%CI: 52.2, 70.4 years). Analysis of these comparative studies confirmed that patients with MINOCA were younger than those with MI-CAD. This analysis may have underscored this difference since the 6 studies included in this comparison recruited MINOCA patients at the upper age spectrum of the overall MINOCA cohort (Table 1).

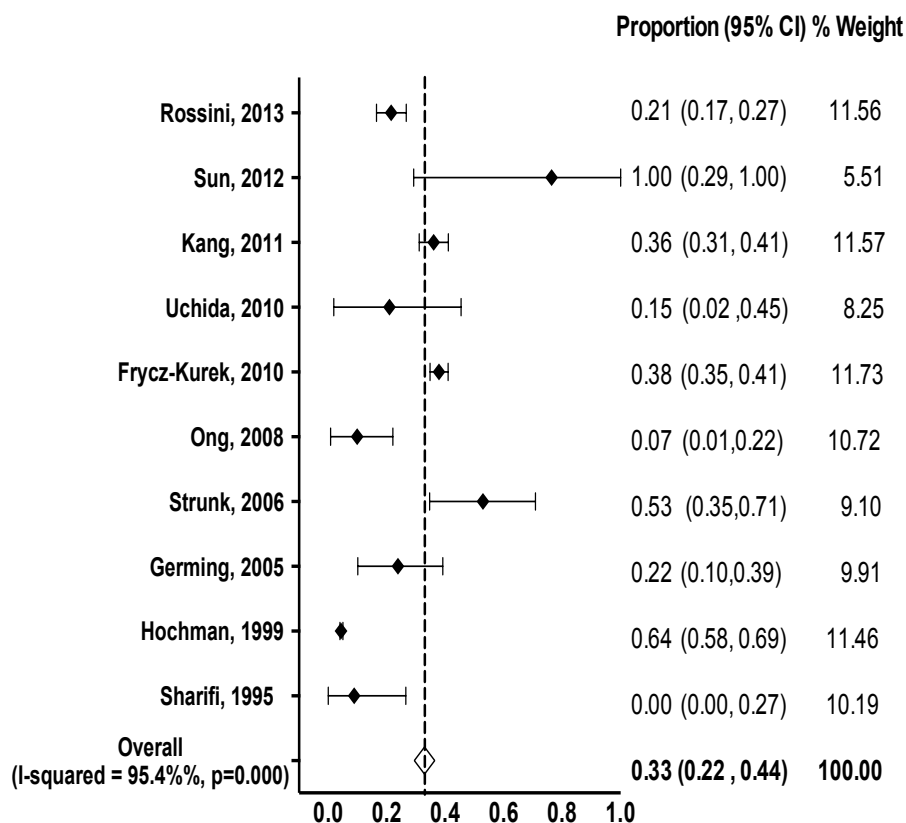
Cardiovascular Risk Factors. By evaluating comparative studies that included both MINOCA and MI-CAD patients, the relative frequencies of cardiovascular risk factors were determined. These are summarized in Table 1 along with the cardiovascular risk profile from all available MINOCA studies. Results reported within this section will be confined to the comparative studies. Compared with MI-CAD patients, those with MINOCA were less likely to have hyperlipidaemia (32% [95%CI: 30-59%] vs. 21% [95%CI: 6-35%], respectively; OR = 0.63, $P < 0.001$). However, it is noteworthy that the prevalence of hyperlipidaemia amongst MINOCA patients in these comparative studies was considerably lower than that observed for the overall MINOCA cohort (33%; 95%CI: 25-41%). Other cardiovascular risk factors including hypertension, diabetes, smoking and family history of premature coronary artery disease were similar between the groups (Table 1).

TABLE 1-CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH MINOCA OR MI-CAD

Risk Factors	Comparative Studies			P value	All MINOCA Studies
	MI-CAD	MINOCA	Mean difference or		
	% (95% CI)	% (95% CI)	OR (95% CI)		
Age	61.3 (52.2-70.4)	58 .8 (51.6-66.1)	4.1 (2.9,5.4)	<0.001	54.7 (50.5-58.7)
Women	24% (19, 30%)	43% (35, 51%)	2.1 (1.7, 2.7)	<0.001	40% (33-46%)
Hyperlipidaemi	32% (15-48%)	21% (6-35%)	0.6 (0.5, 0.7)	<0.001	33% (25-41%)
^a Hypertension	45% (30-59%)	52% (41-62%)	1.3 (0.9, 1.9)	0.183	44% (38-50%)
Diabetes	22% (14-29%)	15% (9-20%)	0.8 (0.5, 1.3)	0.333	13%(11-16%)
Smoking	39% (26-52%)	42% (33-51%)	1.1 (0.7, 1.5)	0.785	42%(36-48%)
Family history	27% (10-43%)	21% (5-38%)	1.0 (0.7, 1.3)	0.794	28%(17-39%)

Data presented as percentage (%) and 95% confidence intervals (CI) with odds ratio (OR) and P values.

Infarct ECG Findings. Ten studies (n=1,998) documented the prevalence of an acute STEMI presentation amongst MINOCA patients. Pooled analysis revealed that 33% (95%CI 22, 44%) presented with features of STEMI (Figure 6). Accordingly, approximately two-thirds of patients were categorized with NSTEMI.



NOTE: weights are from random effects analysis

FIGURE 6: PREVALENCE OF ACUTE STEMI PRESENTATION IN MINOCA.
Forest plot of published studies examining the frequency of STEMI presentation in patients with MINOCA, using a random effects meta-analysis.

Angiographic Findings. By definition, MINOCA patients have <50% lesions on angiography. The relative frequency of smooth vessels (i.e. no lesions visible on angiography) compared with minor irregularities on angiography was assessed in 5 clinical trials with 1,046 MINOCA patients (Figure 7). Amongst the MINOCA patients, the prevalence of smooth vessels on angiography was 51% (95% CI: 39-61%). Importantly, the I^2 test confirmed the presence of significant heterogeneity amongst these studies.

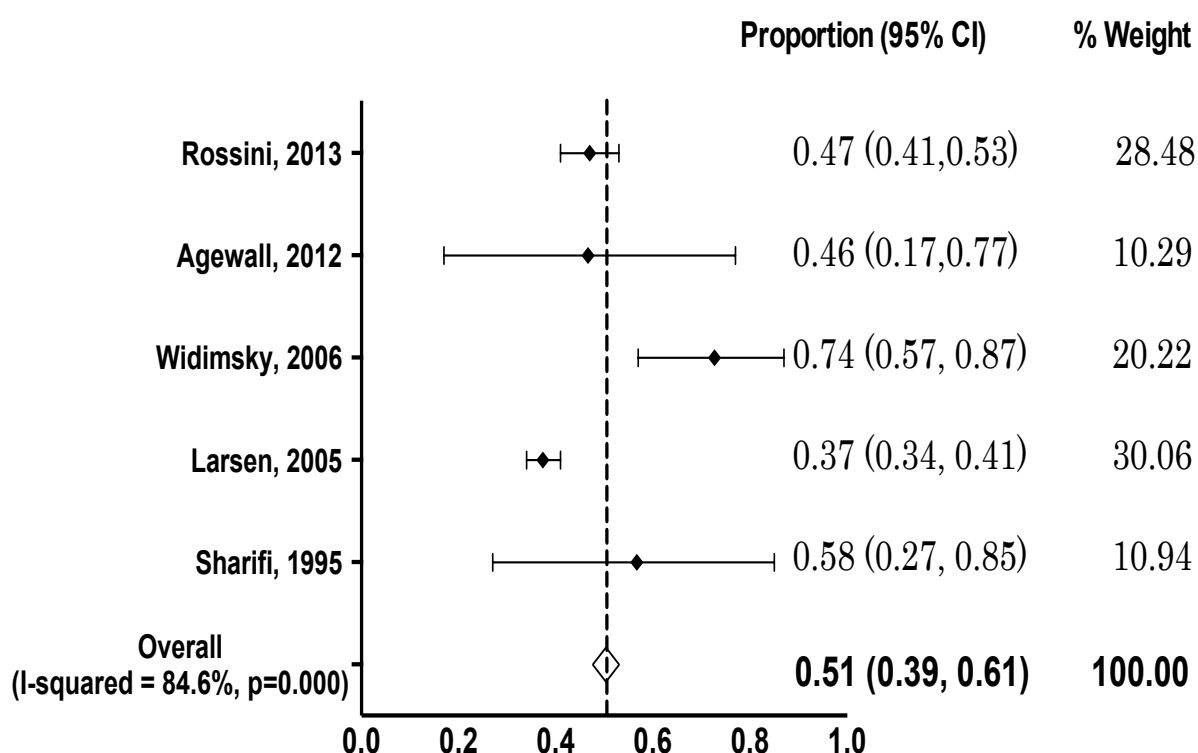


FIGURE 7: PREVALENCE OF ‘NORMAL’ SMOOTH CORONARY ARTERIES IN MINOCA.

Forest plot of published studies examining the frequency of completely smooth coronaries in patients with MINOCA, using a random effects meta-analysis.

Prognosis

Studies assessing prognosis in patients with MINOCA were considerably heterogeneous in their follow-up period and few reported the prevalence of cardiac mortality or re-infarction. Overall 8 studies reported all-cause mortality in patients with MINOCA, including in-hospital (5 studies, n=9,564), and 12-month (4 studies, n=1,924) following MI. Pooled meta-analysis of these studies revealed an all-cause in-hospital and 12-month mortality of 0.9% (95%CI: 0.5, 1.3%), and 4.7% (95%CI: 2.6, 6.9%), respectively. In 6 of these 8 studies, all-cause mortality was assessed in both MINOCA and MI-CAD patients, thereby allowing comparisons of the relative mortality between these forms of MI. As shown in Table 2, although the in-hospital mortality and 12-month mortality were lower in MINOCA patients, the findings remain of concern considering the limited clinical attention received by these patients.

TABLE 2-ALL-CAUSE MORTALITY IN PATIENTS WITH MINOCA OR MI-CAD

All-cause Mortality	Comparative Studies			P value	All MINOCA Studies
	MI-CAD	MINOCA	OR (95% CI)		
	% (95% CI)	% (95% CI)			
In-Hospital	3.2% (1.8%-4.6%)	1.1% (-0.1% -2.2%)	0.37 (0.2-0.67)	0.001	0.9% (0.5%-1.3%)
12-Months	6.7% (4.3%-9.0%)	3.5% (2.2%-4.7%)	0.59(0.41-0.83)	0.003	4.7% ((2.6%-6.9%)

Data presented as percentage (%) and 95% confidence intervals (CI) with odds ratio (OR) and P values.

Potential Pathophysiological Mechanisms

Of the 81 original publications investigating the potential mechanisms responsible for MI in MINOCA patients, 46 utilized three distinct approaches including, (i) assessment of structural myocardial dysfunction with CMR imaging (26 publications), (ii) provocative coronary artery spasm testing (15 publications), and (iii) thrombophilia screening (8 publications, including 3 of the spasm studies). The remaining 35 publications utilized more heterogeneous approaches investigating isolated aspects of MINOCA and therefore not conducive to pooled analysis.

Structural Myocardial Dysfunction. Pooled analyses of the 26 CMR imaging publications involving MINOCA patients, revealed features consistent with a subendocardial infarct on delayed hyper-enhancement in only 24% of 1,801 MINOCA patients studied. The most common finding in the CMR imaging studies was myocarditis, with 33% of the 1,676 MINOCA patients having features of this condition. Other myocardial abnormalities reported in the MINOCA CMR imaging studies included, Tako-tsubo cardiomyopathy (18% of 1,529 patients), hypertrophic cardiomyopathy (3% of 1,074 patients), dilated cardiomyopathy (2% of 625 patients), and other causes (7% of 760 patients) such as pericarditis and amyloidosis. Importantly, 26% of 1,592 MINOCA patients undergoing contrast CMR imaging did not have detectable myocardial abnormalities (Figure 8).

Of the above investigations, 16 CMR studies were undertaken within 6 weeks of the MI (Data Supplement: Supplemental Table 3). These reported similar frequencies in abnormal CMR findings including subendocardial infarct (24%), myocarditis (38%), Tako-tsubo cardiomyopathy (16%) and no significant abnormality (21%).

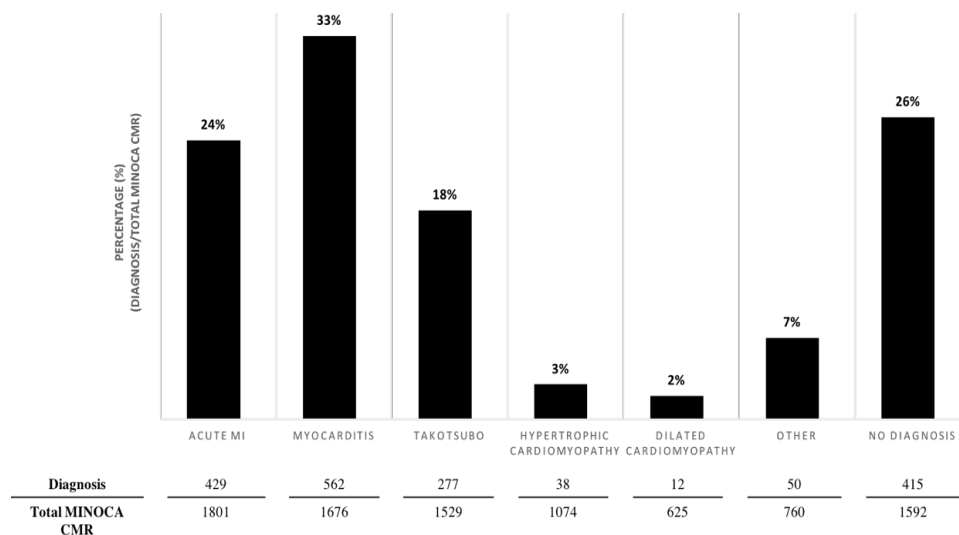


FIGURE 8: CARDIAC MAGNETIC RESONANCE IMAGING FINDINGS IN PATIENTS WITH MINOCA.

Bar graph of published studies showing the diagnostic significance of CMR imaging in MINOCA patients. Data presented as percentage (%).

Coronary Artery Spasm. Provocative spasm testing was undertaken in 14 studies involving MINOCA patients (Table 3). Of the 402 MINOCA patients in the pooled dataset, 28% had inducible spasm. In 8 studies (n=298), provocative testing was performed within 6 weeks of an MI and 28% had inducible spasm. In 4 studies (n=90), provocation testing was undertaken in MINOCA patients with an old myocardial infarct (i.e. MI $\geq$ 6 weeks) and spasm was provoked in 34% of patients (Table 3).

Thrombophilia Disorders. As summarized in Table 4, eight publications examined the presence of inherited thrombotic disorders in patients with MINOCA, with most undertaken in the early post-infarction period. Pooled analyses revealed the following abnormalities within the coagulation pathway: activated Protein C resistance or Factor V Leiden in 12% of 344 patients, Protein C/Protein S deficiency in 3% of 189 patients and Factor XII deficiency in 3% of 163 patients. Overall, 14% of the 378 MINOCA patients who underwent thrombophilia screening had evidence of an inherited thrombotic disorder.

TABLE 3-PROVOCATIVE SPASM TESTING IN PATIENTS WITH MINOCA.

Publications	n	Provocation Test	Spasm Definition	Provoked/ Spontaneous Spasm (%)	
Early Provocative Spasm Testing (within 6 weeks of acute myocardial infarction)					
Bory, 1988	59	iv ergot	≥ 50% constriction on angio	2/59	(3%)
Fukai, 1993	21	iv ergot	≥ 75% constriction on angio	13/16	(81%)
Dacosta, 2001	91	iv ergot	≥ 70% constriction on angio	11/71	(15%)
Wang, 2002	23	ic ergot	≥ 90% constriction on angio	17/23	(74%)
Hung, 2003	19	ic ergot	≥ 70% constriction on angio	18/19	(95%)
Dacosta, 2004	82	iv ergot	≥ 70% constriction on angio	13/82	(16%)
Abid, 2012	21	iv ergot	≥ 70% constriction on angio	5/21	(24%)
Ong 2008	7	ic acetylcholine	≥ 75% constriction on angio	4/7	(57%)
Early spasm				(83/298)	28%
Late Provocative Spasm Testing (≥ 6 weeks following myocardial infarction)					
Legrand, 1982	18	iv ergot	Chest pain & ST elevation	6/18	(33%)
Raymond, 1988	74	iv ergot	≥ 75% constriction on angio	5/16	(31%)
Ammann, 2000	23	Hyperventilate	ST elevation	0/23	(0%)
Kim, 2005	33	iv ergot	RWMA on echocardiography	20/33	(61%)

Late spasm				(31/90)	34%
Undefined timing for Provocative Spasm Testing (relative to myocardial infarction)					
Salem, 1985	10	iv ergot	Chest pain & ST elevation	0/7	(0%)
Verheugt, 1987	21	iv ergot	NR	0/7	(0%)
Provocative Spasm Testing on Cocaine induced MINOCA Patients					
Kossowsky, 1989*	5	cold pressor	NR	0%	
Overall pooled spasm				114/402	28%

Data presented as n (%). iv, intravenous; ic, intracoronary; ergot, Ergonovine; angio, Coronary angiography; RWMA, regional wall motion abnormality; NR, Not recorded.

* Kossowsky et al., 1989 was ignored from the calculations as it represents the cohort of Cocaine abuse patients.

TABLE 4-THROMBOPHILIA SCREENING IN PATIENTS WITH MINOCA.

Publications	n	APCR/ FVL	Protein C/S Deficiency	Factor XII Deficiency	Thrombotic disorders (%)	
Brecker, 1993	12	NE	0	NE	(0 / 12)	0%
DaCosta, 1998*	22	2	1	1	(4 / 22)	18%
Lande, 1998	26	3	2	NE	(5 / 14)	36%
Mansourati, 2000	107	13	NE	NE	(13 / 107)	12%
Van de Water, 2000	60	8	NE	NE	(8 / 60)	13%
DaCosta, 2001	91	7	1	1	(9 / 73)	13%
DaCosta, 2004	82	8	1	3	(12 / 78)	15%
Abid, 2012	21	2	1	0	(4 / 12)	33%
Overall		12% (41/344)	2.6% (5/189)	2.5%(4/163)	14% (51 / 356)	

Data presented as n (%) APCR, Activated Protein C Resistance; FVL, Factor V Leiden; NE, Not Examined.

*Dacosta et al., 1998 was ignored from the calculations as the same patient cohort again used in Dacosta et al., 2004.

Discussion

This detailed systematic review provides the first comprehensive overview of patients with MINOCA. It demonstrates that MINOCA has (a) a 6% prevalence of all MI presentations, (b) no diagnostic distinguishing clinical presentation features compared with MI-CAD, (c) a better 12-month all-cause mortality compared with MI-CAD, although its prognosis should be considered as ‘guarded’, and (d) structural dysfunction, coronary spasm and thrombotic disorders as some potential underlying causes. Given that MINOCA has similar features to MI-CAD, a guarded prognosis and multiple potential aetiologies, it should be considered a ‘working diagnosis’ that requires further evaluation of the potential underlying causes since these may have important clinical implications.

MINOCA patients do not have a distinguishing clinical presentation.

Patients with MINOCA may present with STEMI or NSTEMI, with two-thirds presenting as the latter. Compared with MI-CAD patients, those with MINOCA tend to be younger, predominantly male (although women are over-represented relative to those with MI-CAD, 40% vs 25% respectively) with significant cardiovascular risk factors, although less often have hyperlipidaemia (Table 1). Thus although there are statistical differences in the clinical profile of patients with MINOCA, there are no clinically distinguishing characteristics or risk factors that can easily delineate these patients from those with MI-CAD based upon a systemic review of the literature. Specific future studies examining details of the clinical history may be more discerning. Once coronary angiography is performed and defines the MINOCA patients, completely smooth (i.e. ‘normal’) coronary arteries are observed in only half of the patients, with many having minor irregularities thereby justifying the term ‘MINOCA’. The clinical importance of delineating MINOCA patients with angiographically smooth coronary arteries from those with only mild irregularities needs to be clarified in

future prognostic studies. The only published MINOCA study that has undertaken this comparison examined 12-month all-cause mortality and reported poorer outcomes in those with smooth coronaries, however sample size was small (24 deaths in total)⁸⁸. If future studies demonstrate different outcomes in these angiographic subgroups, then it will be justifiable to clinically delineate them.

MINOCA patients have a ‘guarded prognosis’

Patients with MINOCA have a significantly reduced all-cause mortality compared to those with MI-CAD; including a 63% lower in-hospital mortality and 41% lower 12-month mortality (Table 2).

Although these findings may be reassuring, the 4.7% (95%CI: 2.6-6.9%) 12-month all-cause mortality for patients with MINOCA is of concern when compared to other published prognostic studies. Firstly, the Korean MI Registry⁸⁷ evaluated 12-month all-cause mortality in 8,510 consecutive MI patients, reporting a 3.1% mortality in those with MINOCA, 3.2% in those with single or double vessel coronary artery disease, and 6.5% in those with triple vessel disease or a significant left main coronary artery stenosis. Secondly, patients with stable chest pain (i.e. no prior MI) and normal smooth coronaries on angiography have a 0.2% annual all-cause mortality, while those with only minor luminal irregularities have a 0.3% annual all-cause mortality¹⁵². Accordingly, MINOCA patients appear to have a poorer prognosis than those with stable chest pain and non-obstructive coronary artery disease, and more akin to those with an MI and single/double vessel coronary artery disease. Thus their prognosis should be considered somewhat ‘guarded’ despite being better than those with MI-CAD.

