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Improving the Risk Stratification, Diagnosis and Classification of Patients with Suspected Myocardial Infarction

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To my family

ABSTRACT

Myocardial infarction is a leading cause of morbidity and mortality worldwide. The purpose of this thesis was to develop strategies for the assessment of patients with suspected myocardial infarction using a high-sensitivity cardiac troponin I assay, and to evaluate the relationship between the aetiology of myocardial infarction and long term clinical outcomes to identify opportunities to modify outcomes.

In the United Kingdom, approximately 1 million patients present to hospital with chest pain each year and are assessed for suspected myocardial infarction, yet fewer than 20% of patients receive this diagnosis. Prior clinical standards mandated the admission of patients for serial cardiac troponin testing to identify myocardial necrosis and determine if myocardial infarction had occurred. However, new high-sensitivity assays offer a magnitude improvement in diagnostic precision, and as such provide a novel approach to diagnose or exclude myocardial infarction at an earlier stage.

In our first study, I evaluate the performance of a high-sensitivity cardiac troponin I assay as a risk stratification tool in patients with suspected acute coronary syndrome. A systematic review and individual patient-level data meta-analysis was performed, including prospective studies measuring high-sensitivity cardiac troponin I in patients with suspected acute coronary syndrome, where the diagnosis was adjudicated according to the universal definition of myocardial infarction. The primary outcome was myocardial infarction or cardiac death during the index hospitalization or at 30 days. Meta-estimates for primary and secondary outcomes were derived using a

binomial-normal random effects model. Performance was evaluated in subgroups and across a range of troponin concentrations (2-16 ng/L) using individual patient data.

A total of 22,457 patients were included in the meta-analysis (age 62 [15.5] years; n=9,329 (41.5%) women), of whom 2,786 (12.4%) experienced myocardial infarction or cardiac death at 30 days. Cardiac troponin I concentrations were <5 ng/L at presentation in 11,012 (49%) patients, with a negative predictive value of 99.5% (95% confidence interval [CI] 99.3-99.6) for myocardial infarction or cardiac death at 30 days. Lower thresholds did not improve safety, but did significantly reduce the proportion identified as low risk.

This threshold of 5 ng/L formed the basis for the development of a diagnostic pathway for patients with suspected acute coronary syndrome. In a cohort study of 1,218 patients with suspected acute coronary syndrome who underwent high-sensitivity cardiac troponin I measurement at presentation, 3 and 6 or 12 hours, I derived and validated a novel pathway (rule out myocardial infarction if <5 ng/L at presentation, or change <3 ng/L and <99th centile at 3 hours), and compared this with the established European Society of Cardiology 3-hour pathway (rule out myocardial infarction if <99th centile at presentation, or at 3 hours if symptoms <6 hours). The primary outcome was a comparison of the negative predictive value (NPV) of both pathways for myocardial infarction or cardiac death at 30 days. The primary outcome was evaluated in pre-specified subgroups stratified by age, gender, time of symptom onset and known ischaemic heart disease.

In those <99th centile at presentation, the ESC pathway ruled out myocardial infarction in 28.1% (342/1,218) and 78.9% (961/1,218) at presentation and 3 hours respectively,

missing 18 index and two 30-day events (NPV 97.9%, 95% confidence intervals [CI] 96.9-98.7%). The novel pathway ruled out 40.7% (496/1,218) and 74.2% (904/1,218) at presentation and 3 hours, missing two index and two 30-day events (NPV 99.5%, 95% CI 99.0-99.9%; P<0.001 for comparison). The NPV of the novel pathway was greater than the ESC pathway overall (P<0.001), and in all subgroups including those presenting early or known to have ischaemic heart disease.

There are a number of additional approaches for the rule out of myocardial infarction. Clinical risk scores apply conventional risk factors to estimate the probability of myocardial infarction. The most widely implemented scores, HEART, EDACS, GRACE and TIMI, have been extensively validated when used alongside contemporary troponin assays, however, their impact on pathways applying high-sensitivity cardiac troponin testing is less clear.

In 1,935 patients with suspected acute coronary syndrome, I evaluated the safety and efficacy of our novel pathway or the European Society of Cardiology 3-hour pathway alone, or in conjunction with low-risk TIMI (0 or 1), GRACE (≤108), EDACS (<16) or HEART (≤3) scores. Myocardial infarction or cardiac death at 30-days occurred in 14.3% (276/1,935). The ESC pathway ruled out 70% with 27 missed events giving a negative predictive value (NPV) of 97.9% (95% confidence interval [CI], 97.1 to 98.6%). Addition of a HEART score ≤3 reduced the proportion ruled out by the ESC pathway to 25%, but improved the NPV to 99.7% (95%CI 99.0 to 100%, P<0.001). The novel pathway ruled out 65% with three missed events for a NPV of 99.7% (95%CI 99.4 to 99.9%). No risk score improved the NPV, but all reduced the proportion ruled out (24-47%, P<0.001 for all).

Whilst myocardial infarction due to atherosclerotic plaque rupture and thrombosis (type 1) is well described, the natural disease course of myocardial infarction due to oxygen supply-demand imbalance without atherothrombosis (type 2) is poorly understood. I aimed to define long-term outcomes and explore risk stratification in patients with type 2 myocardial infarction and myocardial injury. Consecutive patients (n=2,122) with elevated cardiac troponin I concentrations (≥0.05 μg/L) were identified at a tertiary cardiac centre. All diagnoses were adjudicated as per the Universal Definition of Myocardial Infarction. The primary outcome was all-cause death. Secondary outcomes included major adverse cardiovascular events (MACE; non-fatal myocardial infarction or cardiovascular death) and non-cardiovascular death. To explore competing risks, cause-specific hazard ratios were obtained using Cox regression models.

The adjudicated index diagnosis was type 1 or type 2 myocardial infarction or myocardial injury in 1,171 (55.2%), 429 (20.2%) and 522 (24.6%) patients, respectively. At five years, all-cause death rates were higher in those with type 2 myocardial infarction (62.5%) or myocardial injury (72.4%) compared with type 1 myocardial infarction (36.7%). The majority of excess deaths in those with type 2 myocardial infarction or myocardial injury were due to non-cardiovascular causes (HR 2.32, 95%CI 1.92-2.81, versus type 1 myocardial infarction). Despite this, the observed crude MACE rates were similar between groups (30.6% versus 32.6%), with differences apparent after adjustment for co-variates (HR 0.82, 95%CI 0.69-0.96). Coronary heart disease was an independent predictor of MACE in those with type 2 myocardial infarction or myocardial injury (HR 1.71, 95%CI 1.31-2.24). Patients with type 2 myocardial infarction were less likely to receive secondary prevention therapy,

suggesting a treatment gap may exist and there may be potential to modify clinical outcomes.

A risk stratification threshold has been defined using high-sensitivity cardiac troponin I which identifies patients at very low risk of myocardial infarction or cardiac death. A diagnostic pathway incorporating this risk stratification threshold appears safer than established guidelines which apply the 99th centile alone. The use of clinical risk scores does not appear to improve the safety of this approach, however, does significantly reduce efficacy. Overall, these findings demonstrate the potential of high-sensitivity cardiac troponin testing to improve the efficiency of the assessment of patients with suspected acute coronary syndrome without compromising patient safety. The observations in those with myocardial injury and infarction have identified a phenotype of patients with type 2 myocardial infarction and coronary artery disease who are at increased cardiovascular risk, and who may benefit from targeted secondary prevention. The studies presented will inform the design of future clinical trials, and may inform international guidelines for the assessment of patients with suspected acute coronary syndrome.

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DECLARATION

This thesis represents research undertaken at the Centre for Cardiovascular Sciences, University of Edinburgh, and the Edinburgh Heart Centre between August 2015 and June 2018. British Heart Foundation Special Project Grant (SP/12/10/29922), Project Grant (PG/15/51/31596), and Clinical Research Training Fellowship Awards (FS/16/75/32533) supported the conduct of these studies.

I was personally involved in the formulation, conduct and data-analysis presented in all aspects of this thesis. In keeping with the nature of collaborative research, this thesis reports findings from patients participating in the High-STEACS clinical trial, designed and led by my supervisor, Professor Nicholas L. Mills. In addition, long-term outcomes of myocardial injury and infarction are reported in a cohort of patients first identified by my colleague, Dr Anoop Shah. Participants contributing to the individual patient-level data-meta analysis were recruited in nine different countries by a number of primary investigators, all of whom obtained the necessary approvals to share patient-level data.

Chapters 1, 3, 4, 5, 6 and portions of Chapter 7 have been published in peer review journals. The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged. All studies were undertaken in accordance with the regulations of the Lothian Research Ethics Committee and with the Declaration of Helsinki of the World Medical Association.

ANDREW RUSSELL CHAPMAN 1ST JUNE 2018

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The research presented within this thesis would not have been possible without the support of my wife, Lindsey, who has made a number of sacrifices to allow me to attend meetings, meet deadlines, and most importantly, maintain perspective. I am very grateful for her love and support, and that of my family. I am certain I would not be in the position I am in today without the support of my parents. Both led by example and showed that anything can be achieved through hard work. I dedicate this thesis to my Dad, Brian, whose influence continues to guide my progress.

ABBREVIATIONS

ACS	Acute coronary syndrome
CABG	Coronary artery bypass grafting
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
GRACE	Global Registry of Acute Coronary Events
HR	Hazard ratio
Hs-cTn	High-sensitivity cardiac troponin
IHD	Ischaemic heart disease
IQR	Interquartile range
LOD	Limit of detection
MACE	Major adverse cardiovascular events
MI	Myocardial infarction
NPV	Negative predictive value
NSTEMI	Non-ST segment elevation myocardial infarction
PCI	Percutaneous coronary intervention
PPV	Positive predictive value
RR	Relative risk
STEMI	ST segment elevation myocardial infarction
TIMI	Thrombolysis in myocardial infarction
URL	Upper reference limit

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CHAPTER 1

INTRODUCTION - ASSESSMENT AND CLASSIFICATION OF PATIENTS WITH MYOCARDIAL INJURY AND INFARCTION

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1.1 Overview

Myocardial infarction remains a leading cause of morbidity and mortality worldwide. Whilst presentations to hospital with chest pain suggestive of myocardial ischaemia are responsible for up to 1 million visits to the emergency department in the United Kingdom each year, fewer than 20% of patients receive a final diagnosis of myocardial infarction. The ability to identify patients without myocardial infarction at an earlier stage has the potential to reduce patient anxiety, to facilitate focused clinical assessment to identify alternative diagnoses and improve resource allocation by reducing unnecessary hospitalisation and testing.

However, the diagnosis of myocardial infarction has been complicated through recognition that this condition may occur in patients without atherosclerotic plaque rupture and intraluminal thrombosis, but with an imbalance in myocardial oxygen supply and demand in the context of another acute illness, such as pneumonia or tachyarrhythmia. Our understanding of the natural disease course of this condition is limited, with no consensus approach to risk stratification and no evidence based strategies for or treatment.

Here, I discuss strategies for the assessment of patients with suspected myocardial infarction using cardiac troponin as a biomarker, evaluate the underlying mechanisms of myocardial necrosis and examine the reported long term outcomes of patients with myocardial injury and infarction. The purpose of this thesis is to provide further evidence and strategies for the assessment and classification of patients with myocardial injury and infarction.

1.2 Classification of myocardial infarction

The definition of acute myocardial infarction has evolved to accommodate increasingly sensitive markers of myocardial necrosis and imaging methods that allow greater understanding of the pathogenic mechanisms of acute coronary syndrome. As such, the universal definition of myocardial infarction proposes that we classify patients with myocardial infarction based on aetiology (***Figure 1.1***) (Thygesen et al., 2012a). While this classification has been used in clinical trials to refine primary and secondary endpoints (Morrow et al., 2009, Bonaca et al., 2012, White et al., 2012), it has not been widely adopted in clinical practice, and the frequency and implications of subtypes of acute myocardial infarction are uncertain (Sandoval et al., 2014a).

The third universal definition of myocardial infarction states the diagnosis of myocardial infarction requires evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia. These criteria require detection of a rise and/or fall in cardiac biomarker levels (preferably cardiac troponin) with at least one value above the 99th percentile upper reference limit, and at least one of the following: (1) symptoms of myocardial ischaemia, 2) new or presumed new significant ST-segment-T-wave changes or new left bundle branch block, 3) development of pathological Q-waves on the electrocardiogram, 4) imaging evidence of loss of viable myocardium or new regional wall motion abnormality or 5) identification of intra-coronary thrombus by angiography or autopsy.

Figure 1.1. Classification proposed by the Third Universal Definition of Myocardial Infarction (Thygesen et al., 2012a).

The classification distinguishes between type 1 myocardial infarction due to thrombosis of an atherosclerotic plaque, and type 2 myocardial infarction due to myocardial oxygen supply-demand imbalance in the context of another acute illness. Myocardial infarction presenting as sudden death (type 3), or after percutaneous coronary intervention (type 4) and coronary artery bypass grafting (type 5) are also defined. Acute myocardial injury is classified where troponin concentrations are elevated with evidence of dynamic change in the absence of overt myocardial ischemia, whereas in chronic myocardial injury troponin concentrations remain unchanged on serial testing. This is an important

distinction, as the underlying pathological mechanisms in acute and chronic myocardial injury are likely to differ.

This classification is contentious and was based on expert consensus rather than evidence from prospective clinical trials. Whilst it has been adopted in research studies, implementation in clinical practice has been less consistent. The most contentious diagnosis is that of type 2 myocardial infarction; a concept based on clinical hypothesis and observation without prospective mechanistic evaluation. Patients classified with type 2 myocardial infarction are heterogeneous and have myocardial ischaemia secondary to a variety of acute medical or surgical conditions. Based on the current criteria, a diagnosis of type 2 myocardial infarction could be applied to patients without coronary artery disease. At present, there is no guidance or consensus on the optimal cardiac investigation, management or treatment strategy for patients with type 2 myocardial infarction. Differentiating between patients with type 2 myocardial infarction and those patients with myocardial necrosis in the absence of ischaemia, in whom the recommended classification is myocardial injury, is challenging (Collinson, 2015). Outcomes for both groups of patients are poor, and investigation and management are inconsistent in practice (Shah et al., 2015c). It is likely that this is at least in part due to variability in interpretation of the guidelines.

The global task force are reviewing the Universal Definition of Myocardial Infarction and recognise the need to provide clearer diagnostic criteria and guidance to ensure consistent adoption in clinical practice (Alpert and Thygesen, 2016, Alpert et al., 2014, Sandoval et al., 2014a). Likewise, in the absence of an accepted definition it is difficult to perform standardised evaluations across different healthcare settings, or to conduct

randomised trials to determine the effectiveness of investigative strategies or preventative treatments for these patients. An improved understanding of the pathogenesis of these conditions is required to refine the universal definition further. If this leads to objective diagnostic criteria and a clear rationale for future investigation, this may improve the applicability and use of the universal definition in clinical practice.

1.3 Mechanisms of myocardial injury

Cardiac troponin is an integral component of the contractile apparatus of the cardiomyocyte, expressed exclusively within the myocardium. It is a complex of three subunits, C, I and T, which regulate calcium mediated excitation-contraction coupling. The majority of cardiac troponin is intracellular, with >90% of troponin isoforms located within the sarcomere, and the remainder unbound within the cytoplasmic pool (White, 2011). In the setting of myocardial injury, cardiac troponin is released into the bloodstream in a time-dependent fashion, and may be detected using biochemical assays. The mechanisms of cardiac troponin release into the circulation are thought to include myocyte necrosis, apoptosis, formation and release of membranous blebs, increased membrane permeability and release of proteolytic troponin degradation products (White, 2011).

It is now recognised that cardiac troponin may be released outwith the context of myocardial ischemia and necrosis, with several purported mechanisms. Cardiomyocytes undergo mechanical stretch in response to pressure or volume overload, and this may trigger activation of intra-cellular proteases associated with intra-cellular degradation of

troponin (Wang et al., 2002). Furthermore, there is evidence that tachycardia may stimulate stress-responsive integrins within the cardiomyocyte, triggering release of intact cardiac troponin I from viable cardiomyocytes in the absence of necrosis (Hessel et al., 2008). Troponin release has also been demonstrated *in vivo* in patients who develop reversible ischaemia during nuclear perfusion imaging with stress testing. Using an ultra-sensitive cardiac troponin I assay with single molecule counting technology, change in cardiac troponin concentration following stress testing was associated with the extent of myocardial ischaemia (Sabatine et al., 2009).

The universal definition makes a distinction between type 2 myocardial infarction and myocardial injury based on the presence or absence of symptoms and signs of myocardial ischaemia, however, there remains considerable overlap and to date there have been no prospective mechanistic studies to evaluate the range of underlying pathophysiology in these patients. Acute myocardial injury may occur in a variety of cardiac and non-cardiac illnesses (***Table 1.1***) as a consequence of myocardial oxygen supply-demand mismatch (hypotension, tachycardia or hypoxemia), due to direct injury in sepsis or viral myocarditis, or as part of the pathophysiological process in acute left ventricular failure. However, in some cases the presenting illness may be associated with a pro-inflammatory and pro-thrombotic state with myocardial injury due to embolisation of platelet aggregates and thrombus from an otherwise silent vulnerable plaque. Furthermore, myocardial injury can occur due to myocardial oxygen supply-demand mismatch in the presence of prognostically important, but unrecognised stable coronary artery disease. It is not therefore appropriate to dismiss episodes of acute myocardial injury as mere bystander phenomenon of no clinical consequence.

Chronic myocardial injury may occur in structural heart disease (hypertensive heart disease, ischaemic or dilated cardiomyopathy) or secondary to other non-cardiac illness such as chronic renal failure. As an example, the detection of chronic myocardial injury may be clinically useful in valvular heart disease, with serum cardiac troponin I concentrations associated with cardiac mass, replacement fibrosis and prognosis in patients with aortic stenosis (Chin et al., 2014).

The presence of chronic elevations in cardiac troponin associated with these conditions may contribute to diagnostic uncertainty in patients with suspected acute coronary syndrome. In recognition of this European guidelines for patients with non-ST-segment elevation myocardial infarction only recommend invasive management where a relative change in cardiac troponin concentration of at least 20% can be demonstrated, or where there is at least a five-fold elevation in cardiac troponin concentrations above the 99th centile on presentation (Roffi et al., 2016, Thygesen et al., 2012b).

Table 1.1. Causes of myocardial necrosis stratified by aetiology.

Primary myocardial ischaemia	**Supply or demand imbalance causing myocardial ischaemia**	**Injury not related to myocardial ischaemia**	**Multifactorial or indeterminate aetiology**
Atherosclerotic Plaque Rupture Intraluminal Coronary Thrombus Distal Microembolisation Coronary Artery Dissection	Anaemia Aortic dissection Aortic valve disease Tachy- or Brady- Arrhythmias Coronary embolism or vasculitis Coronary endothelial dysfunction Coronary vasospasm Hypertension Left Ventricular Hypertrophy Hypertrophic cardiomyopathy Respiratory failure Shock - Cardiogenic - Hypovolaemic - Septic	Ablation Cardiac contusion Cardiac surgery Cardiotoxic drugs Cardioversion Cytokine mediated injury Myocarditis Pacing Rhabdomyolysis	Acute / Chronic Heart Failure Burns Critical Illness Infiltrative diseases - Amyloidosis - Sarcoidosis Pulmonary embolism Pulmonary hypertension Acute Kidney Injury Chronic Kidney Disease Strenuous exercise Takotsubo cardiomyopathy Stroke Subarachnoid Haemorrhage

Adapted from the Third Universal Definition of Myocardial Infarction (Thygesen et al., 2012a).

1.4 Biochemical quantification of myocardial injury

Cardiac troponin is the only recommended biomarker for the detection of myocardial necrosis, and it is integral to the diagnostic criteria for myocardial infarction (Thygesen et al., 2012a).

Our ability to accurately measure cardiac troponin has improved through the development of more sensitive assays, with the latest generation high-sensitivity assays capable of detecting cardiac troponin concentrations in the majority of healthy individuals. This has allowed accurate identification of the normal reference range and the 99th centile upper reference limit (Apple et al., 2012, Shah et al., 2015b, Apple and Collinson, 2012).

The universal definition has recommended the 99th centile as the diagnostic threshold for acute myocardial infarction since 2007, with a rise or fall in cardiac troponin concentrations necessary to confirm the diagnosis (Thygesen et al., 2012a). Improvements in assay precision have identified differences in cardiac troponin concentrations between men and women, with the 99th centile twofold lower in women than men across a range of assays (Apple et al., 2012). The use of high-sensitivity cardiac troponin and sex-specific 99th centile upper reference limits increases the diagnosis of myocardial injury and infarction, particularly in women, and identifies a high-risk group of patients with poor outcomes (Shah et al., 2015b).

There is now widespread adoption of cardiac troponin assays in clinical practice across Europe, with >95% of laboratories using cardiac troponin as the preferred marker for the diagnosis of myocardial infarction (Collinson et al., 2016). In this survey in 2015,

over 50% of European laboratories used the 99th centile upper reference limit as the diagnostic threshold; however, given the widespread availability of high-sensitivity cardiac troponin assays and their prominence in national guidelines, the actual proportion in current practice is likely to be higher.

1.5 High-sensitivity troponin and risk stratification

Recent studies have demonstrated that cardiac troponin concentrations below the 99th centile can help in the risk stratification of patients with suspected acute coronary syndrome (Thygesen et al., 2012a, Shah et al., 2015b, Mills et al., 2011, Body et al., 2011, Rubini Gimenez et al., 2013, Neumann et al., 2016). As such, the latest European Society of Cardiology guidelines include additional pathways incorporating lower thresholds of cardiac troponin for risk stratification and earlier testing (Roffi et al., 2016). We recently demonstrated in consecutive patients with suspected acute coronary syndrome that a high-sensitivity cardiac troponin I concentration <5 ng/L at presentation had a negative predictive value of 99.6% (95% CI 99.3 to 99.8) for myocardial infarction during the index presentation, or myocardial infarction or cardiac death in 30 days (Shah et al., 2015a). Furthermore, patients with troponin concentrations <5 ng/L at presentation had very low rates of adverse cardiac events at 1 year, compared with those with ≥5 ng/L but <99th centile (Shah et al., 2015a). However, alternative thresholds have been proposed, and there is a lack of clinical consensus as to the optimal approach.

The use of cardiac troponin testing in clinical practice is evolving rapidly, with cardiac troponin concentrations increasingly used as a continuous measure of cardiovascular risk, rather than a binary test to identify those patients with and without myocardial infarction. In 2016, the National Institute for Health and Care Excellence (NICE) updated their guidance on the evaluation of patients with suspected acute coronary syndrome. For the first time, they recommended clinicians consider ruling-out myocardial infarction if a patient has very low concentrations of cardiac troponin at presentation when measured using a high-sensitivity assay (NICE, 2016). This guidance could lead to a significant reduction in the proportion of patients who require serial testing, and may tempt clinicians to consider upgrading their infrastructure to facilitate implementation. In the United Kingdom, two high-sensitivity assays are recommended by NICE for use in clinical practice, the Roche Elecsys high-sensitivity cardiac troponin T assay (hs-cTnT) and the Abbott ARCHITECT$_{STAT}$ high-sensitivity cardiac troponin I assay (hs-cTnI). There are important differences in the normal reference range, diagnostic thresholds, levels of imprecision and in the lowest absolute concentrations which can be reliably detected, also known as the limit of detection (LoD). (***Table 1.2***).

Table 1.2. High-sensitivity cardiac troponin assays in use in clinical practice in the United Kingdom

	99th centile (Diagnostic threshold)	**10% coefficient of variation***	**Limit of detection (LoD)**
Roche Elecsys high-sensitivity cardiac troponin T (1)	14 ng/L	13 ng/L	5 ng/L
Abbott ARCHITECT*STAT* high-sensitivity cardiac troponin I (2)	16 ng/L (females) 34 ng/L (males) 26 ng/L (single threshold)	4.7 ng/L	1.2 ng/L

*Lowest concentration where coefficient of variation is <10% (measure of dispersion of replicate sample results around the mean [SD/mean]).
(1) (Giannitsis et al., 2010) (2) (Shah et al., 2015b)

NICE recommend clinicians apply the LoD as a threshold below which myocardial infarction can be safely ruled out at presentation. Such a strategy is only recommended for patients deemed to be at low risk of myocardial infarction *'as indicated by a validated tool'*. During their appraisal, NICE considered evidence from studies including both the Thrombolysis in Myocardial Infarction (TIMI) score and the Global Registry of Acute Coronary Events (GRACE) score. Both scores were derived and validated in patients with confirmed myocardial infarction to confer prognosis, but over time these scores have been implemented for risk stratification in patients with suspected, not confirmed myocardial infarction. Importantly, cardiac troponin concentrations are embedded in both risk scores. NICE ultimately recommend the TIMI score, which has been previously validated in patients with suspected acute coronary syndrome alongside a contemporary troponin assay and serial testing (Than et al., 2012), but not with a high-sensitivity assay and the LoD at presentation alone.

This guidance was recently validated in a pooled study of over 5,000 patients, in five observational cohorts across two continents, with varying prevalence of major adverse cardiovascular events (4.8% to 15.6%), (Carlton et al., 2017). This study demonstrated when a high-sensitivity cardiac troponin T of <5 ng/L (LoD) was applied alongside a TIMI score of 0 and a non-ischaemic electrocardiogram, the sensitivity and NPV were extremely high, at 99.5% (95% CI 98.1 to 99.9%) and 99.6% (95% CI 98.7 to 100%), respectively. The meta-estimate for sensitivity was 98.7% (95% CI 96.5 to 99.6%), with low heterogeneity observed between cohorts (I_2 15.3). For the high-sensitivity cardiac troponin I assay, using the limit of detection (<2 ng/L) and a TIMI score of 0 alongside a non-ischaemic electrocardiogram, the sensitivity was 98.9% (95% CI 97.4 to 99.6%) and NPV was 99.5% (95% CI 98.8 to 99.8%). The meta-estimate for sensitivity was similar (98.5%, 95% CI 95.4 to 99.5%) but the heterogeneity was high (I_2 73.7). The reason for the observed heterogeneity is unclear, but may reflect differences in the assay used for diagnostic adjudication and testing between cohorts. These strategies would identify between 17.9% (95% CI 16.6 to 19.3%) and 21.0% (95% CI 19.9 to 22.2%) of patients as low risk, respectively. However, the use of thresholds above the LoD in combination with the TIMI score (such as <7 ng/L on the hs-cTnT assay, or <5 ng/L on the hs-cTnI assay) was shown to increase in the proportion identified as low risk without compromising sensitivity or NPV.

1.6 Clinical risk scores

Whilst NICE recommend use of the TIMI score in addition to high-sensitivity cardiac troponin testing, the true need for clinical risk scores in this setting is uncertain. A recent meta-analysis of 9,269 patients found a normal electrocardiogram and a hs-cTnT result below the LoD provided excellent NPV (99.3%, 95% CI 97.3 to 99.8%) and sensitivity (98.7%, 95%CI 96.6 to 99.5%) for the diagnosis of myocardial infarction, without the need for additional risk scores (Pickering et al., 2017). There were no deaths at 30 days in patients classified as low risk with the index test. This reflects our understanding that patients classically considered high risk (due to increasing age or cardiovascular risk factors such as diabetes, renal disease or prior ischaemic heart disease) have chronic elevation in high-sensitivity cardiac troponin concentrations (within the normal reference range) and are less likely to have low concentrations to support early discharge. Indeed, the European Society of Cardiology advocate use of the LoD at presentation in conjunction with the electrocardiogram, but do not recommend the addition of clinical risk scores (Roffi et al., 2016).

However, there is undoubtedly an appetite for clinical risk scores in some settings, perhaps due to the additional perceived diagnostic confidence they provide. Unlike the GRACE (Global Registry of Acute Coronary Events) score and the TIMI score which were derived as markers of prognosis in those with confirmed myocardial infarction, the HEART score was developed and validated in a suspected acute coronary syndrome population. This score is based on clinical variables selected a priori (*H*istory, *E*CG, *A*ge, *R*isk factors, cardiac *T*roponin) with arbitrary weighting chosen on a pragmatic basis. A recent meta-analysis of 11,217 patients demonstrated this

score had a sensitivity of just 96.7% (95% CI 94.0 to 98.2%), below the threshold of 99% which most emergency department physicians deem acceptable (Van Den Berg and Body, 2017, Than et al., 2013). Whether use of this score offers additional benefit over risk stratification with troponin alone is unclear. Comparative studies including risk stratification thresholds alone, or in combination with risk scores are required to determine if improvements in safety can be obtained.

The evidence supporting implementation of strategies including low high-sensitivity cardiac troponin concentrations is strong, but some uncertainty remains. All studies on which the NICE recommendations are based were observational in nature (i.e. no patients were discharged from hospital on the basis of a single troponin result), and the same applies to the vast majority of studies in this area. Patients who present early after onset of symptoms are challenging to recruit and therefore under-represented in all observational cohort studies. It is therefore recommended that serial testing is performed in all who present early after onset of symptoms. Similarly, any patient with myocardial ischaemia on the electrocardiogram should not be considered for early rule-out and should undergo serial troponin testing. Of utmost importance is an awareness of the assay in use, the normal reference range, and the appropriate diagnostic and risk stratification thresholds which are not equivalent. Where low concentrations are reported, it is important to ensure appropriate standards for clinical reporting can be met and maintained under routine working conditions. As noted by NICE, implementation of a proposed early rule-out strategy should include clinical audit, with attention paid to the time taken to rule-out the diagnosis and on the clinical outcomes of patients with suspected acute coronary syndrome.

Clinicians should be confident that newer approaches using low concentrations of cardiac troponin are a magnitude safer than prior strategies using the 99th centile alone. High-sensitivity cardiac troponin testing has the potential to improve both the efficiency and safety of healthcare delivery for patients with suspected acute coronary syndrome, however, further investigation to define the optimal approach and to achieve consensus is required.

1.7 Incidence of myocardial injury and type 2 myocardial infarction

The introduction of high-sensitivity cardiac troponin assays and lower diagnostic thresholds into clinical practice may result in a disproportionate increase in the number of patients with type 2 myocardial infarction or myocardial injury compared to type 1 myocardial infarction (Shah et al., 2015b, Thygesen et al., 2012b), and could lead to diagnostic uncertainty with the potential for over treatment in patients who do not have acute coronary syndrome (Shah et al., 2013, Melanson et al., 2008, Makam and Nguyen, 2015).

To date, the majority of studies of patients with type 2 myocardial infarction and myocardial injury have not implemented high-sensitivity cardiac troponin assays and therefore may under recognise the true prevalence of these conditions. The largest reported registry (Baron et al., 2015) assessed all patients with acute myocardial infarction admitted to hospital in Sweden during 2011 (n=20,138). All diagnoses were classified by the attending clinician, with 88.5% of patients classified as type 1

myocardial infarction and 7.1% as type 2 myocardial infarction. Of note, the prevalence of type 2 myocardial infarction varied markedly between different centres (0% to 13%), illustrating the challenge of consistently applying the current diagnostic classification. In studies which classified all patients with elevated cardiac troponin concentrations, the reported prevalence of type 2 myocardial infarction varies between 7% to 37% in unselected hospitalised patients, and from 5% to 74% in patients attending the Emergency Department (***Table 1.3***), (Saaby et al., 2013, El-Haddad et al., 2012, Smith et al., 2013, Sandoval et al., 2014b). Differences in the reported prevalence may in part be explained by the inconsistent approach to distinguishing type 2 myocardial infarction from acute and chronic myocardial injury across studies. It is perhaps unsurprising that the diagnosis of type 2 myocardial infarction has been shown to be less frequent in selected populations with acute coronary syndrome.

Table 1.3. Classification of myocardial injury and infarction in published cohort studies.

				Diagnostic Classification (%) Proportion of all patients with elevation in baseline cardiac troponin				
	Population	Troponin assay and upper reference limit	Number with elevated cardiac troponin concentrations (% of total study population)	Myocardial injury (%)	Type 1 MI (%)	Type 2 MI (%)	Type 3/4/5 MI (%)	Unclassified
(Javed et al., 2009)	Unselected hospital inpatients with cTnI measured (n=2,979)	cTnI (>40 ng/L) ADVIA Immunoassay (Siemens)	701 (23.5%)	461 (65.8%)	143 (20.4%)	64 (9.1%)	9 (1.3%)	24 (3.6%)
(El-Haddad et al., 2012)	Unselected hospital inpatients with cTnI measured (n=807)	cTnI (>160 ng/L) Beckman Access	807 (100%)	Not reported	512 (63.4%)	295 (36.6%)	Nil	Nil
(Saaby et al., 2013)	Unselected hospital inpatients with cTnI measured (n=4,499)	cTnI (>30 ng/L) Architect-STAT (Abbott Diagnostics)	1,961 (43.6%)	1,408 (71.8%)	397 (20.2%)	144 (7.3%)	12 (0.1%)	Nil
(Shah et al., 2015c)	Unselected hospital inpatients with cTnI measured (n=2,165)	cTnI (>50 ng/L) Architect-STAT (Abbott Diagnostics)	2,165 (100%)	522 (24.1%)	1,171 (71.3%)	429 (26.1%)	43 (2%)	Nil

Table 1.3. continued. Classification of myocardial injury and infarction in published cohort studies.

				Diagnostic Classification (%) Proportion of all patients with elevation in baseline cardiac troponin				
	Population	Troponin assay and upper reference limit	Number with elevated cardiac troponin concentrations (% of total study population)	Myocardial injury (%)	Type 1 MI (%)	Type 2 MI (%)	Type 3/4/5 MI (%)	Unclassified
(White et al., 2012)	Cardiology inpatients with recent ACS (2000-2006) (n=2,201)	cTnI, cTnT, CK, CK-MB	169 (7.7%)	Not reported	106 (62.7%)	7 (4.1%)	56 (33.1%)	Nil
(Szymanski et al., 2014)	Cardiology inpatients with ACS (n=2882)	cTn (not specified)	2,882 (100%)	Not reported	2824 (98%)	58 (2%)	Nil	Nil
(Stein et al., 2014)	Cardiology and ICU inpatients with ACS (n=2,818)	Not reported	2,818 (100%)	Not reported	2,691 (95.5%)	127 (4.5%)	Nil	Nil
(Baron et al., 2015)	Hospital inpatients with ACS (n=19,763)	Not reported	19,763 (100%)	Not reported	17,488 (88.5%)	1,403 (7.1%)	141 (0.7%)	731 (3.7%)

Table 1.3. continued. Classification of myocardial injury and infarction in published cohort studies.

				Diagnostic Classification (%) Proportion of all patients with elevation in baseline cardiac troponin				
	Population	Troponin assay and upper reference limit	Number with elevated cardiac troponin concentrations (% of total study population)	Myocardial injury (%)	Type 1 MI (%)	Type 2 MI (%)	Type 3/4/5 MI (%)	Unclassified
(Melberg et al., 2010)	Hospital inpatients with ACS (n=1,093)	cTnT (>30 ng/L) (Roche Elecsys)	1,093 (100%)	Not reported	967 (88.5%)	17 (1.6%)	109 (9.9%)	Nil
(Morrow et al., 2009)	Clinical trial patients with ACS (n=13,608)	Not reported	1,218 (8.9%)	Not reported	397 (32.6%)	43 (3.5%)	778 (63.9%)	Nil
(Sandoval et al., 2014b)	Emergency department patients with cTnI measured (n=1,112)	cTnI (>34 ng/L) OCD Vitros	256 (23%)	Not reported	66 (25.8%)	190 (74.2%)	Nil	Nil
(Smith et al., 2011)	Emergency department patients with cTnI measured (n=662)	cTnI (90 ng/L) Siemens Stratus	139 (20.9%)	Not reported	40 (28.8%)	99 (71.2%)	Nil	Nil

Table 1.3. continued. Classification of myocardial injury and infarction in published cohort studies.

				Diagnostic Classification (%) Proportion of all patients with elevation in baseline cardiac troponin				
	Population	Troponin assay and upper reference limit	Number with elevated cardiac troponin concentrations (% of total study population)	Myocardial injury (%)	Type 1 MI (%)	Type 2 MI (%)	Type 3/4/5 MI (%)	Unclassified
(Smith et al., 2013)	Emergency department patients with suspected ACS (n=1,096)	cTn (not specified)	134 (12.2%)	Not reported	127 (95%)	7 (5%)	Nil	Nil
(Bonaca et al., 2013)	Emergency department presentations with suspected ACS (n=381)	cTnI (>100 ng/L) (Centaur Siemens)	96 (25.2%)	Not reported	86 (90%)	10 (10%)	Nil	Nil
(Shah et al., 2015b)	Unselected patients with suspected ACS (n=1,126)	hs-TnI (F ≥16g/L; M ≥34ng/L) Architect-STAT high-sensitivity (Abbott Diagnostics)	298 (25.4%)	40 (3.6%)	242 (81.2%)	56 (18.8%)	Nil	Nil

A previous study at our centre evaluated all patients with elevated plasma cardiac troponin concentrations irrespective of presenting complaint (n=2,165), admitted during the validation and implementation of a contemporary sensitive cardiac troponin I assay (Shah et al., 2015c). The frequency of type 1 myocardial infarction, type 2 myocardial infarction, and myocardial injury was 54%, 20% and 24% respectively. Type 2 myocardial infarction and myocardial injury were as common as type 1 myocardial infarction in clinical practice, and indeed more common than type 1 myocardial infarction in patients ≥75 years of age (***Figure 1.2***). Lowering the diagnostic threshold with a more sensitive cardiac troponin assay reduced recurrent myocardial infarction or death in patients redefined as having type 1 myocardial infarction, but more than doubled the number of patients with type 2 myocardial infarction or myocardial injury. Despite undergoing additional cardiac investigations, this did not result in changes in treatment, and there was no observed improvement in clinical outcomes (Shah et al., 2015c).

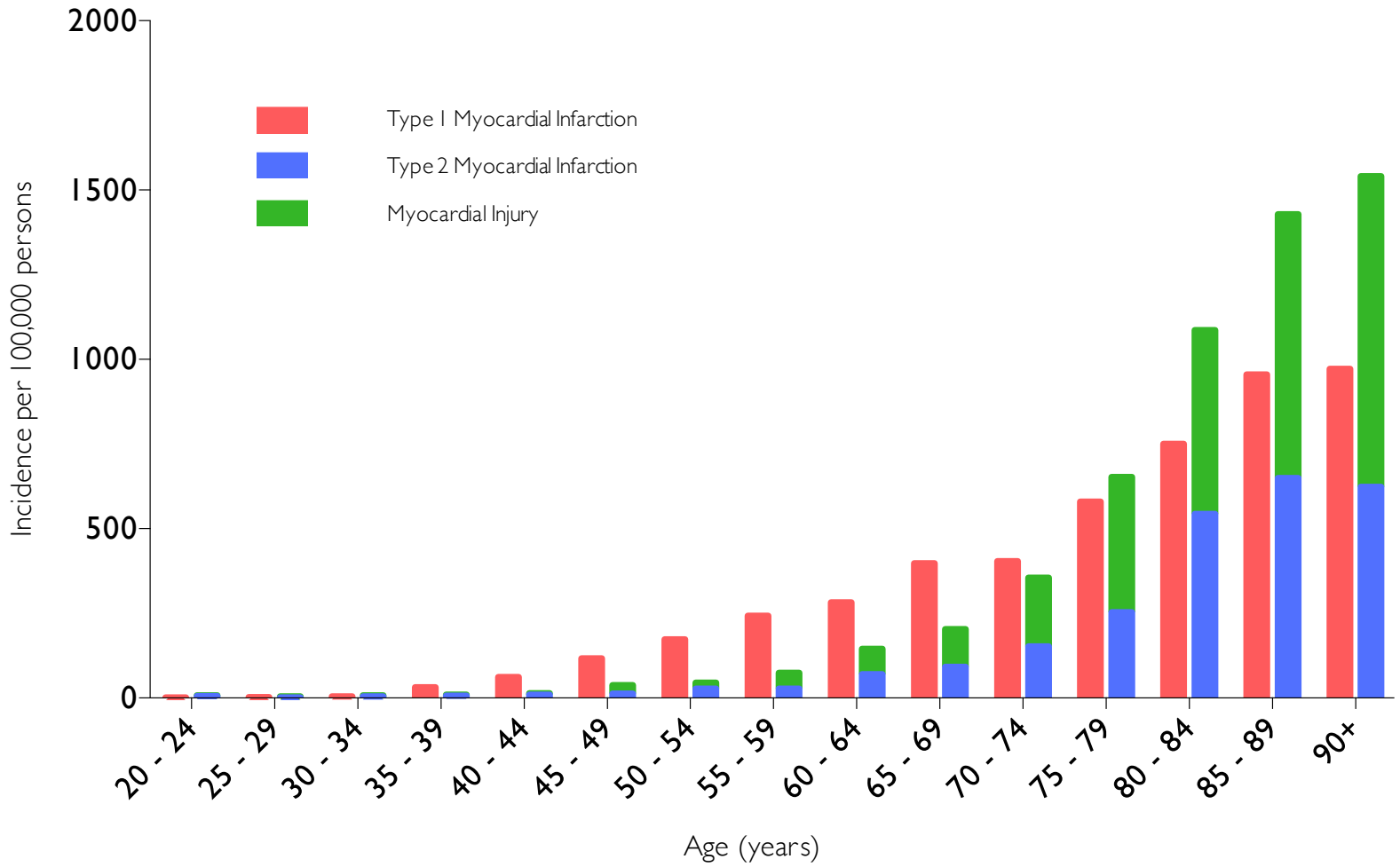


Figure 1.2. Incidence of myocardial infarction and myocardial injury stratified by age in unselected consecutive hospital inpatients with myocardial necrosis. Reproduced with permission (Shah et al., 2015c).

Whether adoption of high-sensitivity troponin assays and the 99th centile for diagnosis of myocardial infarction translates into improvements in clinical outcomes for patients with suspected acute coronary syndrome is being evaluated in a stepped wedge cluster randomised trial across Scotland (High-STEACS, NCT:01852123). If increased sensitivity does not impinge on specificity for the diagnosis of type 1 myocardial infarction, then these assays will improve patient outcomes through better targeting of therapies for coronary artery disease. However, if increased sensitivity leads to poor specificity, then patients may be misdiagnosed and given inappropriate cardiac medications with potentially detrimental outcomes. This trial will establish whether the introduction of high-sensitivity assays into routine clinical practice is detrimental or beneficial to patient management and outcomes; a fundamental and critical assessment for the modern definition of acute myocardial infarction.