MINOCA – A Heterogeneous ‘Working Diagnosis’ with some Treatable Causes

Similar to the diagnosis of heart failure, MINOCA should not be considered as a specific diagnosis but a heterogeneous ‘working diagnosis’ that requires further evaluation to elucidate potential underlying causes. Identifying the cause of MINOCA is important since it may have prognostic implications (e.g. identification of a cardiomyopathy) but even more importantly, it may require institution of specific therapies to treat the underlying cause. Important MINOCA-related diagnoses that may warrant specific targeted therapies include structural myocardial dysfunction (such as cardiomyopathies), coronary spasm and thrombophilia disorders. These are further discussed below.

Structural Myocardial Dysfunction. The detection of structural heart disease with CMR imaging in patients with MINOCA syndrome, can reveal cardiomyopathies such as tako-tsubo, hypertrophic or dilated cardiomyopathy (Figure 8). Although tako-tsubo cardiomyopathy is an important diagnosis considering its prognostic implications, currently there are no specific therapies for this condition. In contrast, hypertrophic and dilated cardiomyopathy (although seldom detected in MINOCA patients) have important management strategies/therapies that can influence patient outcomes. Accordingly, detection of these treatable conditions further justifies the routine use of CMR imaging in patients with MINOCA since it is the optimal diagnostic imaging modality for delineating cardiac structural disorders in this condition.

Myocarditis is an important cause of MINOCA that is optimally diagnosed by CMR imaging. It accounts for approximately a third of MINOCA cases and provides a definitive diagnosis that may have prognostic implications, although generally it requires only conservative management.

Another important MINOCA subgroup detected by CMR imaging are those with a subendocardial infarct pattern on delayed hyper-enhancement images. The infarct may arise from transient occlusive coronary spasm or thrombosis¹⁵³.

Coronary Spasm.

More than a quarter of patients with MINOCA undergoing provocative spasm testing have inducible spasm. Unfortunately there are no suitable studies directly comparing provocative spasm testing between MINOCA and MI-CAD patients, although several have reported inducible spasm in 20-80% of MI-CAD patients^{57, 154}. Thus the relative contribution of coronary spasm to the pathophysiology of MINOCA requires further investigation.

There are several interesting observations in relation to provocative spasm testing findings in patients with MINOCA. Firstly, there is no time-dependence for inducible spasm amongst MINOCA patients (Table 3), whereas MI-CAD patients with a recent (<6 weeks) infarct are more likely to have inducible spasm than those with an old (>6 weeks) infarct. Whether the persistent inducible spasm in MINOCA patients reflects an underlying vasospastic predisposition is open to speculation. Secondly, there appears to be an ethnic predisposition to coronary spasm in patients with a recent myocardial infarct⁵⁷, particularly amongst Japanese patients⁵⁶. Fukai et al¹⁵⁵ from Japan reported an 81% prevalence of inducible spasm in patients with MINOCA, whereas studies from Europe¹⁵⁶⁻¹⁵⁹ and the United States^{160, 161} have a pooled prevalence of only 14%. Interestingly, other Asian-based studies^{162, 163} also report a high prevalence of inducible spasm (Table 3). Finally, cocaine may induce coronary spasm and should be considered as a potential cause of MINOCA however a recent large registry reported that cocaine use was associated with only 0.9% of MI cases¹⁶⁴. The above findings relating to coronary spasm in MINOCA are exploratory and require further investigation since the data are heterogeneous with the studies differing in their study design, provocation

stimulus and coronary spasm definition. In particular, ergonovine provocation is less often used since it is no longer available in some countries and acetylcholine has become the preferred provocation stimulus. Despite these study differences, the importance of coronary spasm as a potential cause of MINOCA must not be overlooked as it appears to occur frequently and the use of calcium channel blockers is an independent determinant of survival in patients with coronary spasm¹⁶⁵.

Thrombophilia Disorders.

As summarized in Table 4, genetic thrombophilia disorders have been observed in MINOCA. Factor V Leiden is a single point mutation with a prevalence of 3-7% in Western populations¹⁶⁶ but was observed in 12% of MINOCA patients. Furthermore, comparative studies with MI-CAD patients also report a higher prevalence in MINOCA et al¹⁶⁷: 4.5% vs 12.1%; and Van de Water et al¹⁶⁸: 4.3% vs 11.7%, respectively). Protein C & S deficiency are autosomal dominant disorders with a population prevalence of 0.1-1%¹⁶⁶, yet occur in 2.6% of MINOCA patients and similarly those with MI-CAD¹⁶⁹.

These associations with the genetic thrombophilia disorders are based upon small studies and require confirmation with larger multicenter prospective studies. Furthermore, investigation of acquired thrombophilia disorders should be considered as these may also occur in the context of acute MI and could exacerbate the genetic disorders. Irrespective of the prevalence of these thrombophilia disorders in MINOCA, their detection may influence subsequent management thereby justifying their routine evaluation in patients with MINOCA.

Limitations

The results from this structured systematic review should be interpreted in the context of several potential limitations. Firstly, the analysis is dependent on the available published data and thus limited by publication bias, patient selection bias, suboptimal definitions for cardiovascular risk factors, retrospective analyses, and applicability of historical publications to contemporary practice. Secondly, there is significant heterogeneity between the studies included in the meta-analysis although the random effects model approach used in this study is less influenced by this pitfall. Thirdly, the second objective, which focused upon pathophysiological studies, did not lend itself to quantitative meta-analysis but a qualitative evaluation of published data. This was necessary as there were differences in patient recruitment, methods of investigation and definitions of a positive result, for each of the respective studies involving CMR imaging, provocative spasm testing, and genetic thrombophilia disorders.

Conclusions

This systematic review provides an important reference point for further research and development of MINOCA. It demonstrates that the condition is not uncommon, has no delineating clinical presentation, a guarded 12-month prognosis, and multiple potential causes with some amenable to specific therapies. Despite this, there are no guidelines regarding the management of these patients and limited insights into the contemporary management undertaken (if any) in affected patients. Based upon the findings of this systematic review, we would propose that MINOCA be considered a ‘working diagnosis’ that requires routine evaluation for treatable underlying causes. This may include CMR imaging, provocative spasm testing, and thrombophilia assessment. Further research is required to

define the optimal therapy in MINOCA patients who do not have an identifiable underlying cause. These strategies may potentially improve the guarded prognosis in these patients.

CHAPTER 3

3 CLINICAL CHARACTERISTICS OF MINOCA

This chapter includes an outline and the manuscript in the form as it appears in the manuscript, “Can chest pain characteristics identify patients with MINOCA (Myocardial infarction with non-obstructive coronary arteries)? authored by Sivabaskari Pasupathy, Rosanna Tavella, Christopher Zeitz, Matthew Worthley, Derek Chew, Margaret Arstall and John F. Beltrame, to be submitted to the Journal of American College of Cardiology.

In keeping with this style of this thesis, the abstract has been removed, the table and figures re-numbered, the references incorporated into the thesis’s master reference list and the manuscript repaginated.

3.1 STATEMENT OF AUTHORSHIP

Comparative study of chest pain characteristics of Myocardial Infarction with non-obstructive coronary arteries (MINOCA) and Myocardial infarction with coronary artery disease (MI-CAD)

Publication Status	Unpublished work written in manuscript style
Publication Details	Not applicable
Principal Author	Sivabaskari Pasupathy
Contribution	Acquisition of data; Analysis and interpretation of data; Drafting of manuscript; Critical revision of the manuscript.
Overall percentage (%)	70%

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature and Date 14/08/2016

Co-Author Contributions

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

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

Chapter 3

Name	Rosanna Tavella
Contribution	Acquisition of data; Analysis and interpretation of data; Critical revision
Signature and Date	12/08/2016

Name	Christopher Zeitz
Contribution	Critical revision
Signature and Date	15/08/2016

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Contribution	Critical revision
Signature and Date	12/08/2016

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Name	Margaret Arstall
Contribution	Critical revision
Signature and Date	12/08/2016

Name	John F Beltrame
Contribution	Study conception and design; Critical revision; Final approval.
Signature and Date	11/08/2016

3.2 STUDY OUTLINE

The systematic review and meta analysis¹⁷⁰ established key details concerning MINOCA from existing literature but is outdated and fragmented, highlighting the limited insights into this condition and the need for contemporary research in this area. The current chapter presents a contemporary update of MINOCA using a clinical registry and aims to determine the possibility of delineating MINOCA patients from MI-CAD patients based on the clinical presentation.

Clinical registries

A clinical registry uses observational study methods to collect uniform data (clinical or other) to evaluate specific features for a population defined by a particular condition, disease or exposure, and serves as a predetermined scientific, clinical or policy purpose^{171, 172}. Registries are classified according to how the population is defined. A disease based registry includes patients with the same diagnosis, for example acute MI, and a procedure-based registry includes patients who have had a common procedure, for example coronary angiography.

Registries have developed as a major resource in support of clinical research in recent years, and are an important complement to randomised controlled trials (RCT). Unlike RCTs, which focus on a very carefully defined population, typically with minimal co-morbidities and few other medications, registries determine real-world outcomes in the practice of medicine. Although limited in their ability to determine efficacy, a registry is especially valuable in determining whether the frequency of adverse effects is in keeping with that identified in clinical trials and in examining the real-world effectiveness of therapies. In addition, registries provide well-documented cohorts, which are effective in determining predictors of

poor prognosis. Furthermore, registries provide data for case-control studies which are useful for determining aetiology or risk factors for diseases.

Although registries can serve many purposes, the focus for this thesis is on a procedure-based registry that provides details on the natural history of the disease, the clinical effectiveness of health services and measures safety and quality of care. These principles serve the underlying role of a clinical quality registry, which aims to improve the quality of health care that patients receive by measuring and benchmarking risk-adjusted outcomes¹⁷³.

The Coronary Angiogram Database of South Australia (CADOSA) is a procedure-based registry designed to achieve the functions of a clinical quality registry as described above. The target population is patients undergoing cardiac catheterisation procedures in the public hospitals in South Australia, Australia (population 1.6 million). Established in 2011, the CADOSA Registry captures all coronary angiography procedures and percutaneous coronary interventions (PCI) performed in each of the four public hospital facilities in South Australia. The data elements for this registry were chosen based on the American College of Cardiology National Cardiovascular Data Registry (NCDR) ®. All data elements and their corresponding data specifications, captured by the CathPCI ®Registry are included in the CADOSA data collection. The data collection procedures include dedicated, trained data abstractors based at each hospital facility, and a central database management team responsible for monitoring, reviewing and reporting the registry data. To ensure optimal target population coverage, an opt-off consent approach is used, and each participating hospital has local human research ethics approval to participate in the registry. The registry activities, scientific oversight and data governance is managed by a Steering Committee which has clinical representation from each participating hospital.

The front page of the data form is shown in Figure 9. Data collected include the details demonstrated in Table 5.

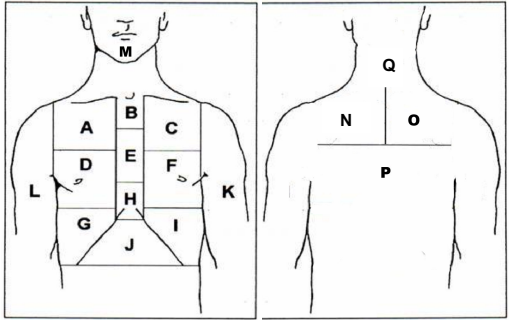
CADOSA 		Coronary Angiogram Database of South Australia Diagnostic Catheterisation and Percutaneous Coronary Intervention Registry		
FACILITY¹⁰¹⁰				
☐ CW		☐ FMC		☐ LMH
☐ RAH		☐ TQEH		
Patient in Follow-up Study? ¹⁰²⁰ ☐ No ☐ Yes				
PART A: DEMOGRAPHICS				
Surname ²⁰⁰⁰ :				
First Name ²⁰¹⁰ :				
Middle Name ²⁰²⁰ :				
Medicare No ²⁰³⁰ :		Patient UR ²⁰⁴⁰ :		
Post Code ³⁰⁰⁵ :		Date of Birth ²⁰⁵⁰ :		Gender ²⁰⁶⁰ :
Postcode N/A ³⁰⁰⁶ : ☐		__ __ __ dd __ __ mm __ __ __ __ yyyy		☐ M ☐ F
Ethnicity:				
☐ Caucasian ²⁰⁷⁰ ☐ Indigenous/Torres Strait Islander ²⁰⁷¹ ☐ Asian ²⁰⁷² ☐ Hispanic ²⁰⁷³				
☐ African ²⁰⁷⁴ ☐ Sub-Continent ²⁰⁷⁵ ☐ Other ^{2076,2077} (specify) _____				
PART B: EPISODE OF CARE and CHEST PAIN EVALUATION				
Arrival to Cath Facility Date ³⁰⁰⁰ :				
__ __ __ dd __ __ mm 20 __ __ __ yyyy				
Time ³⁰⁰¹ :				
__ __ __ hh : __ __ __ mm 24 hr				
Referral Source ³⁰¹⁰ :				
☐ Emergency Department ☐ Admissions Office ☐ Current In-patient ☐ Transfer in From Other Acute Care Facility				
Payer for Episode of Care ³⁰²⁰ :				
☐ Private Health Insurance ☐ Medicare Only ☐ Veteran Gold Card ☐ Other				
Transport to Cath Facility ³⁰⁴⁰ :				
☐ Self ☐ SAAS ☐ Air Ambulance ☐ MedSTAR ☐ Inter-hospital transfer - Air ☐ Inter-hospital transfer – road				
THE FOLLOWING QUESTIONS RELATE TO THE CHEST PAIN SYMPTOMS PROMPTING THIS DIAGNOSTIC CATHETERISATION				
Chest Pain Prompting this Diagnostic Catheterisation ³⁰⁴¹ : ☐ No ☐ Yes ☐ Unknown → If yes, complete below				
Location of Chest Pain: (check all that apply)				
☐ A ³⁰⁴² ☐ B ³⁰⁴³ ☐ C ³⁰⁴⁴				
☐ D ³⁰⁴⁵ ☐ E ³⁰⁴⁶ ☐ F ³⁰⁴⁷				
☐ G ³⁰⁴⁸ ☐ H ³⁰⁴⁹ ☐ I ³⁰⁵⁰				
☐ J ³⁰⁵¹ ☐ K ³⁰⁵² ☐ L ³⁰⁵³				
☐ M ³⁰⁵⁴ ☐ N ³⁰⁵⁵ ☐ O ³⁰⁵⁶				
☐ P ³⁰⁵⁷ ☐ Q ³⁰⁵⁸ ☐ Other ³⁰⁵⁹				
☐ Unknown ³⁰⁶⁰				
				
Quality of Chest Pain:				
☐ Burning ³⁰⁶¹ ☐ Squeezing ³⁰⁶² ☐ Tightness ³⁰⁶³ ☐ Sharp ³⁰⁶⁴ ☐ Heavy ³⁰⁶⁵ ☐ Other ^{3066, 3067} _____ ☐ Unknown ³⁰⁶⁸				
Precipitating Factors:				
☐ Exertion ³⁰⁶⁹ ☐ Meals ³⁰⁷⁰ ☐ Emotional Stress ³⁰⁷¹ ☐ Cold Weather ³⁰⁷² ☐ Nocturnal ³⁰⁷³ ☐ Lying Down ³⁰⁷⁴ ☐ Pleuritic ³⁰⁷⁵ ☐ Only at Rest ³⁰⁷⁶ ☐ Other ^{3077, 3078} _____ ☐ Unknown ³⁰⁷⁹				
Relieving Factors:				
☐ Rest ³⁰⁸⁰ ☐ Nitrates (<5 mins) ³⁰⁸¹ ☐ Nitrates (> 5 mins) ³⁰⁸² ☐ Antacids ³⁰⁸³ ☐ Other ^{3084, 3085} _____ ☐ Unknown ³⁰⁸⁶				
Associated Symptoms:				
☐ Tachypnea ³⁰⁸⁷ ☐ Rapid Palpitations ³⁰⁸⁸ ☐ Pre-syncope/syncope ³⁰⁸⁹ ☐ Post-pain fatigue ³⁰⁹⁰ ☐ Nausea/vomiting ³⁰⁹¹ ☐ Sweating ³⁰⁹² ☐ Chest Wall Tenderness ³⁰⁹³ ☐ Other ^{3094, 3095} _____ ☐ None ³⁰⁹⁶ ☐ Dyspnea ³⁰⁹⁷ ☐ Unknown ³⁰⁹⁸				
Typical Duration ³⁰⁹⁹				
☐ ≤ 15 seconds ☐ > 15 seconds ≤ 15 minutes ☐ > 15 minutes ≤ 30 minutes ☐ > 30 minutes ≤ 60 minutes ☐ > 60 minutes ≤ 2 hours ☐ > 2 hours ≤ 6 hours ☐ > 6 hours ≤ 12 hours ☐ > 12 hours ☐ Unknown				

FIGURE 9: FIRST PAGE OF CADOSA CASE REPORT FORM

TABLE 5-DATA COMPONENTS OF CADOSA REGISTRY

Demographics	Age, Sex, Ethnicity, Postcode
Episode of Care	Referral, Payer, Transport Chest pain characteristics
Risk factors & Clinical History	Cardiovascular risk factors Prior cardiovascular history Non-cardiovascular comorbidities Pre-admission medication
Cath-Lab Visits	Procedure Indication Classification of symptoms Cardiac status Non-Invasive stress or imaging studies
Diagnostic cath procedure	Access site Presentation times Procedure Status Fluoroscopy and contrast Right heart catheterization details Procedural medications
Coronary Angiography findings	Coronary Stenosis Extent of coronary disease Principal cardiac diagnosis
PCI Procedure	PCI Indication PCI Status Lesions treated Devices deployed
Labs	Troponin and CK-MB Creatinine and haemoglobin
Intra/post-procedure events	In-hospital events (infarct/stroke/bleeding)
Discharge	In-hospital mortality Discharge medications Cardiac rehabilitation

The subsequent manuscript utilised the data collected from the CADOSA registry to provide the comprehensive prospective assessment of patients with MINOCA in comparison to MI-CAD.

3.3 MANUSCRIPT: CAN CHEST PAIN CHARACTERISTICS IDENTIFY PATIENTS WITH ISCHAEMIC MINOCA?

Introduction

Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA) refers to a subgroup of myocardial infarcts characterised by the absence of coronary artery disease (CAD) on coronary angiography. With the increasing use of early angiography in the context of AMI, MINOCA has been increasingly recognized and presents a clinical puzzle to the practicing clinician. Patients hospitalized for MINOCA represent a heterogeneous population in terms of clinical presentation, aetiologies and/or comorbidities, consequently implicating various treatment modalities and ambiguous outcomes.

The clinical spectrum of MINOCA with ischaemic causes in unselected populations has not been investigated and this limited information is a major barrier to understanding the true prevalence and prognostic implications of MINOCA. The recent European Society of Cardiology working group position paper on MINOCA by Agewall et al¹⁷⁴ highlighted multiple knowledge gaps on MINOCA in contemporary research and the need for reliable registries to fill such gaps.

MINOCA is a working diagnosis made at coronary angiography and mandates further assessment to identify the underlying cause. These include coronary (eg ischaemic myocardial infarct) and non-coronary causes (eg myocarditis or tako-tsubo cardiomyopathy). A significant proportion of patients with MINOCA are presumed to have an ischaemic aetiology although this has not been extensively investigated in the literature. Moreover, previous studies comparing patients with MINOCA and MI-CAD often included non-

coronary related diagnoses, thereby confounding the analyses that should have focussed on ischaemic causes. It's unclear whether the clinical presentation features in patients with ischaemic MINOCA differ from those with MI-CAD. Chest pain is a key element in the diagnosis of MI; sometimes being pivotal to the diagnosis. It is therefore important to emphasize the evaluation of chest pain. However, there is no literature available discriminating the characteristics of chest pain between these two types of myocardial infarcts. The present study was conducted to evaluate the chest pain features that may potentially delineate patients with ischaemic MINOCA from those with MI-CAD.

Objective: The primary objective of this study is to compare the chest pain characteristics between patients with presumed ischaemic MINOCA and MI-CAD in relation to (i) location, (ii) quality, (iii) precipitating factors, (iv) associated symptoms, and (v) duration. The secondary objective includes a comparison of (i) cardiovascular risk factors, (ii) risk assessment at presentation, (v) discharge medications, and (vi) in-hospital outcomes between MINOCA and MI-CAD patients.

Methods

To achieve the above objectives, a prospectively designed cohort study was undertaken utilising patients from an angiographic registry that recruits all acute MI patients undergoing angiography in the participating institutions.