1.8 Outcomes of myocardial injury and type 2 myocardial infarction

Patients with type 2 myocardial infarction or myocardial injury have poor clinical outcomes, worse than those patients with type 1 myocardial infarction (***Figure 1.3***), with 1 in 3 patients dead at one year (Shah et al., 2015c). In a prospective study of patients with acute coronary syndrome (n=2,818), Stein et al. (2014) found an increased risk of death in those with an adjudicated diagnosis of type 2 *versus* type 1 myocardial infarction at 30 days (13.6% vs. 4.9%, P<0.0001) and at one year (23.9% vs. 8.6%, P<0.0001). Another single centre study by El-Haddad et al. (2012) reported mortality rates 6.9 times greater in type 2 versus type 1 myocardial infarction at one year.

Sarkisian et al. (2016) reviewed 3,762 patients who underwent cardiac troponin testing on clinical indication. Patients with acute myocardial injury were at significantly greater risk of all-cause mortality than those with myocardial infarction at a median follow up of 3.2 years (59% *versus* 39%, P<0.0001 by log-rank test). In a sub-group analysis, they demonstrate no difference in risk of all-cause mortality between patients with type 2 myocardial infarction or myocardial injury (adjusted hazard ratio [HR] 1.28, 95%CI 0.97-1.65). Whether it is possible to improve outcomes in these patients through therapeutic intervention is currently unknown.

The distinction between type 2 myocardial infarction and myocardial injury may however be clinically important, as it has been demonstrated that patients classified as having a type 2 myocardial infarction are twice as likely as those with myocardial injury to be readmitted with a type 1 myocardial infarction at one year (Shah et al., 2015c). This potentially important observation suggests a proportion of patients with type 2 myocardial infarction may benefit from further investigation and treatment for coronary artery disease. Selection of patients for further investigation requires a greater understanding of the clinical features that identify those patients at increased risk of future acute coronary events, and a better understanding of the mechanisms of myocardial injury in this setting.

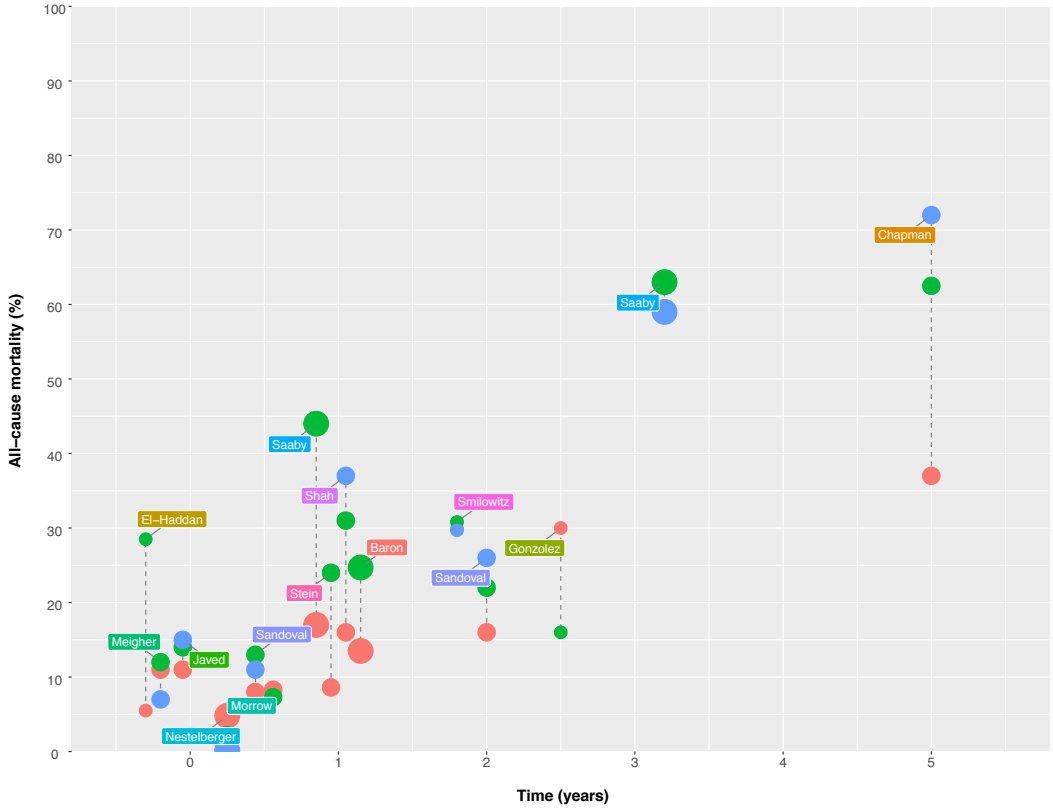


Figure 1.3. All-cause mortality in cohort studies of patients with type 1 or type 2 myocardial infarction, or myocardial injury.
Size of bubble indicates number of patients (small <1000, medium <3000, large >3000). Colour indicates diagnosis (type 1 myocardial infarction = red, type 2 myocardial infarction = green, myocardial injury = blue). Events before time zero are in-hospital death.

Whilst it is well recognised that patients with type 2 myocardial infarction have an increased rate of all-cause mortality, a clear limitation of the majority of studies to date is establishing cause-specific mortality and future cardiovascular risk (Sandoval and Thygesen, 2017). Such analyses are warranted to guide estimates of the proportion of future events that may be modifiable, and to inform the design of future clinical trials.

1.9 Conclusions

The implementation of more sensitive troponin assays in clinical practice has increased our awareness of the spectrum of both acute and chronic myocardial injury. High-sensitivity cardiac troponin assays offer a novel avenue to improve the efficacy and safety of risk stratification in patients with suspected acute coronary syndrome, but consensus as to the optimal approach is required. Whilst the universal definition classifies myocardial infarction by aetiology, inconsistency in the interpretation and application of these guidelines may impact on patient care and outcomes. Identifying patients with acute or chronic myocardial injury, and accurately defining clinical outcomes is a necessary first step. A better understanding of mechanism may guide the need for further investigations and identify those patients with type 2 myocardial infarction who may benefit from preventative therapies.

1.10 Aims and hypotheses

The principle aims of this thesis are to develop a systematic approach to the assessment of patients with suspected acute coronary syndrome using high-sensitivity cardiac troponin testing, and to improve the characterisation of patients with type 2 myocardial infarction and myocardial injury.

In Chapter 3, I will determine the optimal threshold of high-sensitivity cardiac troponin I to rule out myocardial infarction at presentation in a systematic review and individual patient-level data meta-analysis.

In Chapter 4, I will develop a clinical pathway incorporating a risk stratification threshold based on high-sensitivity cardiac troponin concentrations at presentation and serial testing at 3 hours. This will be compared to the established European Society of Cardiology 3-hour rule-out pathway.

In Chapter 5, I will evaluate whether the addition of clinical risk scores such as the GRACE score, TIMI score or HEART score improves the performance of the clinical pathway derived in Chapter 4.

In Chapter 6, I will determine long term outcomes of myocardial injury and infarction by subtype in a cohort of consecutive hospitalised patients. Specifically, I will determine the risk of future cardiovascular events and cause-specific mortality, and determine whether the diagnosis of type 2 myocardial infarction is an independent predictor of cardiovascular risk.

The following hypotheses will be addressed:

1. That a high-sensitivity cardiac troponin I threshold of 5 ng/L will provide the optimal balance between safety and efficacy in the risk stratification of patients with suspected acute coronary syndrome. (Chapter 3).

2. That the integration of a risk stratification threshold at presentation and serial testing at three hours in a clinical pathway will offer improved safety compared to the established European Society of Cardiology 3-hour rule-out pathway (Chapter 4).

3. That the addition of clinical risk scores (GRACE, TIMI and HEART) will not provide additional safety over the clinical pathway incorporating high-sensitivity cardiac troponin risk stratification thresholds (derived in Chapter 4), but will improve the safety of the European Society of Cardiology 3-hour rule-out pathway (Chapter 5).

4. That patients with type 2 myocardial infarction and myocardial injury are at increased risk of all-cause mortality at five years, but are at similar risk of future cardiovascular events as patients with type 1 myocardial infarction (Chapter 6).

CHAPTER 2

METHODS

2.1 Overview

The specific study design and methodology for each of the cohorts are described in detail in the relevant chapters. The following methodology sections will provide an overview of the patient populations studied and the general principles applied.

2.2 Study Populations

This thesis includes patients recruited in a number of different prospective observational cohort studies. Four cohorts were recruited from hospitals in Edinburgh, Scotland, and are described in detail below. An additional 16 cohorts from eight countries worldwide were evaluated as part of an individual patient-level data meta-analysis; the characteristics of these cohorts are detailed in ***Appendix 3***.

High-STEACS Pilot, Edinburgh, Scotland ***(Chapter 3)***

The *High-S*ensitivity *T*roponin in the *E*valuation of patients with suspected *A*cute *C*oronary *S*yndrome (High-STEACS) pilot study prospectively identified consecutive patients presenting to the Royal Infirmary of Edinburgh, United Kingdom, from 1st August to 31st October 2012, in whom the attending doctor suspected an acute coronary syndrome. Serum troponin concentrations were measured on admission and repeated six or 12 hours after the onset of symptoms using both a contemporary sensitive troponin I assay and a high-sensitivity cardiac troponin I assay. Clinical decisions were based on the contemporary assay only, with clinicians blinded to the results of the high-sensitivity assay. To ensure all eligible patients were included and

to avoid selection bias, consent was not sought from individual patients. Patients not resident in the south east of Scotland were excluded from the study.

High-STEACS Validation, Edinburgh, Scotland ***(Chapter 3)***

The High-STEACS validation study prospectively identified consecutive patients with suspected acute coronary syndrome presenting to the emergency departments of two secondary care hospitals (St John's Hospital, Livingston, and Western General Hospital, Edinburgh) and a tertiary care hospital (Royal Infirmary of Edinburgh, Edinburgh) in the southeast of Scotland between the 1st June 2013 and 31st January 2014. Patients were enrolled in the standard care arm of a stepped-wedge cluster randomised clinical trial evaluating the implementation of a high-sensitivity cardiac troponin I assay (High-STEACS, ClinicalTrials.gov number: NCT:01852123). All patients who had cardiac troponin requested by the attending clinician and an electrocardiogram done were included. In order for any request for cardiac troponin to be processed, the requesting healthcare practitioner had to indicate if acute coronary syndrome was suspected, and document the primary presenting symptom and time of symptom onset. Clinical decisions were based on the contemporary assay only, with clinicians blinded to the results of the high-sensitivity assay. To ensure all eligible patients were included and to avoid selection bias, consent was not sought from individual patients. Patients were excluded if they had been admitted previously during the study period, were pregnant, or did not live in Scotland.

High-STEACS Substudy, Edinburgh, Scotland ***(Chapters 3, 4 and 5)***

Patients with suspected acute coronary syndrome were recruited from the emergency department of the Royal Infirmary of Edinburgh, a tertiary care hospital in Scotland, between the 1st June 2013 and the 31st March 2017 into a substudy of the High-STEACS trial. All patients in whom the attending clinician requested cardiac troponin for suspected acute coronary syndrome were eligible for inclusion, using the same electronic request process as for the High-STEACS trial. We did not enrol patients with ST-segment elevation, those who were unable to provide consent or those from outside our region to ensure complete follow-up. Blood samples were obtained at presentation and at 6 to 12 hours for high-sensitivity cardiac troponin testing as part of routine clinical care. Patients provided written informed consent for additional sampling at 3 hours with the results of testing at this time point not used to guide patient care.

Myocardial injury cohort, Edinburgh, Scotland ***(Chapter 6)***

Consecutive hospital inpatients with elevated cardiac troponin I concentrations (≥0.05 μg/L) were identified at a tertiary cardiac centre (Royal Infirmary of Edinburgh, Scotland, UK) during the validation (January 19th to July 31st 2008) and implementation (January 19th to July 31st 2009) phases of a contemporary sensitive cardiac troponin I assay. We included all patients in whom cardiac troponin was requested by the attending clinician, regardless of suspected etiology or hospital department. We excluded patients admitted for elective procedures, those with incomplete electronic hospital records, and patients who were not residents to ensure follow up was complete. To ensure all eligible patients were included and to avoid selection bias, consent was not sought from individual patients.

2.3 Ethical and regulatory considerations

All studies were prospective and conducted in accordance with the Declaration of Helsinki. High-STEACS is a prospective stepped-wedge cluster randomised controlled trial evaluating the implementation of a high-sensitivity cardiac troponin assay in consecutive patients with suspected acute coronary syndrome across secondary and tertiary care hospitals in Scotland. I sought and obtained ethical approval to not obtain individual patient consent in those recruited to the High-STEACS pilot and validation studies. This approach was justified as whilst centres were randomised to the timing of implementation of the high-sensitivity assay, it was planned irrespective of the trial. Secondly, patients undergo no additional research study procedures, with the high-sensitivity assay measured in wastage plasma, and outcomes were recorded using routinely collected data. The High-STEACS study (including pilot, validation and substudy cohorts) was approved by the Scotland A Research Ethics Committee (12/SS/0115) and the Public Benefit and Privacy Panel for Health and Social Care for the National Health Service (NHS) Scotland (PAC 92/14). The conduct of the trial was periodically reviewed by an independent data monitoring committee. All data was collected prospectively from the electronic patient record, deidentified and linked using a unique patient identifier within NHS Scotland.

Ethical approval was not required for patients contributing to the myocardial injury cohort, although the study protocol was reviewed by the chairman and scientific advisor of the Lothian Research Ethics Committee who advised that it represented clinical audit and service evaluation. For this study, approval was sought from the NHS Caldicott Guardian for data collection and record linkage.

2.4 Cardiac troponin assays

2.4.1 High-sensitivity cardiac troponin I assay

The Abbott ARCHITECT$_{STAT}$ high-sensitive cardiac troponin I assay (Abbott Laboratories, Abbott Park, IL) is a two-step chemo-luminescent assay. Briefly, this assay uses a paramagnetic 'capture' antibody to bind cardiac troponin I present in a sample, which is then extracted from the reaction vessel during a wash phase using a magnetic field. A second cardiac troponin I detection antibody is then added. This 'detection' antibody is conjugated to acridinium and activated by a trigger agent, generating fluorescence. This is then detected, with the strength of the signal obtained proportional to the concentration of cardiac troponin I present in the sample.

This assay has a limit of detection (LOD; minimum absolute concentration reliably identified) of 1.2 ng/L and coefficient of variation (measure of dispersion of replicate sample results around the mean) of less than 10% at 6 ng/L. This assay performance has been independently validated across multiple centres under routine laboratory working conditions, with a reported inter-laboratory coefficient of variation of 12.6% at 3.5 ng/L across 33 instruments. The upper reference limit 99th centiles were determined in 4,590 samples from healthy individuals as 16 ng/L for women and 34 ng/L in men (Shah et al., 2015b).

2.4.2 Contemporary sensitive cardiac troponin I assay

Plasma cardiac troponin concentrations were measured using a contemporary sensitive cardiac troponin I assay (ARCHITECT$_{STAT,}$ Abbott Laboratories, Abbott Park, IL), in patients recruited into the myocardial injury cohort ***(Chapter 6)***. This assay has

previously been validated at our institution. According to the manufacturer, the limit of detection is 0.010 μg/L and the upper reference limit (99th centile) as determined in a normal reference population is 0.028 μg/L. However, a diagnostic threshold of ≥0.05 μg/L was implemented in clinical practice as this was the minimum concentration where the coefficient of variation was <10% under local laboratory conditions. This diagnostic threshold was based on a standard assessment of precision using pooled serum at low concentration measured across multiple platforms and reagent lots, and was the lowest concentration that consistently provided a coefficient of variation <10%.

2.5 Diagnostic adjudication

In all cohorts, the final diagnoses were classified as per the third universal definition of myocardial infarction. Patients were classified as having a type 1 myocardial infarction when myocardial necrosis occurred in the context of a presentation with suspected acute coronary syndrome with symptoms of myocardial ischemia, or evidence of myocardial ischemia on the electrocardiogram. Patients with symptoms or signs of myocardial ischemia that were thought to be due to increased oxygen demand (e.g. tachyarrhythmia or hypertrophy) or decreased supply (e.g. hypotension, hypoxia or anaemia) and myocardial necrosis in the context of an alternative clinical diagnosis were classified as having a type 2 myocardial infarction. Myocardial injury was defined as evidence of myocardial necrosis in the absence of any symptoms or signs of myocardial ischemia. The diagnosis of type 1 or type 2 myocardial infarction required evidence of a rise and or fall in cardiac troponin concentration, with at least one value above the 99th centile. For the high-sensitivity cardiac troponin I assay

(Chapters 3, 4 and 5), sex-specific thresholds were applied (>16 ng/L for females, >34 ng/L for males), and for the contemporary sensitive cardiac troponin I assay ***(Chapter 6)*** a single threshold was applied (>0.05 μg/L). Each case was reviewed and classified independently by two cardiologists, and any discrepancies were resolved by consensus through in-depth review of source data. The specific cohort level criteria used by studies in ***Chapter 3*** is available in ***Appendix 3***.

Where we evaluate high-sensitivity cardiac troponin I as a risk stratification tool (***Chapter 4 and 5),*** as the adjudicating clinician was not blinded to cardiac troponin results, there is a risk of incorporation bias. However, the adjudication was completed by a panel of cardiologists prior to undertaking this analysis, and we pre-specified a target negative predictive value rather than a cardiac troponin threshold. Whilst an alternative troponin assay could have been used for adjudication, the agreement between assays is imperfect, and as this could have led to bias against the assay under investigation, this approach was not implemented.

2.6 Statistical analysis

Specific statistical analysis has been described in depth in each chapter, with additional analysis code available in the supplementary appendix. All statistical analyses were performed using R (version 3.2.2).

2.6.1 Evaluation of diagnostic performance

The diagnostic performance of any test can be evaluated using a 2x2 table (***Table 2.1***), illustrating the agreement between the test and the disease state. From this 2x2 table, four key diagnostic metrics may be derived (***Table 2.2***).

Table 2.1. 2x2 table of diagnostic performance

	Condition positive	**Condition negative**
Test positive	True Positive (TP)	False Positive (FP)
Test negative	False Negative (FN)	True Negative (TN)

Table 2.2. Key diagnostic metrics

Sensitivity TP / (TP + FN)	The proportion of patients who have the condition identified by the test
Specificity TN / (TN + FP)	The proportion of patients who do not have the condition identified by the test
Negative predictive value TN / (TN + FN)	The proportion of patients with a negative test who do not have the condition
Positive predictive value TP / (TP + FP)	The proportion of patients with a positive test who have the condition

In the evaluation of cardiac troponin concentrations at presentation for risk stratification in patients with suspected acute coronary syndrome, both sensitivity and negative predictive value have been reported as the primary metric in the literature. Here, a cardiac troponin concentration is not being applied for diagnosis, but for risk stratification in patients in whom the diagnosis is uncertain. NPV is an accepted method to evaluate tests of exclusion, analogous to d-dimer, and is determined in those *without* a diagnosis of myocardial infarction at presentation. Sensitivity is determined in patients *with* a diagnosis of myocardial infarction, including patients >99th centile who are not eligible for risk stratification. We therefore made an *a priori* decision to prioritise negative predictive value over sensitivity in studies evaluating a risk stratification threshold.

As the negative predictive value was expected to be close to 100%, proportions were estimated using a Bayesian approach, sampling from a binomial likelihood with a non-informative Jeffrey's prior (beta distribution with both shape parameters equal to 0.5). This approach has demonstrated good coverage when proportions are close to zero or one (Brown et al., 2001).

Unlike sensitivity and specificity, both negative and positive predictive values are influenced by changes in the prevalence of disease. For example, negative predictive value may be overestimated where the prevalence of disease is low. For this reason, a validation of risk stratification thresholds was undertaken across a range of cohorts in different countries with varying prevalence of myocardial infarction (Chapter 3).

2.6.2 Derivation of clinical risk scores

In ***Chapter 5***, a number of clinical risk scores are evaluated. Using the High-STEACS substudy cohort, the HEART, GRACE, EDACS and TIMI scores were calculated on the basis of prospectively collected data by a trained research nurse at the time of recruitment. Where data were missing for continuous variables (time of symptom onset and creatinine concentration), the median value was imputed. Alternative modelling approaches for handling missing data may have provided a more accurate estimate of the risk score. Derivation of the HEART score was undertaken on a pragmatic basis, with the History aspect of the score based on typical (2) and atypical (1) chest pain. This differs from the original derivation of the HEART score, which was based on high suspicion (2) and slight suspicion (1). It is also noted that this score was not completed by the treating doctor.

CHAPTER 3

THE OPTIMAL RISK STRATIFICATION THRESHOLD OF HIGH-SENSITIVITY CARDIAC TROPONIN I IN PATIENTS WITH SUSPECTED ACUTE CORONARY SYNDROME

Published by

Chapman AR, Lee KK, McAllister DA, Cullen L, Greenslade JH, Parsonage W, Worster A, Kavsak PA, Blankenberg S, Neumann J, Söerensen NA, Westermann D, Buijs MM, Verdel GJE, Pickering JW, Than MP, Twerenbold R, Badertscher P, Sabti Z, Mueller C, Anand A, Adamson P, Strachan FE, Ferry AV, Sandeman D, Gray A, Body R, Keevil B, Carlton E, Greaves K, Korley FK, Metkus TS, Sandoval Y, Apple FS, Newby DE, Shah AS, Mills NL. Association of High-Sensitivity Cardiac Troponin I Concentration With Cardiac Outcomes in Patients With Suspected Acute Coronary Syndrome. *JAMA. 2017;318(19):1913-1924.*

3.1 Summary

Importance

High-sensitivity cardiac troponin I testing is widely used to evaluate patients with suspected acute coronary syndrome. A cardiac troponin concentration <5 ng/L identifies patients at presentation as low risk, but the optimal threshold is uncertain.

Objective

To evaluate the performance of a cardiac troponin I threshold of 5 ng/L at presentation as a risk stratification tool in patients with suspected acute coronary syndrome.

Data Sources

Systematic search of MEDLINE, EMBASE, Cochrane and Web of Science from 1st January 2006 to 18th March 2017.

Study Selection:

Prospective studies measuring high-sensitivity cardiac troponin I concentrations in patients with suspected acute coronary syndrome, where the diagnosis was adjudicated according to the universal definition of myocardial infarction.

Data Extraction and Synthesis

The systematic review identified 19 cohorts. Individual patient-level data was obtained from the corresponding authors of 17 cohorts, with aggregate data from two cohorts. Meta-estimates for primary and secondary outcomes were derived using a binomial-normal random effects model.

Main Outcomes and Measures

The primary outcome was myocardial infarction or cardiac death during the index hospitalization or at 30 days. Performance was evaluated in subgroups and across a range of troponin concentrations (2-16 ng/L) using individual patient data.

Results

Of 11,845 articles identified, 104 underwent full-text review, and 19 cohorts from 9 countries were included. Among 22,457 patients included in the meta-analysis (age 62 [15.5] years; n=9,329 (41.5%) women), the primary outcome occurred in 2,786 (12.4%). Cardiac troponin I concentrations were <5 ng/L at presentation in 11,012 (49%) patients, in whom there were 60 events, giving a NPV of 99.5% (95% confidence interval [CI] 99.3-99.6) for the primary outcome. There were no cardiac deaths at 30 days, and 7 (0.1%) at 1 year, with a NPV of 99.9% (95% CI 99.7 to 99.9%) for cardiac death.

Conclusions and Relevance

Among patients with suspected acute coronary syndrome, a high-sensitivity cardiac troponin I concentration <5 ng/L identified those at low risk of myocardial infarction or cardiac death within 30 days. Further research is needed to understand the clinical utility and cost-effectiveness of this approach to risk stratification.

3.2 Introduction

Chest pain is one of the most common reasons for presentation to hospital worldwide (Makam and Nguyen, 2015). Despite the majority of patients not having myocardial infarction (Goodacre et al., 2005), hospital admission for observation and serial cardiac troponin testing is required in many patients to identify those with and without myocardial infarction (Goodacre et al., 2013). Novel strategies to identify low risk patients at presentation have been proposed in order to reduce hospital admissions, serial testing and resource utilization as well as to improve care for patients (Skinner et al., 2010, Roffi et al., 2016).

High-sensitivity assays are able to quantify cardiac troponin at low concentrations, and provide an opportunity to rule out myocardial infarction at an earlier stage. In a prospective study of consecutive patients with suspected acute coronary syndrome, a risk stratification threshold was defined using a high-sensitivity cardiac troponin I assay. In 4,870 patients, a threshold of <5 ng/L had a negative predictive value (NPV) of 99.6%, misclassifying less than one myocardial infarction for every 200 patients tested (Shah et al., 2015a). This threshold identified more than half of all patients with suspected acute coronary syndrome as low risk, reducing the proportion of patients who require admission for serial testing.

Recent studies have questioned whether <5 ng/L is the optimal threshold to risk stratify patients and have proposed alternative thresholds that may miss fewer patients with myocardial infarction (Carlton et al., 2016, Neumann et al., 2017a, Sandoval et al., 2017a, Goorden et al., 2016). To investigate these concerns, a systematic review of all studies of high-sensitivity cardiac troponin I testing in patients with suspected acute

coronary syndrome was undertaken, and individual patient level data was obtained. Across multiple cohorts with varying prevalence of myocardial infarction, the aim was to evaluate the performance of this threshold, to evaluate other risk stratification thresholds, and to determine the association with other clinical risk characteristics.

3.3 Methods

3.3.1 Search strategy and selection of articles

A systematic search of MEDLINE, EMBASE, Cochrane and Web of Science was performed without language restriction from 1st January 2006 to 18th March 2017 using detailed search terms for: chest pain, acute coronary syndrome, acute myocardial infarction, troponin, high sensitive/sensitivity and emergency department (***Figure 3.1***, ***Appendix 1.1*** for full search strategy). Studies were included if they met the following pre-specified eligibility criteria: 1) prospective studies of patients investigated in the Emergency Department for suspected acute coronary syndrome, 2) cardiac troponin measured using the Abbott ARCHITECT$_{STAT}$ high-sensitive cardiac troponin I assay (Abbott Laboratories, Abbott Park, IL) at presentation, and 3) an adjudicated end-point of myocardial infarction on index hospitalization (***Appendix 1.2 and 1.3***). All findings are reported in accordance with the Preferred Reporting Items for Systematic review and Meta-Analysis of Individual Participant Data (PRISMA-IPD) (Stewart et al., 2015).

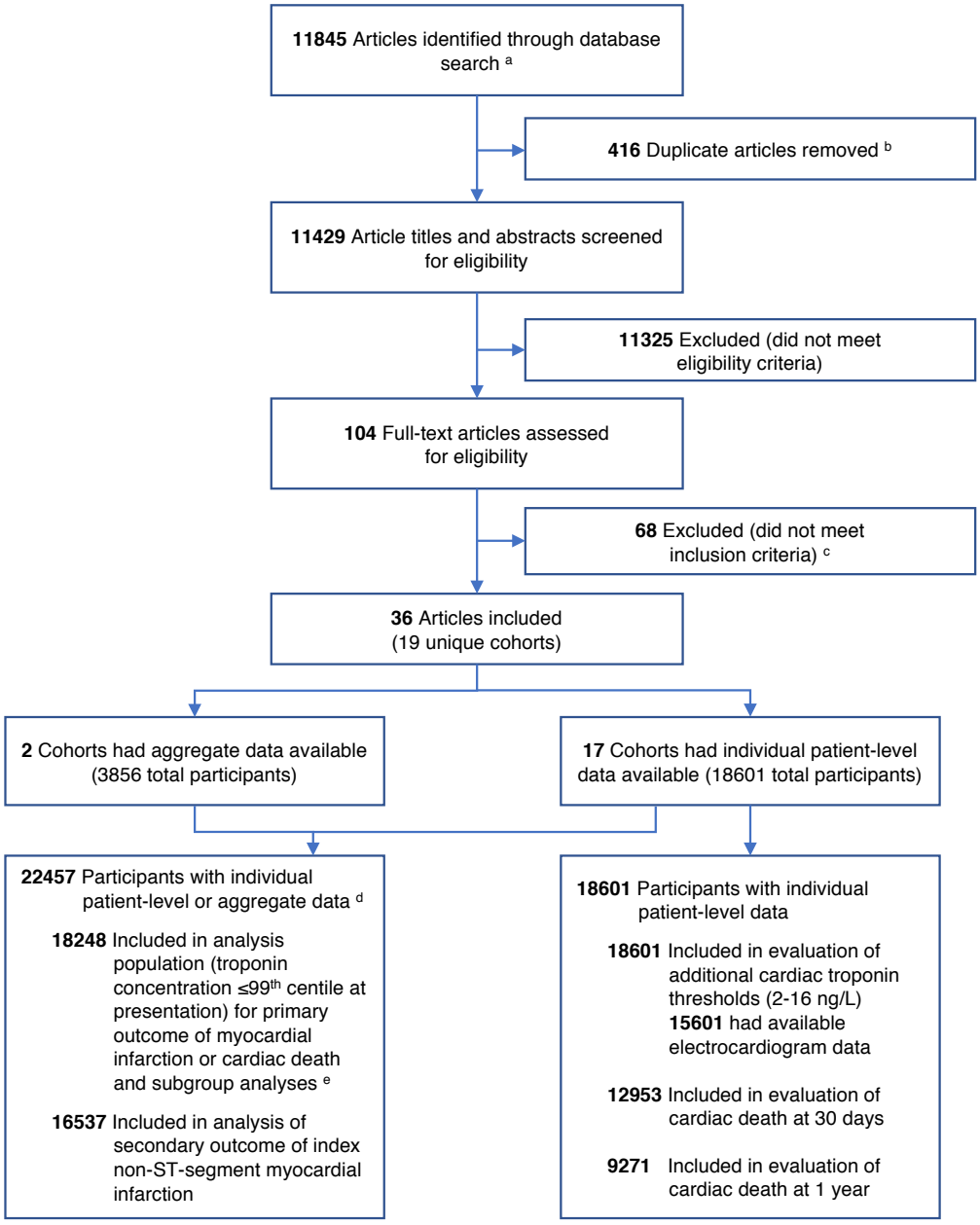
3.3.2 Data extraction

Two investigators (AC, KL) performed the initial screening of titles and abstracts. Full text reports of potentially relevant articles were obtained and assessed by both investigators using our pre-specified protocol (PROSPERO register - CRD42017059128). A third investigator adjudicated all disagreements (AS). Where there were multiple articles from the same cohort, the article that included the largest number of participants was included. The corresponding authors of each eligible cohort were contacted with a request for anonymized data including cardiac troponin

concentrations, adjudicated diagnosis, outcomes and pre-specified covariates (age, sex, chest pain, time from symptom onset to presentation sample, myocardial ischemia on the electrocardiogram, cigarette smoking, diabetes mellitus, hypertension, hyperlipidemia, known angina, previous myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, or stroke). All studies were prospective and conducted in accordance with the Declaration of Helsinki with approval from the regional ethics committee or institutional review board, and written consent obtained where required. This approval permitted each contributor to share individual-level data or aggregate data for inclusion in this meta-analysis. Bias was assessed by two investigators independently, with consensus from a third, using the Quality Assessment of Diagnostic Accuracy Studies version 2 (QUADAS-2) framework (***Appendix 1.4***).

3.3.3 Analysis population and primary outcome

The analysis population comprised patients with cardiac troponin concentrations $\leq 99^{th}$ percentile at presentation, as those $>99^{th}$ percentile have evidence of myocardial injury and are not eligible for risk stratification at presentation. Patients with ST-segment elevation myocardial infarction and those who presented in cardiac arrest were excluded from this analysis. The pre-specified primary outcome was a composite of type 1 myocardial infarction or cardiac death at 30 days. The pre-specified secondary outcomes were recurrent myocardial infarction and cardiac death at 1 year. In addition, we evaluated the performance of cardiac troponin thresholds for the diagnosis of type 1 or type 2 myocardial infarction on index presentation. The number of patients available for each analysis is illustrated in ***Figure 3.1***.



Flow diagram illustrating the systematic database review and screening of articles, level of exclusion, the number of articles included, and the individual patient-level data or aggregate data available for each analysis, based on the PRISMA-IPD guidelines.

a Articles identified through a systematic database search: MEDLINE = 2078; EMBASE = 7116; Cochrane = 390;Web of Science = 2261.

b Any identical publications were removed, but articles from the same cohorts were retained at this stage.

c Articles excluded after full-text review because they evaluated a contemporary cardiac troponin I assay (n = 36), a different high-sensitivity cardiac troponin I assay (n = 14), a high-sensitivity cardiac troponin T assay (n = 5), a different patient population (n = 4), or a different outcome measure (n = 9).

d Authors who did not provide individual patient-level data provided aggregate data for the primary outcome, subgroup analyses, and secondary outcome when available.

e Subgroup analyses were prespecified, with the following data available per group: age (n = 18 248), sex (n = 18 248), diagnosis of ischemic heart disease (n = 14 160), time from symptom onset to troponin sample time (n = 13 404), and electrocardiogram (n = 15 887).

Figure 3.1 Flow diagram of the study population and data analysis

3.3.4 Statistical analysis

Baseline characteristics are summarised as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate. The primary outcome measure was the NPV of a high-sensitivity cardiac troponin I concentration <5 ng/L at presentation. All cardiac troponin concentrations were rounded to integer values in line with clinical standards for reporting. Where individual patient level data were available, this was checked for consistency and completeness, and cohort level summary counts of patients with and without the primary outcome were derived for high-sensitivity cardiac troponin I concentration <5 ng/L at presentation. In the cohorts where raw data were not available, the corresponding authors were asked to provide these summaries. The NPV was calculated at a cohort level using a Bayesian approach, with a binomial likelihood and beta prior (a non-informative Jeffrey's prior with both shape parameters equal to 0.5), as this produces confidence intervals with better coverage when proportions are close to 0 or 1 (Brown et al., 2001). Heterogeneity is reported using the I^2 statistic (Higgins et al., 2003). Survival free from cardiac death at 30 days and one year was reported for patients with cardiac troponin I concentrations <5 ng/L, 5-99th percentile and >99th percentile at presentation.

3.3.5 Pre-specified subgroup analyses

For the primary outcome, the NPV was evaluated in pre-specified subgroups stratified by age (≤65 or >65 years), sex, history of ischaemic heart disease, time of symptom onset (≤2 or >2 hours) and presence of myocardial ischaemia on the electrocardiogram. Most cohorts defined myocardial ischaemia as ≥2 mm ST-segment depression in two consecutive leads, or new T-wave inversion. To explore the clinical implications of differences in performance between subgroups, we undertook these subgroup analyses

in patients without myocardial ischaemia on the electrocardiogram. Studies have demonstrated imperfect calibration between high-sensitivity cardiac troponin I and T assays, with up to 17.5% of patients >99th percentile on the T assay shown to be <99th percentile on the I assay (Wildi et al., 2015). Therefore, a further analysis evaluated whether the assay used to adjudicate the index diagnosis affected the performance of the risk stratification threshold. In addition, we determined whether the assessed risk of bias and site of patient recruitment affected the NPV.

3.3.6 Derivation of meta-estimates

Meta-estimates of the NPV were derived in the analysis population for all primary and secondary outcomes by modelling cohort level proportions *(true negative / [true negative + false negative])* in a binomial-normal random effects model, with an additional term where cohort-level characteristics (adjudication assay, assessment of bias or location of recruitment) were compared. We estimated odds ratios for the difference in NPV between pre-specified sub-groups, meta-analysing this across cohorts to obtain the mean odds ratio and a P-value for the null hypothesis of no association. For cohorts where individual patient level data were available, the cardiac troponin threshold which would identify the highest proportion of patients as low-risk for a NPV ≥99.5% was determined. For this analysis, we pre-specified a NPV of 99.5% as being clinically acceptable and equivalent to a miss rate of 5 per 1,000 low-risk patients (Than et al., 2013). To evaluate how the inclusion of a risk stratification threshold would affect the overall diagnosis in all patients with suspected acute coronary syndrome, meta-estimates of NPV, positive predictive value (PPV) and sensitivity were derived for risk stratification thresholds alone (2-16 ng/L), and in conjunction with a non-ischaemic electrocardiogram at presentation. At each threshold,

the proportion of the total population classified as low-risk and the miss rate per 1,000 patients was reported. All analyses were performed in R (Version 3.2.2), with the meta-analyses performed using the *Metafor* package (Viechtbauer, 2010). The analysis code is available in ***Appendix 1.5.***

3.4 Results

3.4.1 Systematic-review

The initial search identified 11,845 articles, of which 104 articles underwent full text review. A total of 36 articles met inclusion criteria, reporting observations from 19 individual cohorts across nine different countries ***(Figure 3.1)***. Five articles reported outcomes for a high-sensitivity cardiac troponin I concentration of <5 ng/L (Shah et al., 2015a, Carlton et al., 2016, Neumann et al., 2017a, Sandoval et al., 2017a, Shortt et al., 2017).

3.4.2 Study population

All corresponding authors from the 19 individual cohorts identified in the systematic review agreed to provide data for the meta-analysis. Individual patient-level data was obtained from 17 cohorts (Shah et al., 2015a, Chapman et al., 2017b, Carlton et al., 2016, Neumann et al., 2017a, Goorden et al., 2016, Shortt et al., 2017, Shah et al., 2015b, Pickering et al., 2016a, Keller et al., 2011, Than et al., 2012, Cullen et al., 2017a, Korley et al., 2014, Parsonage et al., 2016, Than et al., 2014a, Kavsak et al., 2013b, Body et al., 2014, Body et al., 2017), and aggregate data from two cohorts (Sandoval et al., 2017a, Wildi et al., 2016), for a total study population of 22,457 patients with suspected acute coronary syndrome (age 62 (16) years, 58.5% male; ***Table 3.1***).

In 11 cohorts, data was available for the pre-specified primary outcome of type 1 myocardial infarction or cardiac death at 30 days (***Table 3.2***). In the remainder, the outcome was index type 1 myocardial infarction (n=1) or non-ST-segment elevation myocardial infarction on index presentation (n=5) or at 30 days (n=2). The assessed

risk of bias was high in 11 cohorts due to patient selection or use of a contemporary reference standard (***Appendix 1.3 and 1.4***). Across all cohorts, the proportion with the primary outcome was 12.4% (range 2.4% to 24.0%). The analysis population comprised 18,248 of 22,457 patients where high-sensitivity cardiac troponin I concentrations were <99th percentile at presentation, and the prevalence of the primary outcome was 3.5% (range 0.6% to 6.1%).

Table 3.1. Baseline characteristics of study patients stratified by cohort

	ALL PATIENTS	**High-STEACS-V** (Shah et al., 2015a)	**UTROPIA** (Sandoval et al., 2017a)	**High-STEACS-P** (Shah et al., 2015b)	**High-STEACS-S** (Chapman et al., 2017b)	**EDACS** (Pickering et al., 2016a)
Adjudication assay		***High-sensitivity cardiac troponin I cohorts***				
Number of patients	22457	4701	1630	1064	756[b]	558
Age	62 (15.5)[a]	63.7 (16.3)	57.5 (15.3)	65.6 (15.9)	62 (14.2)	59.2 (11.9)
Male (%)	13128 (58.5)	2651 (56.4)	911 (55.9)	579 (54.4)	462 (61.1)	340 (60.9)
Presentation						
Chest pain (%)	16760 (80.2)	3917 (83.3)	835 (51.2)	880 (82.9)	651 (86.1)	558 (100)
Time from symptom onset to troponin sample (mins)	355 (172-794)	454 (255-814)	352 (114-590)	-	244 (146-644)	210 (115-501)
Myocardial ischemia on ECG (%)	3663 (18.8)	795 (19.5)	126 (7.7)	326 (31.6)	84 (12.3)	25 (4.5)
Cardiovascular risk factors						
Hypertension (%)	11018 (54.3)	1376 (33.3)	1074 (65.9)	570 (53.6)	327 (45.0)	290 (52)
Hyperlipidaemia (%)	9270 (45.7)	1113 (27.0)	696 (42.7)	484 (45.65	291 (40.3)	284 (50.9)
Smoker (%)	6093 (32.6)	842 (32.1)	592 (36.3)	255 (26.2)	149 (20.3)	84 (15.1)
Diabetes mellitus (%)	3703 (18.3)	661 (16.0)	505 (31.0)	173 (16.2)	115 (15.6)	78 (14)
Known angina (%)	4299 (28.7)	1379 (33.3)	264 (16.2)	451 (42.5)	220 (29.8)	139 (24.9)
Previous MI (%)	4319 (21.3)	785 (19.0)	190 (11.7)	284 (26.7)	161 (21.9)	130 (23.3)
Previous PCI (%)	2521 (15.6)	439 (10.6)	150 (9.2)	162 (15.2)	132 (18.1)	-
Previous CABG (%)	1536 (8.4)	242 (5.9)	73 (4.5)	83 (7.8)	37 (5.1)	26 (4.7)
Previous stroke (%)	1603 (8.1)	333 (8.1)	153 (9.4)	136 (12.8)	40 (5.6)	-
High-sensitivity cardiac troponin at presentation						
≤ 99th percentile (%)	18248 (81.3)	3781 (80.4)	1326 (81.3)	828 (77.8)	617 (81.6)	494 (88.5)
> 99th percentile (%)	4209 (18.7)	920 (19.6)	304 (18.7)	236 (22.1)	139 (18.4)	64 (11.5)

Values are number (%) or mean (SD) or median (inter-quartile range). % as a proportion of data available. (-) Data not available.
[a] Summary estimates for age and sample time exclude APACE and UTROPIA as only aggregate data available.
[b] Only unique patients from High-STEACS sub-study cohort are included
Abbreviations: ECG = electrocardiogram; MI = myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery bypass grafting.