Patient population

The Coronary Angiogram Database of South Australia (CADOSA) is a statewide prospective registry of all patients undergoing diagnostic coronary angiography and percutaneous coronary intervention (PCI) in South Australian public hospitals (population 1.6 million). Data elements are compatible with the NCDR® CathPCI® Registry and data collection is

undertaken by trained data abstractors at each hospital site (four facilities). A central database management centre oversees the operation of the registry and conducts regular data audits. The analytic cohort from CADOSA for this study included consecutive acute MI patients hospitalised between January 2012 and December 2013 undergoing coronary angiography. To validate the acute MI diagnosis, 100% of patients identified as MINOCA, and 10% of patients identified as MI-CAD, were independently audited to ensure consistency with the Third Universal definition. Each institution included in the CADOSA Registry has ethics approval for the data collection and research use of the data.

Study Definitions

Acute MI was defined on the basis of (1) a positive cardiac biomarker (Troponin T or Creatinine kinase-myocardial band) exceeding the upper limit of normal according to the individual hospital's laboratory parameters with (2) supporting clinical evidence including either (a) clinical presentation consistent or suggestive of myocardial ischaemia, and/or (b) ischaemic ECG changes as described by Thygesen et al²⁵. ECG features were further classified as ST Elevation Myocardial Infarction (STEMI) with new or presumed ST segment elevation or new left bundle branch block not to be resolved within 20 minutes and Non ST Elevation Myocardial Infarction (NSTEMI) with absence of ECG changes diagnostic of a STEMI. Acute MI patients in whom no significant lesions were identified on coronary angiography (no stenosis or stenosis <50%) were defined as MINOCA. MI-CAD was defined as acute MI patients in whom angiographically significant lesions were found in coronary arteries ($\geq 50\%$ stenosis). Cardiovascular risk factor definitions are listed in Table 6.

To ensure a more select group of patients with ischaemic MINOCA, those with this diagnosis had their discharge diagnosis reviewed. Patients with a diagnosis of tako-tsubo cardiomyopathy, myocarditis, pulmonary embolism or other non-ischaemic causes at the time of discharge were excluded from this study. In addition, patients presenting with cardiac arrest within 24 hours were excluded to encompass a more homogeneous group of 'ischaemic infarcts'. Patients with patent coronaries but previous percutaneous coronary intervention (PCI) were considered as MINOCA if the revascularisation procedure was performed more than 6 months prior to the AMI presentation.

TABLE 6-DEFINITIONS USED FOR CARDIOVASCULAR RISK FACTORS

Risk factor	Definition
Hypertension	Prior medical diagnosis, or current use of antihypertensive agents.
Diabetes	Known history of diabetes with or without active utilization of diabetes medications
Hyperlipidaemia	Total cholesterol greater than 5.17 mmol/L; or LDL greater than or equal to 3.36 mmol/L; or, High-density lipoprotein (HDL) less than 1.03 mmol/L and for patients with known CAD, treatment is initiated if LDL is greater than 2.59 mmol/L
Smoking history	Current smoker: if the patients has been smoking cigarettes currently daily or non-daily.; Former smoker: the patient has not smoked cigarettes during the last year; No smoking history: if the patient has never smoked cigarettes
Family history	Direct relatives who have had any of the following at age less than 55 years for male relatives or less than 65 years for female relatives: Angina, acute MI, sudden cardiac death without obvious cause, coronary bypass grafting surgery (CABG) and PCI.

Data Analysis

Continuous data was summarized using median and inter-quartile range and compared using Mann–Whitney U test between ischaemic MINOCA and MI-CAD patients. Comparison between groups for categorical outcomes (clinical and chest pain characteristics, in-hospital outcomes and discharge management) undertaken using logistic regression with ischaemic MINOCA as the binary independent variable. Analyses were age and gender adjusted where appropriate and the final odds ratio with 95% confidence intervals are reported. Statistical significance was established at an alpha level of 0.05. All statistical analyses were performed using STATA/IC version 11.2 for Mac.

Results

Prevalence of MINOCA

Between January 2012 and December 2013, 3,536 patients presenting with acute MI underwent coronary angiography in South Australian hospitals as identified by CADOSA database. Of these, 4% (153) were excluded since they underwent angiography following an out of hospital cardiac arrest. Consequently 3,383 patients had a diagnosis of acute MI, with 2923 (86%) classified as MI-CAD and 460 (14%) as MINOCA. Following review of the discharge diagnosis amongst the MINOCA patients, only 219 (7%) had no cause identified for the presentation and presumed to be on an ischaemic basis (Figure 10). This constituted the MINOCA study group for the purposes of this investigation.

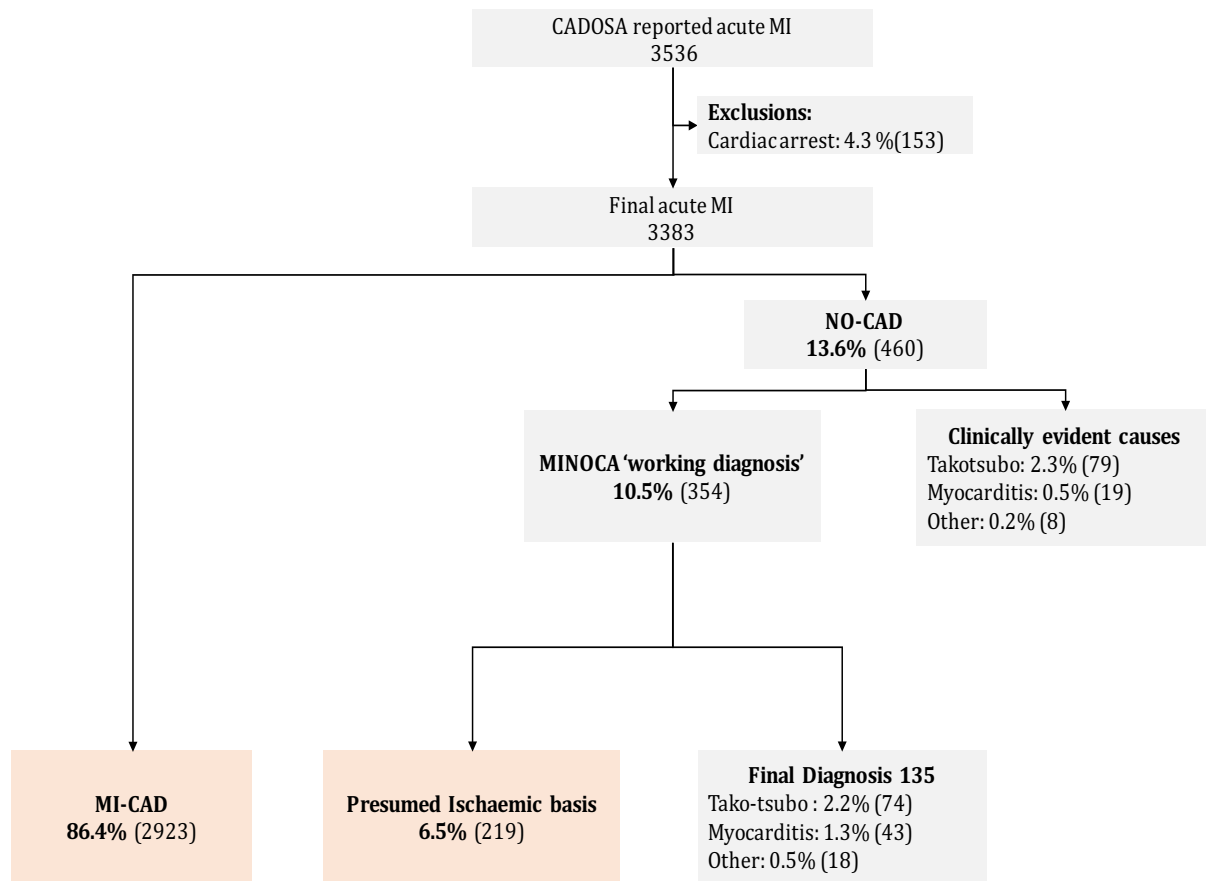


FIGURE 10: PREVALENCE OF ISCHAEMIC MINOCA
Diagnostic percentages are of total final acute MI

Baseline characteristics

The baseline characteristics of the ischaemic MINOCA group compared to MI-CAD group are presented in Table 7. Ischaemic MINOCA patients were younger, more likely to be female, had lower prevalence of cardiovascular risk factors and prior history of acute MI. A small number of ischaemic MINOCA patients (5%) had prior history of MI-CAD with PCI performed. There were no difference between groups in the incidence of prior heart failure, valvular heart disease, stroke, and dialysis use. Non-cardiac comorbidities were similar between ischaemic MINOCA and MI-CAD patients including depression and gastro-oesophageal reflux disease (Table 7).

Presentation Features

Time from symptom onset to hospital arrival was similar for ischaemic MINOCA and MI-CAD patients (509 minutes (132, 1290) vs 360 (109, 1153) minutes, $p>0.05$). The proportion of STEMI were fewer in ischaemic MINOCA compared to MI-CAD and correspondingly ischaemic MINOCA patients had lower peak troponin values. Average GRACE risk score was lower in ischaemic MINOCA patients for both STEMI and NSTEMI presentations. The risk for in-hospital mortality according to GRACE score for ischaemic MINOCA STEMI and MI-CAD STEMI was similar however, the risk for in-hospital mortality was higher for NSTEMI in MI-CAD patients compared to MINOCA (Table 7).

Discharge Therapy

Guideline recommended secondary prevention therapies at discharge were significantly less frequently prescribed in ischaemic MINOCA patients compared to MI-CAD including aspirin, statin, beta-blocker and ACE-inhibitor/ARB blocker. Referral to cardiac rehabilitation was also significantly lower in ischaemic MINOCA patients (Table 7).

In Hospital Outcomes

Unadjusted in-hospital mortality was lower in ischaemic MINOCA patients compared to MI-CAD, although not statistically different. Unadjusted major bleeding rates did not significantly differ between groups, similar to the rates of other procedural complications including stroke and cardiogenic shock. There were no ischaemic MINOCA patients with new onset or acute recurrence of heart failure, however this was observed in a small proportion of MI-CAD patients (Table 7).

TABLE 7: CLINICAL CHARACTERISTICS OF PATIENTS

n	MI-CAD (2,923)		Ischaemic MINOCA (219)		OR (95% of CI)	P
Baseline characteristics						
Age	64 (54, 74)		60 (48,71)			<0.01
Female	26%	(767)	57%	(124)	0.22 (0.16,0.29)	<0.01
Indigenous	6%	(170)	7%	(15)	0.44(0.24, 0.81)	>0.05
Current smoking	34%	(923)	25%	(53)	0.39(0.27, 0.57)	<0.01
Hypertension	66%	(1,880)	61%	(128)	0.93(0.69, 1.27)	>0.05
Hyperlipidaemia	61%	(1,722)	49%	(102)	0.65(0.48, 0.86)	<0.01
Family Hx of CAD	44%	(1,149)	39%	(77)	0.66(0.49, 0.90)	<0.01
Diabetes	32%	(921)	22%	(47)	0.58(0.42, 0.82)	<0.01
Prior MI	21%	(606)	11%	(24)	0.49(0.32, 0.76)	<0.01
Prior heart failure	7%	(200)	7%	(15)	1.13(0.64, 1.98)	>0.05
Prior valvular HD	2%	(61)	2%	(4)	0.94(0.33, 2.66)	>0.05
Prior PCI [#]	12%	(348)	5%	(10)	0.41(0.21, 0.78)	<0.01
Current Dialysis	2%	(54)	3%	(6)	1.22(0.51, 2.94)	>0.05
Prior stroke	8%	(220)	5%	(10)	0.68(0.35, 1.33)	>0.05
History of PAD	6%	(179)	3%	(9)	0.49(0.25, 0.98)	<0.05

Chapter 3

Depression	13%	(353)	19%	(39)	1.33(0.91, 1.94)	>0.05
Active GORD	26%	(718)	33%	(69)	1.49(1.09, 2.03)	0.05
IBS	2%	(51)	2%	(4)	0.79 (0.28, 2.25)	>0.05
Peak troponin*	482	(163, 1504)	166	(91, 482)	-	<0.01
Peak CK-MB*	13	(4.4, 50.5)	7.2	(3.6, 20)	-	<0.01
STEMI	40%	(1,166)	16%	(36)		<0.01
STEMI GRACE risk model- In hospital mortality						
Low <2%	26%	(241)	45%	(13)		<0.01
Intermed-High >5%	74%	(671)	55%	(16)		
NSTEMI GRACE risk model-In Hospital Mortality						
Low <1%	22%	(304)	33%	(46)		<0.01
Intermed-High >3%	78%	(1,062)	67%	(92)		
Discharge medications						
Aspirin	92%	(2631)	73%	(159)	0.23(0.16, 0.33)	<0.01
Statin	88%	(2546)	69%	(149)	0.28(0.21, 0.39)	<0.01
ACE/ARB	82%	(2350)	62%	(135)	0.37(0.28, 0.51)	<0.01
Beta blocker	66%	(1909)	41%	(88)	0.35(0.27, 0.48)	<0.01
Cardiac rehab	54%	(1392)	19%	(36)	0.19(0.13, 0.28)	<0.01

In Hospital Complications

Cardiogenic shock	1.6%	(47)	0	(0)		>0.05*
Stroke	0.6%	(18)	0.9%	(2)	1.60(0.35, 7.29)	>0.05*
Bleeding event	1.4%	(40)	0.5%	(1)	0.29(0.04, 2.16)	>0.05*
Heart failure	1.4%	(40)	0	(0)	-	>0.05
Death	2.0%	(58)	0.5%	(1)	0.25(0.04, 2.02)	>0.05

Values are presented as percentages with numbers (%) or median with interquartile ranges. ACE, angiotensin converting enzyme; ARB, Angiotensin II Receptor Blocker; CABG, coronary artery bypass surgery; CAD, coronary artery disease; CK-MB, creatine kinase-muscle and brain; GORD, gastro oesophageal reflux disease; GRACE, global registry of acute coronary events; MI, myocardial infarction; MI-CAD, myocardial infarction with coronary artery disease; MINOCA, myocardial infarction with Non-Obstructive coronary arteries; NSTEMI, non-ST elevation myocardial infarction; PAD, peripheral artery disease; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction.

*P values are not adjusted for age and gender.

Chest pain characteristics

Overall, there were no major differences observed in the location and distribution of the presenting chest pain characteristics between ischaemic MINOCA and MI-CAD patients (Figure 11). Substernal pain was the most frequently reported pain in both groups (83% vs. 86%, $p>0.05$); followed by left sided chest pain (36% vs. 38%, $p>0.05$ and left arm pain (33% vs. 36%, $p>0.05$). Ischaemic MINOCA were less likely to experience right arm pain (12% vs. 18%, $p>0.05$). Jaw, epigastric and back pain were experienced by minority of the patients and did not exhibit any significant differences between the two groups (Figure 11).

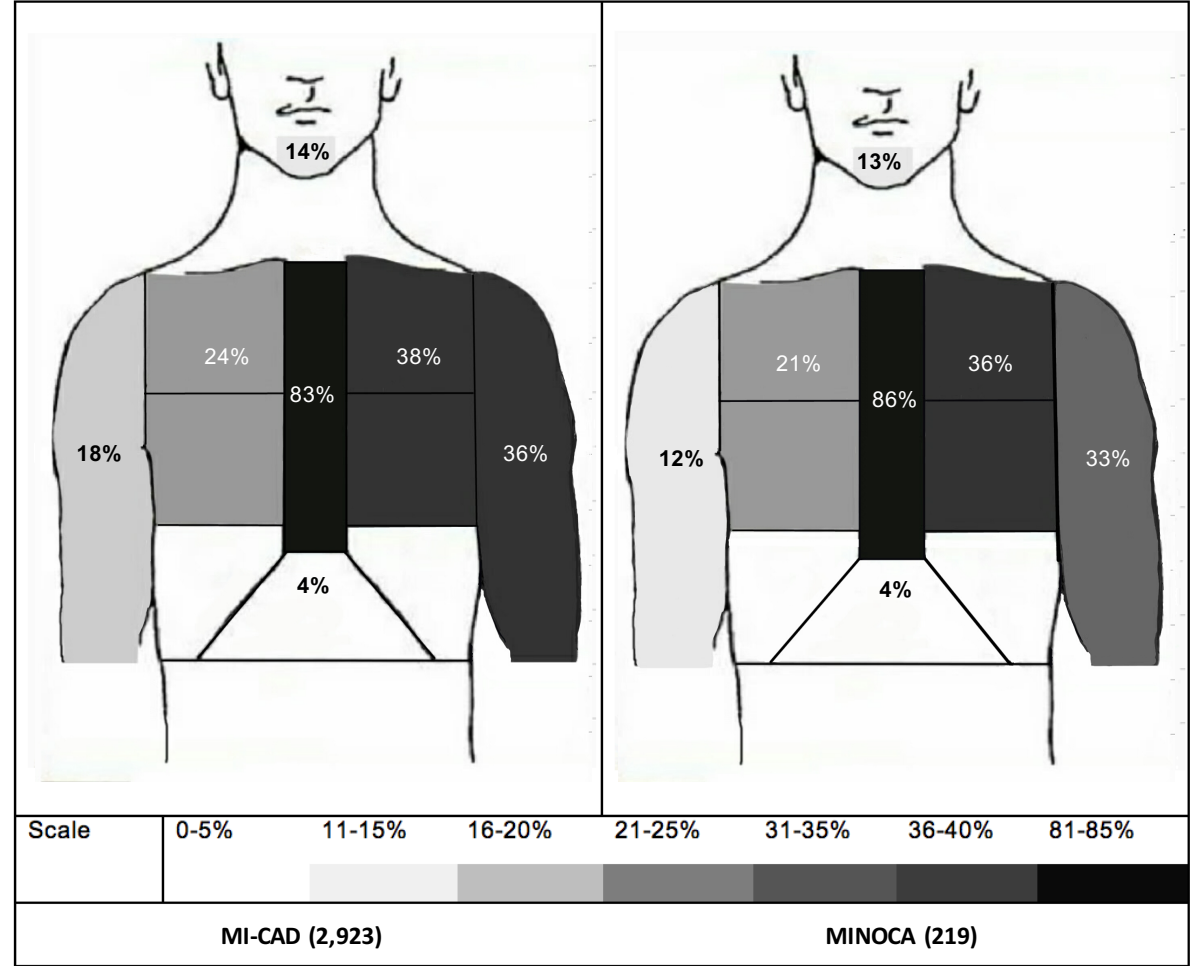


FIGURE 11: LOCATION OF CHEST PAIN COMPARISON.

There were no differences between the groups in regards to the chest pain quality. Tightness (44% vs. 41%, $p>0.05$) and heaviness (41% vs. 40%, $p>0.05$) were commonly reported in ischaemic MINOCA and MI-CAD patients respectively, while a small proportion of the patients experienced sharp, squeezing and burning (Figure 12). In regards to the associated symptoms with the chest pain; sweating, nausea and dyspnoea were observed similarly in both groups (Figure 12). A small, yet higher proportion of MINOCA patients reported emotional stress prior to this acute event (10% vs. 6%, $p<0.05$). Duration of the pain were also similar between the groups with almost 80% of both MINOCA and MI-CAD patients reporting pain lasting longer than 30 minutes (70% vs 75%, $p>0.05$).

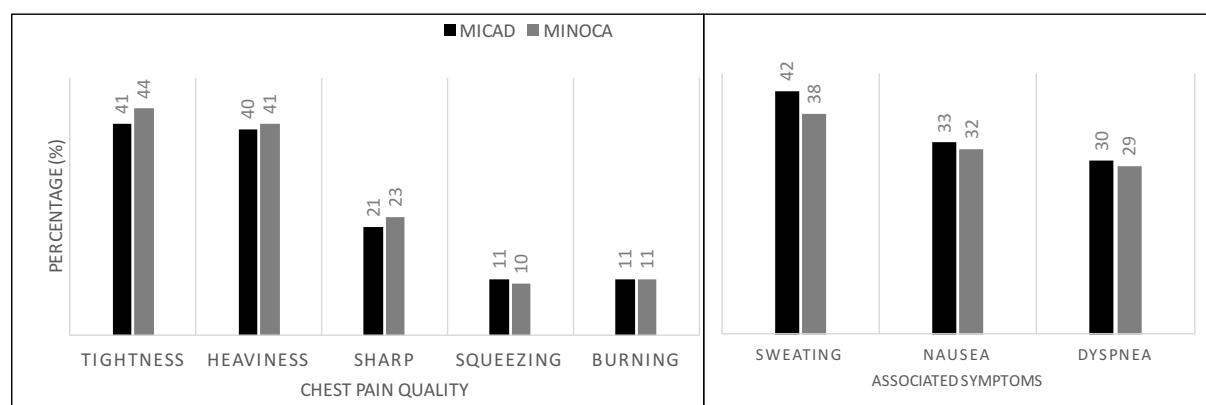


FIGURE 12: CHEST PAIN CHARACTERISTICS.