Table 3.1. continued. Baseline characteristics of study patients stratified by cohort

	Stenocardia (Keller et al., 2011)	**ADAPT-B** (Than et al., 2012)	**IMPACT** (Cullen et al., 2017a)	**ROMI** (Shortt et al., 2017)	**HOPKINS** (Korley et al., 2014)	**ADAPT-C** (Parsonage et al., 2016)	**ADAPT-RCT** (Than et al., 2014a)	**RING** (Kavsak et al., 2013b)
Contemporary cardiac troponin I and T cohorts								
Number of patients	1598	804	1127	1137	808	1106	474	144
Age	61.3 (13.6)	55.2 (15.2)	51.2 (12.6)	66.7 (16.5)	56.6 (13.3)	65.3 (13.0)	60.7 (12.6)	59.7 (13.7)
Male (%)	1046 (65.5)	482 (60.0)	676 (60.0)	535 (47.1)	381 (47.2)	659 (59.6)	297 (62.7)	93 (64.6)
Presentation								
Chest pain (%)	833 (52.1)	690 (85.8)	844 (74.9)	651 (57.3)	479 (59.3)	1106 (100)	474 (100)	134 (93.1)
Time from symptom onset to troponin sample (mins)	295 (150-833)	330 (130-1275)	216 (110-676)	-	669 (348-750)	390 (210-785)	300 (180-525)	210 (140-275)
Myocardial ischemia on ECG (%)	855 (54.1)	51 (6.3)	36 (3.2)	-	-	188 (17.0)	21 (4.4)	-
Cardiovascular risk factors								
Hypertension (%)	1190 (74.5)	403 (50.1)	447 (39.7)	804 (71.2)	509 (63)	679 (61.4)	214 (45.1)	92 (64.3)
Hyperlipidaemia (%)	1178 (73.7)	386 (48)	427 (37.9)	676 (60.6)	340 (42.1)	636 (57.5)	243 (51.3)	79 (56.4)
Smoker (%)	362 (22.8)	188 (23.4)	276 (24.5)	700 (61.6)	290 (35.9)	161 (14.6)	85 (17.9)	95 (66.4)
Diabetes mellitus (%)	246 (15.7)	107 (13.3)	141 (12.5)	333 (29.7)	240 (29.7)	178 (16.1)	70 (14.8)	36 (25.9)
Known angina (%)	-	188 (23.4)	125 (11.1)	305 (27.5)	168 (20.8)	527 (47.6)	100 (21.1)	60 (41.7)
Previous MI (%)	363 (23.2)	138 (17.2)	130 (11.5)	408 (36.6)	153 (18.9)	334 (30.2)	121 (25.5)	52 (36.4)
Previous PCI (%)	335 (25.8)	87 (10.8)	85 (7.5)	251 (22.4)	112 (13.9)	-	-	-
Previous CABG (%)	165 (14.7)	55 (6.8)	44 (3.9)	251 (22.4)	61 (7.5)	122 (11.0)	37 (7.8)	46 (32.2)
Previous stroke (%)	87 (5.5)	74 (9.2)	46 (4.1)	190 (17.0)	117 (14.5)	65 (5.9)	47 (9.9)	11 (7.7)
High-sensitivity cardiac troponin at presentation								
≤ 99th percentile (%)	1193 (74.7)	720 (89.6)	1083 (96.1)	915 (80.5)	636 (78.7)	838 (75.8)	400 (84.4)	122 (84.7)
> 99th percentile (%)	405 (25.3)	84 (10.4)	44 (3.9)	222 (19.5)	172 (21.3)	268 (24.2)	74 (15.6)	22 (15.3)

Values are number (%) or mean (SD) or median (inter-quartile range). % as a proportion of data available. (-) Data not available.
Abbreviations: ECG = electrocardiogram; MI = myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery bypass grafting.

Table 3.1. continued. Baseline characteristics of study patients stratified by cohort

	TI-AMO (Goorden et al., 2016)	**APACE** (Wildi et al., 2016)	**BACC** (Neumann et al., 2017a)	**TRUST** (Carlton et al., 2016)	**Stockport** (Body et al., 2014)	**Manchester** (Body et al., 2017)
High-sensitivity cardiac troponin T cohorts						
Number of patients	1552	2226	1496	867	229	180
Age	67.2 (16.0)	62 (16.0)	62.6 (15.7)	57.9 (13.1)	65.4 (15.6)	57.2 (14.5)
Male (%)	781 (50.3)	1512 (67.9)	955 (63.8)	515 (59.4)	137 (59.8)	116 (64.4)
Presentation						
Chest pain (%)	-	2226 (100)	1206 (80.7)	867 (100)	229 (100)	180 (100.0)
Time from symptom onset to troponin sample (mins)	-	300 (120-720)	-	179 (119-349)	189 (96-513)[c]	197 (84-333)[c]
Myocardial ischemia on ECG (%)	156 (10.5)	476 (21.4)	430 (29.4)	0 (0)	48 (21.0)	46 (25.6)
Cardiovascular risk factors						
Hypertension (%)	-	1383 (62.1)	1015 (68.2)	477 (55.0)	93 (40.6)	75 (41.9)
Hyperlipidaemia (%)	-	1111 (49.9)	592 (39.6)	583 (67.2)	91 (39.7)	60 (33.3)
Smoker (%)	-	1370 (61.5)	352 (23.6)	210 (24.2)	36 (15.7)	46 (26.9)
Diabetes mellitus (%)	-	405 (18.2)	201 (13.6)	145 (16.7)	42 (18.3)	27 (15.0)
Known angina (%)	-	-	-	223 (25.7)	97 (42.4)	53 (29.6)
Previous MI (%)	-	514 (23.1)	240 (16.1)	190 (21.9)	78 (34.1)	48 (27.0)
Previous PCI (%)	-	527 (23.7)	-	168 (19.4)	34 (14.8)	39 (21.8)
Previous CABG (%)	-	211 (9.5)	-	41 (4.7)	30 (13.1)	12 (6.8)
Previous stroke (%)	-	122 (5.5)	102 (6.8)	57 (6.6)	20 (8.7)[d]	3 (1.7)[d]
High-sensitivity cardiac troponin at presentation						
≤ 99th percentile (%)	1156 (74.5)	1801 (80.9)	1202 (80.3)	810 (93.4)	179 (78.2)	147 (81.7)
> 99th percentile (%)	396 (25.5)	425 (19.1)	294 (19.7)	57 (6.6)	50 (21.8)	33 (18.3)

Values are number (%) or mean (SD) or median (inter-quartile range). % as a proportion of data available. (-) Data not available.
[c] Only symptom to presentation time available. [d] Includes patients with transient ischaemic attack.
Abbreviations: ECG = electrocardiogram; MI = myocardial infarction; PCI = percutaneous coronary intervention; CABG = coronary artery bypass grafting.

Table 3.2. Summary of cohort endpoints and prevalence of myocardial infarction

	Primary outcome and prevalence, n (%)			Assay used for Adjudication of Myocardial Infarction	NPV (%) (95% CI)	hs-cTnI <5 ng/L at presentation[a] n (%)
		All patients[a]	*≤99th percentile*[b]			
HighSTEACS-V	Type 1 MI or cardiac death (30d)	662 (14.1)	141 (3.7)	Abbott hs-cTnI	99.6 (99.3-99.8)	2292 (48.8)
UTROPIA	Type 1 MI or cardiac death (30d)	70 (4.3)	22 (1.4)	Abbott hs-cTnI	99.5 (99.0-99.9)	774 (47.5)
HighSTEACS-P	Type 1 MI or cardiac death (30d)	201 (18.9)	46 (5.6)	Abbott hs-cTnI	99.7 (99.0-100)	469 (44.1)
HighSTEACS-S	Type 1 MI or cardiac death (30d)	115 (15.2)	25 (4.1)	Abbott hs-cTnI	99.4 (98.5-99.9)	428 (56.6)
EDACS	Type 1 MI or cardiac death (30d)	66 (11.8)	17 (3.4)	Abbott hs-cTnI	99.1 (97.9-99.8)	378 (67.7)
STENOCARDIA	Index NSTEMI	268 (16.8)	29 (2.4)	Roche cTnT	99.9 (99.7-100)	563 (35.2)
ADAPT-B	Type 1 MI or cardiac death (30d)	48 (6.0)	8 (1.1)	Beckmann Accu-cTnI	99.7 (99.1-100)	532 (66.2)
IMPACT	Type 1 MI or cardiac death (30d)	49 (4.3)	26 (2.4)	Beckmann Accu-cTnI	99.5 (99.0-99.9)	923 (81.9)
ROMI	Index NSTEMI	133 (11.7)	40 (4.4)	Abbott cTnI	99.1 (98.1-99.7)	503 (44.2)
HOPKINS	Index Type 1 MI	19 (2.4)	4 (0.6)	Abbott cTnI	99.4 (98.3-100)	266 (32.9)
ADAPT-C	Type 1 MI or cardiac death (30d)	265 (24.0)	42 (5.0)	Abbott cTnI	99.1 (98.0-99.7)	475 (42.9)
ADAPT-RCT	Type 1 MI or cardiac death (30d)	75 (15.8)	20 (5.0)	Abbott cTnI	99.3 (98.0-100)	228 (48.1)
RING	Index NSTEMI	9 (6.2)	1 (0.8)	Roche c-TnT	99.4 (97.8-100)	88 (61.1)
TI-AMO	Index NSTEMI	90 (5.8)	18 (1.6)	Roche hs-cTnT	99.8 (99.2-100)	613 (39.5)
APACE	Index NSTEMI	399 (17.9)	117 (6.1)	Roche hs-cTnT	99.2 (98.6-99.7)	1801 (49.8)
BACC	Type 1 MI or cardiac death (30d)	181 (12.0)	47 (3.9)	Roche hs-cTnT	98.9 (97.8-99.6)	567 (37.9)
TRUST	Type 1 MI or cardiac death (30d)	66 (7.6)	28 (3.5)	Roche hs-cTnT	98.3 (97.2-99.1)	664 (76.6)
STOCKPORT	NSTEMI (30 days)	43 (18.8)	9 (5.0)	Roche hs-cTnT	99.0 (96.1-100)	48 (21.0)
MANCHESTER	NSTEMI (30 days)	27 (15.0)	5 (3.4)	Roche hs-cTnT	98.4 (95.1-99.9)	93 (51.7)
SUMMARY		2786 (12.4)	645 (3.5)		99.5 (99.3-99.6)	11012 (49.0)

[a]Proportion of total cohort size [b] Proportion of patients <99th centile. MI = Myocardial Infarction, NSTEMI = Non-ST segment Elevation Myocardial Infarction, hs-cTnI = high-sensitivity cardiac troponin I, cTnT = cardiac troponin T, cTnI = cardiac troponin I, hs-cTnT= high-sensitivity cardiac troponin T, 30d = 30 days.

3.4.3 Meta-estimate of the risk stratification threshold

High-sensitivity cardiac troponin I concentrations were <5 ng/L at presentation in 11,012 (49%) patients, with a NPV of 99.5% (95% confidence intervals [CI] 99.3 to 99.6%; ***Figure 3.2, Table 3.2***) for the primary outcome, and a total of 60 missed index or 30-day events (59 index myocardial infarction, one myocardial infarction at 30 days, and no cardiac deaths at 30 days; ***Appendix 1.6***). The NPV was similar across cohorts with varying prevalence of myocardial infarction. The estimate of heterogeneity (I^2) was 31.9%. Cohort level two-by-two summary tables are provided for the analysis population in ***Table 3.3***. Where data were available in the analysis population (n=16,537, 90.6%), we estimated the NPV for the secondary outcome of index non-ST-segment elevation myocardial infarction (type 1 or type 2 myocardial infarction). Cardiac troponin I concentrations were <5 ng/L at presentation in 9,574 (48%) patients, with a NPV of 99.4% (95% CI 99.2 to 99.6%), and a total of 58 missed events.

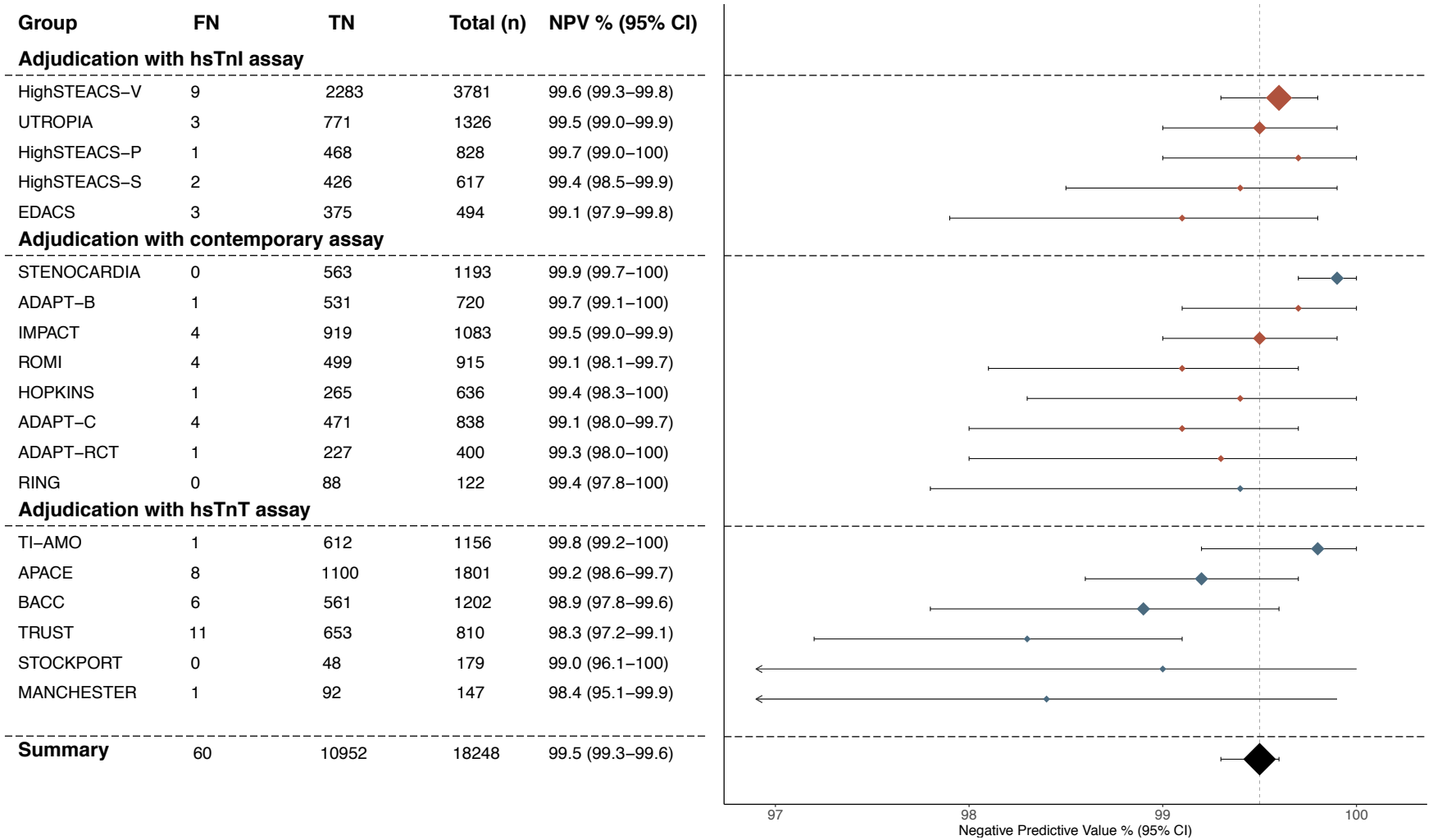


Figure 3.2. Negative predictive value of a cardiac troponin I concentration <5 ng/L at presentation by cohort for myocardial infarction or cardiac death at 30 days.

Diamonds represent the central estimate of NPV with colour corresponding to the assay used to adjudicate myocardial infarction (red for cardiac troponin I, blue for cardiac troponin T), size corresponding to the number of patients per cohort (large diamond >3,000 patients, medium diamond ≥1,000 patients, small diamond <1,000 patients), and whiskers representing 95% confidence intervals. Dotted vertical line represents central estimate of NPV at 99.5%. TN = true negative. FN = false negative. Population (n) = number of patients per cohort <99th percentile at presentation.

Table 3.3. Diagnostic performance of a high-sensitivity cardiac troponin I concentration <5 ng/L for myocardial infarction or cardiac death at 30 days

Cohort	TP	FP	TN	FN	NPV % (95% CI)
HighSTEACS-V	132	1357	2283	9	99.6 (99.3-99.8)
UTROPIA	19	533	771	3	99.5 (99.0-99.9)
HighSTEACS-P	45	314	468	1	99.7 (99.0-100)
HighSTEACS-S	23	166	426	2	99.4 (98.5-99.9)
EDACS	14	102	375	3	99.1 (97.9-99.8)
STENOCARDIA	29	601	563	0	99.9 (99.7-100)
ADAPT-B	7	181	531	1	99.7 (99.1-100)
IMPACT	22	138	919	4	99.5 (99.0-99.9)
ROMI	36	376	499	4	99.1 (98.1-99.7)
HOPKINS	3	367	265	1	99.4 (98.3-100)
ADAPT-C	38	325	471	4	99.1 (98.0-99.7)
ADAPT-RCT	19	153	227	1	99.3 (98.0-100)
RING	1	33	88	0	99.4 (97.8-100)
TI-AMO	17	526	612	1	99.8 (99.2-100)
APACE	109	584	1100	8	99.2 (98.6-99.7)
BACC	41	594	561	6	98.9 (97.8-99.6)
TRUST	17	129	653	11	98.3 (97.2-99.1)
STOCKPORT	9	122	48	0	99.0 (96.1-100)
MANCHESTER	4	50	92	1	98.4 (95.1-99.9)

Abbreviations: TP = true positives; FP = false positives; TN = true negatives; FN = false negatives; NPV = negative predictive value; 95% CI = confidence intervals. Above table refers to population <99th centile at presentation (n=18,248).

3.4.4 Subgroup analysis

Meta-estimates of NPV were obtained in a number of pre-specified subgroups (***Figure 3.3***). The NPV was lower in those with myocardial ischaemia on the electrocardiogram (98.2%, 95% CI 96.4 to 99.1%, n = 2,178) compared to those without (99.7%, 95% CI 99.4 to 99.8%, n = 13,709, P < 0.001), and in those who presented within two hours of symptom onset (99.0%, 95% CI 97.7 to 99.5%, n = 2,303, *versus* 99.6%, 95% CI 99.4 to 99.8%, n = 11,101, P = 0.003). Differences in the NPV were also observed between patients >65 years (NPV 99.1%, 95% CI 98.5 to 99.5%, n = 6,818), compared to those ≤65 years (99.6%, 95% CI 99.4 to 99.8%, n = 11,430, P = 0.02) and in those with a history of ischaemic heart disease (NPV 98.8%, 95% CI 98.1 to 99.3%, n = 3,990) compared to those without (NPV 99.6%, 95% CI 99.4 to 99.7%, n = 10,170, P = 0.03).

Group	FN	TN	Total (n)	NPV (95% CI)	P Value
Age > 65 years	26	2571	6818	99.1 (98.5–99.5)	
					P = 0.02
Age ≤ 65 years	34	8381	11430	99.6 (99.4–99.8)	
Men	38	6043	10920	99.4 (99.1–99.6)	
					P = 0.48
Women	22	4909	7328	99.6 (99.3–99.8)	
Known IHD [a]	22	1834	3990	98.8 (98.1–99.3)	
					P = 0.03
No Known IHD	31	7035	10170	99.6 (99.4–99.7)	
≤ 2hr symptoms [b]	20	1540	2303	99 (97.7–99.5)	
					P = 0.003
> 2hr symptoms	26	6851	11101	99.6 (99.4–99.8)	
Myocardial ischaemia on ECG [c]	15	818	2178	98.2 (96.4–99.1)	
					P < 0.001
No ischaemia on ECG	39	8899	13709	99.7 (99.4–99.8)	

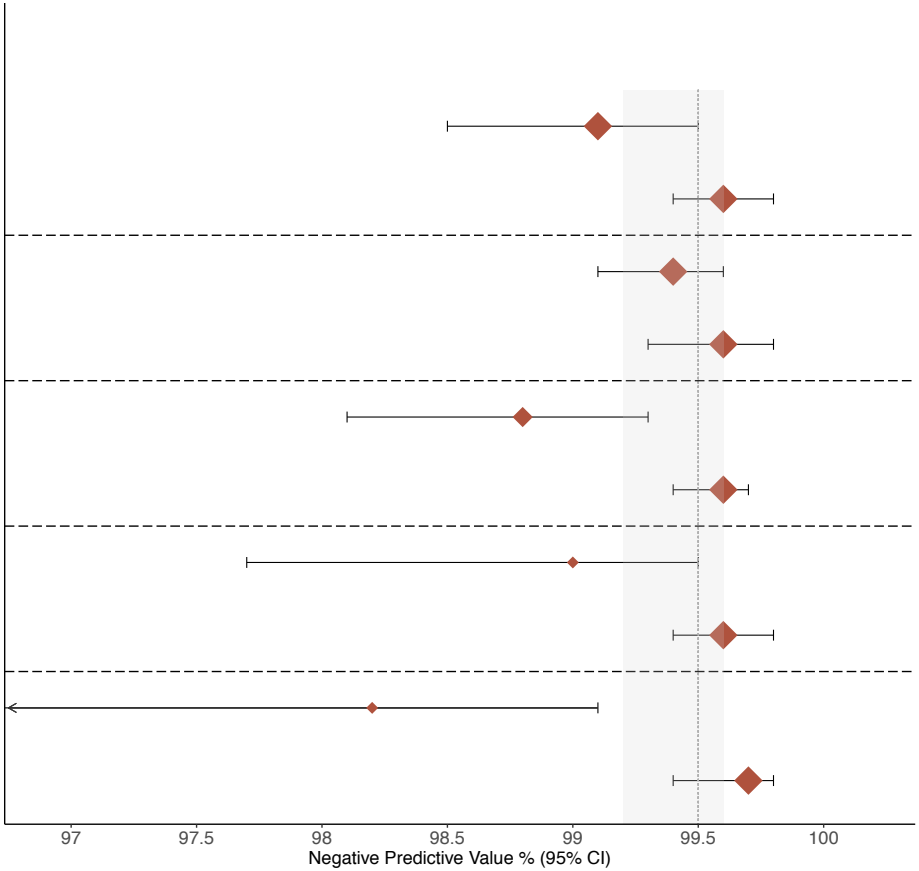


Figure 3.3. Negative predictive value of a cardiac troponin I concentration <5 ng/L at presentation for myocardial infarction or cardiac death at 30 days in pre-specified subgroups

Diamonds represent the central estimate of NPV with size corresponding to the number of patients per cohort (large diamond >3,000 patients, medium diamond ≥1,000 patients, small diamond <1,000 patients), and whiskers representing 95% confidence intervals. The vertical dashed line and shaded areas represent the central estimate and 95% confidence intervals for the full analysis population. TN = true negative. FN = false negative. IHD = ischemic heart disease. All 19 cohorts included unless otherwise specified. a) IHD status available in 16 of 19 cohorts, b) time of symptom onset available in 15 of 19 cohorts, c) electrocardiogram available in 15 of 19 cohorts.

When this analysis was restricted to patients without myocardial ischaemia on the electrocardiogram, estimates of NPV were >99% for all subgroups (***Figure 3.4***). Performance of the risk stratification threshold was similar regardless of the assay used for adjudication (high-sensitivity cardiac troponin I, NPV 99.6%, 95% CI 99.3 to 99.7, n = 7,046; contemporary cardiac troponin I or T, NPV 99.6%, 95% CI 99.3 to 99.7, n = 5,907; high-sensitivity cardiac troponin T, NPV 99.2%, 95% CI 98.6 to 99.6%, n = 5,295, P = 0.27), the assessed risk of bias (high, NPV 99.5%, 95% CI 99.1 to 99.7%, n = 7,043; low, NPV 99.3%, 95% CI 99.3 to 99.6%, n = 11,205, P = 0.37) and the site of patient recruitment (Europe, NPV 99.5%, 95% CI 99.1 to 99.7, n = 11,714; North America, NPV 99.5%, 95% CI 99.0 to 99.8%, n = 2,999 and Asia-Pacific, NPV 99.5%, 95% CI 99.1 to 99.7%, n = 3,535, P = 0.30, ***Figure 3.5***).

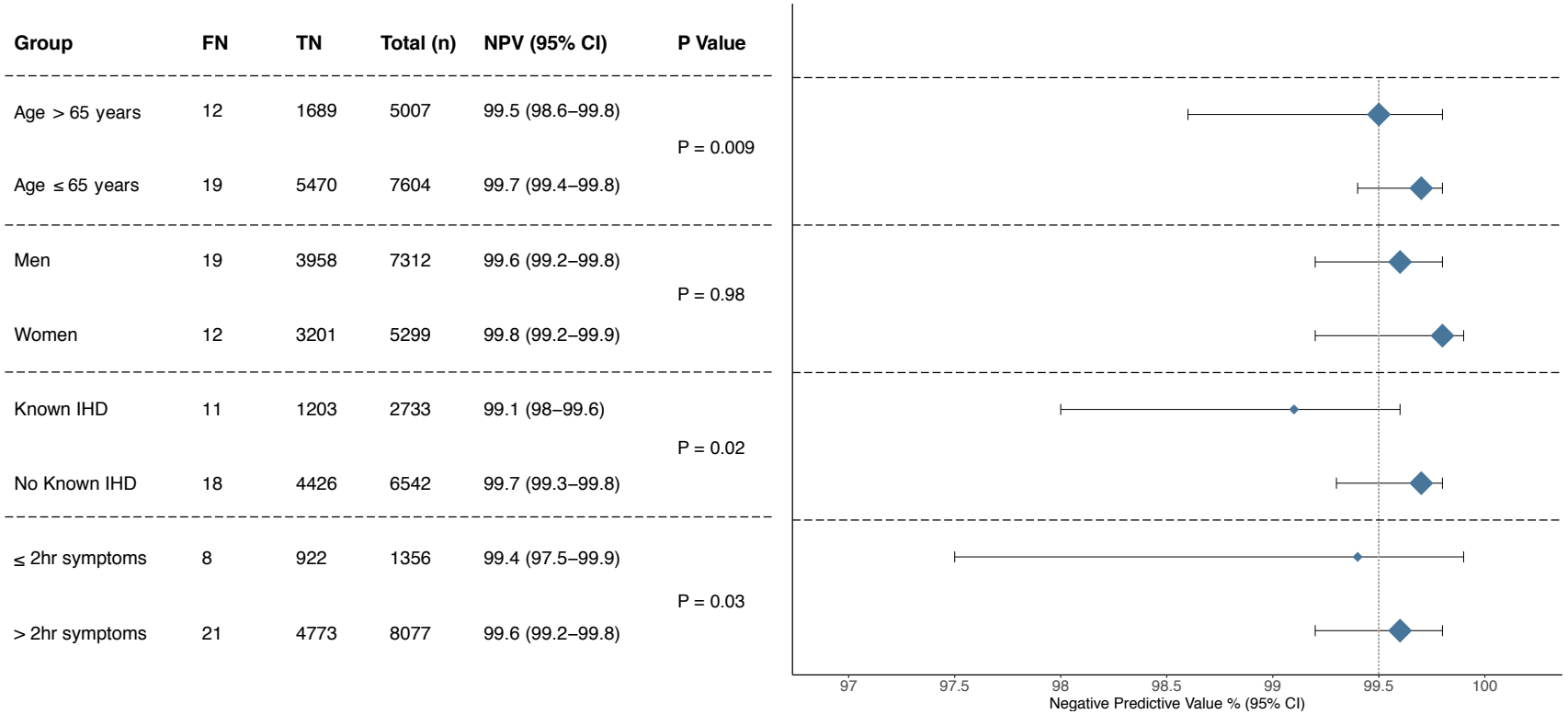


Figure 3.4. Negative predictive value of a cardiac troponin I concentration <5 ng/L at presentation for myocardial infarction or cardiac death at 30 days in patients without myocardial ischaemia on the electrocardiogram by pre-specified subgroups

Diamonds represent the central estimate of NPV with size corresponding to the number of patients per cohort (large diamond >3,000 patients, medium diamond ≥1,000 patients, small diamond <1,000 patients), and whiskers representing 95% confidence intervals. Data obtained from the following cohorts where individual patient-level data (including ECG status) available: HighSTEACS-V, HighSTEACS-S, HighSTEACS-P, ADAPT-B, IMPACT, EDACS, ADAPT-RCT, ADAPT-C, BACC, TIAMO, TRUST, STOCKPORT, MANCHESTER and STENOCARDIA.

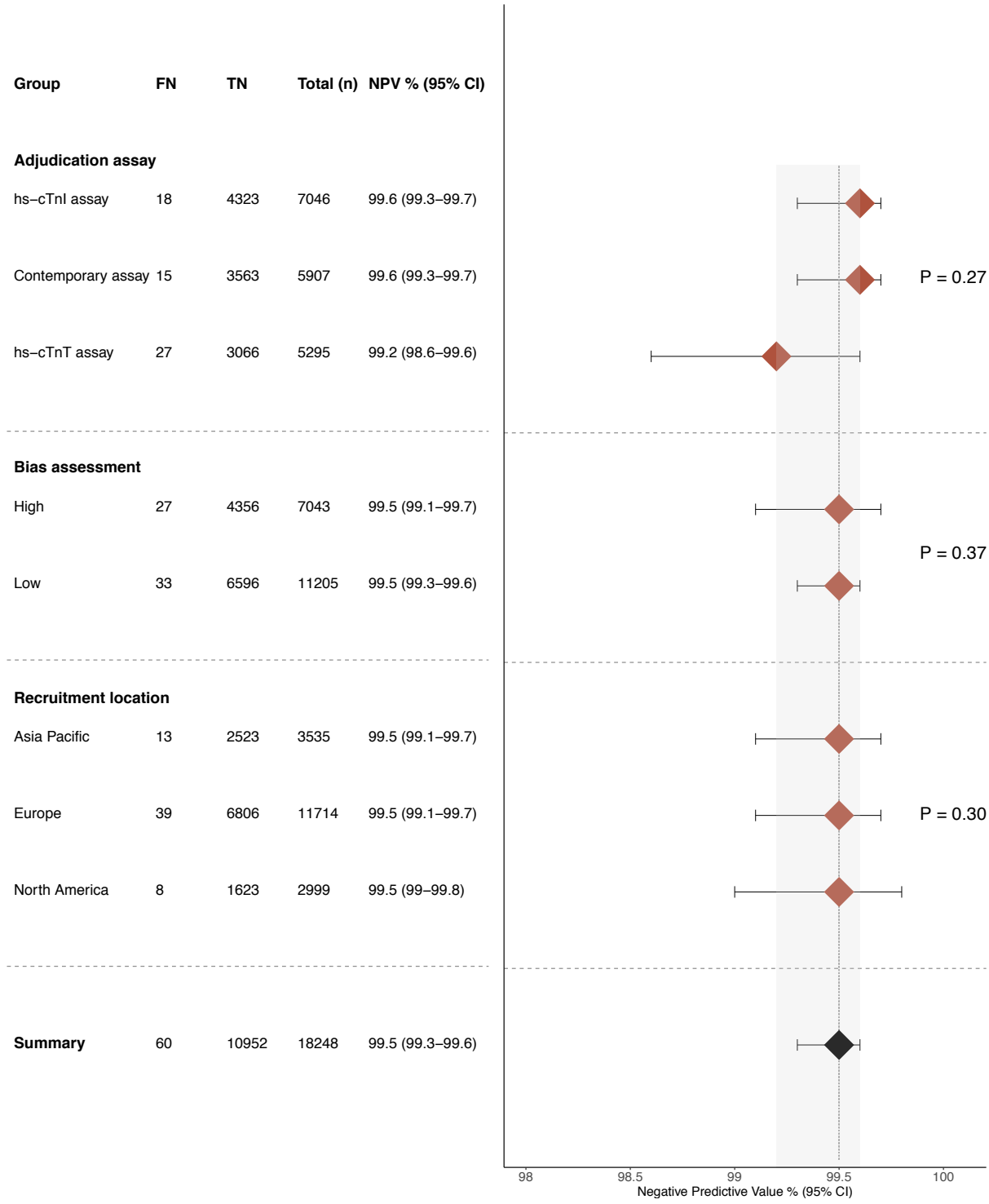


Figure 3.5. Negative predictive value of a high-sensitivity cardiac troponin I concentration <5 ng/L at presentation for myocardial infarction or cardiac death at 30 days, stratified by adjudicating assay, bias, and location.

Diamonds represent the central estimate of NPV and whiskers represent 95% confidence intervals. Black colour indicates overall meta-estimate for negative predictive value.

3.4.5 Short and long term outcomes

Follow up for cardiac death at 30 days and at 1 year was available in 12,953 (57.7%), and 9,271 (41.3%) patients, respectively (***Table 3.4 and 3.5***). In those patients with cardiac troponin concentrations <5 ng/L at presentation (n=6,956), there were no cardiac deaths at 30 days (NPV 100% [95% CI 99.9 to 100%], sensitivity 99.4% [95% CI 97.7-100%]), and 7 cardiac deaths (0.1%) at 1 year (NPV 99.9% [95% CI 99.7 to 99.9%], sensitivity 96.1% [95% CI 92.9 to 98.3%]). In patients with cardiac troponin concentrations between 5 ng/L and the 99th percentile at presentation (n=3,817), there were 19 (0.5%) and 58 (2.1%) cardiac deaths at 30 days and 1 year respectively. By comparison, in those with troponin concentrations above the 99th percentile (n=2,180), there were 62 (2.8%) and 125 (8.2%) cardiac deaths at 30 days and 1 year, respectively. In patients with troponin concentrations <5 ng/L at presentation and an index or 30-day myocardial infarction, there were no cardiac deaths at 30 days or 1 year. As the majority of studies did not adjudicate recurrent myocardial infarction events at 1 year, we were not able to conduct this pre-specified analysis.

Table 3.4. Summary thirty day and one year cardiac death outcomes

Group	30-day follow-up n	30-day cardiac death n (%)	1 year follow-up n	1 year cardiac death n (%)
High-sensitivity cardiac troponin at presentation				
<5 ng/L	6956	0 (0.0)	5054	7 (0.1)
≥5 ng/L and ≤99th centile	3817	19 (0.5)	2698	58 (2.1)
>99th centile	2180	62 (2.8)	1519	125 (8.2)
Total number of patients	12953		9271	

30 day and or 1 year cardiac death outcomes obtained from the following cohorts:
HighSTEACS-V, HighSTEACS-S, HighSTEACS-P, ADAPT-B, IMPACT, EDACS, ADAPT-RCT, ADAPT-C, BACC and TRUST

Table 3.5. Thirty day and one year cardiac death outcomes stratified by cardiac troponin concentration at presentation

Cardiac troponin concentration at presentation	30-day follow-up n	30-day cardiac death n (%)	1 year follow-up n	1 year cardiac death n (%)
<2 ng/L	2024	0 (0.0)	1561	0 (0.0)
<3 ng/L	4528	0 (0.0)	3334	1 (0.0)
<4 ng/L	5912	0 (0.0)	4314	4 (0.1)
<5 ng/L	6956	0 (0.0)	5054	7 (0.1)
<6 ng/L	7623	0 (0.0)	5544	8 (0.1)
<7 ng/L	8187	1 (0.0)	5951	9 (0.2)
<8 ng/L	8558	1 (0.0)	6214	14 (0.2)
<9 ng/L	8892	1 (0.0)	6444	14 (0.2)
<10 ng/L	9144	1 (0.0)	6619	14 (0.2)
<11 ng/L	9347	1 (0.0)	6748	14 (0.2)
<12 ng/L	9506	2 (0.0)	6862	20 (0.3)
<13 ng/L	9678	2 (0.0)	6981	23 (0.3)
<14 ng/L	9807	3 (0.0)	7075	26 (0.4)
<15 ng/L	9937	6 (0.1)	7164	28 (0.4)
<16 ng/L	10053	6 (0.1)	7248	32 (0.4)

30 day and or 1 year cardiac death outcomes obtained from the following cohorts:
HighSTEACS-V, HighSTEACS-S, HighSTEACS-P, ADAPT-B, IMPACT, EDACS, ADAPT-RCT, ADAPT-C, BACC and TRUST

3.4.6 Risk stratification thresholds and diagnosis of myocardial infarction

In all patients with suspected acute coronary syndrome where individual patient-level data were available (n=18,601, 82.8%), we evaluated how different risk stratification thresholds would affect the NPV and sensitivity for the primary outcome. When used in isolation, a troponin concentration of <5 ng/L gave a NPV of 99.5% (95% CI 99.3 to 99.7%) and sensitivity of 98.0% (95% CI 96.4 to 98.9%), identifying 49.1% of patients as low risk with a miss rate of 5.4 (95% CI 4.0 to 7.0) per 1,000 patients. At a threshold of <2 ng/L, the NPV was 99.8% (95% CI 99.0 to 100%) and sensitivity was 100% (95% CI 98.9 to 100%), but the proportion of patients identified as low risk was lower at 13.7%. Whilst the absolute number of missed cases was lower, the miss rate was similar at 4.1 (95% CI 2.0 to 6.9) per 1,000 patients (***Table 3.6***).

Table 3.6. Efficacy and safety of high-sensitivity cardiac troponin I thresholds for myocardial infarction or cardiac death at 30 days

Troponin (ng/L)	TP	FP	TN	FN	FN per 1000 (Mean, 95% CI)	NPV (Mean, 95% CI)	Sensitivity (Mean, 95% CI)	PPV (Mean, 95% CI)	Proportion of all patients tested (%)[a]
High-sensitivity cardiac troponin threshold only									
<2	2307	13744	2540	10	4.1 (2.0-6.9)	99.8 (99.0-100)	100 (98.9-100)	12.7 (9.8-16.3)	13.7
<3	2297	10587	5697	20	3.6 (2.2-5.3)	99.7 (99.4-99.8)	99.3 (98.4-99.7)	16.2 (12.6-20.6)	30.7
<4	2284	8700	7584	33	4.4 (3.0-6.0)	99.6 (99.4-99.8)	98.9 (97.5-99.5)	19.2 (14.9-24.4)	40.9
<5	2268	7203	9081	49	5.4 (4.0-7.0)	99.5 (99.3-99.7)	98.0 (96.4-98.9)	22.5 (17.4-28.7)	49.1
<6	2243	6199	10085	74	7.3 (5.8-9.1)	99.3 (99.0-99.5)	97.0 (94.9-98.3)	25.5 (19.5-32.5)	54.6
<7	2213	5366	10918	104	9.5 (7.8-11.4)	99.1 (98.8-99.4)	95.7 (93.3-97.3)	28.4 (21.9-36.0)	59.3
<8	2195	4803	11481	122	10.6 (8.8-12.5)	99.0 (98.7-99.3)	94.7 (92.0-96.5)	30.6 (23.6-38.6)	62.4
<9	2175	4302	11982	142	11.8 (9.9-13.7)	98.9 (98.5-99.2)	93.8 (90.9-95.8)	33.1 (25.7-41.5)	65.2
<10	2157	3943	12341	160	12.8 (10.9-14.9)	98.8 (98.4-99.2)	92.9 (89.6-95.2)	34.9 (27.1-43.7)	67.2
<11	2129	3636	12648	188	14.7 (12.7-16.8)	98.6 (98.2-99.0)	91.3 (87.9-93.9)	36.8 (28.5-45.9)	69
<12	2106	3395	12889	211	16.1 (14.1-18.4)	98.5 (98.1-98.9)	90.4 (86.8-93.0)	38.2 (29.6-47.5)	70.4
<13	2089	3113	13171	228	17.1 (14.9-19.3)	98.4 (98.0-98.8)	89.5 (85.9-92.3)	40.5 (31.6-50.1)	72
<14	2071	2932	13352	246	18.1 (16.0-20.4)	98.3 (97.9-98.7)	88.8 (85.3-91.5)	41.8 (32.7-51.5)	73.1
<15	2050	2737	13547	267	19.4 (17.1-21.7)	98.2 (97.7-98.6)	87.7 (84.0-90.7)	43.4 (34.0-53.2)	74.3
<16	2030	2575	13709	287	20.5 (18.3-23.0)	98.1 (97.6-98.5)	86.8 (82.8-90.0)	44.6 (35.1-54.6)	75.2

Estimates derived from a binomial-normal random effects model. Analysis includes 18,601 patients from the 17 cohorts where individual participant data was available.

Table 3.6. continued. Efficacy and safety of high-sensitivity cardiac troponin I thresholds for myocardial infarction or cardiac death at 30 days

Troponin (ng/L)	TP	FP	TN	FN	FN per 1000 (Mean, 95% CI)	NPV (Mean, 95% CI)	Sensitivity (Mean, 95% CI)	PPV (Mean, 95% CI)	Proportion of all patients tested (%)[a]
High-sensitivity cardiac troponin threshold and non-ischaemic ECG*									
<2	2082	11538	2044	8	4.1 (1.8-7.3)	99.9 (98.5-100)	100 (96.6-100)	14.2 (11.3-17.6)	13.1
<3	2077	8990	4592	13	2.9 (1.6-4.7)	99.8 (99.5-99.9)	99.7 (98.7-99.9)	18.0 (14.9-21.6)	29.4
<4	2068	7547	6035	22	3.7 (2.3-5.4)	99.8 (99.4-99.9)	99.5 (98.1-99.9)	21.1 (17.5-25.2)	38.6
<5	2059	6423	7159	31	4.4 (3.0-6.0)	99.7 (99.4-99.8)	99.0 (97.3-99.6)	24.5 (20.3-29.2)	45.9
<6	2046	5744	7838	44	5.6 (4.1-7.4)	99.5 (99.2-99.7)	98.5 (96.3-99.4)	27.1 (22.4-32.5)	50.3
<7	2026	5173	8409	64	7.6 (5.9-9.6)	99.3 (99.0-99.5)	97.4 (94.9-98.6)	29.7 (24.5-35.4)	54.1
<8	2013	4798	8784	77	8.7 (6.9-10.8)	99.2 (98.9-99.4)	96.6 (93.8-98.2)	31.4 (25.9-37.5)	56.5
<9	2004	4471	9111	86	9.4 (7.5-11.5)	99.1 (98.8-99.4)	96.0 (93.0-97.7)	33.1 (27.4-39.4)	58.7
<10	1997	4244	9338	93	9.9 (8.0-12.0)	99.1 (98.7-99.4)	95.7 (92.5-97.5)	34.6 (28.6-41.2)	60.2
<11	1980	4054	9528	110	11.5 (9.4-13.7)	98.9 (98.5-99.2)	94.7 (91.3-96.8)	35.9 (29.6-42.7)	61.5
<12	1964	3904	9678	126	12.9 (10.8-15.2)	98.8 (98.4-99.1)	93.8 (90.3-96.1)	36.9 (30.2-44.1)	62.6
<13	1949	3743	9839	141	14.2 (12.0-16.6)	98.6 (98.2-98.9)	92.9 (89.2-95.4)	38.0 (31.2-45.3)	63.7
<14	1937	3645	9937	153	15.2 (12.9-17.7)	98.5 (98.1-98.9)	92.4 (88.5-95.0)	38.7 (31.6-46.2)	64.4
<15	1925	3540	10042	165	16.2 (13.9-18.8)	98.4 (98.0-98.8)	91.5 (87.5-94.4)	39.4 (32.2-47.2)	65.1
<16	1916	3449	10133	174	16.9 (14.5-19.5)	98.4 (97.9-98.7)	91.0 (86.7-94.0)	40.1 (32.6-48.0)	65.8

Estimates derived from a binomial-normal random effects model. Analysis includes 18,601 patients from 17 cohorts where individual participant data was available
[a]Electrocardiographic data not available in 2,929 patients.