Discussion

In comparison to patients with MICAD, this prospectively designed cohort study has demonstrated that patients with ischaemic MINOCA are (i) clinically indistinguishable in relation to their presenting chest pain, (ii) have fewer cardiovascular risk factors (especially hyperlipidaemia, diabetes, smoking and a positive family history), (iii) less likely to have ST elevation on ECG, (iv) lower peak myocardial infarct markers and GRACE scores, and (v) less likely to be discharged on conventional post-infarct medications including aspirin, statins, beta-blockers and renin-angiotensin system inhibitors, as well as cardiac

rehabilitation. Accordingly, although they mimic MICAD patients in their clinical presentation, their apparent low risk profile (fewer risk factors, absence of obstructive atherosclerosis on angiography, smaller peak troponin's and GRACE scores) appears to have influenced their discharge management.

Previous studies.

The overall prevalence of MINOCA in this prospective study was 14%, which is significantly higher than the previously published 6%, as determined in a systematic review of the literature. This was anticipated considering (a) the prospective nature of this study, and (b) more frequent use of angiography in contemporary acute coronary syndrome management. However, only 7% of the total acute MI population were classified as ischaemic MINOCA, based upon the clinical team's discharge diagnosis. This represents half of the MINOCA population.

MINOCA is a working diagnosis made at the time of angiography, when there is no obvious explanation for the acute MI presentation in the absence of obstructive CAD¹⁷⁴. This prospective study identified MINOCA patients at the time of angiography, with subsequent clinical investigation left to the discretion of the attending clinician. These subsequent investigations may reveal non-ischaemic causes for the clinical presentation such as myocarditis and pulmonary embolism. Since this study aimed to compare the clinical presentation and treatment of patients with MINOCA to those with MICAD, a select group of ischaemic MINOCA patients were identified, by excluding the MINOCA patients with an identified non-ischaemic cause for their presentation (as defined by their discharge diagnosis). This approach provides a more appropriate clinical comparison since the non-

ischaemic aetiologies would not be expected to have similar characteristics to MICAD, nor require conventional post-infarct treatments.

In comparison to those with MICAD, patients with ischaemic MINOCA were younger and much more likely to be female (26 vs 57%, Table-7). Hence in this more select ischaemic MINOCA group, the gender difference between MINOCA and MICAD appears to be accentuated and may reflect the higher prevalence of coronary microvascular disorders amongst women.

Chest Pain Characteristics.

Comparison of chest pain characteristics between patients with MICAD and ischaemic MINOCA addresses two important questions, (a) do the later patients represent a ‘false positive infarct’ (as speculated by some sceptics) characterised by atypical chest pain associated with an aberrant troponin rise, and (b) can ischaemic MINOCA patients be clinically identified from those with MICAD on the basis of their chest pain characteristics, thereby allowing early distinction between this groups? This investigation is uniquely positioned to address these questions considering (i) this is a comprehensive and representative study cohort since all acute MI admissions undergoing coronary angiography in the public hospitals of South Australia have been captured in this registry, and (ii) the chest pain characteristics were prospectively collected by trained data collectors who obtained the relevant details directly from the patient. Using this prospective comprehensive evaluation in patients with MICAD and ischaemic MINOCA, this study found the groups to be clinically indistinguishable in their chest pain characteristics in relation to chest pain location (Figure 11), quality (Figure 12), associated symptoms (Figure 12), or duration. Thus clinically, the chest pain experienced by ischaemic MINOCA patients mirrors that experienced by MICAD

patients suggesting that the pain is truly ischaemic in nature. Furthermore, the chest pain characteristics do not allow these acute MI subtypes to be easily distinguished.

Acute Coronary Syndrome Risk Profile

By selecting the subgroup of MINOCA patients with a presumed ischaemic aetiology for their clinical presentation, a more appropriate comparison with MICAD patients can be undertaken to determine the relative risk profile of the MINOCA patients. The MINOCA patients appear to have a lower risk profile compared with MICAD patients considering (a) absence of obstructive CAD – by definition, (b) younger age, (c) lower prevalence of all conventional cardiovascular risk factors except hypertension, (d) fewer presenting with ST elevation on initial ECG, (e) lower peak troponin's, and (f) lower GRACE scores. Hence these patients would be expected to have better outcomes than their MICAD counterparts. Although uninterpretable because of the small sample size, the in-hospital complications are similar or possibly less frequent in the MINOCA group (Table 7).

Discharge Therapy.

Previous observational studies have reported that conventional post-infarct therapy is less often utilised in patients with MINOCA. For example, Patel et al⁸⁹ compared discharge therapies between NSTEMI patients with MICAD and MINOCA, reporting that the latter were less likely to receive aspirin (94% vs 86%), lipid lowering therapy (83% vs 73%), and beta-blockers (88% vs 74%); however they were more likely to receive calcium channel blockers (12% vs 25%). Similarly, Larsen et al¹⁷⁵ compared discharge therapies in all acute MI patients with either MICAD or MINOCA, reporting lower rates of conventional post-infarct therapies in the MINOCA patients; ie aspirin (98% vs 73%), statins (95% vs 56%), and beta blockers (90% vs 50%).

Both of these studies may have been confounded by MINOCA patients who subsequently were found to have non-ischaemic causes for the acute presentation (e.g. myocarditis) and therefore did not warrant consideration of conventional post-infarct therapies. However in the present study, non-ischaemic causes for MINOCA have been clinically excluded by reviewing the discharge diagnosis. Hence the treating clinician presumed the presentation had an ischaemic basis and thus routine post-infarct therapies could be justified. Despite this more selective MINOCA group, the frequency of conventional post-infarct therapies was less common in the ischaemic MINOCA group compared with the MICAD patients (Table 7).

Study Limitations.

This study is unique considering it is a representative sample with consecutive patient recruitment, utilises direct patient interview by trained data collectors for attainment of clinical data, and has defined an appropriate MINOCA subgroup (those with presumed ischaemic MINOCA) to compare with the MICAD patients. Despite these strengths, the study has important limitations that should be considered in interpreting the findings. Firstly, out-of-hospital cardiac arrest patients were excluded from the study, yet potentially this presentation could be ischaemic in nature. However, it was decided to exclude these patients since a troponin rise could be the result of resuscitation efforts rather than ischaemia/infarction. Secondly, investigation of all MINOCA patients to identify the underlying cause was not mandated but left to the discretion of the treating clinician. Thus the diagnosis of ischaemic MINOCA was on the basis of clinical expertise rather than rigorous stratification by a critical pathway as proposed in the recent position paper¹⁷⁴. Indeed cardiac magnetic resonance imaging was undertaken in only 26 of the 219 ischaemic MINOCA patients. Thus it remains possible that the ischaemic MINOCA cohort used in this study may have had patients with undiagnosed myocarditis.

This study did not undertake further stratification of the patients according to the presence of non-significant lesions of <50% or no lesions (i.e. normal coronary arteries).

Conclusion

In conclusion, this comprehensive analysis comparing patients with presumed ischaemic MINOCA with MICAD patients has demonstrated that their chest pain characteristics are indistinguishable suggesting that the former do experience ischaemic chest pain despite the absence of obstructive CAD. However, these ischaemic MINOCA patients have fewer high risk features compared with their MICAD counterparts., Whether this translates to better clinical outcomes requires further prospective studies. These are important to determine if the less aggressive use of post-infarct therapies in ischaemic MINOCA patients is justified. Moreover, studies are required to determine if the benefit of these therapies extend to patients with MINOCA.

CHAPTER 4

4 RISK OF THROMBOSIS IN MINOCA

This chapter includes an outline and the manuscript in the form as it appears in the manuscript, ‘Thrombosis risk in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA)’ authored by Sivabaskari Pasupathy, Susan Rodgers, Rosanna Tavella, Simon McRae and John F. Beltrame, and currently under review in *Coronary Artery Disease*.

In keeping with this style of this thesis, the abstract has been removed, the table and figures re-numbered, the references incorporated into the thesis’s master reference list and the manuscript repaginated.

4.1 STATEMENT OF AUTHORSHIP

Risk of thrombosis in Myocardial Infarction with Non-Obstructive Coronary Arteries (MINOCA)

Publication Status	Under review
Publication Details	Coronary Artery Disease Research
Principal Author	Sivabaskari Pasupathy
Contribution	Acquisition of data; Analysis and interpretation of data; Drafting of manuscript; Critical revision of the manuscript.
Overall percentage (%)	85%

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature and Date 14/08/2016

Co-Author Contributions

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

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

Chapter 4

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4.2 STUDY OUTLINE

The mechanism responsible for acute MI involves an interaction between atherosclerosis, vascular dysfunction and thrombosis as established in Chapter 1. Given the limited burden of atheroma in MINOCA, potential mechanisms are likely to involve microvascular dysfunction and thrombosis in these patients.

As demonstrated in the systematic review, coronary artery spasm and thrombophilia disorders contributed to nearly 30%, and 14% of MINOCA presentations, respectively¹⁷⁰. Clinical studies examining the risk of thrombosis in MINOCA failed to provide detailed, comprehensive assessment between MINOCA and MI-CAD patients, therefore implicating this aspect for further exploration.

The formation of fibrin rich thrombus generally results from plaque rupture which exposes the sub-endothelial layer of the vessel, leading to the accumulation and activation of platelets and the generation of excessive thrombin³⁴. In addition, abnormalities in the coagulation cascade also generate a modest amount of thrombin¹⁷⁷. The coagulation cascade is a complex process involving approximately 30 different proteins. These reactions convert fibrinogen to fibrin (together with platelets) to form a stable thrombus as shown in Figure 13. In detail, vascular injury and tissue factor (TF) initiate the process by activating Factor XII and Factor VII respectively. These processes lead to the activation of Factor X which converts Prothrombin to Thrombin. Activation of factor V (FV), factor VIII (FVIII) and factor XI (FXI) also lead to a thrombin burst^{178, 179}, converting fibrinogen to fibrin. Thrombin generation through the TF – FVIIa pathway is inhibited by the action of tissue factor pathway inhibitor (TFPI)¹⁸⁰, which is thought to be activated by protein S in a protein C-independent manner¹⁸¹. Thrombin not only accelerates its own generation by positive feedback systems

but also inhibits it through its interaction with thrombomodulin. By binding to this endothelial receptor, thrombin loses its procoagulant function and is able to activate protein C. Activated protein C (APC) inhibits activated FVIII (FVIIIa) and activated FV (FVa) with protein S acting as a vital cofactor^{182, 183}. A further major natural anticoagulant inhibiting thrombin generation is antithrombin¹⁸⁴.

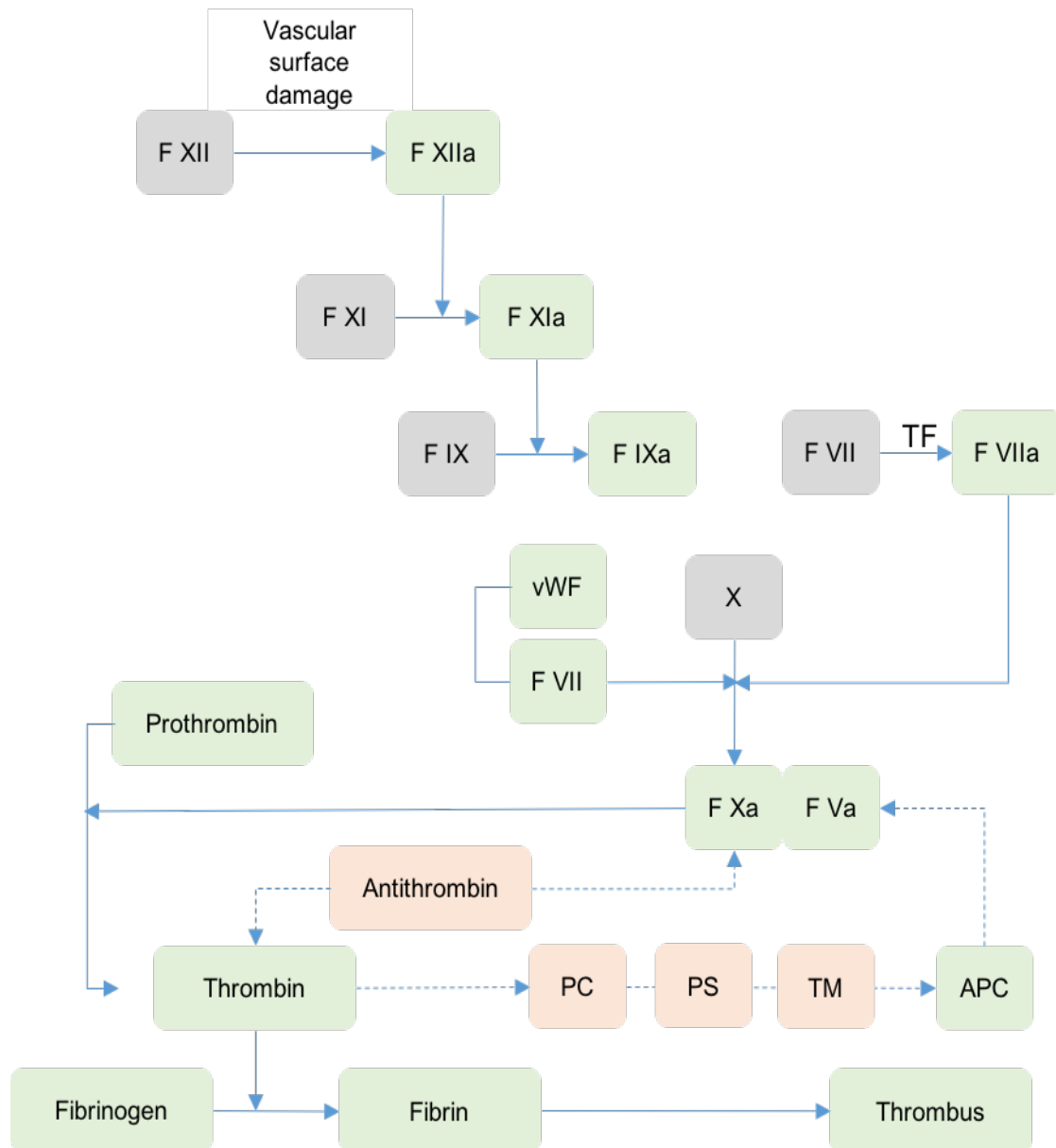


FIGURE13: COAGULATION CASCADE.

‘Thrombophilia’ refers to an inherited and acquired imbalance in the coagulation cascade that leads to an increased risk of thrombosis. As thrombin is the central enzyme in the coagulation cascade, estimating a MINOCA patient’s potential to generate thrombin may provide some insight about the risk of thrombosis as a cause of myocardial infarction in these patients.

Thrombin generation measurement

Traditional coagulation tests, such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), do not assess the whole coagulation system. These tests use clot formation as their endpoint, which occurs when only around 5% of all physiologically relevant thrombin is formed¹⁷⁹. These tests are also insensitive to prothrombotic states. Coagulation factor assays can identify specific deficiencies but these do not always closely correlate with the clinical phenotype. The limitations of the traditional tests are also demonstrated by the observation from Butenas et al¹⁸⁵ who showed that thrombin generation varies up to 40-fold when measurements are undertaken with individual coagulation factors at the extremes of the normal ranges in a synthetic plasma system.

Measurement of an individual’s capacity to generate thrombin, however, captures the end result of the interaction between proteases and their inhibitors, and is therefore potentially more useful as a reflection of a thrombotic risk. MacFarlane et al¹⁸⁶ and Pitney et al¹⁸⁷ pioneered the thrombin measurement in whole blood and plasma respectively. The capacity of plasma to generate thrombin over time has been termed the endogenous thrombin potential (ETP)¹⁸⁸.

Thrombin generation, (The Calibrated Automated Thrombogram (CAT), Thrombinoscope BV, Maastricht, The Netherlands) in a Fluoroscan Ascent fluorometer® (Thermolab systems OY, Helsinki, Finland) using PPP-reagent (5pM tissue factor, 4uM phospholipids, Thrombinoscope) is the technique used in the present study. In thrombin generation test, thrombin is generated upon the addition of PPP-reagent which imitates vessel wall damage in platelet poor plasma and activates the coagulation cascade. Thrombin generation is measured via its reaction with fluorogenic substrate. The parameters measured in the fluorogenic method include the lag time (defined as the moment that the signal deviates by more than 2SD from the horizontal baseline), peak thrombin, the time to peak thrombin, and the ETP. A typical trace is shown in Figure 14.

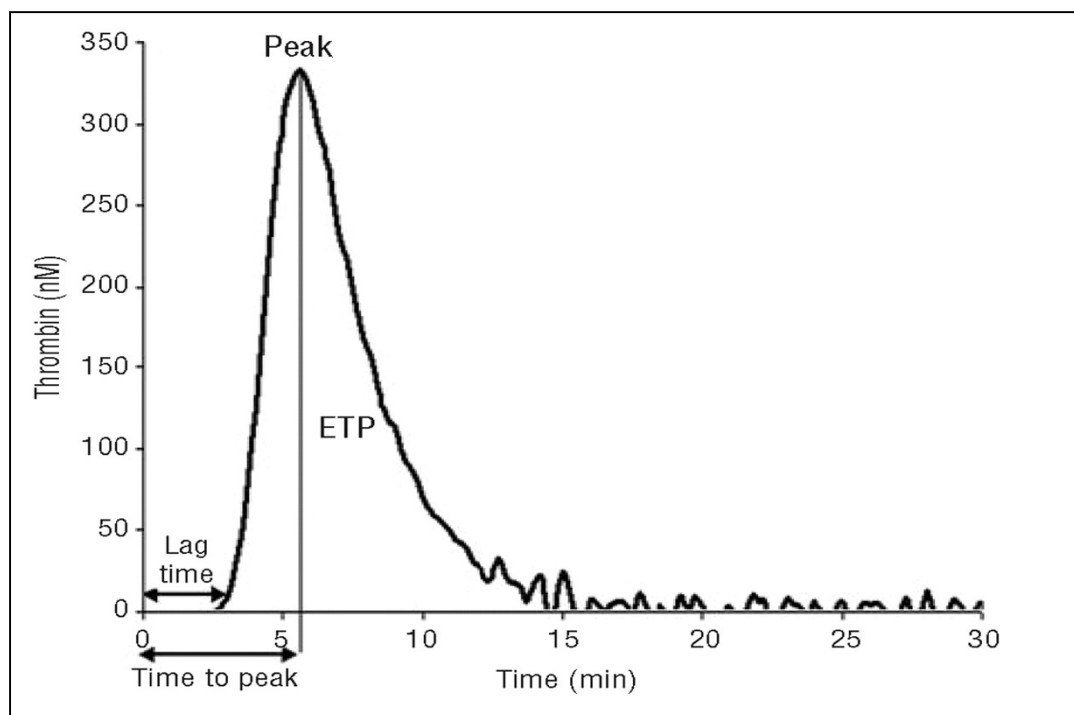


FIGURE14: STANDARD THROMBIN GENERATION CURVE.
ETP, Endogenous thrombin potential

Thrombophilia screening

Hypercoagulability, in which the haemostatic balance is tilted towards thrombus formation, consequently increases the risk of arterial thrombosis¹⁸⁹. An increased risk of myocardial infarction has been reported for high levels of FVIII, fibrinogen, vWF, FX, and FXII¹⁹⁰. Hypercoagulable states causing in-situ thrombosis in coronary arteries is a possible underlying mechanism for MINOCA. From the systematic review of MINOCA, it was shown that hypercoagulability states were implicated in only a proportion of MINOCA patients. However, it is unclear to which extent these findings truly reflect a different role of hypercoagulability in MINOCA and MI-CAD, or whether a difference is only present in this specific patient group, for the differential effect may be limited to specific age and sex categories. The subsequent manuscript utilises thrombin generation test and thrombophilia screen methods to assess the risk of thrombosis in MINOCA patients compared to MI-CAD.

4.3 MANUSCRIPT: THROMBOSIS RISK IN MINOCA

Introduction

Myocardial infarction (MI) with non-obstructive coronary arteries (MINOCA) is considered as a ‘working diagnosis’ for patients presenting with a suspected myocardial infarct in the absence of obstructive coronary artery disease (CAD) on angiography¹⁷⁰. Multiple mechanisms have been proposed for MINOCA and it is imperative that the underlying cause is identified for each patient since this will influence subsequent therapy¹⁹¹. One postulated mechanism for MINOCA is in-situ thrombus formation with subsequent lysis thereby resulting in a morphologically normal angiogram¹⁹² but with the underlying causative pro-thrombotic state potentially predisposing to a further event. A recent systematic review has reported that as many as 14% of patients with MINOCA may have an abnormality detected on thrombophilia screening¹⁷⁰. Congenital thrombophilia disorders detected in patients with MINOCA include factor V Leiden (FVL), prothrombin gene mutation (PGM), protein C and S deficiency¹⁷⁰.

The objective of this study is to compare the pro-thrombotic tendency of patients with MINOCA compared to MI patients with obstructive CAD (MI-CAD), in relation to congenital and acquired thrombophilia disorders, coagulation activation markers, and the thrombin generation assay (a global coagulation marker). The primary study hypothesis is that overall thrombin generation potential, assessed by the thrombin generation test, differs between patients with MINOCA and MI-CAD. Secondary hypotheses propose that MINOCA and MICAD patients differ in the prevalence of congenital thrombophilia states, acquired thrombophilia states and coagulation markers.

Methods

To achieve this objective, we employed a case-control study design recruiting age & gender matched patients with MINOCA and MI-CAD.