In a subgroup analysis combining risk stratification thresholds and a non-ischaemic electrocardiogram (***Figure 3.6***), a cardiac troponin I concentration of <5 ng/L gave a NPV of 99.7% (95% CI 99.4 to 99.8%) and a sensitivity of 99.0% (95% CI 97.3 to 99.6%), identifying 45.9% of patients as low risk with 4.4 (95% CI 3.0 to 6.0) false negatives per 1,000 patients and a PPV of 24.5% (95% CI 20.3 to 29.2%). The combination of a cardiac troponin I concentration <2 ng/L and a non-ischaemic electrocardiogram gave a similar NPV of 99.9% (95% CI 98.5 to 100%) and sensitivity of 100% (95% CI 96.6 to 100%), but identified just 13.1% of patients as low risk, with 4.1 (95% CI 1.8 to 7.3) false negatives per 1,000 patients and a lower PPV of 14.2% (95% CI 11.3 to 17.6%).

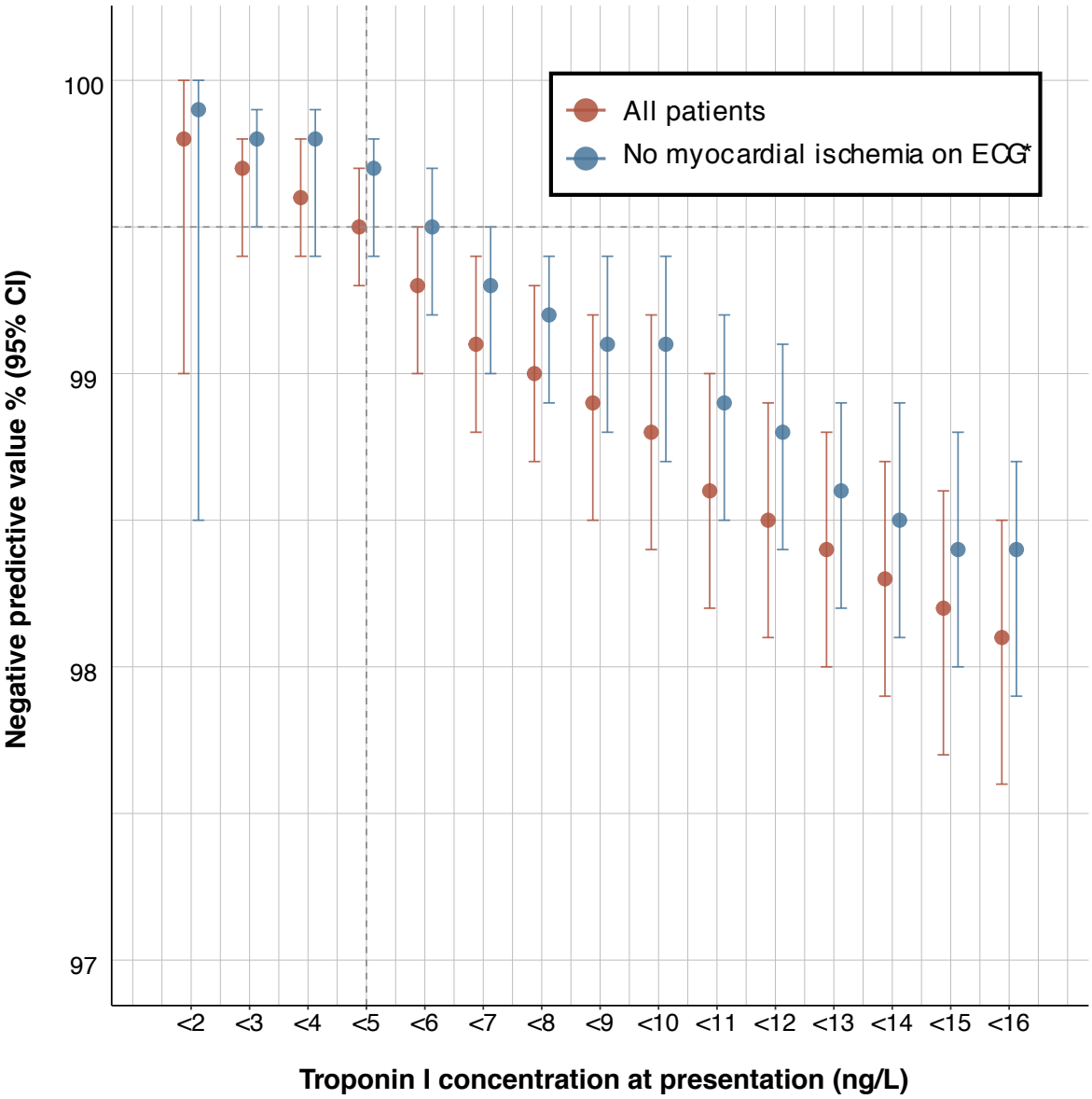


Figure 3.6. The optimal threshold of high-sensitivity cardiac troponin I at presentation to risk stratify patients with suspected acute coronary syndrome for myocardial infarction or cardiac death at 30 days

A). Negative predictive value with 95% confidence intervals across a range of high-sensitivity cardiac troponin I concentrations. Horizontal dotted grey line indicating pre-specified target of 99.5%, and vertical dotted grey line indicating a troponin concentration of <5 ng/L

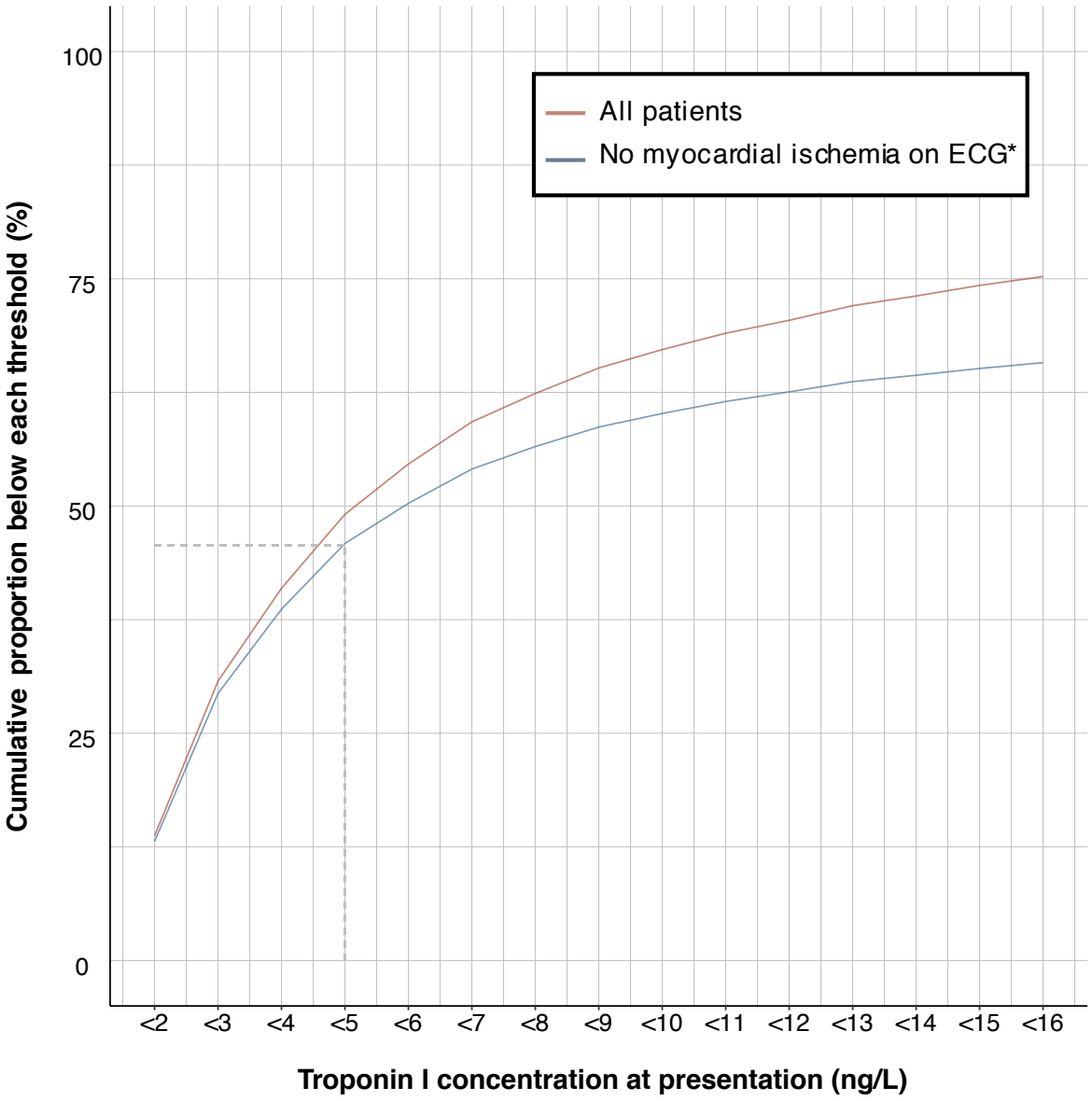


Figure 3.6. The optimal threshold of high-sensitivity cardiac troponin I at presentation to risk stratify patients with suspected acute coronary syndrome for myocardial infarction or cardiac death at 30 days.

B). Cumulative proportion of all patients with suspected acute coronary syndrome classified as low risk, with the dotted vertical line indicating proportion of patients <5 ng/L.

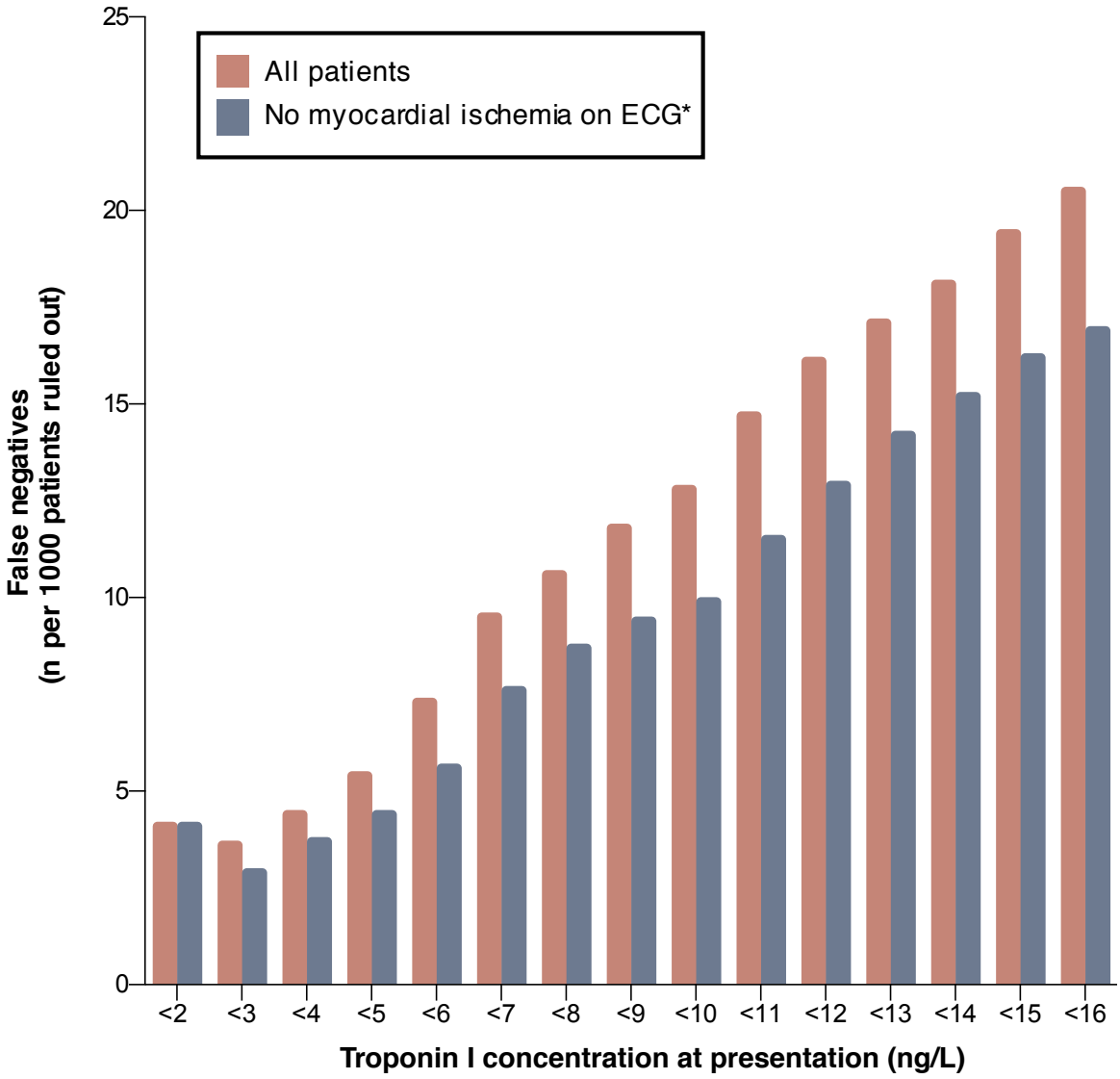


Figure 3.6. The optimal threshold of high-sensitivity cardiac troponin I at presentation to risk stratify patients with suspected acute coronary syndrome for myocardial infarction or cardiac death at 30 days

*C) Number of false negatives per 1,000 patients tested across a range of cardiac troponin thresholds. Panels display performance of cardiac troponin thresholds in all patients (red), and when applied to those patients with a non-ischemic electrocardiogram at presentation (blue). All estimates of NPV derived from binomial-normal random effects model using individual cohort level data (available in 17 cohorts) for each cardiac troponin threshold (n=18,601, Table 3.5). *Electrocardiogram data was not available in 2,929 patients (15.7%)*

3.5 Discussion

In 19 cohorts, across nine countries, encompassing over 22,000 patients, a cardiac troponin concentration <5 ng/L at presentation identified half of all patients with suspected acute coronary syndrome as low risk of myocardial infarction or cardiac death at 30 days, with 5 false negatives per 1,000 patients tested.

There are a number of strengths to the analysis. This was a pre-specified systematic review and meta-analysis that included individual patient-level data from all cohorts identified. The findings were consistent across a range of healthcare settings and geographic regions with considerable differences in the prevalence of myocardial infarction. Individual level data was included from over 22,000 patients, allowing a meaningful analysis of important subgroups. All studies were prospective, and in all the final diagnosis was adjudicated according to the universal definition of myocardial infarction.

Two recent meta-analyses have suggested an approach to risk stratification using the limit of detection of the high-sensitivity cardiac troponin T assay, which identifies up to 31% of patients with a NPV of 99.3% (Zhelev et al., 2015, Pickering et al., 2017). The limit of detection of the high-sensitivity cardiac troponin I assay identifies 19% to 27% of patients as low risk, with a NPV of 99.5% or greater (Carlton et al., 2016, Neumann et al., 2017a, Sandoval et al., 2017a). The major limitation of this approach for both assays is analytical, with biases and analytical variation at the limit of detection associated with rates of misclassification that are twice that observed at 5 ng/L (Lyon et al., 2017, Kavsak et al., 2013a).

In clinical practice, cardiac troponin concentrations are interpreted in conjunction with the electrocardiogram and clinical assessment. When a risk stratification threshold of <5 ng/L was evaluated in the subgroup of patients without myocardial ischaemia on the electrocardiogram, the NPV and sensitivity were excellent. To ensure safety estimates were conservative, performance was evaluated not just for an index diagnosis, but for a composite end-point that included events up to 30 days. Although there were 81 cardiac deaths at 30 days, none occurred in the 6,956 patients with cardiac troponin concentrations <5 ng/L. Furthermore, performance was similar for both spontaneous type 1 and secondary type 2 myocardial infarction. This is relevant as the diagnosis of type 2 myocardial infarction is more challenging and is associated with a worse prognosis (Shah et al., 2015c, Sandoval et al., 2014b).

At a threshold of 5 ng/L, the analytical performance of the high-sensitivity cardiac troponin I assay is excellent (Shah et al., 2015a, Kavsak et al., 2017). The use of lower thresholds did not improve diagnostic accuracy. A miss rate of 5 per 1,000 patients was observed when applying <5 ng/L as the risk stratification threshold, with a miss rate of 4 per 1,000 patients observed at <2 ng/L. Although the true risk of missing an individual patient with myocardial infarction is the same at both thresholds, lower thresholds reduce the proportion of patients classified as low risk; only 1 in 10 patients had a troponin concentration <2 ng/L, compared with 5 in 10 patients <5 ng/L. Use of lower thresholds would result in more patients without myocardial infarction being admitted for serial testing and further investigation, with an increase in healthcare expenditure.

Despite recent changes to guidelines,(Roffi et al., 2016) the majority of clinicians continue to rely on the 99th percentile to rule in and rule out myocardial infarction (Collinson et al., 2016). The use of low concentrations of cardiac troponin to risk stratify patients has been shown to improve safety compared to use of the 99th percentile in multiple studies (Parsonage et al., 2016, Pickering et al., 2016b). This approach to risk stratification using high-sensitivity cardiac troponin assays has major potential to improve both the efficiency of healthcare delivery and patient safety, and is being formally evaluated in a prospective multi-centre clinical trial (NCT:03005158).

3.5.1 Limitations

This study has several limitations. First, not all cohorts used identical protocols, with differences both in the inclusion criteria and diagnostic criteria used for adjudication (***Appendix 1.3***). However, no significant differences in NPV were observed when stratified by adjudicating assay, and the NPV was high across individual cohorts, suggesting these findings are generalizable. Second, the number of patients who presented early after onset of symptoms was low at just 10% of the study population. Despite observing an NPV of 99% in this subgroup, inconsistencies in the documentation of symptom onset across cohorts may affect the analysis, and until further research is available, serial testing is recommended in patients presenting within 2 hours of symptom onset (Roffi et al., 2016). The greatest number of false negatives were observed in the cohort with the shortest median symptom onset to sample time (179 minutes, IQR 119-349, ***Table 3.1***), which may explain the lower NPV and sensitivity reported at this threshold in a previous study (Carlton et al., 2016). Third, whilst it is reassuring that patients with troponin concentrations <5 ng/L had a much lower rate of cardiac death at one year than those patients between 5 ng/L and the 99th percentile, this observation needs to be verified in prospective studies where patient care was guided by this approach.

3.6 Conclusions

Among patients with suspected acute coronary syndrome, a high-sensitivity cardiac troponin I concentration <5 ng/L at presentation identified those at low risk of myocardial infarction or cardiac death within 30 days. Further research is needed to understand the clinical utility and cost-effectiveness of this approach to risk stratification.

CHAPTER 4

COMPARISON OF THE EFFICACY AND SAFETY OF NOVEL RULE-OUT PATHWAYS FOR ACUTE MYOCARDIAL INFARCTION

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4.1 Summary

Background

High-sensitivity cardiac troponin assays enable myocardial infarction to be ruled out earlier, but the optimal approach is uncertain. We compared the European Society of Cardiology (ESC) rule-out pathway, with a pathway that incorporates lower cardiac troponin concentrations to risk stratify patients.

Methods

Patients with suspected acute coronary syndrome (n=1,218) underwent high-sensitivity cardiac troponin I measurement at presentation, 3 and 6 or 12 hours. We compared the ESC pathway (<99th centile at presentation, or at 3 hours if symptoms <6 hours) with a pathway developed in the *High-S*ensitivity *T*roponin in the *E*valuation of patients with *A*cute *C*oronary *S*yndrome (High-STEACS) study population (<5 ng/L at presentation, or change <3 ng/L and <99th centile at 3 hours). The primary outcome was a comparison of the negative predictive value (NPV) of both pathways for index type 1 myocardial infarction, or type 1 myocardial infarction or cardiac death at 30 days. We evaluated the primary outcome in pre-specified subgroups stratified by age, gender, time of symptom onset and known ischaemic heart disease.

Results

The primary outcome occurred in 15.7% (191/1,218) patients. In those <99th centile at presentation, the ESC pathway ruled out myocardial infarction in 28.1% (342/1,218) and 78.9% (961/1,218) at presentation and 3 hours respectively, missing 18 index and two 30-day events (NPV 97.9%, 95% confidence intervals [CI] 96.9-98.7%). The High-STEACS pathway ruled out 40.7% (496/1,218) and 74.2% (904/1,218) at

presentation and 3 hours, missing two index and two 30-day events (NPV 99.5%, 95% CI 99.0-99.9%; P<0.001 for comparison). The NPV of the High-STEACS pathway was greater than the ESC pathway overall (P<0.001), and in all subgroups including those presenting early or known to have ischaemic heart disease.

Conclusion

Use of the High-STEACS pathway incorporating low high-sensitivity cardiac troponin concentrations rules out myocardial infarction in more patients at presentation and misses 5-fold fewer index myocardial infarctions than guideline approved pathways based exclusively on the 99th centile.

4.2 Introduction

Chest pain is a frequent presenting symptom in patients attending the Emergency Department, with significant resource implications for healthcare providers (Makam and Nguyen, 2015). Whilst the majority of patients with chest pain do not have an acute myocardial infarction (Zhelev et al., 2015), prompt and accurate exclusion of this diagnosis remains challenging in clinical practice, and often results in unnecessary hospital admission (Goodacre et al., 2005, Skinner et al., 2010, Goodacre et al., 2013). Guidelines from the European Society of Cardiology (ESC) support the use of high-sensitivity cardiac troponins and earlier testing to rule out myocardial infarction where concentrations are $<99^{th}$ centile upper reference limit (URL) at presentation in those patients with symptoms for more than 6 hours, and at 3 hours in the remainder (Roffi et al., 2016). A similar approach was recommended by the National Institute of Clinical Health and Excellence (NICE), although concerns were raised about the generalisability of the studies evaluating the effectiveness of this approach (NICE., 2014b).

Recent studies have demonstrated that very low cardiac troponin concentrations can help to further risk stratify patients (Thygesen et al., 2012a, Mills et al., 2011, Shah et al., 2015b, Body et al., 2011, Rubini Gimenez et al., 2013, Bandstein et al., 2014, Shah et al., 2015a, Carlton et al., 2016, Thelin et al., 2015, Body et al., 2016, Rubini Gimenez et al., 2015, Mueller et al., 2016, Reichlin et al., 2012, Reichlin et al., 2015, Pickering et al., 2016b). As such, the latest European guidelines include an additional one hour pathway incorporating lower thresholds of cardiac troponin for risk stratification (Roffi et al., 2016). We recently demonstrated in consecutive patients

with suspected acute coronary syndrome that a cardiac troponin concentration <5 ng/L at presentation had a negative predictive value of 99.6% (95%CI 99.3–99.8) for myocardial infarction during the index presentation, or myocardial infarction or cardiac death at 30 days. Furthermore, patients with cardiac troponin concentrations <5 ng/L had very low rates of adverse cardiac events at one year (Shah et al., 2015a).

Whilst it is clear that high-sensitivity cardiac troponins enable myocardial infarction to be ruled out earlier, the optimal approach is uncertain. As such, we compared the safety and efficacy of the ESC pathway based on the 99th centile alone, with our clinical pathway incorporating low cardiac troponin concentrations to risk stratify patients.

4.3 Methods

4.3.1 Study population

Patients with suspected acute coronary syndrome were recruited from the Emergency Department of the Royal Infirmary of Edinburgh, a tertiary care hospital in Scotland, between 1st June 2013 and 31st September 2015 into a sub-study of the *High-S*ensitivity *T*roponin in the *E*valuation of patients with *A*cute *C*oronary *S*yndrome (High-STEACS) trial. All patients in whom the attending clinician requested cardiac troponin for suspected acute coronary syndrome were eligible for inclusion. We did not enrol patients with ST-segment elevation myocardial infarction, those who were unable to provide consent, or those from outside our region to ensure complete follow up. Blood samples were obtained at presentation and at 6-12 hours for high-sensitivity cardiac troponin testing as part of routine clinical care. Patients provided written informed consent for additional sampling at 3 hours with the results of testing at this time-point not used to guide patient care. This pre-specified analysis was restricted to those patients where serial samples were available (***Figure 4.1***). This clinical trial was registered (NCT:01852123), approved by the national research ethics committee, and conducted in accordance with the Declaration of Helsinki.

4.3.2 High-sensitivity cardiac troponin I assay

The Abbott ARCHITECT$_{STAT}$ high-sensitive cardiac troponin I assay (Abbott Laboratories, Abbott Park, IL) is a two-step chemo-luminescent assay with a limit of detection of 1.2 ng/L and coefficient of variation of less than 10% at 6 ng/L (Chin et al., 2014). This assay performance has been independently validated across multiple centres under routine laboratory working conditions, with a reported inter-laboratory

coefficient of variation of 12.6% at 3.5 ng/L across 33 instruments (Shah et al., 2015a). The upper reference limit 99th centiles were determined in 4,590 samples from healthy individuals as 16 ng/L for women and 34 ng/L in men (Shah et al., 2015b), and from 10th December 2013 onwards these thresholds were used in clinical practice.

4.3.3 Baseline characteristics

Patient baseline characteristics, including chest pain characteristics, onset of symptoms, prior medical history, cardiovascular risk factors, medication, and clinical observations, in addition to investigations including serial 12-lead electrocardiography and cardiac imaging, were obtained from a dedicated case record form, patient questionnaire and the electronic patient record (TrakCare, InterSystems, Cambridge, MA). Hyperlipidaemia or hypertension were defined as a history of the condition, or by the use of lipid-lowering or anti-hypertensive therapies, respectively. Ischaemic heart disease was defined as a history of angina, prior myocardial infarction or prior coronary revascularization.

4.3.4 Diagnostic adjudication

The final diagnosis was adjudicated for all patients by two independent physicians (AC/AA), with consensus from a third physician (JA/NM) where there was discrepancy following review of all clinical information, both non-invasive and invasive investigations and outcomes from presentation to 30 days. Patients were classified as having type 1 myocardial infarction, type 2 myocardial infarction or myocardial injury in accordance with the third universal definition of myocardial infarction as previously reported (Roffi et al., 2016, Shah et al., 2015a). Any cardiac troponin I concentration above the sex-specific 99th centile upper reference limit was

considered evidence of myocardial necrosis. Type 1 myocardial infarction was defined as myocardial necrosis in the context of a presentation with symptoms suggestive of acute coronary syndrome or evidence of myocardial ischemia. Patients with symptoms or signs of myocardial ischemia due to increased oxygen demand or decreased supply (e.g. tachyarrhythmia, hypotension or anaemia) secondary to an alternative pathology and myocardial necrosis were classified as type 2 myocardial infarction. Myocardial injury was defined as evidence of myocardial necrosis in the absence of any clinical features of myocardial ischaemia. Agreement for a diagnosis of type 1 myocardial infarction was very good (κ= 0.82, 95%CI 0.75-0.89).

4.3.5 Clinical outcomes

The primary outcome was a composite of index type 1 myocardial infarction, or type 1 myocardial infarction or cardiac death at 30 days. We used regional and national registries in addition to individual patient follow up at 30 days to ensure follow up was complete for the entire study population. All subsequent events were adjudicated using the same approach as for the index presentation. TrakCare software application (InterSystems Corporation, Cambridge, MA, USA) is a regional electronic patient record system, which provides data on all hospital admissions to both tertiary or secondary care hospitals in the southeast of Scotland. All in-hospital and community deaths are recorded in a comprehensive national database, the General Register of Scotland. Cardiac death was defined as any death due to myocardial infarction, arrhythmia or heart failure.

4.3.6 Clinical pathways

We compared the safety and efficacy of two pathways to rule out the composite outcome of index myocardial infarction, and myocardial infarction or cardiac death at 30 days. The ESC pathway rules out myocardial infarction where cardiac troponin concentrations are <99th centile at presentation in patients with symptoms for more than 6 hours. In patients with symptoms for less 6 hours, a second troponin measurement is performed 3 hours from presentation, with myocardial infarction ruled out if cardiac troponin remains <99th centile or is >99th centile without a significant change in concentration (Roffi et al., 2016). Previously published guidance from the ESC Working Group on Acute Cardiac Care recommends use of a change in cardiac troponin concentration >50% of the 99th centile upper reference limit at 3 hours (Thygesen et al., 2012b).

We compared the ESC pathway to the High-STEACS pathway, based on our previous observations, that utilises a risk stratification threshold of 5 ng/L at presentation (Shah et al., 2015a, Shah et al., 2016). This threshold has since been externally validated in separate populations, with a recent a multi-centre study across five independent cohorts finding a troponin concentration of <5 ng/L had a negative predictive value of 99.2% (95%CI 98.8-99.5%) (Carlton et al., 2016). In our pathway, patients with cardiac troponin concentrations <5 ng/L at presentation are considered low risk and myocardial infarction is ruled out without further testing, unless they present early with symptom onset <2 hours from presentation where cardiac troponin is retested 3 hours after presentation (Shah et al., 2015a). Patients with cardiac troponin concentrations ≥5 ng/L at presentation are retested at 3 hours. Myocardial infarction is ruled out at 3 hours if cardiac troponin concentrations are unchanged and remain

<99th centile on retesting. A change in cardiac troponin concentration was defined as an increase or decrease ≥3 ng/L at 3 hours, as this is the lowest measurable concentration within the normal reference range that exceeds analytical variation of the assay (Kavsak et al., 2016). This change in cardiac troponin concentration was internally and externally validated, using data from the APACE cohort (***Appendix 2.1).***

4.3.7 Statistical analysis

Baseline characteristics are summarised as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate. Patients with maximal cardiac troponin concentrations ≤99th centile were compared with those >99th centile using a chi-square test or Wilcoxon rank sum test. The primary outcome was the negative predictive value (NPV) of each pathway, using the composite endpoint of index type 1 myocardial infarction, or subsequent type 1 myocardial infarction or cardiac death at 30 days. As we estimated the NPV would approach 100%, we used a Bayesian approach with a Jeffrey's prior (beta distribution with both shape parameters equal to 0.5) as this is more robust when confidence intervals approach 0 or 1 (Brown et al., 2001). We derived a weighted generalised score statistic to compare the NPV of the ESC and the High-STEACS pathway, as previously described (Leisenring et al., 2000). We evaluated the NPV in pre-specified subgroups stratified by time of symptom onset (<3, <6 or ≥6 hours), age (<65 or ≥65 years), sex, and history of ischaemic heart disease. We determined absolute (hs-TnI$_{3hr}$ – hs-TnI$_{0hr}$) and relative ([(hs-TnI$_{3hr}$ – hs-TnI$_{0hr}$) / hs-TnI$_{0hr}$] x 100) change in cardiac troponin concentration from presentation to 3 hours, and determined sensitivity, specificity and positive predictive value (PPV) with 95% confidence intervals (CI) using a Bayesian approach as per the NPV. In a sensitivity analysis, we evaluated the NPV for a primary outcome encompassing type 1 or type 2

myocardial infarction, or myocardial injury, or myocardial infarction or cardiac death at 30 days. To ensure our findings were generalizable to those centres that do not apply sex-specific diagnostic thresholds, we evaluated the performance of both pathways using a single 99th centile upper reference limit for men and women of 26 ng/L. A further sensitivity analysis evaluated the NPV in patients without evidence of myocardial ischaemia (defined as ≥2mm ST-segment depression or new T-wave inversion) on the presenting electrocardiogram, who were considered intermediate or low risk with a GRACE score of <140 (Roffi et al., 2016). We evaluated pathway efficacy by determining the number of patients ruled out at 0 and 3 hours as a proportion of the total study population, with comparison by McNemar's test for paired proportions. A two-sided $P<0.05$ was considered statistically significant. All analyses were performed using R (Version 3.2.2).

4.4 Results

We identified 1,218 patients with suspected acute coronary syndrome who met our inclusion and exclusion criteria (62.4±14.1 years, 61% male; ***Table 4.1***, ***Figure 4.1***). The adjudicated diagnosis was type 1 myocardial infarction in 15.5% (189/1,218), type 2 myocardial infarction in 5.5% (67/1,218) and myocardial injury in 2.1% (26/1,218). There were six subsequent type 1 myocardial infarcts and six cardiac deaths at 30 days. At presentation, 216 patients had troponin concentrations >99th centile with 11.9% (145/1,218) type 1 and 3.8% (46/1,218) type 2 myocardial infarction, and 2.1% (25/1,218) patients with myocardial injury.

Table 4.1. Baseline demographics stratified by cardiac troponin concentration at presentation

	All Patients (n=1,218)	hs-cTnI <5ng/L (n=692)	hs-cTnI ≤99th centile (n=1,002)	hs-cTnI >99th centile (n=216)	P-value
Age	62.36 (14.1)	57.1 (12.3)	60.97 (13.8)	68.78 (14.1)	<0.001
Male (%)	742 (60.9)	383 (55.3)	622 (62.1)	120 (55.6)	0.088
Primary Symptom					
Chest Pain	1044 (85.7)	614 (88.7)	873 (87.1)	171 (79.2)	<0.001
Collapse	13 (1.1)	7 (1.0)	8 (0.8)	5 (2.3)	0.578
Dyspnoea	40 (3.3)	10 (1.4)	25 (2.5)	15 (6.9)	0.151
Palpitations	20 (1.6)	7 (1.0)	15 (1.5)	5 (2.3)	0.043
Symptom onset					
Minutes since onset	208 (116-616)	196 (111-688)	198 (112-594)	249 (130-744)	0.032
< 2 hours (%)	326 (26.8)	196 (28.4)	279 (27.9)	47 (21.8)	0.081
≥ 2 and < 6 hours (%)	463 (38.0)	250 (36.1)	381 (38.0)	82 (38.0)	1.000
≥ 6 hours (%)	429 (35.2)	246 (35.5)	342 (34.1)	87 (40.3)	0.102
Cardiovascular Risk Factors					
Smoker (%)	255 (20.9)	174 (25.1)	217 (21.7)	38 (17.6)	0.385
Diabetes mellitus (%)	184 (15.6)	75 (11)	140 (14.4)	44 (21.0)	0.024
Hypertension (%)	550 (47.2)	255 (38.4)	443 (46.3)	107 (51.4)	0.203
Hyperlipidaemia (%)	493 (43.3)	233 (33.5)	402 (42.9)	91 (44.8)	0.681
Family history (%)	569 (51.5)	350 (53.8)	479 (52.2)	90 (47.9)	0.312
Known angina (%)	409 (34.6)	180 (26.5)	334 (34.2)	75 (36.4)	0.597
Previous MI (%)	308 (26.1)	112 (16.5)	244 (25.2)	64 (30.6)	0.124
Previous PCI (%)	248 (21.3)	110 (16.4)	205 (21.4)	43 (20.9)	0.942
Previous CABG (%)	87 (7.5)	24 (3.9)	71 (7.5)	16 (7.8)	0.995
Heart failure (%)	42 (3.7)	4 (0.6)	25 (2.7)	17 (8.7)	<0.001
Stroke (%)	81 (7.0)	26 (3.9)	63 (6.6)	18 (8.8)	0.337
PVD (%)	29 (2.6)	7 (1.1)	21 (2.2)	8 (4.0)	0.225
Admission Medication					
Aspirin (%)	442 (38.3)	210 (31.4)	361 (38.0)	81 (39.7)	0.707
Clopidogrel (%)	150 (13.5)	61 (9.5)	119 (13.1)	31 (15.6)	0.409
Warfarin (%)	84 (7.7)	25 (4.0)	66 (7.4)	18 (9.0)	0.52
Beta blocker (%)	353 (31.6)	159 (24.8)	286 (31.2)	67 (33.5)	0.587
ACEi or ARB (%)	379 (33.9)	170 (26.5)	306 (33.3)	73 (36.7)	0.405
CCB (%)	158 (14.4)	72 (11.4)	128 (14.2)	30 (15.2)	0.822
Statin (%)	540 (46.9)	253 (38.4)	444 (46.8)	96 (47.5)	0.91

Values are number (%) or mean (SD) or median (inter-quartile range). Abbreviations: ACE = angiotensin converting enzyme; ARB = angiotensin receptor blocker; MI = myocardial infarction; CCB = calcium channel blocker; PCI = percutaneous coronary intervention; CABG = coronary artery bypass grafting; PVD = peripheral vascular disease.