Patient Selection

Patients admitted for an acute MI at The Queen Elizabeth Hospital, Adelaide, Australia were prospectively screened from May 2013 to March 2015 and were included if the following criteria were met.

Inclusion Criteria

- i. Fulfil the universal diagnostic criteria for an acute MI²⁵ based on troponin elevation with corroborative clinical criteria.
- ii. Coronary angiography performed in the context of MI demonstrating MINOCA non-obstructive (<50% stenosis) coronaries or MI-CAD, obstructive ($\geq$ 50% stenosis) coronaries.

Exclusion criteria

- i. Patients on anticoagulant treatments
- ii. Tako-tsubo cardiomyopathy
- iii. Non cardiac and chronic causes of Troponin elevation such as heart failure, pulmonary disease and chronic kidney disease.

Patient Recruitment

Patients with confirmed MINOCA following coronary angiogram were consecutively approached and prospectively recruited into the study group. Sequential age and gender matched MI-CAD patients were recruited into the control group. All patients gave informed consent. The study was approved by the hospital human research ethics committee.

Plasma processing

Blood was collected four weeks after the initial acute MI presentation, to avoid any influence from acutely administered drugs such as heparin or other anticoagulant agents, or activation of coagulation associated with the acute event. A minimal stasis using a 21 G needle into plastic 3.5 ml Vacuette® tubes (Greiner Bio-One, Austria) containing buffered sodium citrate (final concentration 0.105 mol/l), Serum and EDTA was used. Citrate plasma samples were processed within an hour of blood collection by a single centrifugation for 15 minutes at 2200g (4000 rpm), with the top 2/3 of the plasma then removed, and stored in aliquots at -70 °C.

Assays/Analysis Performed

(i) Thrombin generation test

Thrombin generation was measured using calibrated automated thrombin generation assay (CAT, Thromboscope BV, Maastricht, The Netherlands) in a Fluoroscan Ascent fluorometer® (Thermolab systems OY, Helsinki, Finland) using PPP-reagent (5pM tissue factor, 4uM phospholipids, Thromboscope) as previously described by Rodgers et al¹⁹³. MINOCA samples and matched MI-CAD samples were always tested in the same run, to avoid any effects due to variation between assays. In addition, 2 aliquots of quality control plasma samples and a commercial lyophilized plasma (HemosIL Calibration Plasma, Instrumentation Laboratory, Bedford, MA, USA) were also tested in the same run in each assay for validation. Assays were repeated if the quality control results were not in the desired range. The effect of thrombomodulin (TM) on thrombin generation was tested by the addition of rabbit lung TM (lot 140711, Sekisui, Stamford, CT, USA), which was added into the reaction mixture at a final concentration of 0.35Units/ml (5.89nM). Readings from the

fluorometer were automatically recorded and calculated using dedicated software (Thrombinoscope, Netherlands) that displays thrombin generation curves (time vs. generated thrombin) and calculates endogenous thrombin potential (ETP), peak thrombin, velocity index, lag time, and time to peak.

(ii) Thrombophilia screen and coagulation markers

Congenital thrombophilia states: FVL and PGM were identified by primer extension genotype analysis using the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA). The activities of antithrombin (AT, CV 4.7%) and protein C (PC, CV 2.8%) were assayed using a chromogenic substrate method, and free protein S antigen (PS, CV 4.7%) using latex immune-assay method on a STA-R analyser (Diagnostica Stago, France).

Acquired thrombophilia states: Lupus anticoagulant (CV 4.8%) was measured by a diluted Russell viper venom time assay (STA-Staclot DRVVT Screen, Stago). Anticardiolipin antibodies (CV 3.5%) were detected by a quantitative enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN Medizinische Labordiagnostika AG, Germany) according to the manufacturer's instructions. Factor VIII coagulant activity (FVIII:C, CV4.2% was measured using a two-stage chromogenic assay (Biophen FVIII:C, Hyphen-Biophen, Neuville-sur-Oise, France) von Willebrand factor antigen (VWF:Ag, CV 15%) using latex immunoassay (STA-Liatest for VWF, Stago) and Fibrinogen (CV 6%) using Clauss clotting method (STA-Fibrinogen, Stago) on a STA-R analyser (Diagnostica Stago, France).

Coagulation marker: D-dimer was measured using a immune- turbidimetric method (STA-Liatest D-DI, Stago) on a STA-R analyser (Diagnostica Stago, France).

Statistical analysis

Baseline characteristics, thrombin generation test variables (includes ETP, peak thrombin, lag time and time to peak) and thrombophilia screen results were described in MINOCA patients in comparison to MI-CAD patients. Mean with range or medians with 25th and 75th percentiles were reported for continuous variables, and frequencies for categorical variables. Data were compared using either unpaired t-test with equal SD or Mann-Whitney U test for continuous variables and Fishers exact test for the categorical variables. A p value of <0.05 was considered significant in all comparisons. Statistical analysis was performed using Graph Pad Prism software package for MAC OS X, version 6.0 (San Diego, California, USA).

Results

Baseline characteristics

Patients' baseline characteristics are listed in Table 8. Twenty-four age and gender matched MINOCA (58±3, 50% women) and MI-CAD (60±3, 47% women) patients were recruited in each group. Cardiovascular risk factors were similar between groups. The frequency of STEMI was higher in MI-CAD compared to MINOCA (84% vs. 12%, p<0.05). In addition, MINOCA patients were less likely to receive secondary prevention treatment at discharge. Blood samples were collected at a mean delay of 39 days from the acute presentation.

TABLE 8-BASELINE CHARACTERISTICS

	MINOCA(n=24)	MI-CAD (n=24)	P Value
	Mean (range) or %(n)		
Cardiovascular Risk factors			
Age	58 (18, 87)	60 (34, 50)	0.72
Women	50% (12)	47% (10)	1.00
Hypertension	60% (15)	48% (12)	0.57
Hyperlipidemia	56% (14)	44% (11)	0.57
Diabetes	24% (6)	36% (9)	0.53
Current smoker	24% (6)	32% (8)	0.75
Family History of CAD	24% (6)	20% (5)	1.00
STEMI	12% (3)	84% (21)	< 0.01
Discharge Medications			
Antiplatelet agent	62% (15)	93% (22)	<0.01
Second antiplatelet agent	8% (2)	85% (20)	<0.01
Statin	62% (15)	96% (23)	<0.01
ACE inhibitor	38% (9)	86% (21)	<0.01
ARB	12% (3)	8%(2)	0.6
Calcium channel blockers	46% (11)	38% (9)	0.6
Beta blocker	19% (5)	31% (7)	0.4
Nitrates	19% (5)	28% (6)	0.5

P<0.05 considered statistically significant. ARB, Angiotensin receptor blocker, CAD, Coronary artery disease; MI-CAD, Myocardial infarction with coronary artery disease; MINOCA, Myocardial infarction with non-obstructive coronary arteries; STEMI, ST elevation myocardial infarction.

Thrombin Generation

There was no statistical significant difference in ETP results between the MINOCA and MI-CAD groups (1586 (1380,2000) vs. 1750 (1511,2042) (Table 9). In addition, there were no statistically significant differences observed between MINOCA and MI-CAD in peak thrombin (300 (248, 381) vs. 342 (304, 388)), lag time (3 (2.9,4) vs. 3.3 (3, 3.9), time to peak (6.7 (5.5, 7.4) vs. 5.9 (5.5,6.7) and velocity index (107.1 (67.1, 147.8) vs 128.5 (111.4, 144.9) respectively, although there was a trend for higher values for ETP, peak and velocity in the MI-CAD group. Addition of TM did not yield any statistically significant differences between the two groups either (Table 9)

Thrombophilia screen

Thrombophilia screening results are shown on table 10.

Congenital thrombophilia. Neither mean levels of the congenital inhibitors of coagulation (AT, protein C and protein S) nor the incidence of patients with abnormally low test results differed significantly between MINOCA and MI-CAD patients. The prevalence of patients with the FVL or PGM mutations was low in both the MINOCA and MI-CAD groups (0% vs 4%, 8% vs 4%) and did not significantly differ ($p > 0.05$).

Acquired thrombophilia states. Antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) were not found in any of the patients from both groups. Mean levels of F VIII:C, VWF:Ag and fibrinogen were not significantly different between MINOCA and MI-CAD patients, and the incidence of patients with results above the normal range did not differ significantly. The proportion of patients with elevated D-Dimer level (42% vs. 31%, $p > 0.05$) was similar in both groups, and mean levels did not differ significantly.

TABLE 9-THROMBIN GENERATION TEST PARAMETERS

	-TM			+TM		
	MINOCA	MI-CAD	P Value	MINOCA	MI-CAD	P Value
	Median (25th and 75th Percentiles)			Median (25th and 75th Percentiles)		
ETP (nM.min)	1586 (1380, 2000)	1750 (1511, 2042)	0.55	175 (77, 386)	278 (145, 443)	0.84
PeakThrombin (nM)	300 (248, 381)	342 (304, 388)	0.50	42 (18,90)	63 (32, 101)	0.99
Lag time (min)	3.0 (2.9,4)	3.3 (3.0, 3.9)	0.99	3.2 (2.6,4.6)	3.4 (2.8,4.7)	0.60
Time to Peak (min)	6.7 (5.5, 7.4)	5.9 (5.5,6.7)	0.54	5.7 (5.1,7.3)	6(5.4, 6.7)	0.99
Velocity Index(nM/min)	107.1 (67.05, 147.8)	128.5 (111.4, 144.9)	0.11	16.9 (5.4, 37.9)	25.9 (10.5, 44.5)	0.84

P<0.05 considered significant. ETP, Endogenous thrombin potential; MI-CAD, Myocardial infarction with coronary artery disease; MINOCA, Myocardial infarction with non-obstructive coronary arteries; TM, Thrombomodulin

TABLE 10-INCIDENCE AND EXPRESSION OF THROMBOPHILIA STATES

Tests	MINOCA	MI-CAD	P value
	% (n) or mean and range		
Congenital thrombophilia			
Antithrombin (international units)	101 (72, 134)	100 (82, 122)	0.86
Antithrombin deficiency	8% (2)	0	0.22
Protein C (international units)	121 (65, 293)	116 (78, 189)	0.74
Protein C deficiency	0	0	-
Protein S (international units)	111 (49, 142)	104 (67, 150)	0.18
Protein S deficiency	4% (1)	0	0.47
Factor V Leiden	0	4% (1)	-
Prothrombin gene mutation	8% (2)	4% (1)	0.59
Acquired thrombophilia			
Anticardiolipin antibody (GPL [#] units)	1.5 (1, 4)	2.3 (1, 18)	0.38
Antiphospholipid antibody syndrome	0	0	-
Presence of Lupus anticoagulant	0	0	-
Factor VIII IU/dL	168 (61, 301)	159 (78, 229)	0.72
Above Normal (>180)	42% (11)	31%(9)	0.42
Von willebrand factor antigen IU/dL	151 (60, 299)	147 (57, 274)	0.88
Above Normal(>240)	15% (4)	3% (1)	0.17
Coagulation markers			
Fibrinogen (international units)	3.7 (2.2, 5.4)	3.8 (2.1, 5.7)	0.53
Above normal (>4.0)	42% (11)	34%(10)	0.58
D-Dimer (international units)	0.6 (0.1, 1.9)	0.4 (0.2, 0.9)	0.47
Above Normal (>0.5)	42% (11)	31% (9)	0.41

* Data expressed as either mean with standard deviation or frequencies, P<0.05 considered significant. [#]GPL denotes IgG isotype; dRVVT, dilute Russell's viper venom time; MI-CAD, Myocardial infarction with coronary artery disease; MINOCA, Myocardial infarction with non-obstructive coronary arteries.

Discussion

Underlying thrombophilia resulting in an increased tendency to intravascular thrombosis has been postulated as one of the possible causes of MINOCA in previous studies and reviews^{159, 192}. This is the first study to examine the thrombin generation in this patient group. It demonstrated similar thrombin generation activity in MINOCA patients to a matched MI-CAD population, and no difference in the prevalence of established congenital or acquired thrombophilia states, as well as similar D-dimer findings.

Overall thrombin generation parameters (specific features including, ETP, peak thrombin, lag time, time-to-peak and velocity index) in MINOCA patients were similar to MI-CAD patients. Despite this, thrombosis may play a role in MINOCA with small plaque rupture, not evident on angiography, initiating localised thrombosis resulting in coronary occlusion. Such vessel wall abnormalities may not influence thrombin generation results. The potential role of such a mechanism was highlighted by Reynolds et al¹⁵³, who demonstrated plaque rupture in sixteen of 42 female patients (38%) undergoing IVUS following MINOCA presentation. MINOCA patients may therefore potentially benefit from intravascular ultrasound (IVUS) to screen for plaque/clot rupture. In addition, the role of spasm in these patients could not be tested, but spasm may also initiate a partial stenosis leading to secondary thrombosis or provoke plaque rupture initiating the coagulation cascade.

Evidence of the association between deficiencies of AT, PC or PS and arterial thrombosis is limited to case reports and small studies that are generally hampered by low prevalence of these thrombophilia states similar to the current study. None of the patients demonstrated AT deficiency in studies investigated by Rallidis et al¹⁹⁴ among 70 acute MI patients before the age of 36 years and Dacosta et al¹⁹⁵ among 75 acute MI patients before the age of 45 years.

Dacosta et al^{158, 159} presented only one patient with Protein C or S deficiency in two studies with 73 and 78 MINOCA patients.

Presence of FVL is the most common risk factor for venous thrombosis and has often been associated with MINOCA. DaCosta et al¹⁵⁹, Van De Water et al¹⁶⁸ and Mansourati et al¹⁶⁷ demonstrated around 10% of MINOCA patients have FVL mutation. However, none of the MINOCA patients in the current study exhibited this gene mutation. Mansourati et al¹⁶⁷ demonstrated 12% of 107 MINOCA patients with FVL in comparison to 4.5% of 244 with MI-CAD. Rosendaal et al¹⁹⁶ demonstrated FVL as a risk factor for acute MI young women (under the age of 44). Van De Water et al¹⁶⁸ showed increased frequency of PGM in young MINOCA patients compared to young MI-CAD. It's important to note that the patient cohorts in these studies are primarily younger compared to the present study. Among the studies reporting inherited coagulation disorders in MINOCA, Vandewater et al¹⁶⁸ demonstrated MINOCA patients under the age of 50 are more likely to express either FVL or PGM compared to patients older than 50 years of age (20% vs. 6%, $p<0.05$), whereas the mean age of our MINOCA group was 58 (range 18-87). Ethnicity also plays a crucial role in inherited thrombophilic states. Caucasians are more likely to exhibit FVL compared to any other races outside Europe¹⁹⁷. Our data did not clearly identify the presence of a congenital thrombophilia state as a common risk factor in unselected patients with MINOCA, although previous studies suggest that young patients may be at high risk of developing acute MI in the presence of a congenital thrombophilia state, particularly when classical risk factors such as smoking are present.

Anticardiolipin antibodies were shown to be a rare independent risk factor for MI and recurrent events. The role of anticardiolipin antibodies in the pathophysiology of arterial vascular thrombotic events is well established^{198, 199}. Segev et al 2004²⁰⁰ demonstrated the incidence of anticardiolipin antibodies in 18% of 85 STEMI patients under 50 years in whom percutaneous coronary intervention was performed. Davies et al²⁰¹ presented a case series with five MINOCA patients who found to have lupus anticoagulant and/or anticardiolipin antibodies and suggested MINOCA patients may benefit from screening for antiphospholipid antibodies. Da Costa et al²⁰² also showed a rare case of a MINOCA patient in whom thrombosis was caused by antiphospholipid syndrome. Although, the present study did not identify any patients with antiphospholipid syndrome, screening may benefit some young MINOCA patients.

Elevated FVIII is found in 11% of general adult population²⁰³ and elevated levels are more common in women, patients with blood groups other than O, patients with high body mass index, diabetics and in clinical conditions like chronic inflammation. In many cases there was concomitant elevation in both FVIII and VWF:Ag²⁰⁴. Elevation in acute phases may not return to baseline for several months. There are reported cases of acute coronary syndrome associated with elevated FVIII:C with no other cardiovascular risk factors or significant atheromatous disease^{205, 206}. The relationship between FVIII:C and venous thromboembolism is well documented²⁰⁷ but the role in arterial thrombosis is not clear as yet. Significant elevation of FVIII:C in both groups were noted in the present study, whether this elevation was associated with an acute phase response could not be clearly ruled out, and later testing may have been of benefit.

Elevated plasma fibrinogen levels, whatever their origin, may cause a hypercoagulable state that could influence the degree and duration of thrombus formation at the time of coronary injury. Previous reports suggest fibrinogen is an independent risk factor for premature acute MI²⁰⁸. The present study showed slightly raised levels of fibrinogen in both groups, which could be due to the MI or an underlying vascular disease than as a cause of MI.

D-Dimer, an expression of on-going thrombus formation and lysis, is a marker of substantial incremental value for the early diagnosis of acute coronary syndromes. It adds independent information to the traditional assessment for acute MI. Although, there are no previous studies comparing the elevated D-dimer in MINOCA compared to MI-CAD, because of the wide overlap between the groups, increased D-dimer values are of limited relevance above and beyond other risk factors. Nonetheless, it demonstrates increased fibrinolytic activity four weeks post MI in both groups.

Limitations

The results from this study should be interpreted in the context of several potential limitations. Thrombin generation assay does not measure the cellular components of coagulation, thus giving only a partial perspective of the haemostatic system. We also did not examine thrombin generation using a lower trigger concentration of tissue factor (1 pM). The relatively small sample size may also explain the negative findings, particularly regarding the rarer inherited deficiency states. A sample size calculation for congenital thrombophilia states based on Da Costa et al's findings revealed that to test the proportion difference of 7.5% and 18.2%, at 80% power and 5% significance, 171 patients in each group would be required. It will also be beneficial to compare the MINOCA and MI-CAD cohort to age and gender matched healthy cohort in whom prior MI or CAD is not documented. In addition, the higher incidence of NSTEMI in the MINOCA group is also a limitation.

Conclusion

In summary, overall thrombin generation potential, prevalence of congenital and acquired thrombophilia states, and coagulation markers in this study were not different between MINOCA and MI-CAD patients, suggesting that despite the difference in coronary artery anatomy of the disease progression, acute MI patients generate thrombin in a similar manner in response to local stimuli. Although the role of an abnormal pro-thrombotic tendency is often hypothesized to be causative in the setting of MINOCA, whether testing for such an underlying condition helps in the clinical management of MINOCA is questionable. From our findings, the testing for hereditary thrombophilia would not alter the clinical management of patients however; it could provide important mechanistic insights only in a minority of patients.

CHAPTER 5

5 THE ROLE OF NAC AND GTN IN STEMI: NACIAM TRIAL

This chapter includes an outline and the manuscript in the form as it appears in the manuscript, ‘The early use of N-acetylcysteine (NAC) with glyceryl trinitrate (GTN) in ST-segment Elevation Myocardial Infarction patients undergoing primary percutaneous coronary intervention (NACIAM Trial)’ authored by Sivabaskari Pasupathy, Rosanna Tavella, Suchi Grover, Betty Raman, Yang Du, Gnanadevan Mahadavan, Nathan EK Procter, Irene Stafford, Tamila Heresztyn, Andrew Holmes, Christopher Zeitz, Margaret Arstall, Joseph Selvanayagam, John D Horowitz, John F Beltrame, and currently under review at The Lancet. This study is to be presented as a late breaking trial at the European Society of Cardiology Congress, Rome, Italy 2016.

In keeping with this style of this thesis, the abstract has been removed, the table and figures re-numbered, the references incorporated into the thesis’s master reference list and the manuscript repaginated.

5.1 STATEMENT OF AUTHORSHIP

The early use of N-acetylcysteine (NAC) with Glyceryl Trinitrate (GTN) in ST-segment elevation in myocardial infarct patients undergoing PCI (NACIAM)

Publication Status	Under Review
Publication Details	The Lancet
Principal Author	Sivabaskari Pasupathy
Contribution	Acquisition of data; Analysis and interpretation of data; Drafting of manuscript; Critical revision of the manuscript.
Overall percentage (%)	70%

Certification: This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature and Date 14/08/2016

Co-Author Contributions

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

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
 - ii. permission is granted for the candidate to include the publication in the thesis; and
 - iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution
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Contribution	Study conception and design; Manuscript drafting; Critical revision; Final approval.
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Signature and Date	12/08/2016
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Signature and Date	11/08/2016
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5.2 STUDY OUTLINE

Early myocardial reperfusion using primary PCI remains the optimal treatment strategy for reducing myocardial infarct size while preserving left ventricular (LV) function in STEMI patients. However, it comes at a price, referred to as a ‘myocardial ischaemia reperfusion injury’⁶³, as detailed in Chapter 1. In addition, partial restoration of blood flow following reperfusion, namely no-reflow phenomenon, also significantly influences the final myocardial infarct size¹²³. Advances in PCI technology, anti-platelet agents, and anti-thrombotic agents continue to optimize the reperfusion process. Despite these improvements, mortality associated with STEMI remains significant²⁰⁹, highlighting the need for additional cardio-protective strategies during acute management.