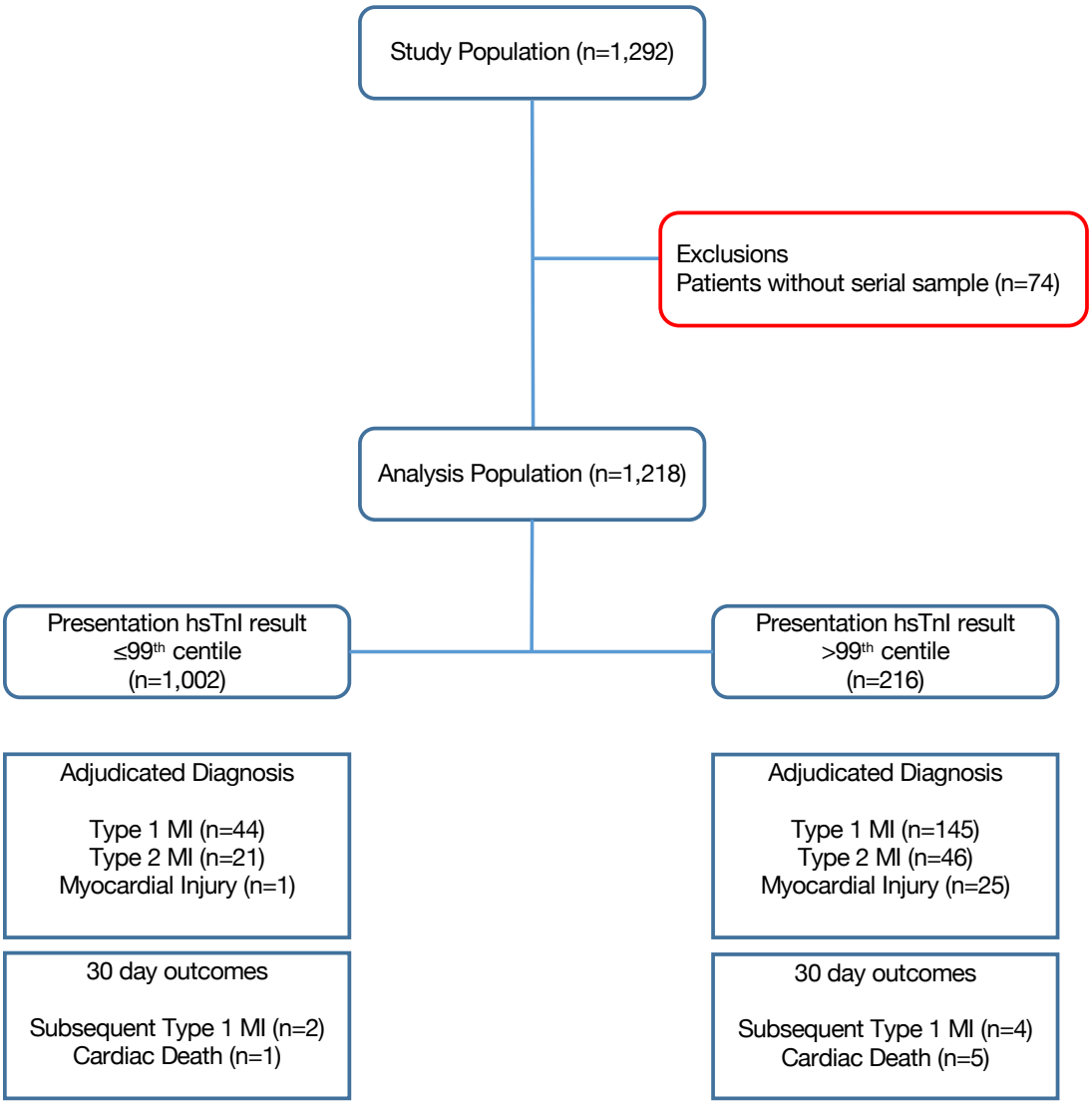

Figure 4.1. Study population, adjudicated diagnosis and 30 day outcomes

4.4.1 ESC pathway

The ESC pathway ruled out 28.1% (342/1,218) of patients at presentation and 78.9% (961/1,218) of all patients by 3 hours. However, this approach missed 18 index type 1 myocardial infarctions (four on presentation, fourteen at three hours) and two subsequent myocardial infarctions within 30 days for an overall NPV of 97.9% [95% CI, 96.9-98.7%; ***Table 4.2***, ***Figure 4.2 and 4.3***]. The sensitivity of this pathway is 89.3% [95% CI, 84.9-93.5%], and a summary of the missed events is provided in ***Appendix 2.2.***

4.4.2 High-STEACS pathway

In comparison, the High-STEACS pathway ruled out 40.7% (496/1,218) of patients at presentation, and 74.2% (904/1,218) of all patients by 3 hours. There were two missed index type 1 myocardial infarction (none at presentation, two at three hours) and two recurrent events for an overall NPV of 99.5% [95% CI, 99.0-99.9]; ***Table 4.2, Figure 4.2 and 4.3***). All events missed by the High-STEACS pathway were also missed by the ESC pathway. The sensitivity of the High-STEACS pathway was 97.7% [95% CI, 95.5-99.5%] and a summary of missed events is provided in ***Appendix 2.3.***

Table 4.2. Diagnostic performance of ESC pathway and High-STEACS pathway for the primary outcome at three hours

		TP	FP	TN	FN	NPV (Mean, 95% CI)	PPV (Mean, 95% CI)	Sensitivity (Mean, 95%CI)	Specificity (Mean, 95%CI)
All patients (n=1,218)	High-STEACS	187	127	900	4	99.5 (99.0-99.9)	59.5 (54.1-64.9)	97.7 (95.5-99.5)	87.6 (85.6-89.6)
	ESC pathway	171	86	941	20	97.9 (96.9-98.7)	66.5 (60.6-72.1)	89.3 (84.9-93.5)	91.6 (89.9-93.3)
Subgroup analysis									
<3 hours since onset (n=544)	High-STEACS	81	60	402	1	99.6 (98.8-100)	57.4 (49.2-65.4)	98.2 (95.3-100)	86.9 (83.8-89.9)
	ESC pathway	73	43	419	9	97.8 (96.2-99.0)	62.8 (53.9-71.3)	88.6 (81.6-94.9)	90.6 (87.9-93.2)
<6 hours since onset (n=789)	High-STEACS	117	98	572	2	99.6 (98.9-99.9)	54.4 (47.7-61.0)	97.9 (95.4-99.9)	85.3 (82.6-88.0)
	ESC pathway	104	66	604	15	97.5 (96.1-98.6)	61.1 (53.7-68.3)	87.1 (81.0-92.8)	90.1 (87.8-92.3)
>6 hours since onset (n=429)	High-STEACS	70	29	328	2	99.2 (98.1-99.9)	70.5 (61.2-79.0)	96.6 (92.4-99.8)	91.8 (88.9-94.5)
	ESC pathway	67	20	337	5	98.4 (96.8-99.4)	76.7 (67.4-84.9)	92.5 (86.4-97.8)	94.3 (91.8-96.6)
Men (n=742)	High-STEACS	122	73	544	3	99.4 (98.5-99.8)	62.5 (55.6-69.1)	97.2 (94.4-99.6)	88.1 (85.5-90.6)
	ESC pathway	110	38	579	15	97.4 (96.0-98.5)	74.2 (66.9-80.8)	87.7 (81.9-93.2)	93.8 (91.8-95.6)
Women (n=476)	High-STEACS	65	54	356	1	99.6 (98.7-100)	54.6 (45.7-63.4)	97.8 (94.2-100)	86.7 (83.4-90.0)
	ESC pathway	61	48	362	5	98.5 (97.0-99.5)	55.9 (46.6-65.0)	91.8 (85.2-97.6)	88.2 (85.0-91.2)

Table 4.2 (continued) Diagnostic performance of ESC pathway and High-STEACS pathway for the primary outcome at three hours

		TP	FP	TN	FN	NPV (Mean, 95% CI)	PPV (Mean, 95% CI)	Sensitivity (Mean, 95%CI)	Specificity (Mean, 95%CI)
Subgroup analysis (continued)									
Age <65 years (n=701)	High-STEACS	78	39	583	1	99.7 (99.2-100)	66.5 (57.8-74.7)	98.1 (95.1-100)	93.7 (91.7-95.5)
	ESC pathway	72	29	593	7	98.8 (97.7-99.5)	71.1 (62.0-79.4)	90.6 (84.2-96.5)	95.3 (93.6-96.9)
Age ≥65 years (n=517)	High-STEACS	109	88	317	3	98.9 (97.5-99.7)	55.3 (48.4-62.2)	96.9 (93.7-99.5)	78.2 (74.2-82.2)
	ESC pathway	99	57	348	13	96.3 (94.1-98.0)	63.4 (55.7-70.7)	88.1 (82.0-93.7)	85.8 (82.4-89.2)
Known ischaemic heart disease (n=518)	High-STEACS	85	77	352	4	98.7 (97.4-99.6)	52.5 (44.8-60.1)	90.5 (90.5-98.9)	82.0 (78.3-85.6)
	ESC pathway	73	52	377	16	95.8 (93.6-97.6)	58.3 (49.6-66.8)	81.7 (73.6-89.3)	87.8 (84.7-90.8)
No known ischaemic heart disease (n=680)	High-STEACS	99	48	533	0	99.9 (99.6-100)	67.2 (59.5-74.5)	99.5 (98.1-100)	91.7 (89.4-93.9)
	ESC pathway	95	33	548	4	99.2 (98.3-99.8)	74.0 (66.2-81.2)	95.5 (91.4-99.0)	94.2 (92.3-96.1)

Abbreviations: TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative, NPV = Negative Predictive Value, PPV = positive predictive value

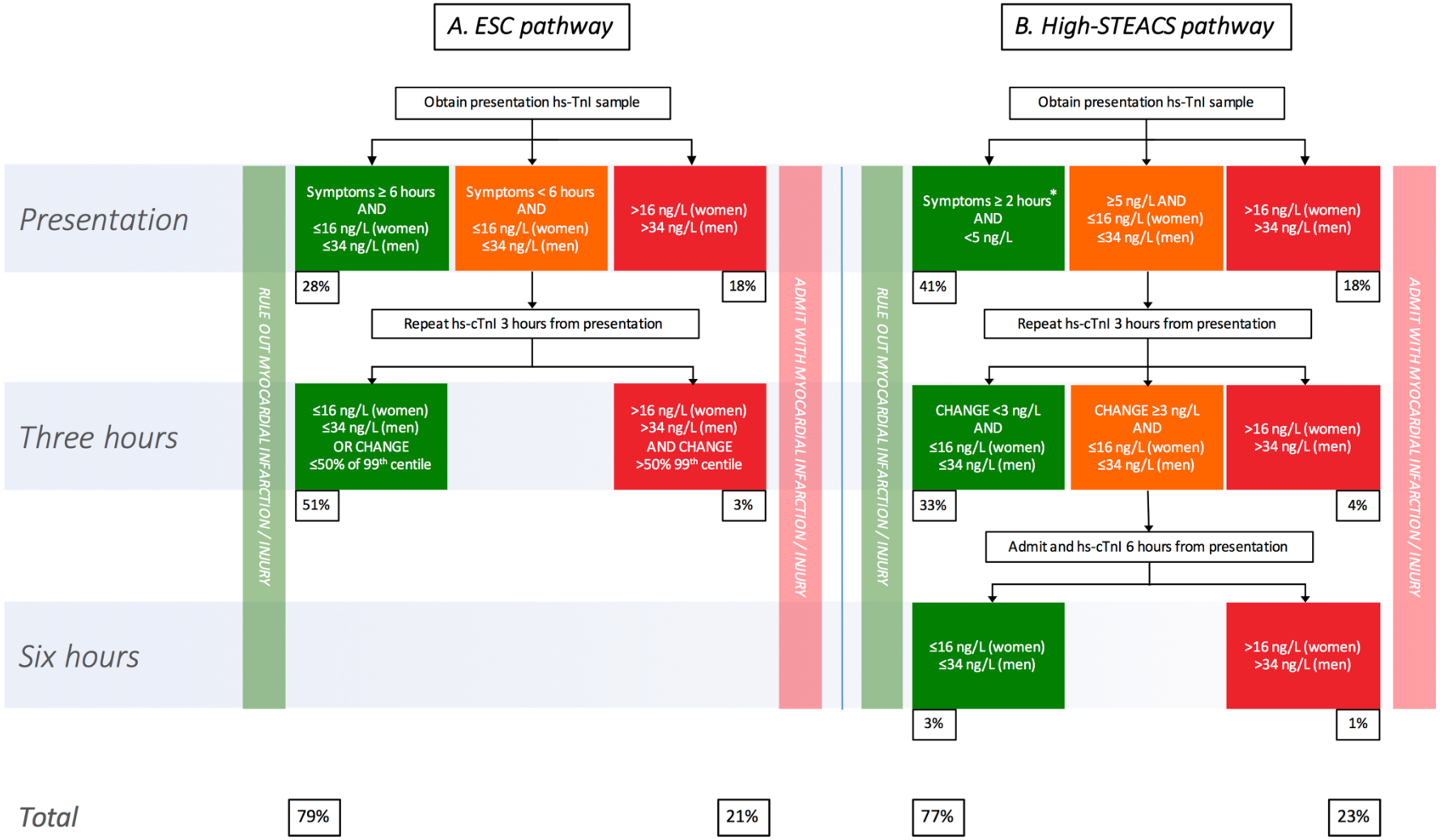

Figure 4.2 Summary of the ESC (A) and High-STEACS (B) rule out pathways. Percentages indicate number of patients ruled out at a given time point, as a proportion of the analysis population (n=1,218). *If symptom onset <2 h retest at 3 hours from presentation.

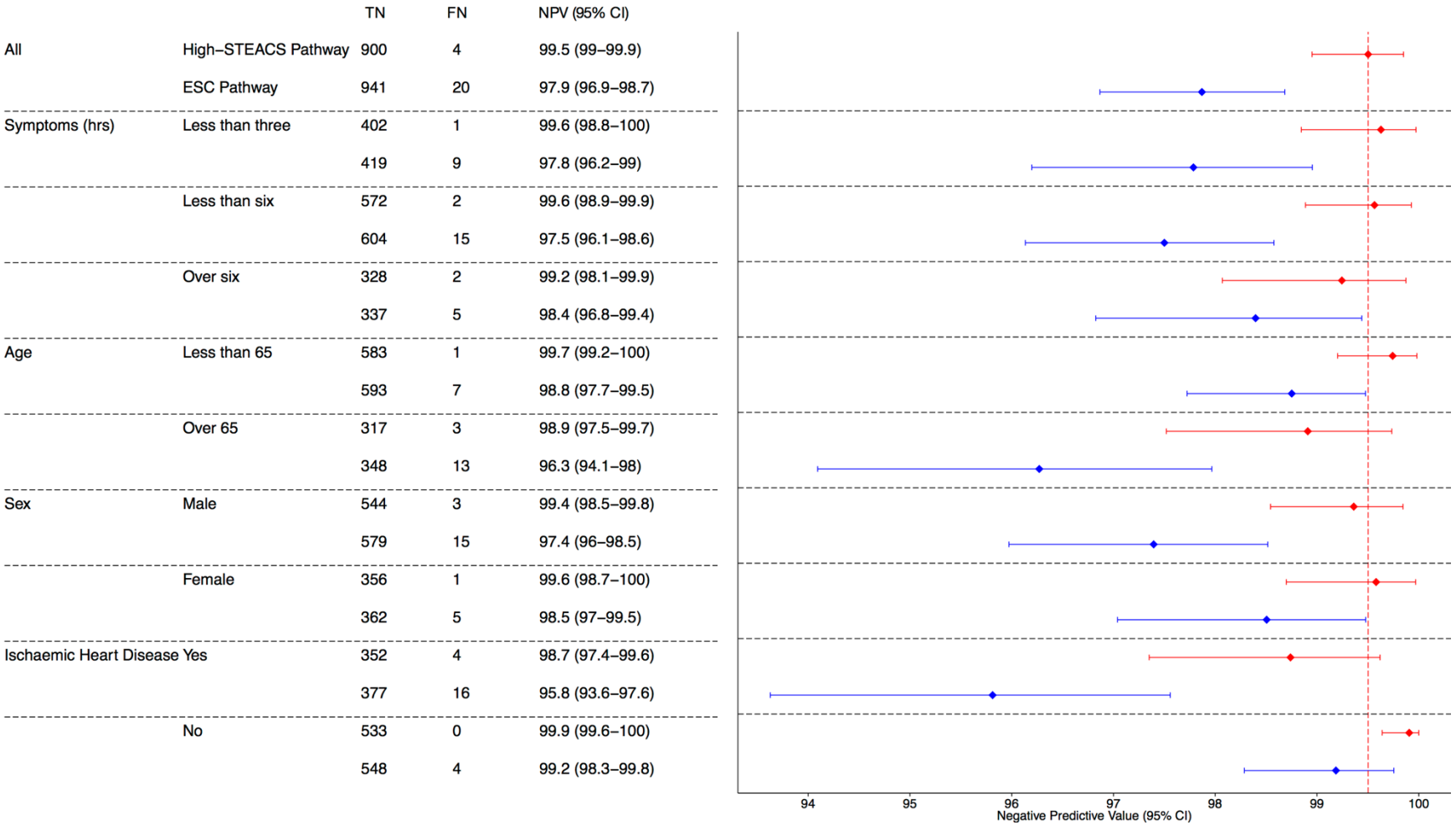

Figure 4.3 Negative predictive value for myocardial infarction or cardiac death at 30 days in the High-STEACS pathway (red) and the ESC pathway (blue).

4.4.3 Comparison of ESC and High-STEACS pathways

The High-STEACS pathway identifies more patients potentially suitable for discharge at presentation following a single cardiac troponin measurement compared to the ESC pathway (40.7% [95% CI, 38.0-43.5%] *versus* 28.1% [95% CI, 25.6-30.7%] respectively, P<0.001; ***Figure 4.2***). At three hours, the High-STEACS pathway ruled our fewer patients than the ESC pathway (74.2% [95% CI, 71.7-76.6%] *versus* 78.9% [95% CI, 76.5-81.1%], P<0.001). In the 57 patients ruled out at three hours by the ESC pathway but not the High-STEACS pathway, there were 13 missed index myocardial infarction (22.8%).

The NPV of the High-STEACS pathway was greater than the ESC pathway overall (99.5% [95%CI 99.0-99.9] *versus* (97.9% [95%CI 96.9-98.9%]; P<0.001), and for all pre-specified subgroups (***Table 4.2, Figure 4.3***). In the subgroup of patients who presented within three hours of symptom onset there were more false negatives and the NPV was lower with the ESC pathway (9 false negatives, NPV 97.8% [95% CI, 96.2-99.0%]) than with the High-STEACS pathway (1 false negative, NPV 99.6% [95% CI, 98.8-100.0%]). Similar differences were apparent in those patients presenting within six hours of symptom onset (ESC *versus* High-STEACS, NPV 97.5% [95% CI, 96.1-98.6%] *versus* 99.6% [95% CI, 98.9-99.9%]). In men, the NPV of the ESC pathway was lower than the High-STEACS pathway (97.4% [95% CI, 96.0-98.5%] *versus* 99.4% [95% CI, 98.5-99.8%]), although both pathways performed similarly in women. The lowest NPV for both the ESC and the High-STEACS pathway was in the subgroup of patients known to have ischaemic heart disease (95.8% [95% CI, 93.6-97.6%] and 98.7% [95% CI, 97.4-99.6%] respectively).

In patients with an index type 1 myocardial infarction missed by the ESC pathway, the median change in cardiac troponin concentration between presentation and 3 hours was 5.5 ng/L (inter-quartile range [IQR] 4.0-13.3 ng/L). The majority of these patients (16/18) were not ruled out at 3 hours by the High-STEACS pathway as the change in cardiac troponin concentration was ≥3 ng/L ***(Table 4.3)*** and further testing at 6 hours is recommended.

Table 4.3. 2x2 table for internal validation of delta criteria at three hours

	Type 1 MI	No Type 1 MI
Change ≥3 ng/L at 3 hours	40	52
Change <3 ng/L at 3 hours	2	216

Patients with cardiac troponin concentrations ≥5 ng/L and <99th centile on presentation are re-tested at three hours. Those with a change in cardiac troponin of <3 ng/L are ruled out if they remain <99th centile.

In an external validation cohort of 2,533 patients with suspected acute coronary syndrome (***Appendix 2.1***), a change in cardiac troponin concentration <3 ng/L at 3 hours ruled out 69.9% of those patients who required retesting (514/735), and missed no patients with an index diagnosis of type 1 myocardial infarction (***Table 4.4).***

Table 4.4. 2x2 table for external validation of delta criteria at three hours

	Type 1 MI	No Type 1 MI
Change ≥3 ng/L at 3 hours	51	170
Change <3 ng/L at 3 hours	0	514

Patients with cardiac troponin concentrations ≥5 ng/L and <99th centile on presentation are re-tested at three hours. Those with a change in cardiac troponin of <3 ng/L are ruled out if they remain <99th centile.

The specificity and PPV of the ESC pathway was greater than the High-STEACS pathway at three hours (specificity 91.6% [95% CI, 89.9-93.3] and PPV 66.5% [95% CI, 60.6-72.1] *versus* specificity 87.6% [95% CI, 85.6-89.6%] and PPV 59.5% [95% CI, 54.1-64.9%], ***Table 4.2***). However, the overall specificity and PPV of the High-STEACS pathway was comparable when patients requiring additional testing at 6 hours were included (specificity 91.4% [95% CI, 89.7-93.1%] and PPV 67.9% [95% CI, 62.3-73.3%], ***Appendix 2.4***).

4.4.4 Sensitivity analyses

In a sensitivity analysis, we evaluated both pathways using a single 99th centile upper reference limit for men and women of 26 ng/L. The performance of both pathways was similar, with a NPV of 97.7% [95% CI, 96.6-98.5%] for the ESC pathway (20 missed index type 1 myocardial infarction and two missed events at 30 days), and 99.4% [95% CI, 98.8-99.8%] for the High-STEACS pathway (three missed index type 1 myocardial infarction and two missed 30 day events). The ESC pathway missed a similar proportion of men and women (10 men, 12 women).

We performed a further sensitivity analysis excluding patients with evidence of myocardial ischaemia on the electrocardiogram or with a GRACE score >140 (n=224), of whom 71 patients had an index type 1 myocardial infarction. The diagnostic accuracy of both pathways improved. The ESC pathway still missed 13 index and one subsequent event (NPV 98.3% [95% CI, 97.3-99.0%]), whereas the High-STEACS pathway missed only one index and one subsequent event (NPV 99.7% [95% CI, 99.2-99.9%] P<0.001).

We evaluated the diagnostic performance of both pathways for a composite endpoint incorporating an index diagnosis of type 1 or type 2 myocardial infarction, or myocardial injury, or myocardial infarction or cardiac death at 30 days. The High-STEACS pathway missed an additional five events, whilst the ESC pathway missed an additional nine events (High-STEACS NPV 99.0% [95% CI, 98.2-99.5%], nine false negatives; two index type 1 and five index type 2 myocardial infarction, two type 1 myocardial infarction at 30 days, *versus* ESC NPV 96.9% [95% CI, 95.8-97.9%], 29 false negatives; 18 index type 1 and nine index type 2 myocardial infarction, two type 1 myocardial infarction at 30 days, respectively).

4.5 Discussion

In patients with suspected acute coronary syndrome, we describe a clinical pathway utilising low cardiac troponin concentrations within the reference range to risk stratify patients. This approach identifies more patients as low risk at presentation, and has a better overall negative predictive value than guideline approved pathways based solely on the 99th centile. Implementation of this pathway has the potential to improve the efficiency and safety of early rule-out approaches for patients with suspected acute coronary syndrome.

We make a number of important and clinically relevant observations. First, we demonstrate the High-STEACS pathway misses fewer patients with an index diagnosis of myocardial infarction, or myocardial infarction or cardiac death events at 30 days than the pathway approved by the European Society of Cardiology (4 missed events *versus* 20 missed events). Second, the negative predictive value of our pathway is 99.5% and better than the existing ESC pathway across all pre-specified subgroups. In particular, the ESC pathway was less effective in men, those with a history of ischaemic heart disease, and those presenting early after the onset of symptoms. Third, in patients without an elevated troponin concentration at presentation, the High-STEACS pathway identified half as low risk with a single measurement, compared to a third identified using the established pathway. This is despite being safer, and missing fewer patients with an index myocardial infarction.

The European Society of Cardiology guideline recommends the use of high-sensitivity cardiac troponin assays, and their central algorithm advises the 99th centile be used as the threshold to rule in and rule out myocardial infarction at presentation and at three

hours (Roffi et al., 2016). However, the 99th centile may not be the optimal threshold to rule out myocardial infarction, and our observations suggest this threshold does not provide an acceptable NPV or sensitivity (97.9% [95%CI 96.9-98.7%] and 89.3% [95%CI 84.9-93.5%] respectively). The performance of the ESC pathway is improved by inclusion of a risk stratification threshold and recognition that changes in cardiac troponin concentration within the reference range are important. In a large external validation cohort, we report that more than two-thirds of patients with troponin concentrations above our risk stratification threshold at presentation can be safely ruled out at 3 hours if troponin concentrations are unchanged (<3 ng/L), with no missed diagnosis of type 1 myocardial infarction.

Our findings are consistent with a recently published evaluation of the ESC pathway which reported a negative predictive value of 99.0% [95%CI 98.1-99.5%], and sensitivity of 93.2% [95%CI 87.5-96.8%] in a pooled analysis of five international cohorts (Pickering et al., 2016b). Importantly, this analysis included lower risk patients without ischaemia on the electrocardiogram. In practice, risk stratification and early rule-out pathways are only likely to be applied to patients without overt myocardial ischaemia on the electrocardiogram (Shah et al., 2016). However, interpretation of the electrocardiogram may be subjective and dependent on clinician experience, and therefore we included all patients in our evaluation to ensure our safety estimates were conservative. Likewise, many clinicians use risk stratification tools to identify patients suitable for early discharge. Whilst the ESC guidelines do not advocate use of GRACE score for this purpose, it is widely used and is recommended to guide further investigation in patients whom myocardial infarction has been ruled out. When we restricted our analysis to patients with no significant ST-segment depression or T-wave

inversion on the electrocardiogram and GRACE scores of <140, we observed a modest improvement in the NPV of the ESC pathway (98.3% [95% CI, 97.3-99.0%]), although even in this lower risk group the ESC pathway was inferior to the High-STEACS pathway (NPV 99.7% [95% CI, 99.2-99.9%]). Whilst the inclusion of all patients in the primary analysis ensures our safety estimates are conservative, it is important to highlight that in clinical practice, careful clinical assessment and risk assessment is mandatory for all diagnostic pathways. In implementing our pathway, we recommend that patients with overt myocardial ischemia on the electrocardiogram at presentation are admitted for further assessment **(*Appendix 2.5*).**

Pickering and colleagues utilized a single diagnostic threshold for myocardial infarction (26 ng/L) in both men and women, although a sensitivity analysis showed the performance of the ESC pathway was similar using sex-specific thresholds (Pickering et al., 2016b). In our analysis, we observed a reduction in the performance of the ESC pathway in men evaluated using the same assay with sex-specific thresholds (34 ng/L in men, 16 ng/L in women; 15 missed events and 5 missed events, respectively). In our sensitivity analysis, use of a single diagnostic threshold of 26 ng/L in men and women did not improve the overall performance of the ESC pathway. In contrast, the safety of the High-STEACS pathway was robust across both sexes and all pre-specified subgroups of patients. Whilst the use of sex-specific thresholds in pathways that rely on the 99th centile remains contentious in clinical practice, risk stratification thresholds are not influenced by sex (Shah et al., 2015a), and therefore a single threshold can be applied equally to risk stratify men and women at presentation.

The efficacy of early rule-out pathways is also an important consideration. We demonstrate that the High-STEACS pathway ruled out a higher proportion of patients than the ESC pathway at presentation (40.7% *versus* 28.1%, P<0.001). Whilst our pathway rules out fewer patients at 3 hours (74.2% *versus* 78.9%), P<0.001), of the additional 57 patients ruled out by the ESC pathway, 1 in 5 (22.8%) were incorrectly ruled out and had an index diagnosis of type 1 myocardial infarction identified on subsequent testing. By identifying those patients with a change in cardiac troponin concentration (≥3 ng/L) from presentation to three hours and undertaking further testing, none of these events would be missed by the High-STEACS pathway. This highlights the value of high-sensitivity cardiac troponin assays, which permit the identification of small, but important changes in troponin concentration within the normal reference range, and allow refinement in the risk stratification of patients with suspected acute coronary syndrome. The only disadvantage of our pathway is that in prioritising safety, the specificity and PPV for a diagnosis of myocardial infarction is lower than the ESC pathway at 3 hours (4% and 7% respectively). Specificity is also important, but in our view it need not be prioritized in early rule out pathways. In patients we identify who require hospital admission, the diagnosis of myocardial infarction is best determined by demonstrating a rise and fall in cardiac troponin concentration over 6-12 hours.

The latest ESC guidelines have introduced a one-hour pathway that incorporates a risk stratification step utilising high-sensitivity cardiac troponin concentrations within the reference range (Roffi et al., 2016). This approach shows promise, and has been validated using both high sensitivity troponin I and high sensitivity troponin T assays, with a NPV of 99.6% [95% CI, 98.4-100] and 99.1% [95% CI, 98.2-99.7%]

respectively (Rubini Gimenez et al., 2015, Mueller et al., 2016). However, to our knowledge no previous studies have directly compared pathways that utilise a risk stratification step with low cardiac troponin concentrations to those based exclusively on the 99th centile. Further studies are needed to compare the efficacy and safety of retesting at 1 and 3 hours in pathways that incorporate a risk stratification threshold.

4.5.1 Limitations

One of the limitations of these studies, including our own, is that they are observational in nature, and enrol selected patients rather than all consecutive patients. Indeed, as no patients were discharged on the basis of pathway decisions, the true efficacy and safety of this approach is unknown. The ESC pathway recommends repeat testing in patients who present within 6 hours of symptom onset. Whilst the inclusion of patients who present early is a strength of our study, fewer patients may be ruled out at presentation by the ESC pathway as a consequence. At present, clinicians do not have evidence from prospective randomised controlled trials to inform their practice (Jaffe, 2016). As such, we are conducting a multi-centred stepped-wedge cluster randomised trial to determine the efficacy and safety of our pathway ***(Appendix 2.5)*** in unselected consecutive patients across Scotland. The outcome of this trial will help to inform our practice, and provide an evidence base for future recommendations on the use of high-sensitivity cardiac troponins to risk stratify patients with suspected acute coronary syndrome.

4.6 Conclusions

The High-STEACS pathway, incorporating low cardiac troponin concentrations to risk stratify patients, rules out more patients on presentation and misses fewer index or recurrent myocardial infarction than guideline-approved pathways based exclusively on the 99th centile. Implementation of this pathway has the potential to improve the efficiency and safety of early rule out approaches for patients with suspected acute coronary syndrome.

CHAPTER 5

HIGH-SENSITIVITY CARDIAC TROPONIN I AND CLINICAL RISK SCORES IN PATIENTS WITH SUSPECTED ACUTE CORONARY SYNDROME

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5.1 Summary

Background: High-sensitivity cardiac troponin assays can help to identify patients who are at low risk of myocardial infarction in the Emergency Department. We aimed to determine if the addition of clinical risk scores would improve the safety of early rule out pathways for myocardial infarction.

Methods and results: In 1,935 patients with suspected acute coronary syndrome, we evaluated the safety and efficacy of two rule out pathways, alone or in conjunction with low-risk TIMI (0 or 1), GRACE (≤108), EDACS (<16) or HEART (≤3) scores. The European Society of Cardiology (ESC) 3-hour pathway uses a single diagnostic threshold (99th centile), whereas the High-STEACS pathway applies different thresholds to rule out (<5 ng/L) and rule in (>99th centile) myocardial infarction.

Myocardial infarction or cardiac death during the index presentation or at 30-days occurred in 14.3% of patients (276/1,935). The ESC pathway ruled out 70% with 27 missed events giving a negative predictive value (NPV) of 97.9% (95% confidence interval [CI], 97.1 to 98.6%). Addition of a HEART score ≤3 reduced the proportion ruled out by the ESC pathway to 25%, but improved the NPV to 99.7% (95%CI 99.0 to 100%, P<0.001). The High-STEACS pathway ruled out 65% with three missed events for a NPV of 99.7% (95%CI 99.4 to 99.9%). No risk score improved the NPV of the High-STEACS pathways, but all reduced the proportion ruled out (24-47%, P<0.001 for all).

Conclusions: Clinical risk scores significantly improved the safety of the ESC 3-hour pathway, which relies on a single cardiac troponin threshold at the 99th centile to rule

in and rule out myocardial infarction. Where lower thresholds are used to rule out myocardial infarction, as applied in the High-STEACS pathway, risk scores halve the proportion of patients ruled out without improving safety.

5.2 Introduction

Chest pain is a common presenting symptom in the Emergency Department, and whilst many patients require investigation for acute coronary syndrome, the majority have alternative diagnoses (Zhelev et al., 2015, Goodacre et al., 2005, Skinner et al., 2010). Earlier identification of patients without myocardial infarction may improve patient experience and healthcare efficiency by reducing hospitalisation for unnecessary investigation, but such strategies can only be implemented if safety is not compromised.

There are several pathways which permit the early rule out of myocardial infarction. The European Society of Cardiology (ESC) 3-hour pathway uses the 99th centile upper reference limit of a cardiac troponin assay to rule in and rule out myocardial infarction (Roffi et al., 2016). However, recent observations have questioned whether this pathway provides adequate diagnostic performance in the era of high-sensitivity cardiac troponin testing (Pickering et al., 2016b, Chapman et al., 2017b, Wildi et al., 2016). The precision of high-sensitivity assays at very low concentrations has been exploited in the development of novel pathways that rule out myocardial infarction using thresholds well below the 99th centile. In an individual patient-level data meta-analysis of 22,457 patients, a cardiac troponin I threshold of <5 ng/L and a non-ischaemic electrocardiogram gave a NPV and sensitivity of 99.7% and 99.0% for myocardial infarction or cardiac death at 30-days, respectively (Chapman et al., 2017c). The same threshold has also been validated for the high-sensitivity cardiac troponin T assay in a recent pooled analysis (Pickering et al., 2017). When this rule out threshold was applied in a pathway that includes serial testing at 0 and 3 hours, five-fold fewer

patients were missed compared to a pathway that relies on the 99th centile to rule out myocardial infarction (Chapman et al., 2017b).

Clinical risk scores provide an alternative approach to identify patients at low risk of myocardial infarction who might be suitable for early discharge, but a number of uncertainties remain (Katus et al., 2017). New risk scores were developed (Backus et al., 2013) or existing scores were incorporated into early rule out pathways primarily to overcome the limitations of contemporary troponin assays (Than et al., 2012). However, the role of clinical risk scores in pathways that incorporate high-sensitivity cardiac troponin testing is unclear, particularly in those pathways that apply different thresholds to rule out and rule in myocardial infarction. Here, we evaluate the safety and effectiveness of two established early rule out pathways, with and without the addition of clinical risk scores.

5.3 Methods

5.3.1 Study population

Patients with suspected acute coronary syndrome were recruited from the Emergency Department of the Royal Infirmary of Edinburgh, a tertiary care hospital in Scotland, between 1st June 2013 and 31st March 2017 into a sub-study of the *High-S*ensitivity *T*roponin in the *E*valuation of patients with *A*cute *C*oronary *S*yndrome (High-STEACS) trial. All patients in whom the attending clinician requested cardiac troponin for suspected acute coronary syndrome were eligible for inclusion. We did not enrol patients with ST-segment elevation myocardial infarction, those who were unable to provide consent, or those from outside our region to ensure complete follow up. Blood samples were obtained at presentation and at 6-12 hours for high-sensitivity cardiac troponin testing as part of routine clinical care. Patients provided written informed consent for additional sampling at 3 hours with the results of testing at this time-point not used to guide patient care. This clinical trial was registered (NCT:01852123), approved by the national research ethics committee, and conducted in accordance with the Declaration of Helsinki.

5.3.2 High-sensitivity cardiac troponin I assay

The Abbott ARCHITECT$_{STAT}$ high-sensitive cardiac troponin I assay (Abbott Laboratories, Abbott Park, IL) is a two-step chemo-luminescent assay with a limit of detection of 1.2 ng/L and coefficient of variation of less than 10% at 6 ng/L (Chin et al., 2014). This assay performance has been independently validated across multiple centres under routine laboratory working conditions, with a reported inter-laboratory coefficient of variation of 12.6% at 3.5 ng/L across 33 instruments (Shah et al., 2015a).

The upper reference limit 99th centiles were determined in 4,590 samples from healthy individuals as 16 ng/L for women and 34 ng/L in men (Shah et al., 2015b), and from 10th December 2013 onwards these thresholds were used in clinical practice.

5.3.3 Baseline characteristics

Patient baseline characteristics, including chest pain characteristics, onset of symptoms, prior medical history, cardiovascular risk factors, medication, and clinical observations, in addition to investigations including serial 12-lead electrocardiography and cardiac imaging, were obtained from a dedicated case record form, patient questionnaire and the electronic patient record (TrakCare, InterSystems, Cambridge, MA). Hyperlipidaemia or hypertension were defined as a history of the condition, or by the use of lipid-lowering or anti-hypertensive therapies, respectively. Ischaemic heart disease was defined as a history of angina, prior myocardial infarction or prior coronary revascularisation.

5.3.4 Diagnostic adjudication and clinical outcomes

The final diagnosis was adjudicated for all patients by two independent cardiologists, with consensus from a third cardiologist where there was discrepancy following review of all clinical information, both non-invasive and invasive investigations and outcomes from presentation to 30 days. Patients were classified as having type 1 myocardial infarction, type 2 myocardial infarction or myocardial injury in accordance with the third universal definition of myocardial infarction as reported previously (Roffi et al., 2016, Shah et al., 2015a). Any high-sensitivity cardiac troponin I concentration above the sex-specific 99th centile upper reference limit was considered evidence of myocardial necrosis. Type 1 myocardial infarction was defined as

myocardial necrosis in the context of a presentation with symptoms suggestive of acute coronary syndrome or evidence of myocardial ischemia. Patients with symptoms or signs of myocardial ischemia due to increased oxygen demand or decreased supply (e.g. tachyarrhythmia, hypotension or anaemia) secondary to an alternative pathology and myocardial necrosis were classified as type 2 myocardial infarction. Myocardial injury was defined as evidence of myocardial necrosis in the absence of any clinical features of myocardial ischaemia. Further details on the adjudication process are available in a supplementary appendix. Agreement for a diagnosis of type 1 myocardial infarction was good (κ=0.77; 95% CI 0.69-0.84).

The primary outcome was a composite of index type 1 myocardial infarction, or type 1 myocardial infarction or cardiac death at 30 days. We used regional and national registries in addition to individual patient follow up at 30 days to ensure follow up was complete for the entire study population. All subsequent events were adjudicated using the same approach as for the index presentation. TrakCare software application (InterSystems Corporation, Cambridge, MA, USA) is a regional electronic patient record system, which provides data on all hospital admissions to both tertiary or secondary care hospitals in the southeast of Scotland. All in-hospital and community deaths are recorded in a comprehensive national database, the General Register of Scotland. Cardiac death was defined as any death due to myocardial infarction, arrhythmia or heart failure (ICD-10 codes I20-25, I34-37, I42, I43, I46, I48-51).

5.3.5 Clinical pathways

We evaluated the safety and efficacy of the European Society of Cardiology 3-hour pathway and the High-STEACS pathway (***Figure 5.1***), with and without the addition of clinical risk scores, to rule out the composite outcome of index type 1 myocardial infarction, and type 1 myocardial infarction or cardiac death at 30 days. These pathways were selected as they represent examples of approaches using troponin as a continuous variable, or as a binary decision tool applying the 99th centile alone. To improve generalizability, where samples were available, we also evaluate the European Society of Cardiology 1-hour pathway.

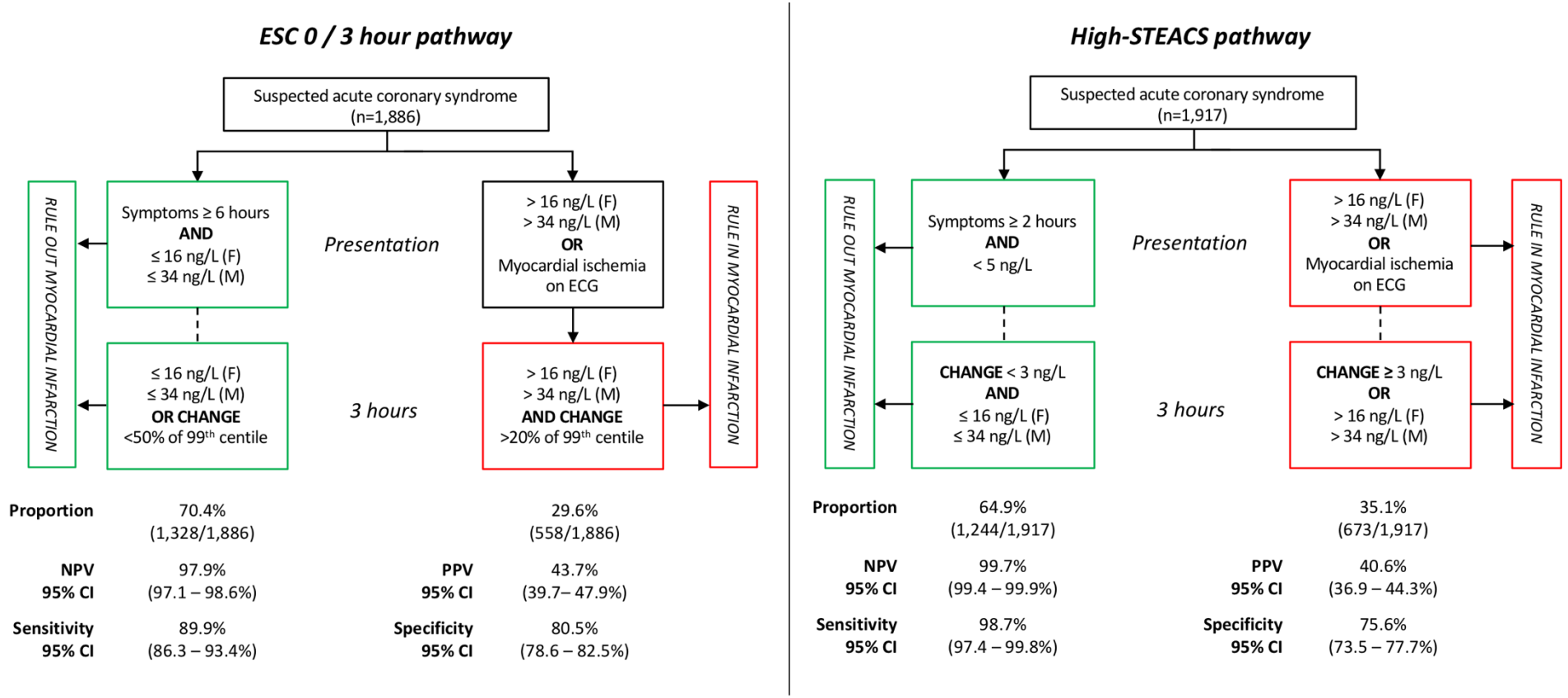

Figure 5.1. Illustration of the European Society of Cardiology 3-hour pathway, and the High-STEACS pathway, with associated diagnostic metrics.

The ESC 3-hour pathway rules out myocardial infarction in patients without ischaemia on the electrocardiogram where cardiac troponin concentrations are <99th centile at presentation in patients with symptoms for more than 6 hours. In patients with symptoms for less than 6 hours, a second troponin measurement is performed 3 hours from presentation, with myocardial infarction ruled out if cardiac troponin remains <99th centile or is >99th centile without a significant change in concentration (Roffi et al., 2016). Previously published guidance from the ESC Working Group on Acute Cardiac Care recommends use of a change in cardiac troponin concentration >50% of the 99th centile upper reference limit at 3 hours where the initial concentration is ≤99th centile, or >20% when the initial concentration was >99th centile (Thygesen et al., 2012b). The ESC pathway recommends a GRACE score of <140 in those who are pain free as a final step prior to discharge.

The derivation and validation of the High-STEACS pathway has been reported previously (Chapman et al., 2017b). This pathway was based on previous observations (Shah et al., 2015a, Chapman et al., 2017c), and utilises a risk stratification threshold of 5 ng/L at presentation. Patients without myocardial ischaemia on the electrocardiogram and cardiac troponin concentrations <5 ng/L at presentation are considered low risk, with myocardial infarction ruled out without further testing, unless they present early with symptom onset <2 hours from presentation where cardiac troponin is retested 3 hours after presentation. Patients with cardiac troponin concentrations ≥5 ng/L at presentation are retested at 3 hours. Myocardial infarction is ruled out at 3 hours if cardiac troponin concentrations are unchanged (delta <3 ng/L) and remain ≤99th centile.

The ESC 1-hour pathway rules out myocardial infarction in patients without ischemia on the electrocardiogram where cardiac troponin concentrations are <2 ng/L at presentation and symptoms are present for more than 3 hours. In all other patients, myocardial infarction is ruled out if cardiac troponin concentrations are <5 ng/L at presentation with a change of <2 ng/L after one hour.

5.3.6 Clinical risk scores

We derived GRACE, TIMI, HEART and EDACS scores using prospectively collected clinical information documented in the case record form by the research nurse at the time of recruitment (***Figure 5.2***). We calculated the GRACE score for in hospital death; this algorithm is available online (Anderson and FitzGerald, 2010). In line with prior recommendations, a GRACE score of ≤108 (estimated in hospital mortality of <1%) (Hamm et al., 2011), a HEART score of ≤3 (Backus et al., 2013), a TIMI score of 0 or 1 (Antman et al., 2000) or an EDACS score of <16 were considered low risk (Than et al., 2014b). For comparison, we provide the diagnostic performance of the HEART, GRACE, TIMI and EDACS scores alone in the appendix.

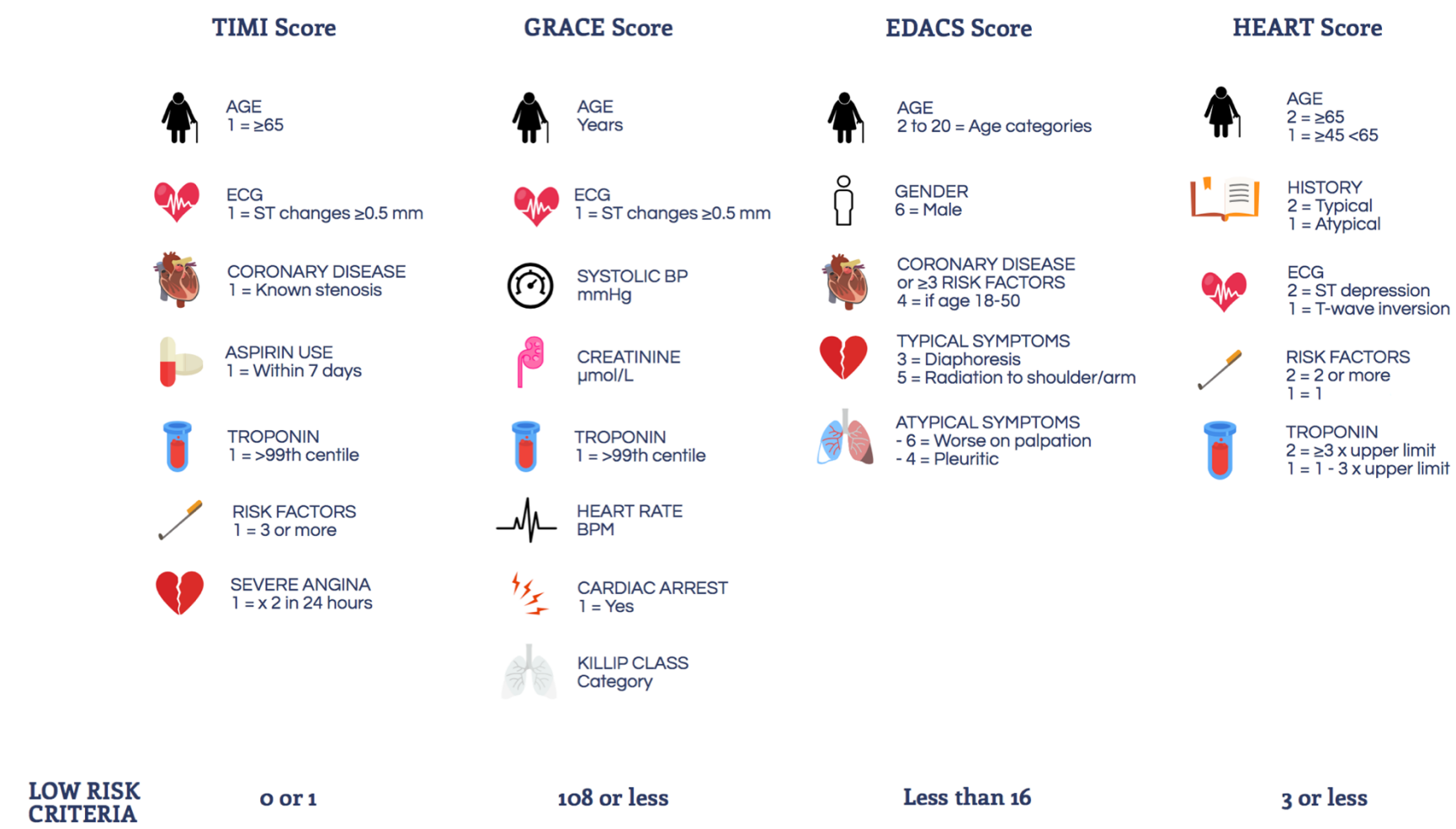

Figure 5.2. Illustration of the components of the HEART, GRACE, EDACS and TIMI score.