Over the last three decades, a number of studies have investigated methods to minimize the infarct size following STEMI. As explained in Chapter 1, following coronary occlusion, experimental studies have identified several factors that act in concert to mediate the eventual infarct size including oxidative stress, intracellular Ca^{2+} overload, the rapid restoration of physiological pH at the time of reperfusion and inflammation, to name a few.

There are several mechanistic studies utilising pharmacological agents which have provided promising insights into reducing the reperfusion related myocardial injury. However, many of these studies failed to translate the effects in the clinical setting. Adenosine²¹⁰, atrial natriuretic peptide²¹¹, atorvastatin²¹², erythropoietin²¹³, exenatide²¹⁴, glucose insulin potassium (GIK)²¹⁵, cyclosporine²¹⁶ and sodium nitrite²¹⁷ are a few of the pharmacological agents that failed to show clinical significance following reperfusion.

Oxidative stress significantly contributes to ischaemia reperfusion injury. Re-introduction of blood flow during reperfusion reintroduces and leads to generation of ROS as established in Chapter 1, section 1.7. Several studies have documented the release of ROS within myocardium during reperfusion after sustained myocardial ischaemia^{218, 219}. Previous studies suggest that ROS may mediate myocardial dysfunction (or stunning) post MI,^{220, 221} leading to lipid peroxidation during ischaemia-reperfusion that may produce membrane defects, resulting in calcium overload and contractile dysfunction^{63, 222}. The present study explores a potential method to reduce the oxidative stress produced by ROS and increase the tissue reperfusion in STEMI patients undergoing primary PCI.

NAC and GTN in STEMI

NAC is a sulfhydryl donor, which has two major potential beneficial effects in acute STEMI, namely, (a) free radical scavenging properties²²³ and (b) potentiation of the hemodynamic and platelet ant aggregatory effects of GTN^{224, 225}. Accordingly, the early use of NAC may further reduce oxidative stress which may reduce ischaemia reperfusion injury, while the potentiation of GTN may increase infarct artery patency, and consequently increase tissue reperfusion. These beneficial effects may reduce the size of evolving infarcts (Figure 15).

In animal models, NAC has reduced infarct size²²⁶ and myocardial stunning²²⁷. In humans, it has been shown to reduce the progression to acute MI in patients with severe unstable angina when co-administered with GTN²²⁸. In STEMI patients managed with thrombolytic reperfusion therapy, NAC together with GTN reduced oxidative stress with a trend towards more rapid ST segment resolution and better preservation of LV function²²⁹. In addition, Marenzi et al²³⁰ demonstrated that NAC has renoprotective effects in primary PCI.

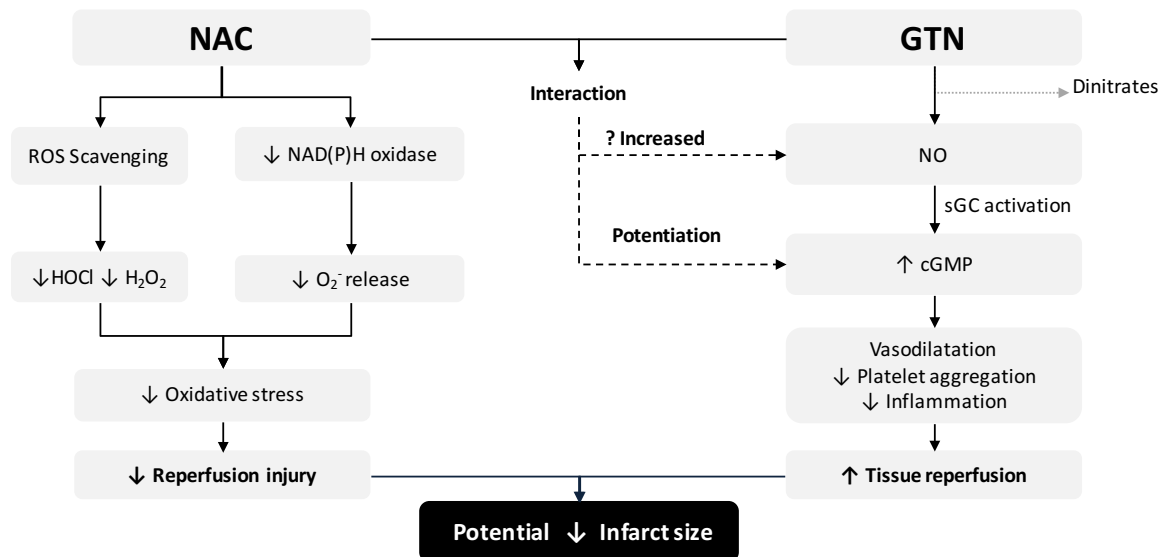


FIGURE 15: PUTATIVE MECHANISMS OF NAC AND GTN.

cGMP, Cyclic guanosine monophosphate; H₂O₂, Hydrogen peroxide; HOCl, hypochlorous acid; O₂^{·-}, Superoxide; NO, Nitric oxide; ROS, Reactive oxygen species; sGC, Soluble guanylate cyclase

CMR Imaging

The non-invasive assessment of infarct size in the post-infarct period plays a central role in patient management and prognostication²³¹. Although, ECG, cardiac biomarkers, and echocardiography are used in clinical practice to identify and characterise myocardial infarct size^{232, 233}, there are many advantages to CMR imaging compared to these modalities. Firstly, the images yielded from CMR allow for non-invasive assessments of reperfusion therapy through comprehensive evaluation of wall motion, global function, perfusion and viability. CMR is considered the clinical gold standard for viability imaging as it provide images that accurately depict the transmural and extent of infarction²³⁴. In short, CMR allows for the rigorous detection and quantification of myocardial infarct size, microvascular obstruction (MVO)²³⁵, area at risk, myocardial salvage and left ventricular function²³⁶.

Stone et al²³⁷ showed that every 5% reduction in infarct size assessed by CMR is associated with a significant reduction in subsequent all-cause mortality, independent of age, sex and cardiovascular risk factors. Consequently, it was recommended that infarct size assessed by CMR should serve as the primary endpoint in clinical trials examining potential drugs to reduce infarct size.

Cine Images

Cine images are acquired using a steady state in free precession (SSFP) sequence. It produces images of high spatial and temporal resolution and the soft tissues can be clearly defined without the need for contrast agents. As a tomographic technique, it can acquire images in any desired imaging plane and is not limited by chest wall anatomy. As any plane can be acquired, the entirety of both ventricles can be imaged (Figure 16) and so a full three-dimensional dataset of ventricular volumes (end-diastolic volume (EDV), end-systolic volume (ESV), LV-ejection fraction (LV-EF)) and myocardial mass can be obtained. The accuracy of volume measurement has been demonstrated by comparing the left and right stroke volumes in normal subjects^{238, 239}. Measurement of LV mass by CMR has been validated in both human autopsy and animal models^{240, 241}.

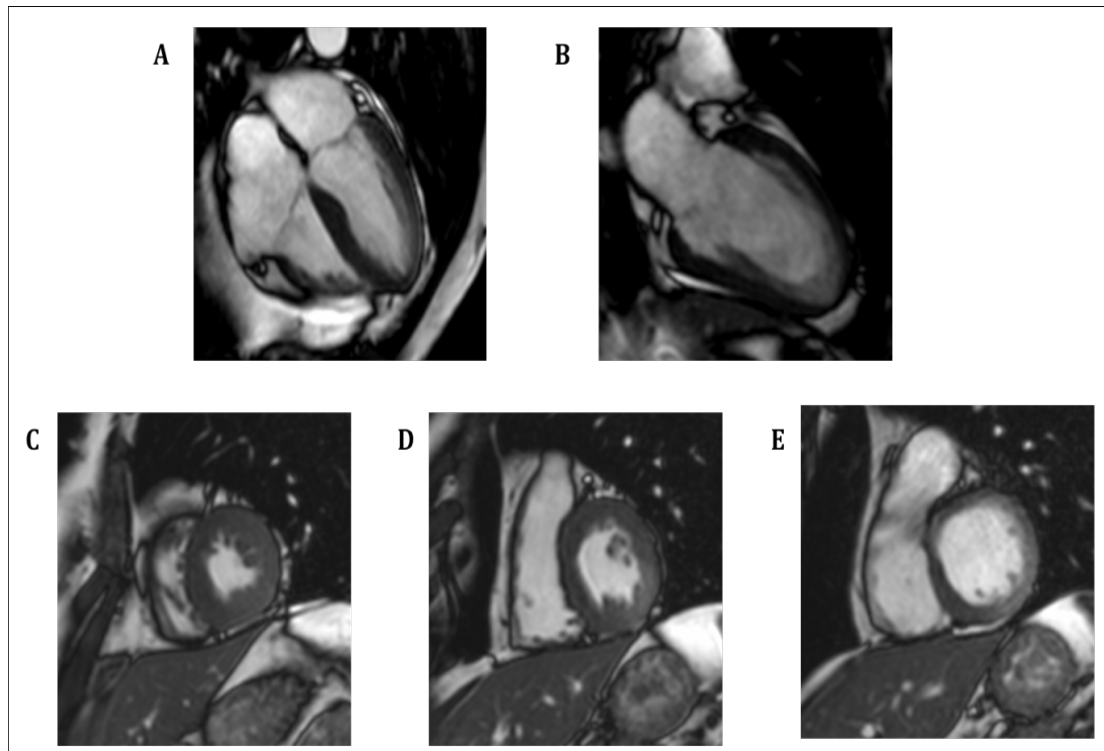


FIGURE 16: STANDARD PLANES OF CINE IMAGE ACQUISITION ON CMR ON AN ACUTE MI PATIENT.

(A) Four chamber view of heart; (B) two chamber view of LV; (C) apex of LV on short axis; (D) mid LV on short axis; (E) base of LV on short axis

Late gadolinium enhancement (LGE) Images

The unique ability of CMR in the evaluation of myocardial viability via late gadolinium enhancement (LGE) is accepted as the gold standard method for assessing fibrosis/infarct size in acute MI²⁴². LGE-CMR technique relies on the administered contrast agent's distribution in the extracellular space. Gadolinium based contrast agents are used, which is barred from intracellular space by intact cellular membranes. However, in the setting of acute MI, myocardial cell necrosis and subsequent cell membrane ruptures, enable gadolinium to penetrate into the affected myocardium²⁴³. This results in an accumulation of gadolinium in the affected myocardium which appears 'hyper-enhanced' in CMR imaging, and is referred to as infarct size²⁴² (Figure 17).

Myocardial infarct size is measured by delineating endocardial and epicardial walls of the left ventricle. Upon tracing, the infarct size is computed as a percentage of total myocardial mass by specialised software. The present study utilized CMR42 software (CMR42 v3.2 (Circle Cardiovascular Imaging, Canada). The infarct area is defined as the region of hyper-enhanced myocardium with signal intensity above five standard deviations of healthy myocardium²⁴⁴ (Figure 18 A & B). In the current study, inter and intra-observer variability, assessed by the Bland-Altman method, demonstrated a mean bias of -1.2% (95% limits of agreement -6.7, 9.1) and -1.5% (95% limits of agreement -8.7, 5.7), respectively without significant variability according to mean value (Figure 18).

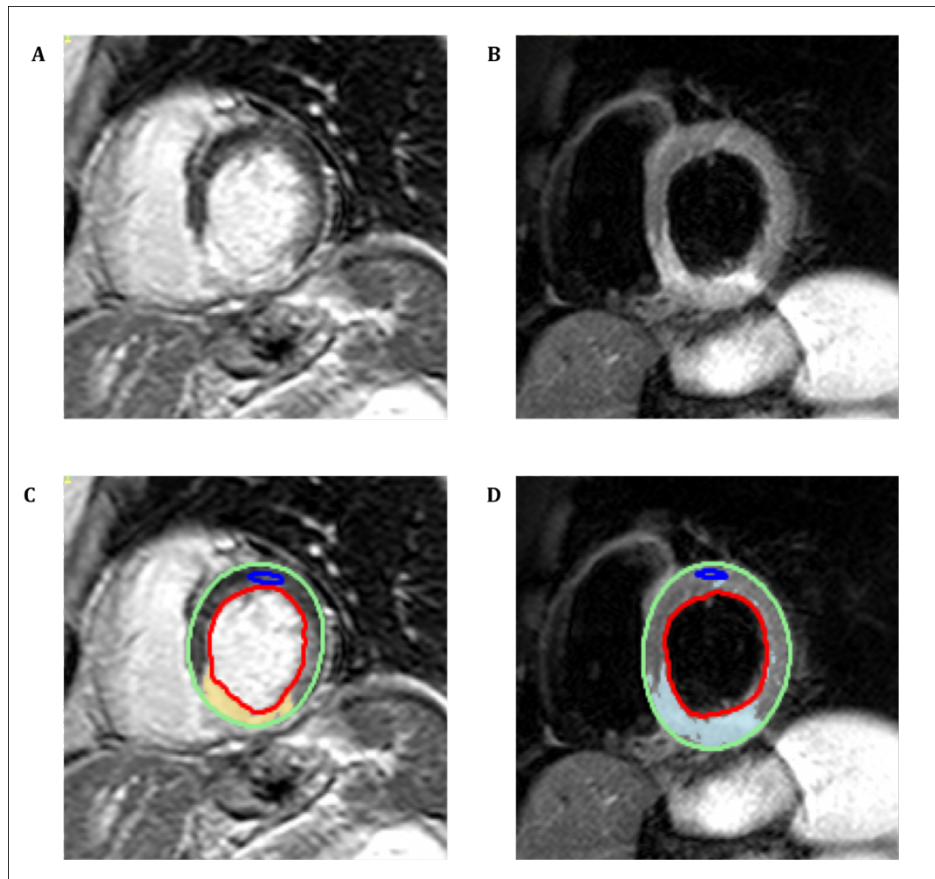
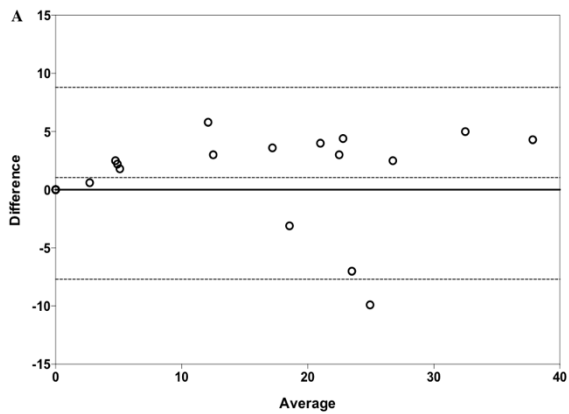
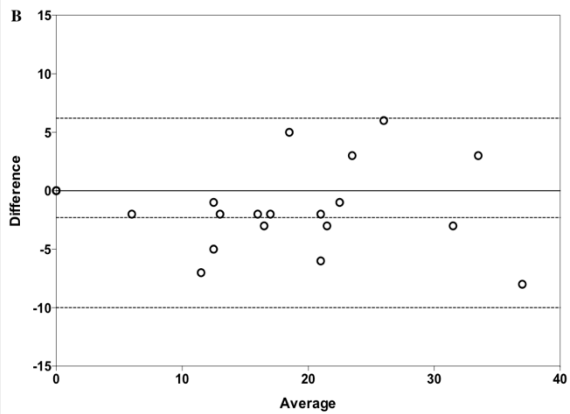


FIGURE 17: EXAMPLE SHORT-AXIS CMR IMAGES OF A STUDY PATIENT PRESENTING WITH INFERIOR MI.

Top Row: Original (A) LGE and (B) T2W image. Bottom row: following the endocardial (red) and epicardial (green) border tracing, infarct area is highlighted in yellow (C) and area at risk in light blue (D).



Inter-observer variability	
Bias $\pm$ SD	1.19 $\pm$ 4.04
95% limits of agreement	-6.7 to 9.1



Intra-observer variability	
Bias $\pm$ SD	-1.5 $\pm$ 3.68
95% limits of agreement	-8.7 to 5.7

FIGURE18: BLAND-ALTMAN PLOTS OF INTER (A) AND INTRA (B) OBSERVER VARIABILITY FOR INFARCT SIZE MEASUREMENT.

T2-Weighted imaging

The T2-Weighted (T2W) sequence primarily allows for the assessment of tissue oedema. Since the acute MI has oedema, the combination of LGE imaging with T2W imaging helps to differentiate acute from chronic MI²⁴⁵⁻²⁴⁷. The oedematous tissue in acute MI is thought to reflect the area-at-risk (AAR), allowing for quantitative assessment of salvaged myocardium after reperfusion therapy²⁴⁸⁻²⁵⁰. Myocardial salvage is measured as the difference between T2W-AAR and LGE infarct size. In the current study, inter and intra-observer variability, assessed by the Bland-Altman method, demonstrated a mean bias of -0.1% (95% limits of agreement -11.5, 11.3) and -0.5% (95% limits of agreement -9.8, 8.9), respectively without significant variability according to mean value (Figure 19).

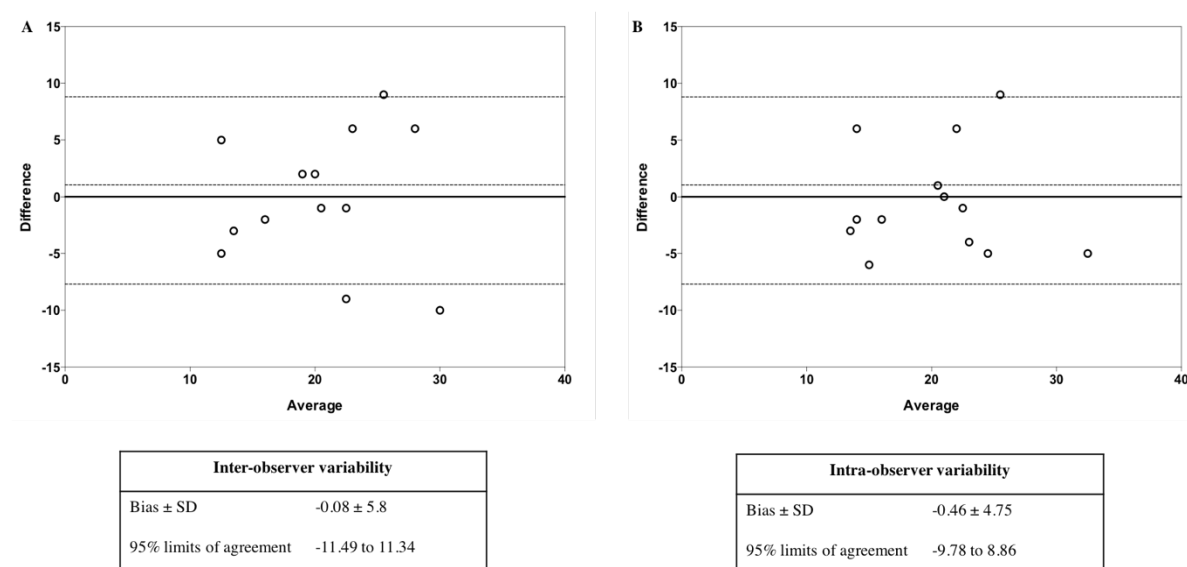


FIGURE 19: BLAND-ALTMAN PLOTS OF INTER (A) AND INTRA (B) OBSERVER VARIABILITY FOR AAR MEASUREMENT.

5.3 MANUSCRIPT: THE EARLY USE OF NAC WITH GTN IN STEMI PATIENTS UNDERGOING PCI

Introduction

Myocardial infarct size is a major determinant of cardiac outcome in patients presenting with acute ST-segment elevation acute myocardial infarction (STEMI)^{237, 251}. Infarct size is conventionally dependent upon the myocardium at risk, duration of coronary occlusion and collateral blood flow²⁵². Early reperfusion therapy (thrombolysis and angioplasty/stenting) rapidly restores coronary patency but not necessarily myocardial perfusion, primarily due to “ischaemia-reperfusion injury”, engendered by increased release of reactive oxygen species immediately following restoration of reperfusion²²². Consequently, potential adjunctive treatments to reperfusion therapy have targeted ischaemic-reperfusion injury utilising strategies such regional vasodilation (Eg: adenosine), reperfusion injury salvage kinase pathway activation, or protection of mitochondrial integrity and thus cellular energetics²⁵³. Despite promising results in vitro and in vivo animal experiments, none of these adjunctive treatments have consistently demonstrated clinical efficacy.

Use of organic nitrates, such as intravenous (IV) glyceryl trinitrate (GTN), in acute STEMI improves myocardial function²⁵⁴ but has not convincingly been shown to improve cardiac outcomes²⁵⁵. Adding complexity to this therapy is the uncertainty of the ‘correct’ infusion rate and the rapid development of nitrate tolerance with high infusion rates²⁵⁶. However a consensus has been established that low GTN infusion rates (2.5 to 10µg/min) are preferable²⁵⁷.

GTN is a pro-drug that releases nitric oxide via an enzymatic process which requires sulfhydryl cofactors^{258, 259}. A number of studies have shown that N-acetylcysteine (NAC), a sulfhydryl-containing antioxidant, potentiates the vasodilator and anti-aggregatory effects of GTN^{224, 225}. Clinically, this interaction has shown promise in the management of unstable angina²²⁸, acute pulmonary oedema²⁶⁰ and in STEMI patients undergoing thrombolysis^{229, 261}. However, the efficacy of NAC therapy has not been systematically tested in STEMI patients in the PCI era.

We now report the results of the NACIAM (N-AcetylCysteine In Acute Myocardial infarction) trial, which was designed to evaluate the efficacy of addition of IV high dose NAC to low dose GTN for reduction of infarct size in STEMI patients.

Methods

Study design

NACIAM, utilised a randomised, double-blind, placebo-controlled multicentre trial design and was conducted in three South Australian tertiary hospitals with a 24-hour primary PCI service (Flinders Medical Centre, Lyell McEwin Health Service & The Queen Elizabeth Hospital) between March 2010 and March 2015 including a minimum of 2 years' follow-up for all patients. The study was registered (ANZCTRN12610000280000) and the institutional Human Research Ethics Committee of each participating hospital approved the study protocol, with all patients providing written informed consent.

Study Recruitment

Patients presenting with STEMI were screened for the following inclusion criteria: age ≥ 18 years, chest pain duration between 30 minutes and 12 hours, electrocardiographic features consistent with acute STEMI (ST elevation, ≥ 1 mm in ≥ 2 limb leads or ≥ 2 mm in ≥ 2 contiguous precordial leads), and a clinical decision by the attending cardiologist to undertake primary PCI. Exclusion criteria were prior history of MI, left bundle branch block, IV thrombolytic administration prior to angiography, cardiogenic shock (systolic blood pressure < 90 mmHg, unresponsive to fluid load), intra-aortic balloon pump insertion prior to initial angiography, permanent pacemaker or implantable defibrillator (cardiac magnetic resonance (CMR) imaging contra-indicated) and a clinical decision to administer open-label NAC (e.g. for renoprotection^{230, 262}). All patients (both placebo- and NAC-treated patients) received infusions of GTN through non-adsorbent tubing, with infusion rate held at $2.5 \mu\text{g}/\text{min}$ for 48 hours.

Randomisation protocol

Eligible patients were assigned to receive NAC or placebo in a 1:1 ratio utilising a computer-generated algorithm with randomization blocks of 10, balanced for each participating institution. Treatment assignment was blinded to both patients and treating staff. Patients received either placebo or IV NAC infusion at $20 \text{ mg}/\text{min}$ in the first hour followed by $10 \text{ mg}/\text{min}$ for the remaining 47 hours, representing a total of 15g over 24 hours^{229, 261}. Both NAC and placebo were delivered in a 5% dextrose solution.

Clinical management

All patients underwent emergency coronary angiography, with angioplasty/stenting, anti-aggregatory therapy (including aspirin, a P2Y₁₂ receptor antagonist ± IV glycoprotein IIb/IIIa inhibitors) at the discretion of the interventional cardiologist. Intravenous NAC/Placebo and GTN were continued as per protocol for 48 hours. The GTN therapy was titrated down only in the event of hypotension (systolic BP < 90mmHg) or titrated up in the event of severe angina. After 48 hours of IV therapy, GTN infusion was ceased and oral nitrates were prescribed at the discretion of the treating cardiologist. Following catheterization, patients were transferred to the coronary care unit for the next 48 hours or more.

Study endpoints

The primary endpoint was myocardial infarct size (% infarcted myocardium relative to total left ventricle (LV) mass) as measured by CMR imaging within 7 days (early) from the acute presentation. The secondary efficacy endpoints were: (i) myocardial salvage, determined by CMR imaging (ii) area under the creatine kinase (CK) release-time curve (for all randomized patients), (iii) resolution of chest pain, and (iv) Pre-PCI infarct-related artery patency. Safety endpoints were: (i) incidence of adverse events: symptomatic hypotension, bleeding and contrast induced nephropathy, and (ii) long term clinical outcomes including 2-year mortality and readmission.

Data acquisition

CMR

CMR imaging was performed 5±2 days (early CMR imaging) and 94±17 days (late CMR imaging) post STEMI admission using either a 1.5T system (Philips Intera CV, Best, The Netherlands) or a 3T system (Siemens Medical Solutions, Erlangen, Germany) with vector

electrocardiographic imaging. The imaging sequences were standardised between centres as much as possible, with the following CMR parameters assessed: (a) infarct size determined from early CMR imaging using delayed hyper-enhancement, (b) area at risk assessed from early CMR imaging with T2-weighted images, (c) myocardial salvage calculated from infarct size and area at risk, and (d) left ventricular volumes and ejection fraction calculated at early and late CMR imaging.

Functional assessment of the LV was performed pre-contrast using cine CMR with steady-state free precession imaging. Imaging was performed in multiple short breath-holds. The in-plane resolution was approximately 2mm (26 μ l/voxel), and the temporal resolution was 40 to 50ms within the cardiac cycle. The heart was imaged in multiple parallel short-axis planes 8mm thick separated by 3mm gaps, as well as in the two-chamber, three-chamber, and four-chamber long-axis views.

CMR analyses were performed offline, using CMR42 software (CMR42 v3.2 (Circle Cardiovascular Imaging, Canada)). The CMR images were examined and interpreted by two independent experts. In case of disagreement, a third CMR imaging specialist was consulted for agreement. End-diastolic volume (EDV), end-systolic volume (ESV), LV ejection fraction (LVEF) and LV mass were driven from the software after the contours were drawn in the endocardial and epicardial border of the left ventricle at end-diastole and end-systole. All assessments included papillary muscles and trabeculations when contiguous with the LV.

Infarct assessment using late gadolinium enhancement (LGE) CMR imaging was performed 10min after injection of 0.1mmol/kg gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA, Magnevist, Schering, Berlin, Germany). Infarct area was defined as the region of

hyperenhanced myocardium with signal intensity of 5 SD above mean signal intensity of the remote normal myocardium²⁶³. Following the delineation of endocardial and epicardial borders, infarct size was expressed as a percentage of total myocardial mass.

For area at risk, the same algorithm was used with the threshold of 2SD above healthy remote myocardial signal²³⁶. Microvascular obstruction was defined as presence of an area of hypo-enhancement within the infarcted myocardium and delineated manually and agreed by consensus. Myocardial salvage (%) was defined as: $\frac{\text{Area at risk} - \text{Infarct size}}{\text{Area at risk}} \times 100$. Regional wall motion score index was calculated using a 16-segment model. The score for each segment was graded according to the following system: normal, 1; hypokinesia, 2; akinesia, 3; dyskinesia, 4. The RWMSI was calculated by dividing the total wall motion score by 16²⁶⁴.

In addition, the following clinical parameters were assessed in the NACIAM Trial:

- Cardiovascular Risk Factors and co-morbidities – as documented in the case record.
- Chest Pain Resolution – patients were asked to report on chest pain intensity at baseline, 4, 12 and 24 hours, with ‘0’ being no pain and ‘10’ being the most severe pain they have ever experienced.
- ECG-determined Area at Risk – as assessed on the initial ECG utilizing the Wilkins score method²⁶⁵
- Pre-PCI Infarct-related Artery Patency - assessed as either occluded or patent.
- Symptomatic Hypotension – defined as a systolic BP < 90mmHg with associated symptoms, potentially necessitating a reduction GTN infusion rate
- Bleeding- Bleeding associated with clinically significant fall in in hemoglobin values.
- Contrast-induced nephropathy - defined as an increase in serum creatinine concentration of $\geq 25\%$ from baseline values within 72 hours after PCI²³⁰.

- Two-year Clinical Outcomes – including all-cause mortality and hospital readmission for an acute coronary syndrome, heart failure or stroke, as determined from administrative case-mix data sets.
- Creatine Kinase (CK)-assessed infarct size - Plasma samples for the CK assay were collected at baseline and repeated at 4, 8, 12, 16 and 24 hours.

Statistical methods

Sample sizes calculations for the primary end-point were based upon previous studies reporting a mean early infarct size ranging from 5 to 13% of LV mass²⁶⁶⁻²⁶⁸, with pilot data suggesting an infarct size of 13±3%. Thus to detect an absolute 2% difference in early CMR-determined infarct size between groups for a power of 80% at the alpha = 0.05 level, 36 patients per group would be required. Patient recruitment was continued until sufficient CMR imaging studies were performed, since it was anticipated that a significant number of patients would fail to proceed to CMR imaging or may have inadequate scans for infarct quantification.

Statistical analyses were performed with STATA statistical software (STATA/IC for Mac), version 11.2 and the statistical package for the social sciences (SPSS, IBM) version 22.0. Figures were generated using Prism 6 for Mac OS X. Frequency distributions were analysed using Fisher's exact test. Comparisons between groups were analysed via independent t-test or Wilcoxon rank-sum test as appropriate. Backwards stepwise multiple linear regression analysis was also performed to identify correlates of variation in infarct size, CK release and myocardial salvage. The model included factors known to modulate infarct size (age, gender, diabetes, area at risk, total ischaemia time, and systolic blood pressure on admission), variables that fulfilled the $p < 0.25$ univariate threshold criteria (Smoking status) and the

randomized study treatment. Area under the CK-time curve was calculated using the trapezoid method. Variation in clinical or biochemical parameters with time was analysed by analysis of variance (ANOVA) and treatment-specific variation via analysis of covariance (ANCOVA). Normally distributed data are expressed as mean $\pm$ SD, and skewed data as median with interquartile range.

Results.

Study population and clinical characteristics

Between March 2010 and March 2013, 141 presumptive STEMI patients were screened in the 3 participating hospitals (Figure 20). Of these, 132 patients were randomised with the remainder declining participation. Following randomisation, a further 20 were excluded from further analysis due to alternate ‘non-infarct’ diagnoses (tako-tsubo cardiomyopathy in 7 and pericarditis in 5 patients) and 8 patients withdrawing consent. The remaining 112 randomised patients (mean age 64 ± 14 years, 75% male) were included in the study and had CK data available. Early CMR imaging was performed in 75 patients (mean age 63 ± 14 years, 80% male) with the primary endpoint of infarct size assessed in 37 and 38 NAC- and placebo-treated patients respectively.

Baseline clinical characteristics of the study population are presented in Table 11 for (a) the 112 randomised patients and (b) the 75 patients who had early CMR imaging data available. Treatment groups were well-matched for both comparisons (Table 11) and there were no differences in clinical characteristics between patients undergoing and not undergoing CMR. Only a small proportion of patients had a previous history of angina. Median total ischaemic time (i.e., coronary occlusion duration) was 2.4 hours in both groups. Only 16% of patients received glycoprotein IIb/IIIa inhibitors. IV GTN was up-titrated in 1 patient and down-titrated in 3 patients, during the first 48 hours.

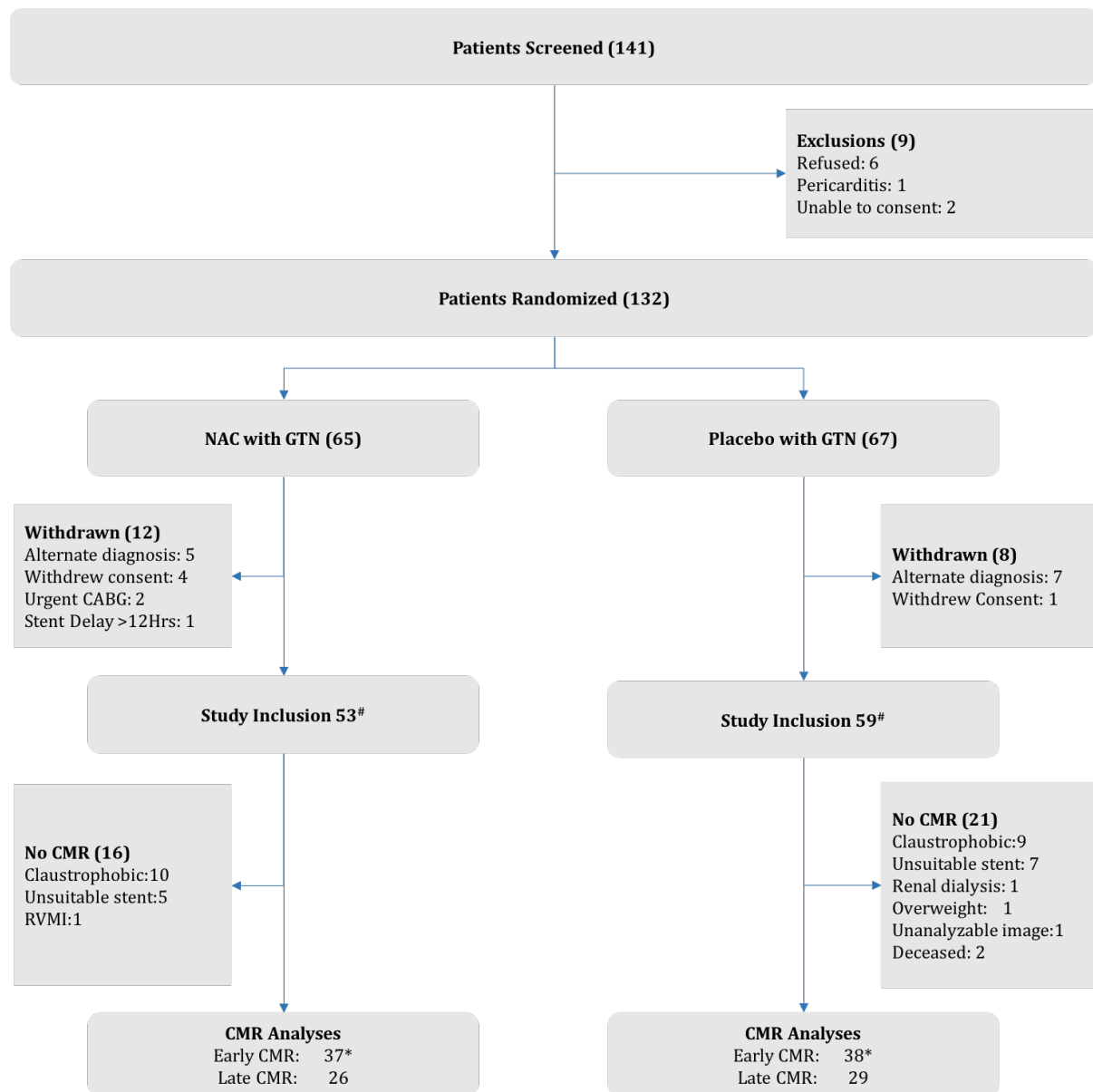


FIGURE 20: CONSORT DIAGRAM: SPECIFICS OF NACIAM TRIAL.

CABG, Coronary artery bypass grafting; CMR, Cardiac magnetic resonance imaging; GTN, Glyceryl trinitrate; NAC, N-Acetylcysteine; RVMI, Right ventricular myocardial infarction (Infarct limited to right ventricle on Echocardiography); Numbers Of Patients Treated/Analysed Are Indicated.

TABLE 11-BASELINE CHARACTERISTICS.

		Randomised			Early CMR analysis performed		
		NAC(53)	Placebo(59)	p	NAC (37)	Placebo	p
Age (years)	mean	63 ± 14	64 ± 15	0.59	62 ± 12	65 ± 16	0.51
Female	% (N)	20 (11)	28 (17)	0.38	11 (4)	29 (11)	0.08
BMI (Kg/m ²)	mean ±	27.6 ± 4	27.7 ± 4	0.84	26.8 ± 4	27.2 ± 4	0.76
Hypertension	% (N)	31 (16)	47 (28)	0.11	27 (10)	42 (16)	0.22
Hypercholesteromia	% (N)	34 (17)	31 (18)	0.83	25 (9)	32 (12)	0.61
Family History	% (N)	35 (18)	38 (21)	0.84	41 (15)	47 (16)	0.63
Diabetes	% (N)	14 (7)	19 (11)	0.61	11 (4)	16 (6)	0.73
Current smoker	% (N)	43 (22)	40 (23)	0.84	41 (15)	41(15)	1.00
History of angina	% (N)	8 (4)	4 (2)	0.41	3 (1)	0	0.49
Prior PCI	% (N)	4 (2)	2 (1)	0.59	3 (1)	3 (1)	1.00
PAD	% (N)	2 (1)	0	0.46	3 (1)	0	0.49
CVD	% (N)	2 (1)	5 (3)	0.62	0	8 (3)	0.24
Door to balloon	median	51(42,67)	59(48,71)	0.12	53 (37,68)	56 (48,68)	0.51
Total Ischaemic	median	144	141	0.97	144(103,185)	144	0.76
Pre PCI							
Extent of VD							
1 vessel disease	% (N)	50 (26)	56 (33)	0.57	52 (17)	52 (17)	1.00
2 vessel disease	% (N)	30 (16)	24 (14)	0.53	27 (9)	27 (9)	1.00
3 vessel disease	% (N)	20 (11)	20 (12)	1.00	18 (6)	21 (7)	1.00
Artery Patency							
Occluded	%(N)	61 (30)	73 (39)	0.28	62 (22)	75 (25)	0.43
Patent	%(N)	39 (19)	27 (14)		37 (12)	25 (8)	
PCI							
Balloon only	% (N)	7 (4)	6 (4)	1.00	19 (7)	19 (7)	1.00
Stent insertion	% (N)	91 (48)	94 (55)	0.43	81 (30)	82 (31)	1.00
No intervention	% (N)	2 (1)*	0	-	3 (1)*	0	-
Post procedure	Mean ±	2.9 ± 0.36	2.9 ± 0.19	0.54	2.9 ± 0.04	3.0 ± 0.03	0.56
Treatment at PCI							
Gp IIb/IIIa	%(N)	29 (12)	42 (20)	0.27	32 (12)	42 (16)	0.47
Discharge medication							
Aspirin	% (N)	93 (49)	96 (57)	0.65	95 (35)	97 (37)	0.62
P2Y ₁₂ Inhibitors	%(N)	100 (53)	98 (58)	1.00	100 (37)	97 (37)	1.00
Statin	% (N)	100 (53)	100 (59)	-	100 (37)	100 (38)	-
Beta-Blocker	% (N)	27 (14)	45 (26)	0.08	27 (10)	50 (19)	0.06
ACE inhibitor	% (N)	100 (53)	92 (54)	0.12	100 (37)	95 (36)	0.49
ARB	% (N)	2 (1)	2 (1)	1.00	3 (1)	0	0.49
CCB	% (N)	46 (24)	37 (21)	0.51	43 (16)	29 (11)	0.23
Organic nitrates	% (N)	39 (21)	40 (23)	1.00	35 (13)	39 (15)	0.81

Data expressed as number (%) unless otherwise stated. ACE, Angiotensin converting enzyme; ARB, Angiotensin II receptor blockers; AUC, Area under the curve; BMI, Body Mass Index; CCB, Calcium channel blocker; CK, creatine kinase; CVD, Cerebrovascular Disease; iqr, interquartile range; Gp, glycoprotein; min, Minutes; NAC, N-acetylcysteine; PAD, Peripheral Arterial Disease; PCI, Percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction score; VD, vessel disease.

* The patient was referred for CABG after more than 48 hours.

Early CMR Imaging - Primary endpoint: Infarct size

Myocardial infarct size was significantly lower in the NAC-treated patients compared to the placebo-treated (11% (4.1, 16.3) vs. 16.5% (10.7, 24.2), $p=0.02$), despite similar baseline area at risk assessed by both CMR imaging (25% (17, 37) and 23% (18, 31), respectively; Figure 21). The 95% confidence intervals for the (median 5.5%) reduction in infarct size were 0.7% and 10.2%. There was no significant difference between treatment groups in coronary artery patency at the pre-PCI angiogram. There was a close and direct relationship between area at risk and infarct size. However, NAC was associated with smaller infarct size per unit area at risk (Figure 22A). In the multiple regression model, treatment with NAC ($p=0.001$), smaller area at risk ($p<0.01$) and current smoking ($p=0.04$) were significantly associated with a smaller infarct size.

Secondary efficacy endpoints

Myocardial salvage was approximately doubled in patients randomised to the NAC group (60% (37, 79)) compared to the placebo group (27% (14, 42)) ($p<0.001$) (Table 12). The beneficial effect of NAC on salvage was reduced with total ischaemic time (Figure 22B). In a multiple regression model, treatment with NAC ($t=4.9$, $p<0.001$) and current smoking ($t=2.27$, $p=0.03$) were directly related to myocardial salvage, while area at risk was an inverse correlate ($t=-2.1$, $p=0.04$).

Median CK areas under the curve were 22,000 IU.hours and 38,000 IU.hours in the NAC and placebo groups, respectively ($p=0.08$) (Figure 22C). In a multiple regression model, this effect of NAC treatment remained at borderline statistical significance ($p=0.052$). In addition, NAC treatment was associated with more rapid resolution of chest pain ($p=0.04$) (Figure 22D).

CMR-assessed left ventricular parameters including LVEF and end systolic volumes did not vary significantly between groups (Table 12). Late CMR imaging was performed on 55 patients and continued to show a significantly reduced infarct size in the NAC group without any significant differences in LVEF (Table 12).