5.3.7 Sensitivity analyses

We evaluated the NPV of all approaches for a primary outcome encompassing type 1 or type 2 myocardial infarction, or myocardial injury, or cardiac death at 30 days. As the High-STEACS pathway was derived in the first 1,218 participants included in our dataset, we repeated our analyses and excluded these subjects. In a further analysis, we tested both pathways excluding any patients who underwent invasive or non-invasive cardiac testing within 30 days of index presentation.

5.3.8 Statistical analyses

Baseline characteristics are summarised as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate. Where there were missing data for continuous variables, we imputed the median value. The primary outcome was the negative predictive value (NPV) of each pathway, using the composite endpoint of index type 1 myocardial infarction, or subsequent type 1 myocardial infarction or cardiac death at 30 days (Shah et al., 2015a). As we estimated the NPV would approach 100%, we used a Bayesian approach with a Jeffreys prior (beta distribution with both shape parameters equal to 0.5) as this is more robust when confidence intervals approach 0 or 1 (Brown et al., 2001). We determined absolute ($\text{hs-TnI}_{3hr} - \text{hs-TnI}_{0hr}$) and relative ($[(\text{hs-TnI}_{3hr} - \text{hs-TnI}_{0hr}) / \text{hs-TnI}_{0hr}] \times 100$) change in cardiac troponin concentration from presentation to 3 hours, and determined sensitivity, specificity and positive predictive value (PPV) with 95% confidence intervals (CI) using a Bayesian approach as per the NPV. We derived a weighted generalised score statistic to compare the NPV of each pathway with and without the addition of clinical risk scores (Leisenring et al., 2000). We evaluated pathway efficacy by determining the number of patients ruled out with the pathway alone, or with the combination of pathway and

risk score as a proportion of the total study population, with comparison by McNemar's test for paired proportions. A two-sided $P<0.05$ was considered statistically significant. All analyses were performed using R (Version 3.2.2).

5.4 Results

We enrolled 1,951 patients with suspected acute coronary syndrome, of whom 1,935 had a cardiac troponin I result available from presentation (***Table 5.1, Table 5.2 and Appendix 3.1*** respectively)**.** The adjudicated diagnosis was type 1 myocardial infarction in 273 patients (14.1%), type 2 myocardial infarction in 77 patients (4%) and myocardial injury in 31 patients (1.6%), with 6 deaths from a cardiac cause at 30 days (***Figure 5.3***).

Table 5.1. Baseline characteristics of the study population stratified by troponin concentration

	All patients (n=1,935)	<5 ng/L at presentation (n=1,142)	≤99th centile at presentation (n=1,639)	>99th centile at presentation (n=296)
Baseline characteristics				
Age	61.5 (14.2)	56.6 (12.6)	60.2 (14)	68.7 (13.6)
Male (%)	1182 (61.1)	637 (55.8)	1012 (61.7)	170 (57.4)
Chest pain (%)	1623 (84.1)	983 (86.4)	1387 (84.9)	236 (79.9)
Symptom to arrival time (mins)	199 (118-464)	199 (114-462)	199 (115-445)	199 (138-528)
Past medical history				
Diabetes mellitus (%)	286 (14.8)	124 (10.9)	229 (14.0)	57 (19.3)
Hypertension (%)	769 (39.7)	358 (31.3)	630 (38.4)	139 (47.0)
Hyperlipidaemia (%)	765 (39.5)	376 (32.9)	641 (39.1)	124 (41.9)
Family History (%)	927 (47.9)	575 (50.4)	793 (48.4)	134 (45.3)
Ischaemic Heart Disease (%)	582 (30.1)	252 (22.1)	484 (29.5)	98 (33.1)
Previous MI (%)	460 (23.8)	172 (15.1)	374 (22.8)	86 (29.1)
Previous PCI (%)	366 (18.9)	154 (13.5)	308 (18.8)	58 (19.6)
Previous CABG (%)	117 (6.0)	31 (2.7)	96 (5.9)	21 (7.1)
Previous Heart Failure (%)	66 (3.4)	10 (0.9)	45 (2.7)	21 (7.1)
Previous Stroke (%)	119 (6.1)	45 (3.9)	97 (5.9)	22 (7.4)
Smoker (%)	385 (19.9)	266 (23.3)	330 (20.1)	55 (18.6)
Medication history				
Aspirin (%)	655 (33.9)	305 (26.7)	545 (33.3)	110 (37.2)
Clopidogrel (%)	250 (12.9)	100 (8.8)	203 (12.4)	47 (15.9)
Beta-blocker (%)	522 (27.0)	240 (21.0)	431 (26.3)	91 (30.7)
ACE Inhibitor (%)	582 (30.1)	271 (23.7)	487 (29.7)	95 (32.1)
Statin (%)	824 (42.6)	398 (34.9)	690 (42.1)	134 (45.3)
Long Acting Nitrate (%)	369 (19.1)	156 (13.7)	296 (18.1)	73 (24.7)
Calcium Channel Blocker (%)	242 (12.5)	109 (9.5)	199 (12.1)	43 (14.5)
Warfarin (%)	105 (5.4)	34 (3.0)	83 (5.1)	22 (7.4)
Electrocardiogram findings				
ST depression (%)	112 (5.8)	27 (2.4)	65 (4.0)	47 (15.9)
ST elevation (%)	58 (3.0)	28 (2.5)	42 (2.6)	16 (5.4)
T-wave inversion (%)	300 (15.5)	113 (9.9)	218 (13.3)	82 (27.7)
Physiological parameters				
Systolic BP (mmHg)	137 (124-152)	137 (124-152)	137 (124-152)	137 (121-151)
Diastolic BP (mmHg)	77 (68-88)	79 (70-89)	78 (69-88)	74 (66-85)

Heart rate (bpm)	75 (64-87)	75 (65-86)	75 (65-86)	76 (63-91)
Temperature	36.5 (36.1-36.9)	36.5 (36.1-37)	36.5 (36.1-36.9)	36.5 (36-36.8)
Respiratory rate	16 (16-18)	16 (16-18)	16 (16-18)	16 (16-18)
Oxygen saturations	97 (96-98)	98 (96-99)	97 (96-99)	97 (96-98)
Creatinine	74(67-84)	73 (66-78)	74 (67-82)	76 (69-94)
Clinical risk scores				
HEART Score	5 (3-6)	4 (3-5)	4 (3-5)	7 (6-8)
GRACE Score	100 (79-107)	91 (79-100)	100 (79-129)	129 (107-151)
TIMI Score	1 (0-3)	1 (0-2)	1 (0-2)	3 (2-4)
Adjudicated index diagnosis				
Type 1 myocardial infarction (%)	273 (14.1)	6 (0.5)	65 (4.0)	208 (70.3)
Type 2 myocardial infarction (%)	77 (4.0)	5 (0.4)	24 (1.5)	53 (17.9)
Myocardial injury (%)	31 (1.6)	0 (0.0)	1 (0.1)	30 (10.1)
Outcomes at 30 days				
Type 1 myocardial infarction (%)[a]	8 (0.4)	0 (0.0)	3 (0.2)	5 (1.7)
Cardiac death at 30 days (%)	6 (0.3)	0 (0.0)	2 (0.1)	4 (1.4)
Type 1 myocardial infarction or cardiac death at 30 days (%)	276 (14.3)	6 (0.5)	68 (4.1)	208 (70.3)

Values are mean (SD),median (IQR) or n(%). MI – myocardial infarction. PCI – percutaneous coronary intervention. CABG – coronary artery bypass grafting. ACE – angiotensin converting enzyme, BP – blood pressure. [a] Excluding index events.

Table 5.2. Investigation and management of patients stratified by troponin concentration at presentation

	All patients (n=1,935)	**<5 ng/L at presentation (n=1,142)**	**≤99th centile at presentation (n=1,639)**	**>99th centile at presentation (n=296)**
In-hospital management				
Aspirin (%)	1055 (56.3)	564 (50.9)	839 (52.9)	216 (74.7)
Clopidogrel (%)	217 (11.9)	27 (2.5)	54 (3.5)	163 (57.2)
Ticagrelor (%)	6 (0.4)	1 (0.1)	2 (0.1)	4 (1.6)
Heparin (%) *	157 (8.6)	11 (1.0)	25 (1.6)	132 (46.6)
Cardiology referral (%)	888 (47.9)	378 (34.8)	627 (40.2)	261 (89.7)
Investigation and management within 30 days of presentation				
Echocardiography (%)	183 (9.5)	36 (3.2)	90 (5.5)	93 (31.4)
Coronary angiography (%)	220 (11.4)	24 (2.1)	72 (4.4)	148 (50.0)
Percutaneous coronary intervention (%)	138 (7.1)	9 (0.8)	36 (2.2)	102 (34.5)

* including unfractionated or low molecular weight heparin

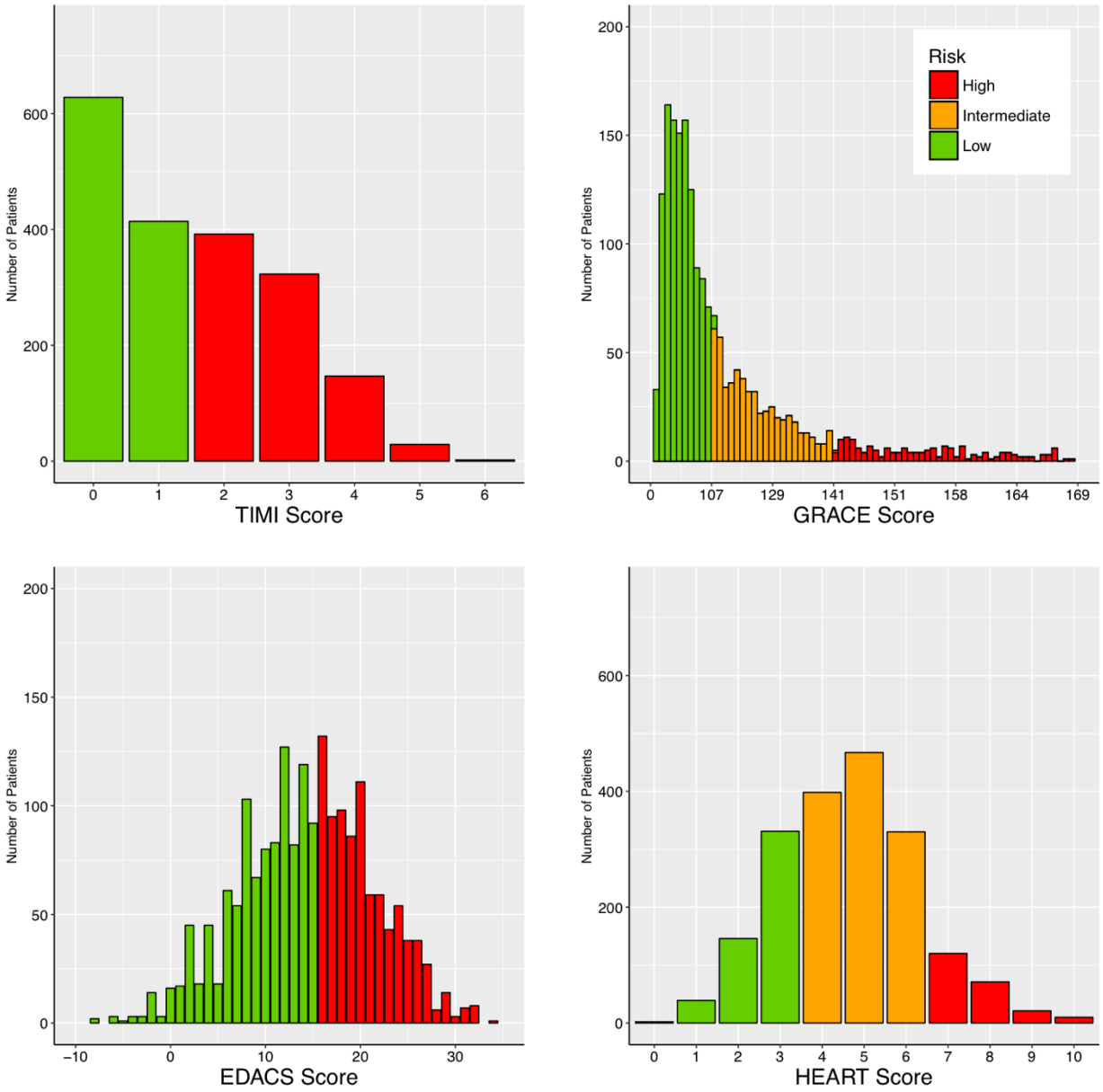

Figure 5.3. Number of patients in each risk category by TIMI, GRACE, EDACS and HEART risk scores.

5.4.1 ESC 3-hour pathway

A total of 1,886 patients (97.5%) were included, with 49 patients excluded due to missing 3-hour samples which were required for the ESC pathway (***Figure 5.1***). The ESC pathway identified 70% (1,328/1,886) of patients as low risk, with 27 missed events (25 index type 1 myocardial infarction, one type 1 myocardial infarction and one cardiac death at 30 days, ***Appendix 3.2***) for a negative predictive value (NPV) of 97.9% (95% confidence interval [CI] 97.1 to 98.6%) and sensitivity of 89.9% (95%CI 86.3 to 93.4%; ***Table 5.3***).

5.4.2 ESC 3-hour pathway plus clinical risk scores

When a HEART score of ≤3 was applied alongside the ESC pathway, the proportion identified as low risk fell from 70% to 24.8% (468/1,886, P<0.001). However, the NPV improved to 99.7% (95%CI 99.0 to 100%, P<0.001). A similar improvement in safety was observed when an EDACS score of <16 was applied, with a NPV of 99.2% (95%CI 98.5 to 99.7%, P<0.001), identifying 42.4% as low risk (800/1,886, P<0.001). A TIMI score of 0 or 1 gave a NPV of 99.2%, (95%CI 98.5 to 99.7%, P<0.001), and a GRACE score of ≤108 gave a NPV of 99.0% (95%CI 98.2 to 99.5%, P<0.001), with a reduction in the proportion identified as low risk to 43.5% (844/1,886, P<0.001) and 49% (924/1,886, P<0.001) of patients, respectively. When a higher GRACE score of <140 was applied as recommended in the guideline, the NPV and sensitivity were lower at 98.1% (95%CI 97.3 to 98.8%) and 91.3% (95%CI 87.8 to 94.4%), respectively, with 23 missed index or 30-day events.

Table 5.3. Diagnostic metrics for the European Society of Cardiology 3-hour pathway with and without clinical risk scores

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95% CI)	Sensitivity (95% CI)	Positive predictive value (95% CI)	Specificity (95% CI)	Proportion low risk (%)
ESC 3-h pathway	244	314	1301	27	97.9 (97.1-98.6)	89.9 (86.3-93.4)	43.7 (39.7-47.9)	80.5 (78.6-82.5)	70.4
ESC 3-h + TIMI (0/1)	265	777	838	6	99.2 (98.5-99.7)	97.6 (95.5-99.1)	25.5 (22.9-28.1)	51.9 (49.5-54.3)	44.8
ESC 3-h + GRACE ≤108	262	700	915	9	99.0 (98.2-99.5)	96.5 (94.0-98.3)	27.3 (24.5-30.1)	56.7 (54.2-59.1)	49.0
ESC 3-h + EDACS <16	265	821	794	6	99.2 (98.5-99.7)	97.6 (95.5-99.1)	24.4 (21.9-27.0)	49.2 (46.7-51.6)	42.4
ESC 3-h + HEART ≤3	270	1148	467	1	99.7 (99.0-100)	99.4 (98.3-100)	19.1 (17.1-21.1)	28.9 (26.7-31.2)	24.8

ESC – European Society of Cardiology, TIMI – Thrombolysis In Myocardial Infarction, GRACE – Global Registry of Acute Coronary Events, EDACS = Emergency Department Assessment of Chest pain Score CI – confidence interval

5.4.3 High-STEACS pathway

A total of 1,917 patients (99.1%) were included, with 18 patients excluded due to missing 3-hour samples which were required for the High-STEACS pathway ***(Figure 5.1)***. The High-STEACS pathway identified 64.9% (1,244/1,917) of patients as low risk, with three missed events (two index type 1 myocardial infarction and one type 1 myocardial infarction at 30 days, ***Appendix 3.3***) for a NPV of 99.7% (95%CI 99.4 to 99.9%) and sensitivity of 98.7% (95%CI 97.4 to 99.8%; ***Table 5.4***).

5.4.4 High-STEACS pathway plus clinical risk scores

When a HEART score ≤3 was applied alongside the High-STEACS pathway, the proportion identified as low risk fell to 24.3% (465/1,917, P<0.001). There was no improvement in the NPV (99.9%, 95%CI 99.6 to 100%, P=0.083). Similarly, no improvements in NPV were observed when the High-STEACS pathway was applied in conjunction with an EDACS score of <16 (NPV 99.7%, 95%CI 99.2 to 99.9%, P=0.912), a TIMI score of 0 or 1 (NPV 99.8%, 95%CI 99.4 to 100%, P=0.313), or a GRACE score of ≤108 (NPV 99.7%, 95%CI 99.3 to 100%, P=0.815). All risk scores reduced the proportion of patients identified as low risk (EDACS 41%, TIMI 44% and GRACE 47%, P<0.001 for all; ***Figure 5.4***).

Table 5.4. Diagnostic metrics for the High-STEACS pathway with and without clinical risk scores

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95% CI)	Sensitivity (95% CI)	Positive predictive value (95% CI)	Specificity (95% CI)	Proportion low risk (%)
High-STEACS Pathway	273	400	1241	3	99.7 (99.4-99.9)	98.7 (97.4-99.8)	40.6 (36.9-44.3)	75.6 (73.5-77.7)	64.9
High-STEACS + TIMI (0/1)	275	808	833	1	99.8 (99.4-100)	99.5 (98.3-100)	25.4 (22.9-28)	50.8 (48.3-53.2)	43.5
High-STEACS + GRACE ≤108	274	734	907	2	99.7 (99.3-100)	99.1 (97.7-99.8)	27.2 (24.5-30)	55.3 (52.9-57.7)	47.4
High-STEACS + EDACS <16	274	851	790	2	99.7 (99.2-99.9)	99.1 (97.7-99.8)	24.4 (21.9-26.9)	48.1 (45.7-50.6)	41.3
High-STEACS + HEART ≤3	276	1176	465	0	99.9 (99.6-100)	99.8 (99.3-100)	19.0 (17.1-21.1)	28.3 (26.2-30.6)	24.3

High-STEACS = High-Sensitivity Troponin in the Evaluation of patients with Acute Coronary Syndrome, TIMI – Thrombolysis In Myocardial Infarction, EDACS – Emergency Department Assessment of Chest pain Score, GRACE – Global Registry of Acute Coronary Events, CI – confidence interval

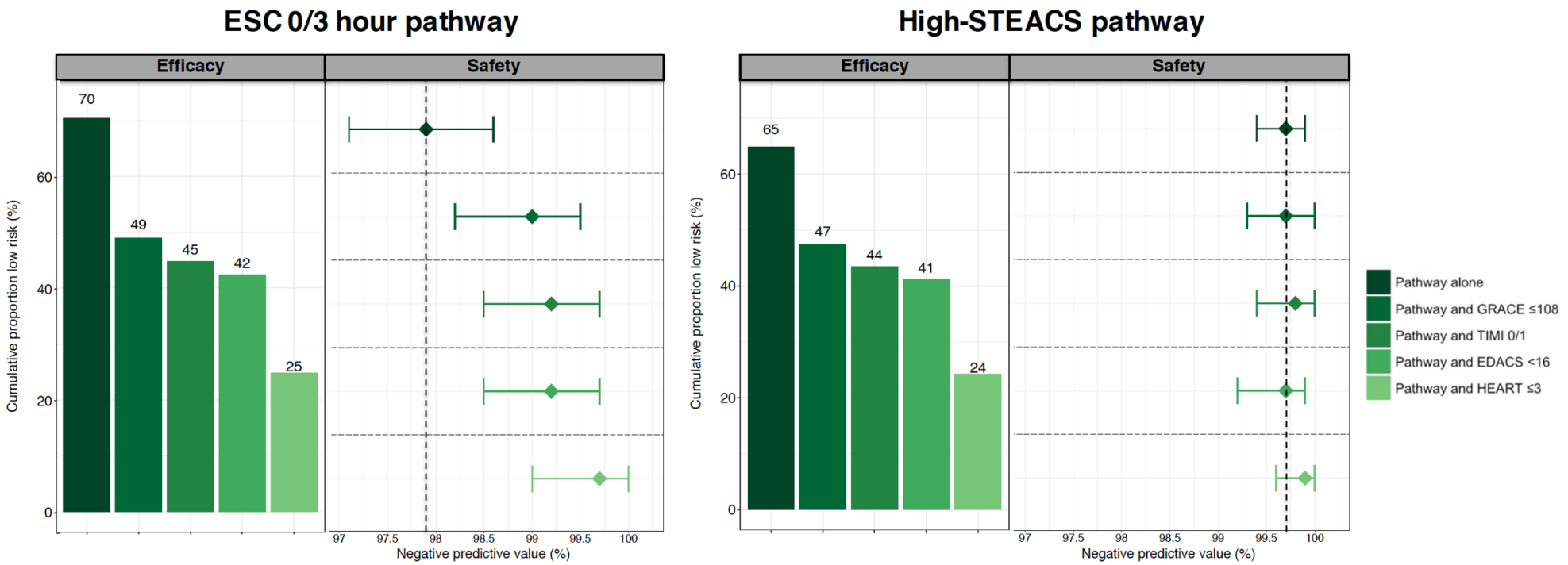

Figure 5.4. Proportion of patients identified as low risk by pathway with or without addition of clinical risk score.

5.4.5 Sensitivity analyses

We repeated our analyses of the High-STEACS and ESC 3-hour pathways for a composite endpoint including type 1 or type 2 myocardial infarction, or myocardial injury, with similar performance observed ***(Appendix 3.4 and 3.5).*** As the High-STEACS pathway was derived in the first 1,218 participants of this cohort study, we repeated our analyses and excluded these patients, with no differences observed in diagnostic performance (***Appendix 3.6***). In a further sensitivity analysis, we excluded any patients who underwent invasive or non-invasive cardiac testing, and observed no differences in safety or efficacy (***Appendix 3.7***). The diagnostic metrics for each risk score alone are provided in ***Appendix 3.8***.

5.4.6 ESC 1-hour rule out pathway

Where samples were available at presentation and one hour (406/1,935), we evaluated the performance of the ESC 1-hour rule out pathway (***Appendix 3.9***). In this population, the prevalence of the primary outcome was 8.1% (33/406). The ESC 1-hour pathway identified 37.7% (153/406) of patients as low risk at presentation, and a total of 71.4% (290/406) of patients as low risk at one hour, with no missed cases, for a NPV of 99.8% (95%CI 99.3 to 100%) and sensitivity of 98.5% (95%CI 94.4 to 100%). As no cases were missed, no risk score improved safety, but all significantly reduced the proportion identified as low risk. When we applied the High-STEACS pathway in the subgroup of patients with one hour samples available, there were also no missed events.

5.5 Discussion

In a prospective observational cohort study of patients with suspected acute coronary syndrome, we have evaluated the performance of high-sensitivity cardiac troponin testing in the European Society of Cardiology (ESC) 3-hour pathway, and the High-STEACS pathway which applies a lower threshold to rule out myocardial infarction, with and without the addition of clinical risk scores. We make a number of clinically relevant observations.

When used in isolation, the ESC pathway identifies a high proportion of patients as low risk, but the negative predictive value and sensitivity were poor, missing 27 index or 30-day events. The addition of a clinical risk score markedly improves safety, with the combination of ESC pathway and a HEART score ≤3 missing just one index event. However, this strategy identifies 3-fold fewer patients as low risk. Conversely, the High-STEACS pathway incorporates a lower threshold of 5 ng/L to rule out myocardial infarction, and has both a high negative predictive value and sensitivity, missing just three index or 30-day events and identifying two-thirds of patients as low risk. There was no improvement in diagnostic performance when the High-STEACS pathway was applied in conjunction with the TIMI, GRACE, EDACS or HEART scores, but there was a 2 to 3-fold reduction in the proportion of patients identified as low risk.

The European Society of Cardiology 3-hour pathway was first introduced in the 2011 Non-ST Elevation Acute Coronary Syndrome (NSTE-ACS) guideline, and has been a central component of our evaluation of patients with suspected acute coronary syndrome (Hamm et al., 2011, Roffi et al., 2016). This pathway was devised in an era

of contemporary cardiac troponin assays, where the upper reference limit was up to five-fold higher than that applied with current generation high-sensitivity assays (Mills et al., 2011). It is perhaps unsurprising that when evaluated with more sensitive assays, the diagnostic performance of this pathway is worse. Several recent studies have demonstrated a low negative predictive value, with diagnostic sensitivities <95%, well below the level deemed clinically acceptable (Pickering et al., 2016b, Chapman et al., 2017b, Wildi et al., 2016). Although the ESC guideline includes a low risk GRACE score (≤140) as a final step prior to discharge, there is a lack of clarity as to the intended strategy for this approach in clinical practice. In the present analysis, we demonstrate a GRACE score ≤140 is not effective, but a more conservative GRACE score of ≤108 does improve the NPV and sensitivity, although the latter remained at 96%.

The GRACE and TIMI scores were derived in patients with confirmed myocardial infarction and were designed to guide prognostication and management. These scores have been extrapolated for use as risk stratification tools in patients with suspected acute coronary syndrome, and both improved the performance of the ESC pathway. In contrast, both the HEART and the EDACS scores were derived and validated in patients with suspected, not confirmed myocardial infarction.

The HEART score has been shown to perform better than GRACE and TIMI in patients with suspected acute coronary syndrome (Backus et al., 2013). In this analysis, we demonstrate the greatest improvement in the safety of the ESC pathway when a HEART score of ≤3 was included. The ESC pathway and the HEART score appear synergistic, with the combination of strategies offering an improved safety profile than either in isolation. Our observation is consistent with a recent meta-analysis of 11,217

patients with suspected acute coronary syndrome, in whom a HEART score ≤3 gave a sensitivity of 96.7% (Van Den Berg and Body, 2017). The current HEART score uses troponin as categorical variable based on multiples of the upper reference limit. One option to improve the performance of the HEART score further would be to incorporate high-sensitivity cardiac troponin concentrations as a continuous marker of risk (Roffi et al., 2016), and to harness rather than omit this information to aid risk stratification. Similar improvements in the safety of the ESC pathway were observed when applied in conjunction with a low risk EDACS score. However, this approach identified almost twice as many patients as low risk. This observation may influence clinicians if considering which approach to implement in practice.

The High-STEACS pathway applies a cardiac troponin threshold of <5 ng/L in conjunction with a non-ischemic ECG as an initial risk stratification step, with serial testing at 3 hours in all other patients. This pathway performs well in patients with suspected acute coronary syndrome, and we demonstrate no improvement in safety with the addition of clinical risk scores. When applied in isolation, the High-STEACS pathway ruled out 1,244 patients with 3 missed events (a miss rate of less than 1 in 400 patients). The safety of pathways incorporating low concentrations of cardiac troponin for risk stratification is high and not improved by additional risk scores. One of the reasons this approach is so effective is that cardiac troponin concentrations are increased in patients with risk factors for acute coronary syndrome (such as hyperlipidemia, hypertension or renal impairment) or in those with subclinical coronary or structural heart disease that may not be evident at presentation to the Emergency Department (Chin et al., 2014, Shah et al., 2017, Miller-Hodges et al., 2018, Twerenbold et al., 2018).

There are several alternatives to the two rule out pathways presented in this analysis (Cullen et al., 2017b). The European Society of Cardiology introduced a 0 and 1-hour rule out pathway in their 2015 guideline (Roffi et al., 2016). This pathway has excellent diagnostic performance and has been validated in a number of settings, including a subgroup analysis of the present study (Mueller et al., 2016, Neumann et al., 2016, Reichlin et al., 2015, Pickering et al., 2016a). However, the practicality of delivering presentation troponin results and obtaining serial testing at 1 hour may be challenging in many healthcare settings, and until point of care solutions that facilitate rapid turnaround times with similar test characteristics to laboratory troponin assays, safe alternative pathways with serial testing at 2 or 3 hours are required. In such settings, we believe the ESC 3-hour pathway should be applied with a clinical risk score. Alternatively, the EDACS score alone provides excellent safety and efficacy when applied with a non-ischemic electrocardiogram and serial cardiac troponin testing at 0 and 2 hours, as recommended by the authors (***Appendix 3.8***) (Than et al., 2014b)

In settings where high-sensitivity cardiac troponin I testing is available, the High-STEACS pathway is a more effective alternative approach, identifying more patients as low risk without compromising safety. The performance of this pathway is currently being evaluated in a stepped-wedge cluster randomized trial of ~30,000 patients in Scotland (NCT:03005158).

5.5.1 Limitations

There are important limitations to the data presented. This is a single center observational cohort study. However, as a large tertiary cardiology center, we believe our findings are likely to be generalizable. The High-STEACS pathway was derived in the first 1,218 patients included in this population, and whilst the performance was identical in our sensitivity analysis excluding these patients, further external validation studies are required. As the majority of patients underwent serial sampling at 3 hours (***Appendix 3.10***), we were only able to evaluate the ESC 1-hour pathway in a minority of patients. As this strategy includes low concentrations of cardiac troponin to risk stratify patients, it is likely that the performance would be similar to the High-STEACS pathway in the full population. To date, no comparison studies of these approaches have been undertaken. We were not able to undertake evaluation of other validated rule out pathways such as ADAPT or T-MACS. Our analysis of the ESC 3-hour pathway focused on high-sensitivity cardiac troponin I. However, similar findings have been documented in studies of high-sensitivity cardiac troponin T (Pickering et al., 2016b). Finally, as with all observational cohort studies, no patients were discharged on the basis of the pathways evaluated, and differences in management may have influenced outcomes. Whilst we did not have information on rates of exercise tolerance testing or nuclear testing, patients with low troponin concentrations were less likely to undergo transthoracic echocardiography or invasive coronary angiography (***Table 3.2***) and when we repeated the primary analysis removing all patients who underwent these investigations (***Appendix 3.7***), we observed no reduction in safety. Nevertheless, the results of implementation studies are necessary to guide clinical practice and future guidelines.

5.6 Conclusions

Clinical risk scores significantly improve the safety of the European Society of Cardiology 3-hour pathway that relies on the 99th centile to rule out myocardial infarction. Where lower cardiac troponin I concentrations are used to rule out myocardial infarction, the risk scores evaluated half the proportion of patients ruled out without further improvements in safety.

CHAPTER 6

LONG TERM OUTCOMES IN PATIENTS WITH TYPE 2 MYOCARDIAL INFARCTION AND MYOCARDIAL INJURY

Published by

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6.1 Summary

Background

Type 2 myocardial infarction and myocardial injury are common in clinical practice, but long-term consequences are uncertain. We aimed to define long-term outcomes and explore risk stratification in patients with type 2 myocardial infarction and myocardial injury.

Methods

We identified consecutive patients (n=2,122) with elevated cardiac troponin I concentrations (≥0.05 μg/L) at a tertiary cardiac centre. All diagnoses were adjudicated as per the Universal Definition of Myocardial Infarction. The primary outcome was all-cause death. Secondary outcomes included major adverse cardiovascular events (MACE; non-fatal myocardial infarction or cardiovascular death) and non-cardiovascular death. To explore competing risks, cause-specific hazard ratios were obtained using Cox regression models.

Results

The adjudicated index diagnosis was type 1 or type 2 myocardial infarction or myocardial injury in 1,171 (55.2%), 429 (20.2%) and 522 (24.6%) patients, respectively. At five years, all-cause death rates were higher in those with type 2 myocardial infarction (62.5%) or myocardial injury (72.4%) compared with type 1 myocardial infarction (36.7%). The majority of excess deaths in those with type 2 myocardial infarction or myocardial injury were due to non-cardiovascular causes (HR 2.32, 95%CI 1.92-2.81, *versus* type 1 myocardial infarction). Despite this, the observed crude MACE rates were similar between groups (30.6% *versus* 32.6%), with

differences apparent after adjustment for co-variates (HR 0.82, 95%CI 0.69-0.96). Coronary heart disease was an independent predictor of MACE in those with type 2 myocardial infarction or myocardial injury (HR 1.71, 95%CI 1.31-2.24).

Conclusions

Despite an excess in non-cardiovascular death, patients with type 2 myocardial infarction or myocardial injury have a similar crude rate of major adverse cardiovascular events to those with type 1 myocardial infarction. Identifying underlying coronary heart disease in this vulnerable population may help target therapies that could modify future risk.

6.2 Introduction

The diagnostic criteria for acute myocardial infarction were updated to accommodate the introduction of more sensitive cardiac troponin assays, and in recognition of the wide range of conditions that are associated with myocardial injury (White et al., 2014). The third universal definition of myocardial infarction recommends a classification based on etiology, where type 1 myocardial infarction is due to plaque rupture or erosion with atherothrombotic consequences, and type 2 myocardial infarction due to myocardial oxygen supply-demand imbalance in the absence of atherothrombosis. Patients with elevated cardiac troponin concentrations who do not have overt myocardial ischemia are classified as having myocardial injury (Thygesen et al., 2012a). Whilst these diagnostic categories are considered distinct in guidelines, implementation in clinical practice has been challenging due to similarities between patients with type 2 myocardial infarction and myocardial injury, with the implications of these diagnoses uncertain.

The Global Task Force is reviewing the classification of myocardial infarction, and recognizes the need to provide greater clarity for clinicians in practice (Alpert and Thygesen, 2016). Whilst patients with type 2 myocardial infarction and myocardial injury have higher crude rates of all-cause death compared with those with type 1 myocardial infarction (Shah et al., 2015c, Javed et al., 2009, El-Haddad et al., 2012, Sarkisian et al., 2016, Saaby et al., 2014, Stein et al., 2014), differences do not always persist in adjusted analyses (Sandoval et al., 2017b, Neumann et al., 2017b) and few studies report cause of death or risk of future cardiovascular events (Gaggin et al., 2017). If patients with type 2 myocardial infarction are at increased risk of

cardiovascular events attributable to atherosclerotic disease, then targeted investigation and preventative therapies have the potential to modify outcomes.

In consecutive patients with elevated cardiac troponin concentrations measured using a sensitive assay, we previously observed that the diagnosis of type 2 myocardial infarction or myocardial injury was as common as type 1 myocardial infarction (Shah et al., 2015c). Here we report outcomes for these patients, and determine the clinical features associated with major adverse cardiovascular events, with the aim of improving risk stratification in patients with type 2 myocardial infarction or myocardial injury.

6.3 Methods

6.3.1 Study population

Consecutive hospital inpatients with elevated cardiac troponin I concentrations (≥0.05 μg/L) were identified at a tertiary cardiac centre (Royal Infirmary of Edinburgh, Scotland, UK) during the validation (January 19th to July 31st 2008) and implementation (January 19th to July 31st 2009) phases of a contemporary sensitive cardiac troponin I assay (Shah et al., 2015c, Mills et al., 2011). We included all patients in whom cardiac troponin was requested by the attending clinician, regardless of suspected etiology or hospital department. All clinical details were obtained using an electronic patient record (TrakCare, InterSystems, Cambridge, MA). We excluded patients admitted for elective procedures, those with incomplete electronic hospital records, and patients who were not residents to ensure follow up was complete.

6.3.2 Cardiac troponin assay

Plasma cardiac troponin concentrations were measured using a contemporary sensitive cardiac troponin I assay (ARCHITECT$_{STAT}$, Abbott Laboratories, Abbott Park, IL). The study was divided into validation and implementation phases (Shah et al., 2015c, Mills et al., 2011). Only cardiac troponin concentrations above the diagnostic threshold of the previous generation assay (≥0.20 μg/L) were reported to clinicians during the validation phase, whereas concentrations above a revised diagnostic threshold (≥0.05 μg/L) were reported during the implementation phase. The 99th centile of this assay is 0.028 μg/L; however, a diagnostic threshold of ≥0.05 μg/L was implemented as this was the minimum concentration where the coefficient of variation

was <10% under local laboratory conditions. All troponin results were available to the research team irrespective of study phase.

6.3.3 Diagnostic classification

All diagnoses were classified as per the third universal definition of myocardial infarction (Thygesen et al., 2012a, Shah et al., 2015c). Patients were classified as having a type 1 myocardial infarction when myocardial necrosis occurred in the context of a presentation with suspected acute coronary syndrome with symptoms of myocardial ischemia, or evidence of myocardial ischemia on the electrocardiogram. Patients with symptoms or signs of myocardial ischemia that were thought to be due to increased oxygen demand (e.g. tachyarrhythmia or hypertrophy) or decreased supply (e.g. hypotension, hypoxia or anaemia) and myocardial necrosis in the context of an alternative clinical diagnosis were classified as having a type 2 myocardial infarction. Myocardial injury was defined as evidence of myocardial necrosis in the absence of any symptoms or signs of myocardial ischemia. For this analysis, we excluded patients classified as having type 3, type 4a or 4b, or type 5 myocardial infarction. Each case was reviewed and classified independently by two cardiologists, and any discrepancies were resolved by consensus through in-depth review of source data. Further information on the adjudication process is provided in ***Appendix 4.1***.

6.3.4 Clinical outcomes

Clinical outcomes were identified using local and national population registries. We determined death using TrakCare (InterSystems, Cambridge, MA) and the National Register of Scotland (NRS), with future hospitalization for myocardial infarction or heart failure identified using an extract from the Scottish Morbidity Record (SMR01).

We defined death from a cardiovascular cause where one of the following ICD10 codes were listed as primary cause of death: I20-25, I34-37, I42-43, I46, I48-51 and I60-69 ***(Appendix 4.2).*** The primary outcome was all cause death. Secondary outcomes included major adverse cardiovascular events (MACE; defined as cardiovascular death or subsequent myocardial infarction), non-fatal myocardial infarction, fatal myocardial infarction, hospitalization with heart failure, and non-cardiovascular death. We obtained follow up for all patients until the primary outcome or date of censoring (16th November 2015).

6.3.5 Ethical considerations

The parent study protocol evaluated the implementation of a sensitive cardiac troponin assay, and was deemed to fall under the remit of audit and service evaluation by the NHS Lothian Regional Ethics Committee, therefore formal ethical approval was not required. For this study, we received approval from the Caldicott guardian to obtain long term follow up through local and national registries.

6.3.6 Statistical analysis

Baseline characteristics were summarized as mean (SD) or median (IQR) as appropriate, with patients grouped based on the classification of myocardial infarction. Crude incidence rates for primary and secondary outcomes were calculated, with risk ratios obtained using a generalized linear model with a log link, Poisson error distribution and robust variance estimates.(Yelland et al., 2011) We adjusted for clinically relevant covariates including age, sex, renal function (estimated glomerular filtration rate, eGFR), hemoglobin (g/L), diabetes mellitus, hypertension, coronary heart disease (defined as previous myocardial infarction, coronary revascularization or

known angina pectoris), stroke, peripheral vascular disease or cigarette smoking. The study period included a lowering of the upper reference limit for cardiac troponin from 0.20 μg/L (validation phase) to 0.05 μg/L (implementation phase), and we therefore included study phase in all models. We repeated these analyses among only those patients who survived 30 days after presentation, defining the start of the follow-up period as 30 days post presentation. To explore competing risks, cause-specific hazard ratios were obtained using Cox regression models for type 1 myocardial infarction versus type 2 myocardial infarction or myocardial injury for MACE and non-cardiovascular death. Penalised splines were used to accommodate departures from linearity. We examined for non-proportional hazards graphically and via the method proposed by Grambsch and Therneau (1994). In patients who survived to 30 days, we explored associations between covariates and future risk of MACE. Cumulative incidence plots were produced for secondary cardiovascular outcomes, which also illustrate the competing risk of non-cardiovascular death. We report 95% confidence intervals for all estimates, with all analyses performed using R (Version 3.2.2) using the survival and cmprsk packages (Team., 2015). The analysis code for this study has been made available online ***(Appendix 4.3).***

6.4 Results

We identified 2,929 consecutive patients with elevated cardiac troponin concentrations (≥0.05 µg/L) of whom 807 met our exclusion criteria **(*Figure 6.1*).** In the study population (n=2,122), the adjudicated diagnosis was type 1 myocardial infarction in 1,171 patients (55.2%), type 2 myocardial infarction in 429 patients (20.2%) and myocardial injury in 522 patients (24.6%; ***Table 6.1***).

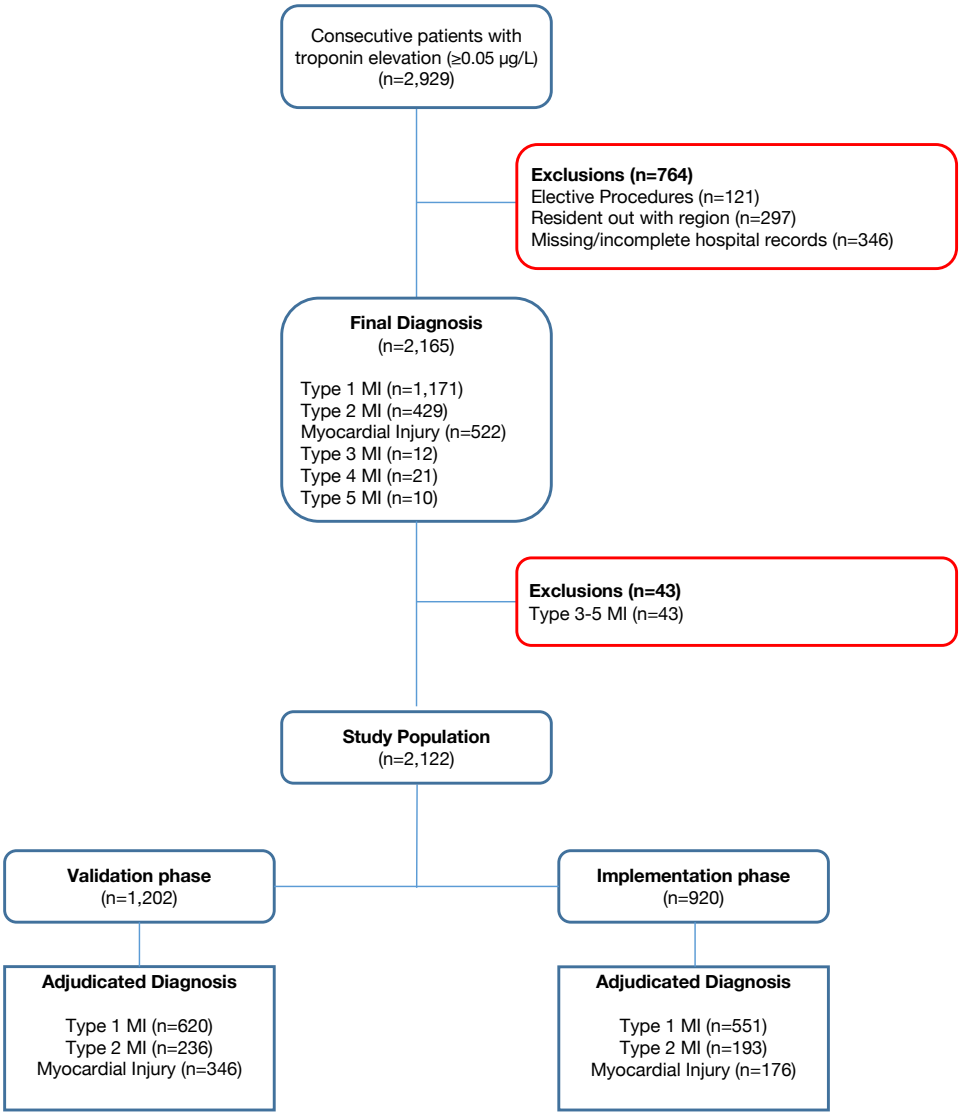

Figure 6.1 Consort diagram with identification of the study population.

6.4.1 Clinical characteristics

Patients with type 2 myocardial infarction or myocardial injury were older, and there were a higher proportion of women than men compared to patients with type 1 myocardial infarction. Anaemia or renal impairment was more common in patients with type 2 myocardial infarction or myocardial injury. A history of previous coronary revascularization was more frequent in those with type 1 myocardial infarction. At presentation, the prescription of anti-platelet, anti-hypertensive and lipid lowering therapies was similar across all patients **(*Table 6.1*).** The most common diagnoses in patients with type 2 myocardial infarction or myocardial injury were cardiac arrhythmia, decompensated left ventricular failure, pneumonia or long bone fracture, with variation in prevalence by classification ***(Appendix 4.4).***

Table 6.1. Baseline characteristics of the study population

	Type 1 Myocardial Infarction (n=1,171)	Type 2 Myocardial Infarction (n=429)	Myocardial Injury (n=522)
Baseline Characteristics			
Age	68 (14)	75 (14)	76 (13)
Male (%)	709 (60.5)	222 (51.7)	260 (49.8)
Past Medical History			
Diabetes Mellitus (%)	185 (16.7)	93 (21.7)	96 (18.7)
Hypertension (%)	533 (48.2)	254 (59.3)	303 (58.9)
Hyperlipidaemia (%)	539 (48.6)	177 (41.5)	202 (39.5)
Family History (%)	193 (18.1)	14 (3.3)	10 (2.0)
Ischaemic Heart Disease (%)	497 (44.7)	191 (44.6)	186 (36.3)
Previous MI (%)	231 (23.9)	109 (26.0)	107 (20.9)
Previous Stroke (%)	92 (8.3)	48 (11.2)	86 (16.8)
Previous PVD (%)	85 (7.7)	29 (6.8)	39 (7.6)
Previous PCI (%)	153 (14.7)	17 (4.0)	23 (4.5)
Previous CABG (%)	62 (6.3)	30 (7.1)	32 (6.2)
Smoker (%)	380 (34.0)	62 (14.5)	73 (14.0)
Admission Medication			
Aspirin (%)	413 (49.7)	175 (44.1)	207 (45.9)
Clopidogrel (%)	100 (12.2)	25 (6.3)	26 (5.8)
Beta-blocker (%)	257 (31.2)	101 (25.7)	111 (24.6)
ACE Inhibitor (%)	300 (36.4)	136 (34.4)	158 (35.1)
Statin (%)	384 (46.5)	156 (39.5)	191 (42.4)
Long Acting Nitrate (%)	124 (15.1)	48 (12.2)	43 (9.6)
Calcium Channel Blocker (%)	165 (20.1)	65 (16.5)	67 (14.9)
GTN Spray (%)	250 (30.3)	76 (19.3)	63 (14.0)
Diuretic (%)	230 (27.9)	170 (43.0)	196 (43.6)
Warfarin (%)	35 (4.5)	38 (9.7)	52 (11.6)
Baseline Investigations			
Haemoglobin (g/L)	133.9 (20.4)	121.4 (25)	120.2 (22.1)
Urea (mmol/L)	8.2 (9.4)	10 (7.1)	12.02 (11.5)
Creatinine (mmol/L)	106.8 (59.8)	132.5 (108.9)	155 (172.2)
Corrected eGFR (ml/min)	69 (26)	58 (28)	54 (32)
Cholesterol (mmol/L)	4.8 (1.3)	4.3 (1.2)	4.3 (1.4)
Troponin I (μg/L)	2.42 (0.27-15.23)	0.14 (0.07-0.66)	0.13 (0.06-0.39)

Values are mean (SD),median (IQR) or n(%). MI – myocardial infarction. PVD – peripheral vascular disease, PCI – percutaneous coronary intervention. CABG – coronary artery bypass grafting. ACE – angiotensin converting enzyme. GTN – glyceryl trinitrate, eGFR – estimated glomerular filtration rate, Ischaemic Heart Disease – previous myocardial infarction or angina pectoris.

6.4.2 Clinical outcomes at five years in all patients

During 8,809 person years follow up (median 4.9 years), death from any cause occurred in 1,231 patients (58%). In patients with type 2 myocardial infarction, at five years, the observed risk of death was higher compared to those with type 1 myocardial infarction (62.5% *versus* 36.7%, unadjusted relative risk (RR) 2.15, 95% confidence intervals (95%CI) 1.82-2.55. After incorporating age, sex, renal function, hemoglobin and other clinically relevant co-variates, the adjusted RR fell to 1.51, (95%CI 1.21-1.87, ***Figure 6.2, Table 6.2***).

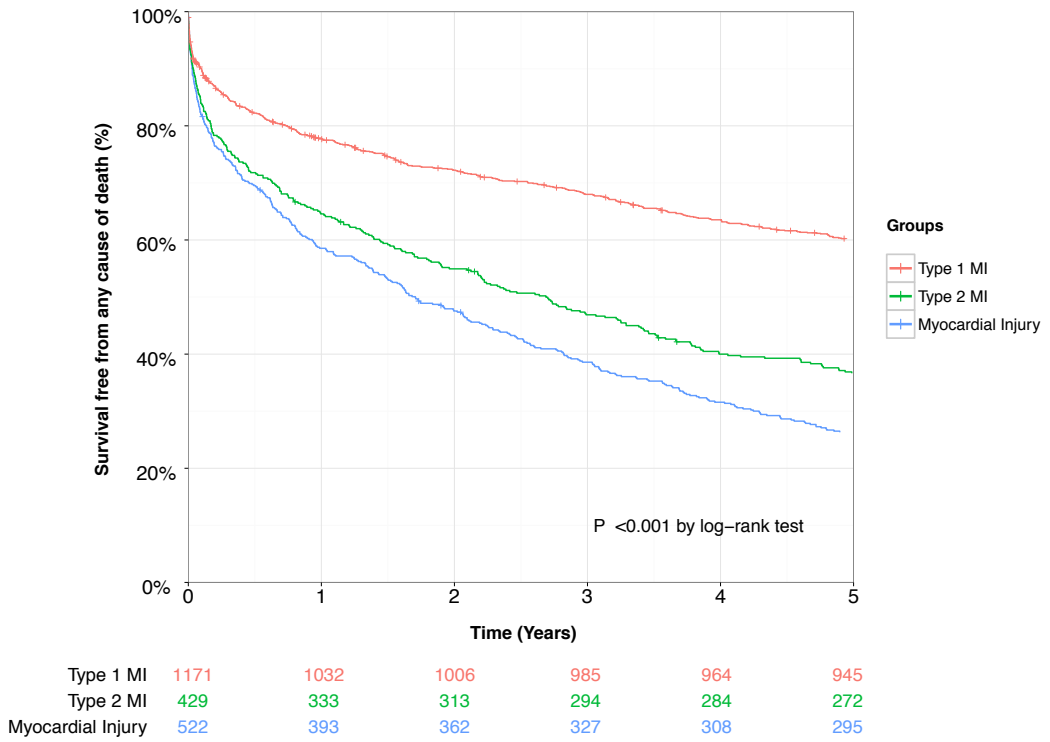

Figure 6.2 Kaplan-Meier curves illustrating risk of death from any cause at five years stratified by index diagnosis, with table of number at risk. Pair-wise comparison of groups obtained using the log-rank test.

Table 6.2. Death and major cardiovascular events at 5 years stratified by diagnosis

	Type 1 MI (n=1,171)	Type 2 MI (n=429)	Myocardial injury (n=522)	Type 2 MI versus Type 1 MI		Myocardial Injury versus Type 1 MI	
				Unadjusted RR (95% CI)	**Adjusted RR (95% CI)**	**Unadjusted RR (95% CI)**	**Adjusted RR (95% CI)**
Death from any cause	430 (36.7%)	268 (62.5%)	378 (72.4%)	2.15 (1.82-2.55)	1.51 (1.21-1.87)	2.88 (2.43-3.40)	2.09 (1.72-2.55)
MACE	382 (32.6%)	129 (30.1%)	162 (31.0%)	0.92 (0.77-1.09)	0.74 (0.62-0.88)	0.95 (0.81-1.11)	0.77 (0.66-0.89)
Non-fatal MI	209 (17.8%)	43 (10.0%)	35 (6.7%)	0.60 (0.45-0.79)	0.58 (0.44-0.77)	0.43 (0.31-0.58)	0.44 (0.32-0.60)
Cardiovascular death	253 (21.6%)	104 (24.2%)	145 (27.8%)	1.11 (0.92-1.34)	0.85 (0.70-1.03)	1.25 (1.07-1.46)	0.92 (0.79-1.07)
Fatal MI	32 (2.7%)	9 (2.1%)	18 (3.4%)	0.81 (0.45-1.46)	0.64 (0.37-1.11)	1.17 (0.81-1.71)	0.93 (0.64-1.34)
Heart failure hospitalization	103 (8.8%)	25 (5.8%)	48 (9.2%)	0.71 (0.50-1.02)	0.77 (0.54-1.12)	1.03 (0.81-1.32)	1.08 (0.86-1.35)
Non-cardiovascular death	155 (13.2%)	153 (35.7%)	218 (41.8%)	2.33 (1.99-2.71)	1.66 (1.40-1.98)	2.54 (2.33-2.89)	1.84 (1.61-2.11)

Event rates (number, %) for primary and secondary outcomes with adjusted relative risk (RR) and 95% confidence intervals (95% CI) at five years. MACE = major adverse cardiovascular events (non-fatal type 1 myocardial infarction or cardiovascular death), MI = myocardial infarction. For the composite of MACE, patients who experienced non-fatal myocardial infarction and subsequent cardiovascular death are counted once. Cause of death was not determined in 48 patients due to missing data.

The five-year risk of non-fatal myocardial infarction or cardiovascular death (MACE) was similar in patients with type 2 compared to type 1 myocardial infarction (30.1% *versus* 32.6%, unadjusted RR 0.92, 95% CI 0.77-1.09, ***Figure 6.3***), but lower after adjustment for age, sex and other co-variates (adjusted RR 0.74, 95% CI 0.62-0.88). Adjusting for the same co-variates, the cause-specific hazard ratio for MACE (with non-cardiovascular mortality as the competing outcome) was similar to the relative risk (HR 0.82 95% CI 0.69-0.96, ***Table 6.3, Appendix 5.5***).

Table 6.3. Cause-specific hazard ratio for MACE and non-cardiovascular death in patients with type 2 myocardial infarction *or* myocardial injury versus type 1 myocardial infarction in unadjusted and fully adjusted Cox-regression models.

	Major Adverse Cardiovascular Events	
	csHR (95% CI)	P value
Model 1	1.16 (1.00-1.34)	0.052
Model 2	0.84 (0.72-0.98)	0.024
Model 3	0.74 (0.63-0.87)	<0.001
Model 4	0.82 (0.69-0.96)	0.016
	Non-Cardiovascular Death	
	csHR (95% CI)	P value
Model 1	3.73 (3.15-4.41)	<0.001
Model 2	2.63 (2.21-3.12)	<0.001
Model 3	2.27 (1.90-2.72)	<0.001
Model 4	2.32 (1.92-2.81)	<0.001

Model 1 – Unadjusted. Model 2 – Adjusted for Age and Sex. Model 3 – As per Model 2 with adjustment for estimated glomerular filtration rate. Model 4: As per Model 3 with adjustment for haemoglobin, smoking, diabetes, hypertension, coronary artery disease, stroke, peripheral vascular disease and study phase. csHR- cause specific hazard ratio. Type 1 myocardial infarction as the referent group. P-value for inclusion of index diagnosis term.

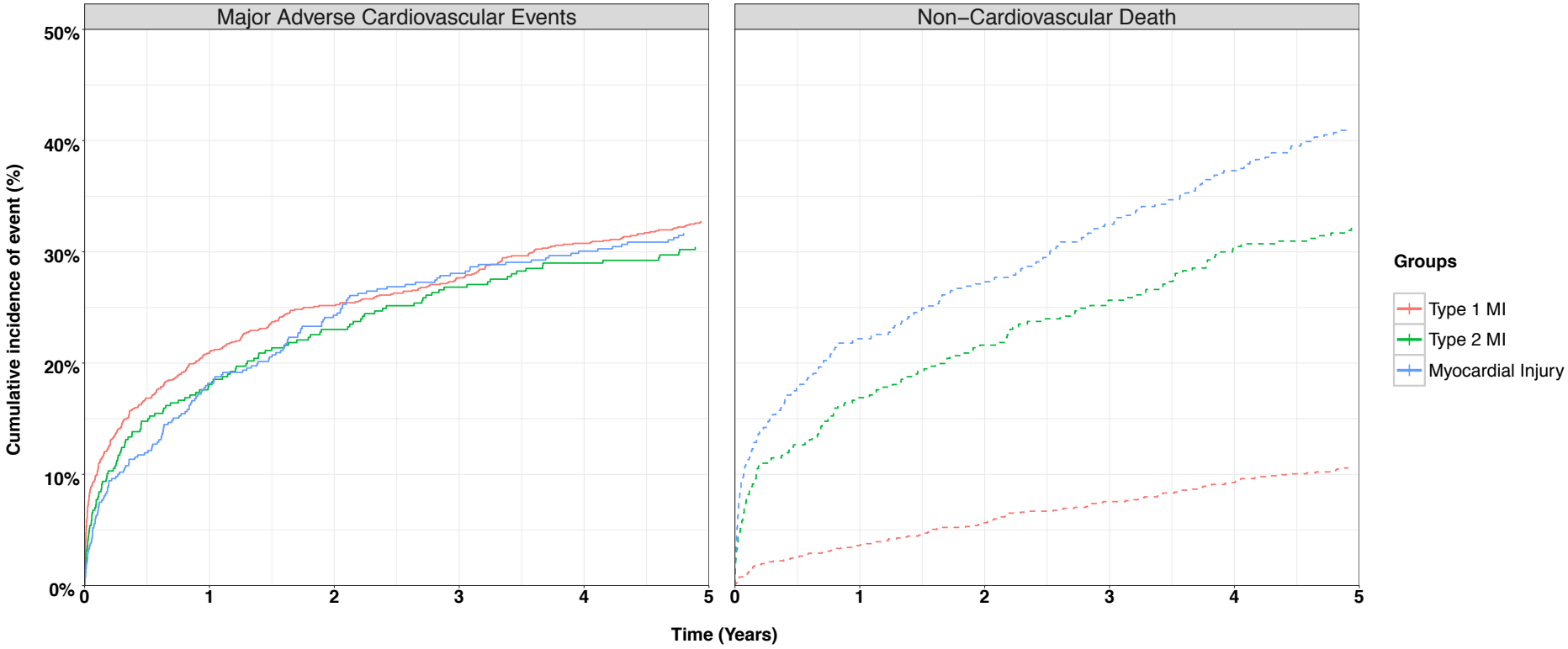

Figure 6.3 Cumulative incidence curves illustrating risk of major adverse cardiovascular events (MACE; type 1 myocardial infarction or cardiovascular death) and competing risk of non-cardiovascular death at five years stratified by index diagnosis.

For the individual components of MACE, the risk of non-fatal myocardial infarction was lower in those with type 2 myocardial infarction compared to type 1 myocardial infarction (10.0% *versus* 17.8%, adjusted RR 0.58, 95% CI 0.44-0.77). Whilst the crude rates of cardiovascular death were higher for type 2 myocardial infarction compared to type 1 myocardial infarction (24.2% *versus* 21.6%) the adjusted relative risk was lower at 0.85 (95% CI 0.70-1.03). Risks of fatal-myocardial infarction and hospitalization with heart failure were comparable across groups **(*Table 6.2*).** Non-cardiovascular death was higher in patients with type 2 myocardial infarction compared to type 1 myocardial infarction (35.7% *versus* 13.2%, adjusted RR 1.66, 95% CI 1.40-1.98, ***Figure 6.3)***.

We found similar relative risks for patients with myocardial injury compared to type 1 myocardial infarction for most primary and secondary outcomes, but a lower risk of non-fatal myocardial infarction and higher risk of non-cardiovascular death were observed. Patients with myocardial injury had a higher risk of all-cause death and heart failure hospitalization than patients with type 2 myocardial infarction ***(Appendix 4.6***).

6.4.3 Clinical outcomes at five years in those who survive to 30 days

In patients who survived from their initial presentation to 30 days, death from any cause occurred in 31% (333/1,074) of patients with type 1 myocardial infarction, 56.1% (207/368) of patients with type 2 myocardial infarction and 67% (293/437) of patients with myocardial injury (***Table 6.4***). The adjusted relative risk of death for patients with type 2 myocardial infarction versus type 1 myocardial infarction was similar to that observed in the total population (adjusted RR 1.52, 95% CI 1.21-1.92). For all but one of the secondary outcomes, the relative risks were similar to those obtained in the main analysis. However, the association between type of myocardial infarction and risk of MACE was weaker than was observed in the whole population, occurring in 27.4% (101/368) of patients with type 2 myocardial infarction and 27.7% (298/1,074) of patients with type 1 myocardial infarction, with an adjusted RR of 0.80 (95% CI 0.65-0.98).

Table 6.4. Death and major cardiovascular events at 5 years stratified by diagnosis in those who survived index hospitalization

	Type 1 MI (n=1,074)	**Type 2 MI (n=368)**	**Myocardial injury (n=437)**	**Type 2 MI versus Type 1 MI**	**Myocardial Injury versus Type 1 MI**
				Adjusted RR (95% CI)	**Adjusted RR (95% CI)**
Death from any cause	333 (31.0%)	207 (56.1%)	293 (67.0%)	1.52 (1.21-1.92)	1.95 (1.60-2.39)
MACE	298 (27.7%)	101 (27.4%)	135 (30.9%)	0.80 (0.65-0.98)	0.87 (0.73-1.02)
Non-fatal MI	198 (18.4%)	41 (11.1%)	34 (7.8%)	0.60 (0.45-0.81)	0.46 (0.34-0.64)
Cardiovascular death	172 (16.0%)	77 (20.9%)	118 (27.0%)	0.95 (0.76-1.18)	1.07 (0.90-1.27)
Fatal MI	32 (3.0%)	9 (2.4%)	17 (3.9%)	0.65 (0.38-1.14)	0.90 (0.61-1.31)
Heart failure hospitalization	92 (8.6%)	22 (6.0%)	39 (8.9%)	0.86 (0.58-1.26)	1.18 (0.91-1.52)
Non-cardiovascular death	145 (13.5%)	121 (32.8%)	162 (37.1%)	1.55 (1.28-1.88)	1.61 (1.38-1.88)

In patients with type 2 myocardial infarction or myocardial injury, age, declining renal function, a history of diabetes mellitus, peripheral vascular disease and coronary artery disease were independent predictors of MACE at five years **(*Table 6.5***). The presence of coronary artery disease was associated with an increase in the cause-specific hazard ratio for MACE at five years (HR 1.71, 95% CI 1.31-2.24), compared to those without coronary artery disease. When compared to patients with type 1 myocardial infarction, patients with type 2 myocardial infarction or myocardial injury with coronary artery disease had a higher risk of MACE (RR 1.56, 95% CI 1.29-1.88). The adjusted cause-specific hazard ratio for MACE, which accounts for competing risk from non-cardiovascular death, was 1.05 (95% CI 0.85-1.30, ***Figure 6.4***).

Table 6.5. Cause-specific hazard ratios for major adverse cardiovascular events in patients with type 2 myocardial infarction or myocardial injury alone *who survive from their initial presentation to 30 days*; unadjusted and fully adjusted cox-regression models.

	Major Adverse Cardiovascular Events (MACE)	
	Unadjusted HR (95% CI)	***Adjusted HR (95% CI)***
Age (per 10-year increase)	1.56 (1.39-1.75)	1.53 (1.34-1.75)
Sex (male)	1.08 (0.84-1.38)	1.26 (0.97-1.64)
Haemoglobin (per 10 g/L reduction)	1.10 (1.04-1.16)	1.04 (0.99-1.10)
eGFR (per 10 ml/min reduction)	1.16 (1.10-1.21)	1.11 (1.05-1.17)
Smoking	0.86 (0.60-1.23)	1.39 (0.94-2.05)
Diabetes Mellitus	1.79 (1.36-2.35)	1.50 (1.12-2.01)
Hypertension	1.61 (1.24-2.10)	1.02 (0.76-1.36)
Stroke	1.54 (1.12-2.13)	1.12 (0.80-1.55)
Peripheral Vascular Disease	2.43 (1.68-3.50)	1.82 (1.21-2.74)
Validation phase	1.19 (0.92-1.53)	1.25 (0.96-1.63)
Coronary Artery Disease	2.21 (1.73-2.83)	1.71 (1.31-2.24)

eGFR = estimated glomerular filtration rate. Patients without coronary artery disease as referent group.

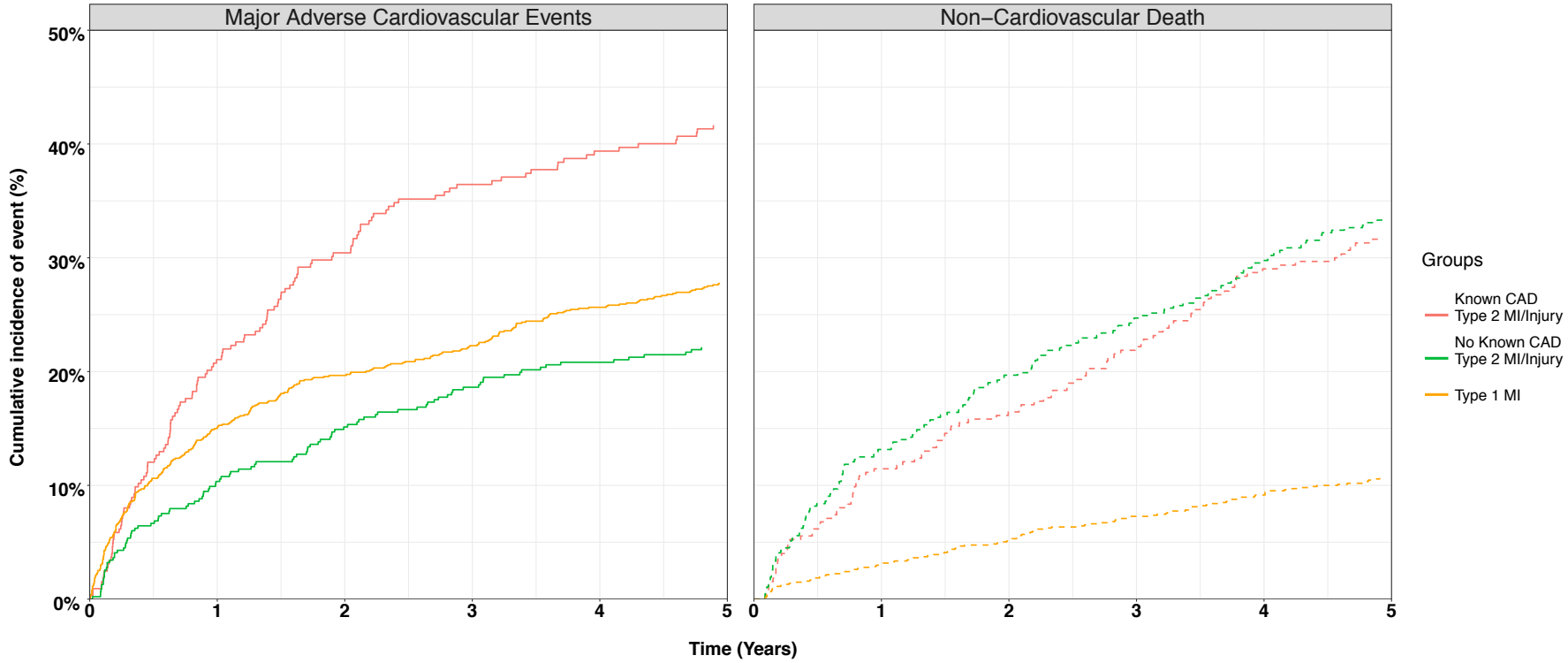

Figure 6.4. Cumulative incidence curves illustrating risk of major adverse cardiovascular events (MACE; type 1 myocardial infarction or cardiovascular death) and competing risk of non-cardiovascular death in those who survive to 30 days in patients with type 1 myocardial infarction, and in those with type 2 myocardial infarction or myocardial injury stratified by known coronary artery disease (CAD).

On discharge from hospital, patients with type 2 myocardial infarction or myocardial injury and a history of coronary artery disease were less likely than those with type 1 myocardial infarction to be prescribed aspirin (66.2% *versus* 90.7%), a statin (69.2% *versus* 86.0%) or an ACE inhibitor (52.9% *versus* 71.3%, P<0.001 for all, ***Table 6.6***).

Table 6.6 Recommended therapies at discharge in patients with type 1 myocardial infarction, type 2 myocardial infarction and myocardial injury who survive to 30 days, stratified by the presence of coronary artery disease.

	Type 1 Myocardial Infarction	*Type 2 Myocardial Infarction or Myocardial Injury*	*Type 2 Myocardial Infarction or Myocardial injury*	*P value*
		Known coronary artery disease	*No known coronary artery disease*	
	(n=1,074)	*(n=325)*	*(n=467)*	
Aspirin	896 (90.7%)	190 (66.2%) *	148 (37.7%)	<0.001
Clopidogrel	823 (80.7%)	52 (17.6%) *	31 (7.6%)	<0.001
Beta-blocker	651 (64.2%)	126 (42.6%) *	97 (23.7%)	<0.001
ACE Inhibitor	724 (71.3%)	156 (52.9%) *	124 (30.2%)	<0.001
Statin	872 (86.0%)	204 (69.2%) *	120 (29.3%)	<0.001
Long acting nitrates	143 (14.1%)	77 (26.1%) *	12 (2.9%)	<0.001
GTN Spray	671 (66.0%)	121 (41.0%) *	23 (5.6%)	<0.001
CC Blockers	165 (16.3%)	67 (22.7%)	43 (10.5%)	<0.001
Warfarin	33 (3.4%)	44 (15.0%) *	64 (15.6%)	<0.001

*P values obtained from group-wise comparison using Chi-square test. *P<0.001 in post hoc analysis comparing patients with type 2 myocardial infarction or myocardial injury with coronary artery disease versus patients with type 1 myocardial infarction.*

6.5 Discussion

In a cohort of consecutive hospitalized patients with elevated cardiac troponin concentrations, we classified the diagnosis of myocardial infarction according to the universal definition and report outcomes after five years follow up. We make several observations that have implications for clinical practice. First, over two-thirds of patients with type 2 myocardial infarction or myocardial injury are dead at five years. This mortality rate was twice that of patients with type 1 myocardial infarction, with differences primarily due to an excess in non-cardiovascular deaths. Second, major adverse cardiovascular events occurred in one-third of patients, and rates were similar irrespective of diagnostic classification. In those patients with type 2 myocardial infarction or myocardial injury, the presence of coronary heart disease was one of the strongest predictors of MACE. Those patients with type 2 myocardial infarction or myocardial injury with known coronary artery disease were less likely to receive secondary prevention therapies compared to those with type 1 myocardial infarction. Identifying patients with elevated cardiac troponin concentrations in the context of an acute illness who have underlying coronary heart disease may provide an opportunity for clinicians to improve the targeting of preventative therapies and reduce the risk of cardiovascular events.

Several studies demonstrate that the diagnosis of type 2 myocardial infarction is common in clinical practice, responsible for between 2% and 37% of all elevations in cardiac troponin in unselected hospitalized patients and between 5% to 71% in unselected patients attending the Emergency Department (Melberg et al., 2010, Shah et al., 2015b, Smith et al., 2013, Sandoval et al., 2014b, Baron et al., 2016). Myocardial

injury has been reported in up to 70% of unselected patients (Javed et al., 2009, Saaby et al., 2013), but as the frequency of diagnosis is not reported by the majority of studies, failure to classify patients according to the criteria set out in the universal definition may inflate the incidence of type 2 myocardial infarction (Baron et al., 2015). Both type 2 myocardial infarction and myocardial injury increase the risk of all-cause death at up to three years (Javed et al., 2009, El-Haddad et al., 2012, Sarkisian et al., 2016, Saaby et al., 2014, Stein et al., 2014, Baron et al., 2016, Baron et al., 2015, Morrow et al., 2009, Bonaca et al., 2012). We now provide outcome data at five years demonstrating that two-thirds of patients with type 2 myocardial infarction or myocardial injury are dead with twice the event rate of patients with type 1 myocardial infarction.

One of the key limitations of prior analyses is the majority have not reported the specific cause of death, and therefore estimates of the proportion of events which may be attributable to cardiovascular disease, are lacking (Sandoval and Thygesen, 2017, Sandoval, 2017). We found the excess in all-cause death in patients with type 2 myocardial infarction or myocardial injury was largely attributable to a three-fold increase in non-cardiovascular death. As patients with type 2 myocardial infarction or myocardial injury are older, and have a higher prevalence of anaemia, renal impairment, and other co-morbidities, this is perhaps unsurprising. Nonetheless, it is notable that the crude risk of MACE in patients with type 2 myocardial infarction or myocardial injury was similar to that in patients with type 1 myocardial infarction. In models taking into account the differences in age, sex and other characteristics between patients with different index diagnoses, the risk of subsequent cardiovascular events was around 25% lower in patients with type 2 myocardial infarction or

myocardial injury than in patients with type 1 myocardial infarction. This may in part be attributable to competing risks, with the much higher rates of non-cardiovascular death reducing the pool of patients at risk of having a cardiovascular event. However, competing risks are not the only explanation for the lower rates of MACE in patients with type 2 myocardial infarction or myocardial injury, as in an adjusted analysis taking into account competing risks and other clinical variables, a difference in the cause-specific hazard ratio was still apparent between the groups.

The diagnostic distinction between patients with type 2 myocardial infarction and myocardial injury is challenging, but worthwhile if the diagnosis conveys important prognostic information, or influences treatment decisions (Sarkisian et al., 2016, Collinson and Lindahl, 2015, Chapman and Mills, 2017, Chapman et al., 2017a). In our analysis, the recommended classification of type 2 myocardial infarction or myocardial injury did not differentially identify those patients at risk of MACE. This observation is consistent with previous studies and suggests alternate strategies for risk stratification may be required. In patients with type 2 myocardial infarction, the presence of obstructive coronary artery disease may influence prognosis. Outcomes from the SWEDEHEART registry of 41,817 patients with type 1 or 2 myocardial infarction demonstrated an increased risk of all-cause death in patients with type 2 myocardial infarction with obstructive coronary artery disease compared to those without (Baron et al., 2016). Similarly, in a recent analysis of the APACE cohort, Nestelberger et al. (2017) found patients with type 2 myocardial infarction and coronary artery disease had a 90 day cardiovascular mortality of 3.6%, with no deaths observed in those without coronary artery disease. Our analysis supports these findings, with coronary artery disease one of the strongest predictors of MACE in patients with

type 2 myocardial infarction or myocardial injury. The prevalence of coronary artery disease in patients with type 2 myocardial infarction or myocardial injury was 42% in our cohort, and varies between 36% to 78% in previous reports (Sarkisian et al., 2016, Neumann et al., 2017b, Baron et al., 2016, Saaby et al., 2013, Ambrose et al., 2012). However, estimates obtained from registry studies are hindered by selection bias as those who undergo angiography will have a higher pre-test probability of coronary artery disease, and the true prevalence of coronary artery disease in this group of patients remains uncertain (Januzzi and Sandoval, 2017).

Importantly, patients with type 2 myocardial infarction or myocardial injury receive fewer prescriptions for preventative therapies compared to those with type 1 myocardial infarction (Stein et al., 2014, Sandoval et al., 2017b, Sandoval et al., 2014b, Baron et al., 2016, Saaby et al., 2013, Baron et al., 2015). To date, there have been no randomized controlled trials evaluating secondary prevention in this population, and there are no formal recommendations for risk assessment or treatment. Given the current heterogeneity in application of the Universal Definition of Myocardial Infarction, the feasibility of delivering such a study with comparable observations across multiple healthcare settings is uncertain. Primary prevention guidelines recommend statin therapy where the predicted ten year risk of adverse cardiovascular events exceeds 10% (NICE., 2014a). In our study, patients who survive their initial presentation with type 2 myocardial infarction and are not already known to have coronary artery disease, the rate of MACE exceeds 10% at one year. Whilst this may be partially attributable to age and the presence of co-morbidities, a significant proportion may have unrecognized coronary artery disease and may benefit from further investigation or preventative therapies.

We believe clinicians should adopt a pragmatic approach, and risk stratify individual patients based on their likelihood of coronary artery disease. There are no risk assessment tools validated for use in this setting, therefore clinicians must review the presenting symptoms, medical history, cardiovascular risk factors, serial 12-lead electrocardiograms and any available imaging findings and apply clinical judgement. Where the probability of coronary disease is high, it may be reasonable to commence secondary prevention with aspirin and a statin in the absence of contraindications. If patients with type 2 myocardial infarction are found to have obstructive coronary artery disease, revascularization could plausibly reduce the risk of future cardiac events, but this strategy has not been evaluated. Where the probability of coronary disease is intermediate or low, further investigation (invasive or CT coronary angiography) should be considered to identify patients with underlying coronary artery disease, where the benefits of secondary prevention are well recognized. The optimal timing for investigation in this group of patients is also uncertain. Where the probability of type 1 myocardial infarction is high, invasive assessment should be considered on an urgent basis in line with standard practice. In those patients where myocardial injury or infarction is secondary to oxygen supply-demand imbalance, further assessment may need to be deferred until the patient has recovered from their primary illness. Furthermore, a recognition that these patients are at increased risk of non-cardiovascular events may lead to an improvement in outcomes, through better monitoring or intensification of treatment of the primary presenting condition.

6.5.1 Limitations

There are important limitations to the data presented. The study population was identified on the basis of an elevated troponin I concentration measured using a contemporary sensitive assay with a diagnostic threshold of 0.05 μg/L, and the true prevalence of myocardial injury and infarction could be higher using a lower threshold or a high-sensitivity cardiac troponin assay. Whilst two cardiologists adjudicated all index diagnoses using all available clinical information, with excellent intra-observer agreement, there remains potential for misclassification, particularly for type 2 myocardial infarction and myocardial injury. There is likely to be variation in the in-hospital treatments received which we could not adjust for, nor could we adjust for illness severity. As previously reported, a low proportion of patients with type 2 myocardial infarction or myocardial injury underwent inpatient coronary angiography (Shah et al., 2015c). We therefore defined coronary artery disease based on a diagnosis of angina, previous myocardial infarction or previous coronary revascularization, which is likely to significantly underestimate the prevalence of coronary artery disease. Finally, subsequent hospitalizations and cardiovascular or non-cardiovascular death were determined using ICD-10 coding obtained from regional and national registry data, where there is the potential for both diagnostic and coding error. We were therefore not able to determine the incidence of subsequent type 1 or type 2 myocardial infarction.

6.6 Conclusions

Over two-thirds of patients admitted to hospital with type 2 myocardial infarction or myocardial injury die within five years, with the majority of deaths due to non-cardiovascular causes. Nonetheless, major adverse cardiovascular events occur in one-third of patients with elevated cardiac troponin concentrations, irrespective of whether myocardial necrosis was spontaneous or secondary to another acute illness. Whilst patients with type 1 myocardial infarction were at highest risk, there was no separation of risk between those with a diagnosis of type 2 myocardial infarction or myocardial injury. In contrast, those patients with type 2 myocardial infarction or myocardial injury known to have coronary artery disease are at highest risk of cardiovascular events, and efforts to diagnose coronary artery disease may provide opportunities to target preventative therapies and improve patient outcomes.

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Summary of findings

Chest pain is a common symptom in patients presenting to the Emergency Department, and often results in hospital admission for further testing despite as few as 20% of patients receiving a final diagnosis of acute coronary syndrome. Diagnostic delay introduces patient anxiety and is an inefficient use of healthcare resources. Cardiac troponin is a central component of the diagnostic criteria for myocardial infarction, and improvements in assay sensitivity offer both opportunities and challenges for clinical practice.

The purpose of this thesis was to study the use of a clinically available high-sensitivity cardiac troponin I assay for the evaluation of patients with suspected acute coronary syndrome in practice. I hypothesised that a high-sensitivity cardiac troponin I risk stratification threshold of 5 ng/L would provide the optimal balance between safety and efficacy, and integration into a clinical pathway would offer improved performance over pathways based entirely on the 99th centile, with no improvement when contemporary clinical risk scores were applied. Furthermore, I investigated the long term outcomes of patients with myocardial infarction when classified by the universal definition, and hypothesised patients with type 2 myocardial infarction or myocardial injury would be at similar risk of future cardiovascular events.

7.1.1 Optimal risk stratification in suspected acute coronary syndrome

Whilst there are a number of recommended risk stratification thresholds, there has been a lack of consensus as to the optimal approach for use in clinical practice. In a systematic review and meta-analysis, a high-sensitivity cardiac troponin I threshold of 5 ng/L was shown to provide an excellent balance between safety and efficacy. When applied alongside a non-ischaemic electrocardiogram, this approach identifies nearly half of all patients as low risk, with a miss rate of less than one in two hundred patients. Although some statistical differences in negative predictive value were noted in pre-specified subgroup analyses, this reflects the very high negative predictive value in low risk patient groups, and is of no clinical significance. The robust performance of this threshold across multiple healthcare settings with varying prevalence of myocardial infarction gives confidence that this approach is safe and generalisable in practice.

7.1.2 Comparison of the efficacy and safety of novel rule out pathways

Whilst there are several guideline approved pathways for the rule out of myocardial infarction in patients with suspected acute coronary syndrome, the most widely used approach recommended by the European Society of Cardiology was designed in the era of contemporary cardiac troponin assays and applies the upper reference limit (99th centile) as a threshold both to rule in and rule out myocardial infarction. In a prospective observational cohort study, I derived and validated a clinical pathway incorporating a risk stratification threshold of 5 ng/L at presentation and serial testing at 3 hours to identify patients with changing troponin concentrations, within the normal

reference range, who are at increased risk of myocardial infarction. This novel pathway identified more patients on presentation as suitable for rule out, and missed five-fold fewer myocardial infarction or cardiac death events at 30-days than the guideline approved pathway based exclusively on the 99th centile.

7.1.3 Clinical risk scores in suspected acute coronary syndrome

There are a number of additional approaches for the rule out of myocardial infarction. Clinical risk scores apply conventional risk factors for cardiovascular disease to estimate the probability of myocardial infarction. The most widely implemented scores, HEART, GRACE or TIMI, have been extensively validated when used alongside contemporary troponin assays, however, their impact on pathways applying high-sensitivity cardiac troponin testing is less clear. The safety of the European Society of Cardiology 3-hour pathway, which applies the 99th centile alone, could be significantly improved by the addition of a clinical risk score. Conversely, no risk score improved the performance of a novel pathway incorporating a risk stratification threshold. The excellent performance of the novel pathway our understanding that patients with low troponin concentrations have fewer risk factors for coronary or structural heart disease, and are more likely to be admitted for serial testing. Whilst not undertaken in this analysis, additional modelling such as determining the improvement in discrimination (C-statistic), calibration (Hosmer-Lemeshow Test), or calculating the net reclassification index could provide further insight to the additive benefit of such risk scores. All risk scores reduce efficacy, but this is justifiable for the ESC pathway where clear improvements in safety are apparent.

7.1.4 Long term outcomes of myocardial injury and infarction

The universal definition of myocardial infarction suggests a classification based on aetiology, with type 1 myocardial infarction due to atherosclerotic plaque rupture, intraluminal thrombosis and distal microvascular occlusion, and type 2 myocardial infarction occurring due to myocardial oxygen supply or demand imbalance in the context of another illness. In a prospective observational cohort study, all consecutive patients with myocardial necrosis measured using a contemporary sensitive cardiac troponin assay were classified as per the universal definition. Patients with type 2 myocardial infarction or myocardial injury were at significantly increased risk of all cause death, which persisted after adjustment for a number of clinically important co-variates. Whilst not undertaken in this analysis, propensity score matching may have facilitated more accurate estimation of the effect size by adjusting for confounding by indication. Crude rates of cardiovascular events were comparable between groups, with patients with type 2 myocardial infarction or myocardial injury and known coronary artery disease at highest risk of adverse outcomes. Despite this, less than half of patients received optimal secondary prevention, suggesting a treatment gap exists, with the potential opportunity to improve clinical outcomes.