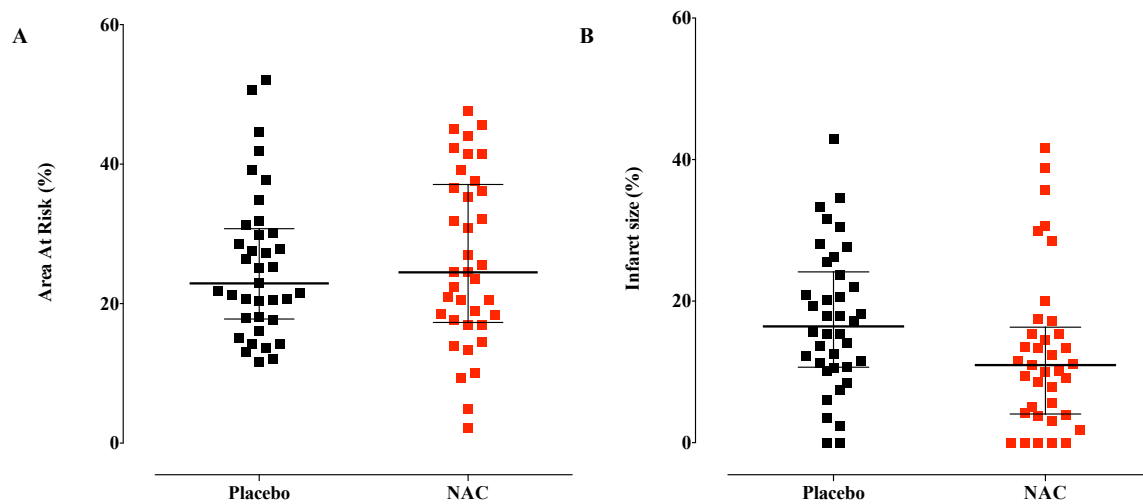


FIGURE 21: INDIVIDUAL PATIENT DATA.
(A) Area at risk and (B) Infarct size comparison between placebo and NAC.

TABLE 12-EARLY AND LATE CMR RESULTS.

		NAC (37)	Placebo (38)	p
Early CMR Analyses				
Myocardial mass	(g)mean $\pm$ SD	146.2 $\pm$ 29.1	142.5 $\pm$ 35.5	0.62
Anterior Infarct	% (N)	35 (13)	39 (15)	0.81
Area At Risk	% median (iqr)	24.5 (17.3, 37.1)	22.9 (17.8, 30.8)	0.76
Infarct size	% median (iqr)	11 (4.1, 16.3)	16.5 (10.7, 24.2)	0.02
Myocardial Salvage	% median (iqr)	60 (37.2, 79.4)	27.3 (14.4, 41.9)	0.001
MVO	% (N)	46 (17)	45 (17)	1.00
MVO	mean $\pm$ SD	0.95 $\pm$ 1.8	1.1 $\pm$ 1.8	0.66
Transmural infarct	% (N)	54 (20)	79 (30)	0.02
EDV (ml)	mean $\pm$ SD	148.5 $\pm$ 26.9	147.2 $\pm$ 40.1	0.87
ESV (ml)	mean $\pm$ SD	65.4 $\pm$ 24.6	68.5 $\pm$ 29.4	0.62
SV (ml)	mean $\pm$ SD	83.2 $\pm$ 17.3	78.6 $\pm$ 20.8	0.31
EF	% mean $\pm$ SD	56.9 $\pm$ 11.6	54.8 $\pm$ 11.7	0.42
RWMSI	Mean $\pm$ SD	1.34 $\pm$ 0.2	1.37 $\pm$ 0.24	0.10
Late CMR Analyses				
Infarct size	% median (iqr)	5 (0.7, 12.4)	10.2 (6.8, 14.8)	0.02
EDV	mean $\pm$ SD	153.3 $\pm$ 46.6	155.5 $\pm$ 38.7	0.85
ESV	mean $\pm$ SD	64.3 $\pm$ 34.5	67.9 $\pm$ 25.7	0.66
SV	mean $\pm$ SD	88.9 $\pm$ 26.5	87.7 $\pm$ 25.7	0.86
EF	% mean $\pm$ SD	59.6 $\pm$ 11.1	56.7 $\pm$ 10.5	0.33
RWMSI	mean $\pm$ SD	1.31 $\pm$ 0.32	1.32 $\pm$ 0.25	0.32

Data expressed as mean $\pm$ SD or median with interquartile ranges. EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; ESV, End Systolic Volume; MVO, Microvascular obstruction; NAC, N-acetylcysteine; RWMSI, Regional wall motion score index; SV, Stroke Volume

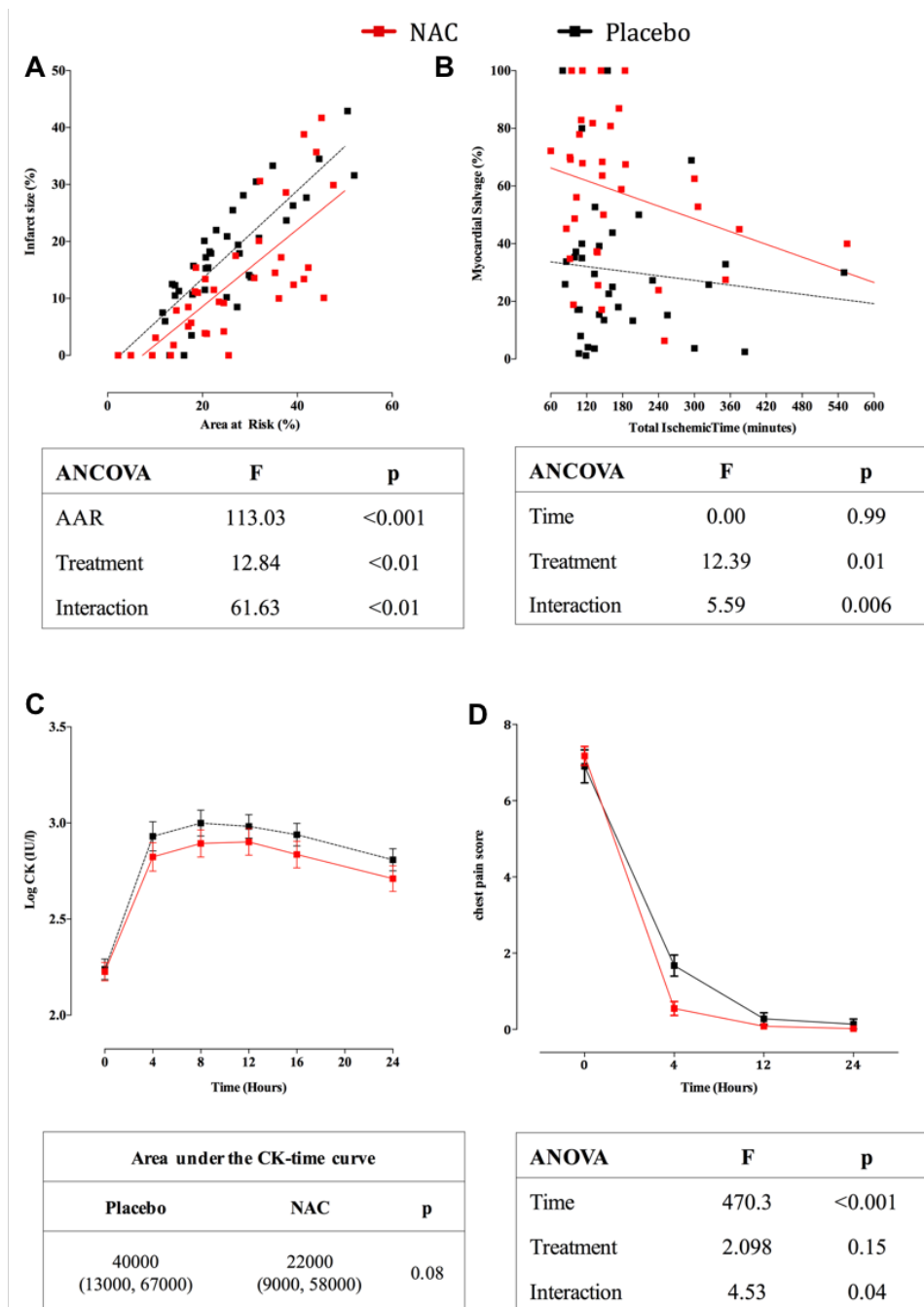


FIGURE 22: EFFECTS OF TREATMENT ON INDICES OF INFARCT SIZE, MYOCARDIAL SALVAGE AND RESOLUTION OF ISCHAEMIA.

(A) Relationship between treatment group and infarct size per unit area at risk; (b) differential myocardial salvage related to duration of ischaemia; (c) progressive CK release; (d) resolution of chest pain. AAR, area at risk; ANOVA, analysis of variance; ANCOVA, analysis of covariance; CK, creatine kinase; GTN, glyceryl trinitrate; NAC, n-acetylcysteine.

Safety endpoints

Early IV administration of NAC did not change the incidence of the pre-specified safety endpoints. There was no differential effect on systolic blood pressure with time ($p=0.88$). Incidence of adverse events including hypotension (26% vs. 27%), bleeding (6% vs. 7%) and contrast-induced nephropathy (6% vs. 9%) were similar between NAC and placebo groups. There were 2 in-hospital deaths in the placebo group and none in the NAC group. Moreover, in the 2 years following admission, the NAC-treated patients experienced fewer readmissions (2 vs 9 patients) and deaths (1 vs 7 patients) than the placebo-treated group. Indications for hospital readmissions for the NAC and placebo treated groups included recurrent acute myocardial infarction (2 vs 1), unstable angina (0 vs 7) and heart failure (2 vs 1, respectively).

Discussion

This randomised, double-blind, placebo-controlled multicentre study is the first to demonstrate a reduction in myocardial infarct size, using high dose IV NAC in patients presenting with an acute STEMI and managed with low-dose IV GTN and primary PCI. No analogous data are available for GTN alone, and indeed most large trials of organic nitrates are notionally neutral²⁵⁵. Extrapolating from previous data examining the relationship between CMR-estimated infarct size and cardiovascular outcomes in primary PCI²³⁷, the median extent of reduction in infarct size (5.5%, 95% CI 0.7%, 10.2%) could translate into a substantial (perhaps 20%) reduction in all-cause mortality at 12 months. Consistent with this expectation, this study also demonstrated that IV NAC administration was associated with more rapid chest pain resolution, improved myocardial salvage, a favourable in-hospital safety profile, sustained infarct size reduction at 3 months post-STEMI, and promising clinical outcomes at 2 years.

There have been a number of previously reported investigations involving the use of IV NAC in combination with GTN in acute coronary syndromes. In patients with unstable angina, use of NAC was associated with a reduced frequency of acute myocardial infarction²²⁸. In STEMI patients undergoing thrombolysis, there was a trend towards smaller myocardial infarcts, as measured by CK release, and a diminution of oxidative stress^{229, 261}. In patients with acute pulmonary oedema, NAC treatment improved the rate of recovery in arterial oxygenation²⁶⁰. Furthermore, Marenzi et al²³⁰ utilised a mixed IV/oral regimen of NAC in STEMI patients, noting a reduction in acute mortality rates, but did not measure infarct size.

The relative reduction in infarct size associated with NAC was approximately 33% on early CMR and 50% at late CMR imaging. This extent of therapeutic effect is surprisingly large, particularly as no similar benefits over those of PCI have been consistently recorded in humans with ancillary utilisation of any other pharmacological agent²⁶⁹. While the effects of NAC on salvage (approximately 50% improvement) diminished with longer coronary occlusion duration, they persisted for at least 6 hours. Although there was no significant reduction in area under the CK-time curve, the point estimates were consistent with a 50% reduction, with marked inter-individual variability. NAC treatment was also associated with more rapid resolution of chest pain, suggesting more effective tissue reperfusion. The finding that current smokers tended to have smaller infarcts is consistent with results of previous investigations²⁷⁰.

NAC may have exerted its beneficial effects via potentiation of GTN at the level of vasomotor tone²²⁴, coronary blood flow^{271, 272} and/or platelet aggregability²⁷³⁻²⁷⁵. It is unlikely that GTN tolerance induction was a major problem with the regimen utilized²⁵⁷. However, this trial was not designed to address these mechanisms and potentially the benefits of NAC

may be independent of GTN. It is, however, unlikely that pre-PCI restoration of coronary patency via potentiation of GTN anti-aggregatory effect played a major role, given the lack of significant difference in vessel patency status²⁷³⁻²⁷⁵.

NAC, in combination with GTN, may theoretically increase the risk of hypotension and bleeding by potentiating the vasodilator and anti-aggregatory effects of GTN^{224, 225}. However, this did not occur, perhaps because great care was taken not to increase GTN infusion rates or to give standard doses of sublingual GTN²²⁸. Overall, therefore, NAC seems haemodynamically safe using this regimen.

Alternatively, a major component of the beneficial effects of NAC may have been GTN-independent, reflecting, for example, scavenging of hypochlorous acid released predominantly via activation of myeloperoxidase. In support of this concept, myocardial salvage in NAC-treated patients was directly and significantly related to myeloperoxidase levels in plasma (Appendix 2, Supplementary Figure 1B).

Irrespective of the relative roles of NAC alone versus NAC/GTN potentiation, it is quite likely that a component of benefit may have included the activation of conditioning mechanisms, including both pre-and post-conditioning, as has been previously reported for NO donors.²⁷⁶ However, this study was not designed to evaluate this putative mechanism of benefit.

Limitations

The main limitation of this study is the potential for type II error by virtue of its relatively small size. Indeed, type II error probably accounts for the failure to demonstrate differences in CK release. While the possibility that the finding of a reduction in infarct size is a “false positive” (Type I error) is relatively remote (risk 2%), especially in view of the late CMR results, the magnitude of effect has relatively wide 95% confidence intervals. Moreover, considering the nexus between myocardial infarct size and subsequent mortality²³⁷, the findings of this study should be considered as pilot data for a large randomised controlled study evaluating the impact of IV NAC administration on cardiac events, in acute STEMI.

Although the safety profile with IV NAC observed in this study is reassuring, it should be noted that glycoprotein IIb/IIIa inhibitor use was low in the cohort and therefore it is uncertain whether these agents can be used safely in patients receiving NAC.

Finally, it must be emphasized that the results of the investigation may not apply if either the delivery of GTN were less consistent or if NAC were delivered orally (given its very low oral bioavailability^{277, 278}), or at lower IV doses.

Conclusions

This study demonstrates a reduction in myocardial infarct size by a third when high dose IV NAC is administered to acute STEMI patients undergoing primary PCI on low dose IV GTN. This may translate to a reduction in all-cause mortality at 12 months, which should be substantiated in an adequately-powered randomised controlled trial assessing major adverse cardiac endpoints.

CONCLUSIONS

The past 50 years have seen significant advancements in the understanding and management of patients with acute MI. Registries have evolved as an important tool to identify incidence, risk factors, and adverse events. Large scale multicentre clinical trials have examined therapeutic innovations to optimise outcomes. Despite these advances, acute MI remains a global burden and a major cause of mortality and morbidity. This thesis has utilised systematic reviews, clinical registries, mechanistic studies and a therapeutic clinical trial to further advance the clinical insights into acute MI. The studies detailed in each chapter are summarised below.

In Chapter 2, the systematic review and meta-analysis provides an important reference point, by establishing MINOCA patients with a distinct identity, and calling for further research to develop an improved understanding of the condition. MINOCA is recognised as a common presentation, with no delineating cardiovascular risk factors, and a guarded prognosis at 12-months. As there are no guidelines regarding the appropriate management of these patients, it was proposed that MINOCA should be considered a ‘working diagnosis’, prompting further routine evaluation for patients to identify the underlying pathophysiology and thus directing subsequent treatment. The systematic review implicates multiple potential causes for MINOCA and identifies coronary spasm testing and CMR imaging technique as diagnostic tools to guide appropriate management in MINOCA patients.

In Chapter 3, a well-established coronary angiographic registry in South Australia was utilized to provide the first comprehensive contemporary understanding of the clinical

profile of presumed ischaemic presentations of MINOCA. Notably, the presenting chest pain characteristics of presumed ischaemic MINOCA patients do not differ from those with MI-CAD. Although identifying a clinical profile for MINOCA patients may aid in the clinical delineation of these patients from those with MICAD, this chapter affirms that presumed ischaemic MINOCA patients are almost clinically indistinguishable from the traditional MI-CAD presentation. This supports the concept that these ischaemic MINOCA patients have experienced a myocardial infarct and should not be labelled as ‘false positive infarcts’. The study also demonstrated that these MINOCA patients are low risk compared with the MICAD patients and less often receive conventional post-infarct therapies.

In Chapter 4, a well-recognised method was employed to explore the risk of thrombosis in presumed ischaemic MINOCA patients compared to MI-CAD. The study showed overall thrombin generation potential and inherited or acquired thrombophilia states were not different between ischaemic MINOCA and MI-CAD suggesting that ischaemic MINOCA patients generate thrombin in a comparable manner. This data represents the first prospective examination of the role of thrombin generation in MINOCA, and while it provided important insights, it was not conclusive, and thus should be considered as preliminary given the small sample size. Larger scale studies encompassing adequate power and utilising alternative sensitive methodology will provide further insights.

Chapter 5 explored a novel pharmacological intervention to optimise outcomes for STEMI patients. This multicentre, randomized, double-blind, clinical trial demonstrated a significant reduction in myocardial infarct size assessed by CMR

when high dose NAC together with low dose GTN was administered to acute STEMI patients undergoing primary PCI. However, an improvement in function was not observed over three months. This pilot study suggests that the antioxidant properties of NAC together with the potentiation components of GTN may have a potential therapeutic role to enhance the outcomes of acute STEMI patients. A larger scale study is required to further investigate this concept.

Over the past 30-40 years, the treatment of acute MI has become evidenced-based with clinical trials demonstrating the benefit of thrombolytic therapy, primary PCI, early angiography in NSTEMI, and the use of anti-platelet agents in reducing adverse cardiac events. These clinical trials are constructed on the basis of a common mechanism being responsible for the acute MI so that a single therapeutic approach is all that is required to arrest the disease process. However, as William Osler (1849-1919) famously quoted “the good physician treats the disease; the great physician treats the patient who has the disease”. Hence this astute physician recognised that therapies need to be adapted to the specific patient’s needs rather than a ‘one-size fits all’ approach. Today this concept is herald with the advent of ‘personalised medicine’.

This thesis has provided important insights into MINOCA, which can be conceptually extended to the understanding of MICAD. The chapters on MINOCA in this thesis reveal that it is a heterogeneous disorder with multiple potential causes. Thus although these patients fulfil the Universal criteria for acute MI, the underlying mechanism responsible for the presentation may be non-ischaemic (eg myocarditis) or even non-cardiac (eg pulmonary embolism). These causes along with other coronary mechanisms (eg coronary spasm or embolism) have only been considered because of

the surprising finding of no obstructive coronary artery disease on angiography. However, these mechanisms could equally apply in patients with MICAD (especially in the absence of an occluded culprit artery) and the CAD may only be an incidental finding. Thus we should learn from the experience with MINOCA and adopt a more ‘personalised medicine’ approach in patients with MICAD and consider the underlying mechanism.

The authors of the universal definition of acute MI have made some advances in this approach by introducing ‘type of infarcts’, which may require more specific mechanistic-directed therapies. For example, Type-1 infarcts are believed to be the most common and due to plaque rupture, whereas Type-2 infarcts may due to supply & demand imbalance from causes such as a tachyarrhythmia or coronary spasm. Thus it could be proposed that statins are more useful in preventing plaque rupture and therefore of use in type-1 infarcts but may be of limited use if a tachyarrhythmia is primarily responsible for the infarct. These subtypes require further refinement since even type-2 infarcts may have multiple mechanisms and thus effective therapies. For example beta-blockers may be useful in preventing a tachyarrhythmia precipitated infarct but calcium channel blockers would be essential if coronary spasm was responsible. Thus we should learn from the lessons of MINOCA and perhaps even patients with MICAD should be worked up to reveal the underlying mechanism and then therapy ‘personalised’ to the responsible mechanism.

This same concept can be extended to reperfusion injury and the use of NAC as in the NACIAM study. Do all patients with STEMI experience reperfusion injury and therefore will benefit from NAC therapy? Clearly there is a need to be able to identify

patients who are likely to experience reperfusion injury and then initiate therapies like NAC before establishing reperfusion.

The novel clinical insights of acute MI arising from this thesis provide much value in regards to the contemporary classification and management of acute MI patients. The studies in MINOCA highlight the limitations in our understanding and approach in the treatment of acute MI and the need to adopt a ‘personalised medicine’ approach in the management of our patients. Thus while answering some issues, this thesis also stresses the importance of further research in acute MI.

Pasupathy, S., Air, T., Dreyer, R., Tavella, R. & Beltrame, J.F. (2015).
Systematic Review of Patients Presenting with Suspected Myocardial Infarction
and Non-Obstructive Coronary Arteries (MINOCA).
Circulation, 131(10), 861-870.

NOTE:

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It is also available online to authorised users at:

<http://dx.doi.org/10.1161/CIRCULATIONAHA.114.011201>

Pasupathy, S., Air, T., Dreyer, R., Tavella, R. & Beltrame, J.F. (2015). Response to Letter Regarding Article, "Systematic Review of Patients Presenting With Suspected Myocardial Infarction and Non-obstructive Coronary Arteries" *Circulation*, 132(19), e232.

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