7.2 Future directions

The results presented within this thesis have generated discussion, led to further research questions and have formed the basis of applications for research funding. Three key studies are described below, two of which have received funding and are in progress with the final proposal under review

7.2.1 Prospective evaluation of the High-STEACS pathway

We have demonstrated a clinical pathway incorporating a risk stratification threshold and serial testing offers improved performance over guideline approved pathways based on the 99th centile. However, as with the majority of studies conducted in this area, these findings are based on observational data, with no patients discharged on the basis of a cardiac troponin concentration.

To address this key limitation and provide robust evidence of safety in clinical practice, we are conducting a prospective, multi-centre, stepped-wedge, cluster randomised controlled trial evaluating the safety and efficacy of the High-STEACS pathway in clinical practice (NCT:03005158). This trial is recruiting all consecutive patients in whom a high-sensitivity cardiac troponin concentration is requested in the Emergency Department for suspected acute coronary syndrome across six centres in Scotland. Patients are prospectively identified using electronic healthcare records. When the attending clinician requests a cardiac troponin, they provide a minimum data set for the order to be processed, including presenting complaint, time of symptom onset, and whether an acute coronary syndrome is suspected. There are three six-month phases to the study (***Figure 7.1***).

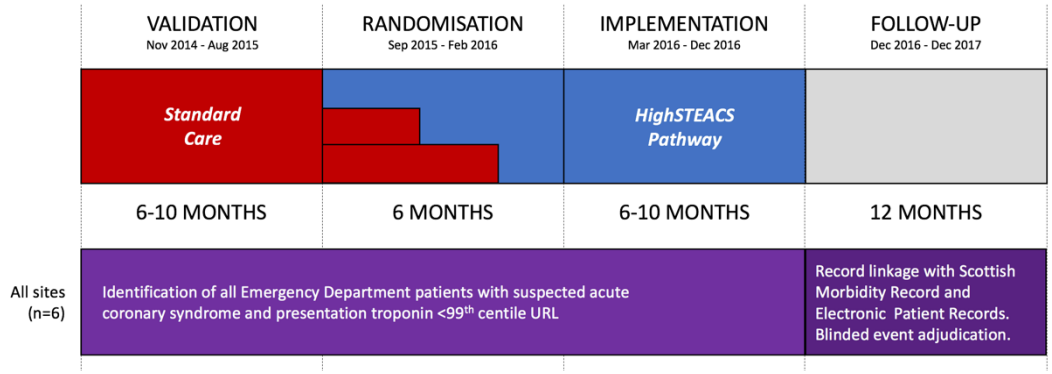

Figure 7.1. Outline of study phases.

During the validation phase, patients will be managed according to the established assessment protocol (standard care), which rules out myocardial infarction where cardiac troponin concentrations are <99th centile at presentation in patients with symptoms for more than 6 hours. In patients with symptoms for less 6 hours, a second troponin measurement is performed 12 hours from symptom onset, with myocardial infarction ruled out if cardiac troponin remains <99th centile. This will be followed by a 6-month randomisation phase where participating centres will be randomly stepped-in to use the early rule-out pathway, with stratification by hospital size. A final calendar matched implementation phase will follow in which all sites will use the early rule-out pathway.

The study design uses a co-primary endpoint with sequential hypothesis testing. The primary efficacy endpoint is the length of hospital stay, defined as the length from initial presentation to the Emergency Department until final discharge from hospital in minutes. The primary safety endpoint is the rate of type 1 or type 4b myocardial infarction or cardiac death after discharge and within 30 days of index presentation. For the primary efficacy endpoint, we hypothesise that implementation of the early

rule out pathway will reduce the overall length of stay compared to standard care. For the primary safety endpoint, we hypothesise the proportion of patients with type 1 or type 4b myocardial infarction or cardiac death after discharge and within 30 days of index presentation will not differ by more than 0.5%.

The anticipated total study population is approximately 39,000. Based on simulation methods, a sample size of 38,994 patients will provide 99% power at the two-sided 5% level of significance to detect a difference of at least 60 minutes in arithmetic mean length of stay (primary efficacy endpoint), and 90% power to demonstrate non-inferiority assuming an event rate of 0.4% at 30-days (primary safety endpoint). All three phases of this trial are now complete, with blinded endpoint adjudication in progress and an aim to report in late 2018.

7.2.2 Evaluating the mechanisms of type 2 myocardial infarction

The definition of acute myocardial infarction has evolved to accommodate increasingly sensitive markers of myocardial necrosis and imaging methods that allow greater understanding of the pathogenic mechanisms of acute coronary syndrome. As such, the universal definition of myocardial infarction now proposes that we classify patients with myocardial infarction based on aetiology (Thygesen et al., 2012a). Whilst this classification has been used in clinical trials to refine clinical outcomes (Morrow et al., 2009, Bonaca et al., 2012, White et al., 2012), it has not been widely adopted in clinical practice, and the frequency and implications of subtypes of acute myocardial infarction are uncertain. I believe the diagnostic criteria for type 2 myocardial infarction require clarification and that this is necessary to encourage clinicians to adopt the proposed classification. This can only be achieved through prospective and

systematic evaluation of the clinical presentation, pathophysiological mechanisms and outcomes of unselected patients with acute myocardial injury in clinical practice.

In a prospective observational cohort study (EVEREST-MI, ClinicalTrials.gov number: NCT:03338504), I am systematically evaluating the mechanisms of acute myocardial injury in unselected patients who present to hospital with an alternative primary illness likely to cause myocardial oxygen supply or demand imbalance (***Appendix 5***).

All participants will undergo cardiac magnetic resonance imaging of the myocardium where there are no contraindications, in addition to evaluation of coronary anatomy by either invasive or non-invasive coronary angiography dependent on co-morbidities or the individual participant preference. For example, frail patients or those with severe peripheral vascular disease in whom intra-vascular access may be challenging would be more likely to undergo non-invasive imaging

Cardiovascular magnetic resonance (CMR) will be performed using a 3T scanner (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany) at the Clinical Research Imaging Centre (CRIC), Edinburgh. The MRI scan will consist of localisers, axial and coronal HASTE images, standard breath-held and ECG-gated cine sequences in 2 chamber, 4 chamber and short axis views. Short-axis cine images will be obtained using a balanced steady-state free precession sequence from the mitral valve annulus to the apex (8 mm parallel slices with 2 mm spacing) for the assessment of left ventricle function and volumes. Left ventricle volumes, mass and ejection fraction will be assessed using dedicated software (Argus Ventricular Function, Siemens AG Healthcare Sector, Erlangen, Germany) and values indexed to body

surface area. Breath-held, ECG-gated T2 mapping sequences of the myocardium will be performed in the long-axis as a marker of myocardial inflammation. T1-weighted imaging of the coronary arteries will be performed to look for evidence of recent intraplaque thrombus or haemorrhage using the CATCH sequence. All patients will then receive 0.4mg (5ml) of peripheral Regadenoson (Rapiscan™) as a stress perfusion agent, and a bolus of 0.2 mmol/kg Gadolinium (Gadovist™), to quantify areas of myocardial ischaemia. Stress-perfusion imaging will not be undertaken in patients with severe asthma or chronic obstructive pulmonary disease, those on dipyridamole, theophylline or aminophylline, or those with atrioventricular block. Patients will be asked to withhold caffeine intake for 12 hours prior to imaging. This will be followed by standard late-gadolinium enhancement sequences. The late gadolinium enhancement and T2 mapping techniques will identify regions of new or old myocardial infarction as well as other patterns of injury such as the mid-wall pattern associated with myocarditis.

Coronary angiography will be performed via the femoral or radial artery with 6F arterial catheters. In patients with one or more stenoses in a major epicardial vessel, a coronary pressure guidewire (PressureWire™ Aeris™, St. Jude Medical, St. Paul, Minnesota) will be used to determine distal coronary pressure and the fractional flow reserve (FFR) calculated at maximal adenosine-induced (intravenous 140 μg/kg/min) hyperaemia. As previously described, frequency domain optical coherence tomography (FD-OCT) will be performed in all three coronary vessels using a FastView® coronary imaging catheter (Terumo, Tokyo, Japan) with automated pullback at 20 mm/s to identify features consistent with vulnerable plaque or recent plaque rupture (Gudmundsdottir et al., 2015). If there is evidence of inducible

myocardial ischaemia due to coronary artery stenosis, revascularisation with percutaneous coronary intervention may be considered if in the patients best interests and on discussion with the patients attending clinician.

Where invasive coronary angiography is contraindicated, CT coronary angiography will be performed according to previously published methodology (Newby, 2015). Imaging will be performed using a 128 multidetector row CT (Siemens Biograph, Siemens Healthcare, Erlangen, Germany). Patients with a heart rate exceeding 65 beats/min will receive oral beta-blockade (50 or 100 mg metoprolol) 1 hour before computed tomography. Additional intravenous beta blockers will be given depending on heart rate at the time of imaging. All patients will receive sublingual glyceryl trinitrate (300 μg) immediately prior to dual cardiac and respiratory-gated computed tomography imaging of the coronary arteries. We will quantify total plaque burden using CT calcium scoring. A bolus of 80-100 mL of contrast (400 mg/mL; Iomeron, Bracco, Milan, Italy) will be injected intravenously at 5 mL/s. CT angiography will be evaluated jointly by a Radiologist and a Cardiologist with suitable training to determine the extent of coronary atherosclerosis. An assessment of the functional consequences of coronary artery stenosis will be made using the computed tomography fractional flow reserve (CT-FFR) technique, using the HeartFlow^{TM} platform.(Min et al., 2012)

Serial blood samples will be obtained on enrolment to the study, at 24 hours, and at last point of contact to the research team (on discharge from hospital or at outpatient visit for study imaging) in two 9 mL lithium-heparin tubes, two 9mL EDTA plasma

tubes and two 9mL serum tubes. Samples obtained will facilitate development of novel biomarkers using proteomic and genomic approaches.

All patients will provide informed consent in line with routine clinical practice. We will report the results of all investigations to the patients attending clinician so therapy may be modified where this is felt to be of benefit. The target for completion of all diagnostic studies is 28 days from index presentation, with the aim to perform imaging as early as is feasible.

I hypothesise that the majority of patients with myocardial injury secondary to oxygen supply or demand imbalance will have evidence of underlying obstructive coronary artery disease. I aim to recruit 100 patients as this is a feasible sample size, and will complete recruitment over 12-18 months. As this is a pilot study, no formal power calculations have been performed.

7.2.3 Novel biomarkers to distinguish type 1 and type 2 myocardial infarction

Cardiac troponin is the only recommended biomarker for the detection of myocardial necrosis, and it is integral to the diagnostic criteria for myocardial infarction. However, as the cellular mechanisms of myocardial injury are thought to differ between subtypes of myocardial infarction, there may be opportunities to identify other candidate cardiovascular biomarkers which may distinguish subtypes of myocardial infarction, improving diagnostic accuracy.

In patients with type 2 myocardial infarction, in the absence of plaque rupture, platelet aggregation, embolisation and microvascular obstruction, the profile of sarcomeric and inflammatory proteins released from the myocardium may differ compared to that

observed in patients with type 1 myocardial infarction. In patients recruited to the EVEREST-MI study in whom serial blood samples are obtained, I will undertake a systematic evaluation of the kinetics of cardiac troponin release, and will compare patients with type 2 myocardial infarction with an existing cohort of patients with confirmed type 1 myocardial infarction recruited at the time of primary PCI (***Appendix 6***).

EDTA plasma from 90 subjects (45 type 1 myocardial infarction, 45 type 2 myocardial infarction) will be transferred to Olink Proteomics (Upsalla Science Park, Upsalla, Sweden) for analysis. The Olink Proteomics Inflammation $^{(96x96)}$, Olink Proteomics Cardiovascular II $^{(96x96)}$ and Olink Proteomics Cardiovascular III $^{(96x96)}$ biomarker panels are high-throughput, multiplex immunoassay systems capable of analysing multiple proteins associated with inflammatory or cardiovascular disease, simultaneously. A total 276 candidate proteins will be evaluated. This technology is facilitated by novel proximity extension assay (PEA) technology. Each panel contains 92 oligonucleotide-labelled antibody pairs which bind to the target when present in the sample. Binding by antibody pairs triggers the formation of double-stranded DNA amplicons, which are quantified by real-time polymerase chain reaction (PCR). The resultant relative values are normalized and log transformed, where a high value correlates to a high protein content. PEA is a robust and reproducible technique, with intra-assay coefficient of variation (CV) ranging between 5% and 13%, and inter-assay CV between 9% and 39% dependent on assay type.

We will explore the relationship between high-sensitivity cardiac troponin concentration and candidate biomarkers using pairwise correlation, illustrated in a

correlation matrix and ordered by hierarchical clustering. We anticipate a range of candidate biomarkers will be elevated in patients with type 1 and type 2 myocardial infarction, but some may distinguish between diagnoses. The association between biomarker value and diagnosis (type 1 or type 2 myocardial infarction) will be explored by fitting univariate generalised linear models with a binomial link. Candidate biomarkers which are predictive in univariate analysis will be evaluated in a multi-variate generalised linear model with adjustment for age, gender and renal function. A final model will be derived using backward elimination, including co-variates with a P-value of <0.05. We will assess the discriminatory performance of the model by evaluating sensitivity, specificity, and area under the receiver operator curve (C statistic). For cardiac troponin, and candidate biomarkers which show significant associations with both diagnoses, we will evaluate the time course of biomarker release using a polynomial linear mixed effects model, with a random term for subject. Where candidate biomarkers show high discriminatory ability, we will perform validation studies using immunoassay and ELISA technology in stored samples.

Understanding the mechanisms of acute myocardial injury in hospitalised patients and the contribution of coronary artery disease will lead to the development of a diagnostic algorithm and framework for clinicians to base their assessment, and will help to guide future therapeutic trials.

7.3 Clinical perspective

The novel approaches to risk stratification described in this thesis demonstrate the potential of high-sensitivity cardiac troponin assays to improve diagnostic safety and efficiency in patients with suspected acute coronary syndrome, and complement prior research. By safely ruling out the diagnosis of myocardial infarction in nearly half of all patients presenting to the emergency department, we have the opportunity to improve patient experience and healthcare resource utilization at a time of increasing pressures on the National Health Service. Further work is ongoing to provide prospective, randomised controlled trial data in consecutive patients; the results of which have the potential to change our practice.

Perhaps more relevant to the hospital physician is the diagnosis of type 2 myocardial infarction, which is twice as common as type 1 myocardial infarction in patients over the age of 75, and for which we have no evidence based recommendations for investigation or treatment. The task force for the universal definition of myocardial infarction recognise and acknowledge the lack of clear guidance for clinicians in practice. The EVEREST-MI study will provide insight into the underlying mechanism of myocardial injury in patients with type 2 myocardial infarction. This may guide recommendations for investigational strategy and facilitate prospective trials of secondary prevention therapies known to reduce future cardiovascular events in patients with type 1 myocardial infarction. Such studies are necessary if we are to improve clinical outcomes in this understudied patient group.

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APPENDIX 1

THE OPTIMAL RISK STRATIFICATION THRESHOLD OF HIGH-SENSITIVITY CARDIAC TROPONIN I IN PATIENTS WITH SUSPECTED ACUTE CORONARY SYNDROME

Appendix 1.1. Search Strategy

I. Ovid MEDLINE(R) <1946 to Present>

1. exp Chest Pain/
2. (chest adj2 pain*).ti,ab.
3. chest discomfort.ti,ab.
4. exp Acute Coronary Syndrome/
5. acute coronary syndrome*.ti,ab.
6. ACS.ti,ab.
7. exp Coronary Artery Disease/
8. coronary artery disease.ti,ab.
9. exp Angina, Unstable/
10. unstable angina.ti,ab.
11. Myocardial Infarction/di
12. myocardial infarction.ti,ab.
13. heart attack.ti,ab.
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
15. exp Troponin/du
16. troponin*.ti,ab.
17. hs-ctn*.ti,ab.
18. 15 or 16 or 17
19. exp Emergency Service, Hospital/
20. (emergency adj (room* or department*)).ti,ab.
21. (ER or ED).ti,ab.
22. (presenting or presented or presentation).ti,ab.
23. (admission* or admitted).ti,ab.
24. chest pain unit*.ti,ab.
25. 19 or 20 or 21 or 22 or 23 or 24
26. 14 and 18 and 25
27. limit 26 to yr="2006 -Current"

II. EMBASE <1974 to present>

1. exp Chest Pain/
2. (chest adj2 pain*).ti,ab.
3. chest discomfort.ti,ab.
4. exp Acute Coronary Syndrome/
5. acute coronary syndrome*.ti,ab.
6. ACS.ti,ab.
7. exp Coronary Artery Disease/
8. coronary artery disease.ti,ab.
9. exp Angina, Unstable/
10. unstable angina.ti,ab.
11. Myocardial Infarction/di
12. myocardial infarction.ti,ab.
13. heart attack.ti,ab.
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13

15. exp Troponin/du
16. troponin*.ti,ab.
17. hs-ctn*.ti,ab.
18. 15 or 16 or 17
19. exp Emergency Service, Hospital/
20. (emergency adj (room* or department*)).ti,ab.
21. (ER or ED).ti,ab.
22. (presenting or presented or presentation).ti,ab.
23. (admission* or admitted).ti,ab.
24. chest pain unit*.ti,ab.
25. 19 or 20 or 21 or 22 or 23 or 24
26. 14 and 18 and 25
27. limit 26 to yr="2006 -Current"

III. Web of Science

"chest pain" OR "acute coronary syndrome" OR "coronary artery disease" OR "unstable angina" OR "myocardial infarction"
AND
"troponin"
[limit publication year from 2006 to present]

IV. The Cochrane Central Register of Controlled Trials

#1 MeSH descriptor: [Myocardial Ischemia] explode all trees
#2 MeSH descriptor: [Troponin] explode all trees
#3 #1 and #2: publication year from 2006 to present

Appendix 1.2: Characteristics of the cohort studies included in the meta-analysis

Study	Study type, country	Inclusion criteria	Exclusion criteria
HighSTEACS-V [1]	Multi-centre, UK	Consecutive patients in whom the attending clinician requested cardiac troponin for suspected acute coronary syndrome	Patients with ST-segment-elevation myocardial infarction (STEMI), already admitted during the study period, non-resident in Scotland.
UTROPIA [2]	Single-centre, USA	Consecutive patients presenting to ED in whom cardiac troponin measurements were obtained on clinical indication.	Patients <18 years old, with evidence of STEMI, pregnancy, trauma, declined to participate in research, without death date available, did not present through ED or were transferred from an outside hospital.
HighSTEACS-P [3]	Single-centre, UK	Consecutive patients in whom the attending clinician suspected an acute coronary syndrome.	Patients not resident in the south east of Scotland.
HighSTEACS-S [4]	Single-centre, UK	Consecutive patients in whom the attending clinician requested cardiac troponin for suspected acute coronary syndrome.	Patients with STEMI, those who were unable to provide consent or those from outside the region.
EDACS [5]	Single-centre, New Zealand	Patients 18 years or over with at least five minutes of symptoms consistent with an acute coronary syndrome. Enrollment was consecutive during the hours of the available research nurse (normally 8AM to 11PM, 7 days a week)	Patients with STEMI, a clear cause other than acute coronary syndrome (ACS), inability to provide informed consent, staff considered recruitment to be inappropriate, transfer from another hospital, pregnancy, previous enrolment, or inability to be contacted after discharge.
STENOCARDIA [6]	Multi-centre, Germany	Consecutive patients between 18 and 85 years of age presenting with acute angina pectoris or equivalent symptoms.	Major surgery or trauma within the previous 4 weeks, pregnancy, intravenous drug abuse, and anemia (haemoglobin level <10g/dL)

Appendix 1.2 continued: Characteristics of the cohort studies included in the meta-analysis

Study	Study type, country	Inclusion criteria	Exclusion criteria
ADAPT-B [7]	Multi-centre, Australia	Consecutive patients 18 years or over, with at least five minutes of symptoms consistent with an acute coronary syndrome where the attending physician planned to perform serial cardiac troponin tests.	Patients with STEMI, a clear cause other than ACS, inability to provide informed consent, staff considered recruitment to be inappropriate, transfer from another hospital, pregnancy, previous enrolment, or inability to be contacted after discharge.
IMPACT [8]	Single-centre, Australia	Patients 18 years or over with at least five minutes of symptoms consistent with an acute coronary syndrome where the attending physician planned to perform serial cardiac troponin tests.	Patients with STEMI, a clear cause other than ACS, inability to provide informed consent, staff considered recruitment to be inappropriate, transfer from another hospital, pregnancy, previous enrolment, or inability to be contacted after discharge.
ROMI [9]	Multi-centre, Canada	Patients 18 years or over, presenting to the ED with symptoms of and investigated for ACS (cardiac troponin ordered by an Emergency Department physician)	Patients with STEMI, death (all-cause) or serious ventricular cardiac dysrhythmia before troponin testing. Patients who had traumatic chest pain, including surgery or cardiac complications, NSTEMI, pulmonary embolus, known active malignancy, sepsis within the previous 30 days or previously enrolled or transferred from another primary care facility.
HOPKINS [10]	Single-centre, USA	Patients with non-diagnostic initial ECGs, chief complaints of chest pain or shortness of breath and cardiac troponin ordered by treating clinician for possible acute myocardial infarction. Enrolment of patients occurred on weekdays from 9.00am to 9.00pm.	Patients who left against medical advice or if initial blood samples were not obtained.

Appendix 1.2 continued: Characteristics of the cohort studies included in the meta-analysis

ADAPT-RCT [12]	Single-centre, New Zealand	Consecutive patients 18 years or older who present acutely to the Emergency Department with possible cardiac chest pain. Recruitment occurred between 8AM and 10AM, 7 days a week.	Patients with STEMI, an initial clear cause other than acute coronary syndrome, inability to provide informed consent, staff considered recruitment to be inappropriate, chest pain symptoms began more than 12 hours before presentation, persisting chest pain, transfer from another hospital, pregnancy, previous inclusion in the study, or inability to be contacted after discharge.
RING [13]	Single-centre, Canada	Patients ≥18 years old with onset of ACS symptoms in the past 6 hours, blood sample collection ordered by the ED physician for cardiac troponin measurement, informed consent obtained, and availability for telephone follow-up.	Patients with STEMI, those referred directly to surgery, trauma patients, those with more than 6 hours of symptoms and previously enrolled patients
TI-AMO [14]	Single-centre, The Netherlands	Patients with symptoms suggestive of acute myocardial infarction for at least 3 hours in whom a high sensitivity troponin measurement was performed.	Patients with STEMI who underwent primary intervention elsewhere, onset of symptoms >12 hours prior to presentation or incomplete data collection.
APACE [15]	Multi-centre, New Zealand and Australia	Consecutive patients presenting to the Emergency Department with symptoms suggestive of AMI within 12 hours	Patients with STEMI, terminal kidney failure on chronic dialysis, baseline troponin measurement were not available or where it was not possible to accurately adjudicate the final diagnosis.
APACE [15]	Multi-centre, New Zealand and Australia	Consecutive patients presenting to the Emergency Department with symptoms suggestive of AMI within 12 hours	Patients with STEMI, terminal kidney failure on chronic dialysis, baseline troponin measurement were not available or where it was not possible to accurately adjudicate the final diagnosis.

Appendix 1.2 continued: Characteristics of the cohort studies included in the meta-analysis

Study	Study type, country	Inclusion criteria	Exclusion criteria
BACC [16]	Single-centre, Germany	Patients >18 years of age with suspected acute myocardial infarction, with the ability to provide written informed consent.	STEMI.
TRUST [17]	Single-centre, UK	Consecutive patients attending the Emergency Department at least 18 years old and at least five min of chest pain suggestive of acute coronary syndrome, and for whom the attending physician determined inpatient evaluation was required.	Patients with STEMI, new left bundle branch block, ECG changes diagnostic of ischemia, arrhythmias, hs-TnT sample not suitable for analysis, age ≥80 years, atypical symptoms in the absence of chest discomfort, a clear non-ACS cause for chest pain at presentation, another medical condition requiring hospital admission, refusal and inability to give informed consent, non-English speaking, pregnancy, renal failure requiring dialysis or inability to be contacted after discharge.
STOCKPORT [18]	Single-centre, UK	Adult patients presenting to the Emergency Department with suspected cardiac chest pain.	Patients with STEMI, new left bundle branch block, new-onset ECG changes diagnostic of ischemia (ST-segment depression ≥1mm or T-wave inversion consistent with ischemia), significant arrhythmias, age <18 years, a clear cause of the symptoms other than acute coronary syndromes, pregnancy, declined to take part, did not have capacity to provide informed consent, did not speak English, prisoner, already included and those deemed inappropriate for researcher to approach patient (eg, terminal illness), follow-up considered impossible.

Appendix 1.2 continued: Characteristics of the cohort studies included in the meta-analysis

Study	Study type, country	Inclusion criteria	Exclusion criteria
MANCHESTER [19]	Single-centre, UK	Consecutive adult patients presenting to the Emergency Department with suspected cardiac chest pain occurring in the previous 24 hours.	Patients with STEMI, new left bundle branch block, new-onset ECG changes diagnostic of ischemia (ST-segment depression ≥1mm or T-wave inversion consistent with ischemia), pregnancy, significant arrhythmias, age <18 years, pregnancy, patients with another medical condition necessitating hospital admission, did not have capacity to provide informed consent, those with renal failure requiring dialysis, patients with chest trauma and suspected myocardial contusion, those who did not speak English, prisoners, patients for whom all means of follow-up would be impossible and those deemed inappropriate for researcher to approach patient (eg, terminal illness).

Study title acronyms:
HighSTEACS-V: High-sensitivity cardiac troponin in the evaluation of patients with Suspected Acute Coronary Syndrome - Validation
HighSTEACS-S: High-sensitivity cardiac troponin in the evaluation of patients with Suspected Acute Coronary Syndrome - Sub-study
UTROPIA: Use of Abbott high sensitivity cardiac troponin I assay in acute coronary syndromes
HighSTEACS-P: High-sensitivity cardiac troponin in the evaluation of patients with Suspected Acute Coronary Syndrome - Pilot
EDACS: Emergency department assessment of chest pain score
IMPACT: Improved assessment of chest pain trial
ADAPT: 2-hour accelerated diagnostic protocol to assess patients with chest pain symptoms using contemporary troponins as the only biomarker
ROMI: Rule out of myocardial infarction
RING: Reducing the time interval for identifying new guideline
BACC: Biomarkers in acute cardiovascular care
APACE: Advantageous predictors of acute coronary syndromes evaluation
TRUST: Triage rule-out using high-sensitivity troponi

Appendix 1.3: Criteria used to adjudicate the diagnosis of myocardial infarction by study

Study	Reference assay	Reference assay cutoff (ng/L)	Study protocol intended time of troponin sampling (hours from presentation)
HighSTEACS-V [1]	Abbott hs-TnI	16 in women and 34 in men	0, 6-12
UTROPIA [2]	Abbott hs-TnI	16 in women and 34 in men	0, 3-24
HighSTEACS-P [3]	Abbott hs-TnI	16 in women and 34 in men	0, 6-12
HighSTEACS-S [4]	Abbott hs-TnI	16 in women and 34 in men	0, 6-12
EDACS [5]	Abbott hs-TnI	16 in women and 26 in men	0, 2
STENOCARDIA [6]	Abbott c-TnI	32	0, 3, 6
ADAPT-B [7]	Beckman Coulter Accu c-TnI	40 + ≥20% change	0, 2, 6-12
IMPACT [8]	Beckman Coulter Accu c-TnI	40 + ≥20% change	0, 2, 6-12
ROMI [9]	Abbott c-TnI	30 + ≥30 change if <100, or ≥20%	0, 3-6
HOPKINS [10]	Abbott c-TnI	60 + ≥30% change	0, 3-9
ADAPT-C [11]	Abbott c-TnI (New Zealand)	30 + ≥20% change	0, 2, 6-12
ADAPT-RCT [12]	Abbott c-TnI	32	0, 2, 6-12
RING [13]	Roche Elecsys c-TnT	40 + ≥3SD change if <100, or 20%	0, 1.5, 3
TI-AMO [14]	Roche hs-TnT	14 + ≥7 change	0, 2.5-4.5
APACE [15]	Roche hs-TnT	14 + ≥10 change	0, 1, 2, 3, 6
BACC [16]	Roche hs-TnT	14	0, 3
TRUST [17]	Roche hs-TnT	14	0, 6
STOCKPORT [18]	Roche hs-TnT	14	0, 12
MANCHESTER [19]	Roche hs-TnT	14	0, 12

Appendix 1.4: Assessment of bias based on the QUADAS-2 Framework.

Study	Risk of Bias				Applicability Concerns		
	Patient selection	**Index Test**	**Reference Standard**	**Flow and timing**	**Patient selection**	**Index test**	**Reference standard**
HighSTEACS-V [1]	Low	Low	Low	Low	Low	Low	Low
UTROPIA [2]	Low	Low	Low	Low	Low	Low	Low
HighSTEACS-P [3]	Low	Low	Low	Low	Low	Low	Low
HighSTEACS-S [4]	Low	Low	Low	Low	Low	Low	Low
EDACS [5]	Low	Low	Low	Low	Low	Low	Low
STENOCARDIA [6]	Low	Low	High	Low	Low	Low	Low
ADAPT-B [7]	Low	Low	High	Low	Low	Low	Low
IMPACT [8]	Low	Low	High	Low	Low	Low	Low
ROMI [9]	Low	Low	High	Low	Low	Low	Low
HOPKINS [10]	Low	Low	High	Low	Low	Low	Low
ADAPT-C [11]	Low	Low	High	Low	Low	Low	Low
ADAPT-RCT [12]	Low	Low	High	Low	Low	Low	Low
RING [13]	Low	Low	High	Low	Low	Low	Low
TI-AMO [14]	Low	Low	Unclear	Low	Low	Low	Low
APACE [15]	Low	Low	Unclear	Low	Low	Low	Low
BACC [16]	Low	Low	Unclear	Low	Low	Low	Low
TRUST [17]	High	Low	Unclear	Low	Low	Low	Low
STOCKPORT [18]	High	Low	Unclear	Low	Low	Low	Low
MANCHESTER [19]	High	Low	Unclear	Low	Low	Low	Low

Patient Selection: Consecutive or random patient sample recruited without significant exclusions = low risk
Index test: Pre-specified threshold using index text under study = low risk
Reference standard: Interpretation of independent reference standard without knowledge of index test result = low risk
Flow and timing: Appropriate interval between index and reference test without exclusions = low risk
Patient selection: Included patients and setting match the review standard = low risk
Index test: Index test, conduct and interpretation consistent with the review question = low risk
Reference standard: Target condition matches the reference standard = low risk
There was no evidence of publication bias (Rank correlation test for funnel plot asymmetry P=0.54)

Appendix 1.5: Analysis code

All analysis was performed using R (version 3.2.2) using the *metafor* package. For transparency, the analysis code detailing all steps taken is available open source via GitHub.

Link: https://github.com/a-r-chapman/hsTnI_IPD_meta_analysis

Appendix 1.6. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay used for adjudication

Age	Sex	Presenting symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
High-sensitivity cardiac troponin I cohorts													
High-STEACS Validation [1]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >34ng/L (M)**					
64	M	Chest pain	85	Yes	Hypertension Previous MI Previous PCI	1	850	1	850	Type 1 MI	PCI to RCA and LAD	No	No
70	F	Chest pain	799	Yes	Previous MI	3	70	3	70	Type 1 MI	PCI to LAD	No	No
48	F	Chest pain	86	No	Hypertension Hyperlipidaemia Previous MI Previous PCI	4	6,480	4	6,480	Type 1 MI	PCI to RCA	No	No
80	M	Chest pain	75	No	Hypertension Angina	3	534	3	534	Type 1 MI	Medical	No	No
62	M	Chest pain	164	-	Smoker Diabetes Mellitus Hypertension Hyperlipidaemia	4	78	4	78	Type 1 MI	PCI to RCA	No	No
69	F	Chest pain	164	No	Hypertension Previous MI Previous PCI	4	852	4	852	Type 1 MI	Medical	No	No
69	F	Chest pain	237	No	Previous MI Previous PCI	1	17	1	17	Type 1 MI	Medical	No	No
44	M	Chest pain	63	Yes	Smoker Hyperlipidaemia	2	14,008	2	14,008	Type 1 MI	Medical	No	No
56	F	Chest pain	200	No	Family history	1	3,689	1	3,689	Type 1 MI	PCI to LAD and LCx	No	No

The reference assay results relate to the assay used for diagnostic adjudication. In high-sensitivity cardiac troponin I cohorts, this assay was also used in clinical practice.
Abbreviations: (-) data not available. MI – myocardial infarction, PCI – percutaneous coronary intervention, LAD – left anterior descending artery, RCA – right coronary artery, LCx – circumflex artery.

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
UTROPIA [2]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >34ng/L (M)**					
60	F	Chest pain	-	Yes	Smoker Hypertension	2	1,190	2	1,190	Type 1 MI	PCI	No	No
80	M	Chest pain	968	Yes	Diabetes mellitus Hypertension Angina	4	267	4	267	Type 1 MI	CABG	No	No
73	F	Chest pain	-	No	Diabetes Mellitus Hypertension Hyperlipidaemia Previous PCI	1	37	1	37	Type 1 MI	PCI	No	No
High-STEACS Pilot study [3]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >34ng/L (M)**					
76	M	Chest pain	480	Yes	Hypertension Hyperlipidaemia Diabetes mellitus Previous CABG	3	1,094	3	1,094	Type 1 MI	Medical	No	No
High-STEACS Sub-study [4]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >34ng/L (M)**					
60	M	Chest pain	113	Yes	Smoker Angina Previous PCI	2	2,932	2	2,932	Type 1 MI	PCI to RCA and D1	No	No
43	M	Chest pain	146	No	Smoker Diabetes Mellitus Hypertension Hyperlipidaemia	4	6,594	4	6,594	Type 1 MI	PCI to LCx	No	No
EDACS [5]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >26ng/L (M)**					
63	F	Chest pain	170	No	Hypertension Hyperlipidaemia Angina Previous MI	3	6	3	6	Type 1 MI at 4 days	Medical	No	-

The reference assay results relate to the assay used for diagnostic adjudication. In high-sensitivity cardiac troponin I cohorts, this assay was also used in clinical practice.
Abbreviations: (-) data not available. MI – myocardial infarction, PCI – percutaneous coronary intervention, CABG – coronary artery bypass graft, RCA – right coronary artery, D1 – diagonal artery, LCx – circumflex artery.

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
EDACS continued [5]								**Reference assay (URL): Abbott hsTnI >16ng/L (F) >26ng/L (M)**					
70	M	Chest pain	245	No	Smoker Diabetes Mellitus Hypertension Hyperlipidaemia	2	215	2	215	Type 1 MI	Angiography No PCI	No	-
44	M	Chest pain Dyspnoea	235	No	Smoker	3	1,244	3	1,244	Type 1 MI	Angiography No PCI	No	-
Contemporary cardiac troponin I and T cohorts													
ADAPT Brisbane [7]								**Reference assay (URL): Beckmann Coulter AccuTnI >40 ng/L**					
65	F	Chest pain	570	No	Hypertension Hyperlipidaemia	4	4	10	200	NSTEMI	Medical	No	-
IMPACT [8]								**Reference assay (URL): Beckmann Coulter AccuTnI >40 ng/L**					
44	M	Chest pain Dyspnoea	100	No	Diabetes Mellitus Hypertension Hyperlipidaemia	3	2	20	2,000	NSTEMI	PCI	No	No
63	M	Chest pain Dyspnoea	120	No	None	2	5	90	100	NSTEMI	PCI	No	-
57	M	Chest pain Dypsnoea	45	No	None	2	8	10	120	NSTEMI	CABG	No	No
44	M	Chest pain Presyncope	1,370	No	Smoker	3	5	10	160	NSTEMI	PCI	No	No
ROMI [9]								**Reference assay (URL): Abbott cTnI >30 ng/L**					
71	M	Back pain Diaphoresis	-	-	Hypertension Hyperlipidaemia Angina	3	3	10	600	Type 1 MI	Medical	-	-

The reference assay results relate to the assay used for diagnostic adjudication. In high-sensitivity cardiac troponin I cohorts, this assay was also used in clinical practice.
Abbreviations: (-) data not available. MI - myocardial infarction, NSTEMI – Non-ST segment elevation myocardial infarction, PCI – percutaneous coronary intervention, CABG – coronary artery bypass graft

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
ROMI continued [9]								**Reference assay (URL): Abbott cTnI >30 ng/L**					
40	M	Chest pain	-	-	Smoker Hypertension Hyperlipidaemia	4	4	10	5,200	Type 1 MI	PCI	-	-
50	F	Chest pain	-	-	Smoker Hypertension	1	434	10	920	Type 1 MI	PCI	-	-
55	F	Chest pain	-	-	Smoker	4	84	10	100	Type 1 MI	Angiogram Medical	-	-
HOPKINS [10]								**Reference assay (URL): Abbott cTnI >60 ng/L**					
63	F	Chest pain	91	-	Smoker Hypertension Hyperlipidaemia	4	16,007	<LOD	9,530	Type 1 MI	PCI	-	-
ADAPT Christchurch [11]								**Reference assay (URL): Abbott cTnI >30 ng/L**					
81	M	Chest pain	540	No	Hypertension Hyperlipidaemia Angina Previous MI	2	423	310	330	Type 1 MI	PCI to RCA	No	-
40	M	Chest pain	645	No	Hyperlipidaemia Angina Previous MI	4	5	50	60	Type 1 MI	PCI to LAD	No	-
60	M	Chest pain	120	Yes	Hypertension Hyperlipidaemia	2	2	40	220	Type 1 MI	PCI to LCx and LAD	No	-
71	M	Chest pain	230	No	Angina Previous PCI	4	4	150	180	Type 1 MI	Angiogram No PCI	No	-
ADAPT RCT [12]								**Reference assay (URL): Abbott cTnI >32 ng/L**					
68	F	Chest pain	130	No	Hypertension Hyperlipidaemia Previous MI	2	312	2	415	Type 1 MI	Medical Normal Echo Normal MPS	No	-

Abbreviations: (-) data not available. MI - myocardial infarction, PCI – percutaneous coronary intervention, LAD – left anterior descending artery, RCA – right coronary artery, LCx – circumflex artery, MPS – myocardial perfusion scan

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
High-sensitivity cardiac troponin T cohorts													
APACE [14]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
73	M	Chest pain	240	No	Hypertension Hyperlipidaemia Previous MI	3	4	33	33	Type 2 MI	Medical	No	No
76	M	Chest pain	240	No	Hypertension Previous MI Previous PCI Previous CABG	4	5	20	20	Type 2 MI	Medical	No	No
74	F	Chest pain	60	No	Smoker Hypertension Hyperlipidaemia	3	11	10	17	Type 1 MI	PCI	No	No
44	M	Chest pain	120	No	Smoker Hypertension Hyperlipidaemia	4	257	5	74	Type 1 MI	PCI	No	No
75	M	Chest pain	60	No	Hypertension Hyperlipidaemia Angina	3	9	6	15	Type 1 MI	Medical	No	No
93	F	Chest pain	540	No	Hypertension Hyperlipidaemia Angina	4	4	41	41	Type 1 MI	None	No	No
71	M	Chest pain	120	No	Hypertension Hyperlipidaemia Angina Previous MI Previous PCI	4	14	9	93	Type 1 MI	PCI	No	No
79	F	Chest pain	60	Yes	Diabetes mellitus Hypertension Hyperlipidaemia Previous MI Previous CABG	4	7	18	22	Type 2 MI	None	No	No

Abbreviations: (-) data not available. MI – myocardial infarction, PCI – percutaneous coronary intervention, CABG – coronary artery bypass graft

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	Myocardial ischaemia on ECG	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
TRUST [15]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
73	F	Chest pain	199	No	Hypertension Hyperlipidaemia Angina	4	-	15	26	Type 1 MI	Medical	No	No
68	M	Chest pain	2915	No	Diabetes Mellitus Hypertension Previous MI	1	-	24	18	Type 1 MI	Medical	No	No
64	F	Chest pain	120	No	Hypertension Hyperlipidaemia Angina Previous PCI	1	-	7	16	Type 1 MI	Medical	No	No
73	M	Chest pain	130	No	Hypertension Hyperlipidaemia Previous MI Previous PCI	3	-	28	20	Type 1 MI	Medical	No	No
64	M	Chest pain	99	No	Hypertension Hyperlipidaemia Previous MI Previous PCI	3	-	11	15	Type 1 MI	Medical	No	No
61	M	Chest pain	111	No	Diabetes Mellitus Hyperlipidaemia	1	-	29	53	Type 1 MI	PCI	No	No
57	F	Chest pain	150	No	Diabetes Mellitus Hypertension Hyperlipidaemia Angina	3	-	6	18	Type 1 MI	Medical	No	No
56	F	Chest pain	70	No	Hyperlipidaemia Previous MI	1	-	6	437	Type 1 MI	PCI	No	No
75	F	Chest pain	4540	No	Diabetes Mellitus Hypertension Hyperlipidaemia	1	-	20	25	Type 1 MI	PCI	No	No
48	M	Chest pain	472	No	Smoker Hypertension Hyperlipidaemia	1	-	21	16	Type 1 MI	Medical	No	No

Abbreviations: (-) data not available. MI – myocardial infarction, PCI – percutaneous coronary intervention

Appendix 1.6. continued. Missed outcomes in patients with high-sensitivity cardiac troponin I concentration <5 ng/L by assay

Age	Sex	Symptom	Symptom onset to sample (minutes)	ECG Ischaemia	Risk Factors	First hsTnI (ng/L)	Peak hsTnI (ng/L)	Reference first (ng/L)	Reference peak (ng/L)	Diagnosis	Management	Cardiac death at 30 days	Cardiac death at 1 year
TRUST continued [15]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
72	M	Chest pain	90	No	Hypertension Hyperlipidaemia Angina Previous PCI	4	-	19	15	Type 1 MI	Medical	No	No
TIAMO [16]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
52	M	Chest pain	-	Yes	-	4	-	3	-	Type 1 MI	PCI	-	-
BACC [17]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
56	F	Chest pain	-	Yes	Hypertension Hyperlipidaemia Previous MI	4	7,782	3	1,797	Type 1 MI	PCI to LAD	No	-
59	M	Chest pain	-	Yes	Smoker	4	110	7	50	Type 1 MI	Angiogram No PCI	No	No
61	M	Chest pain	-	No	Diabetes Mellitus Hypertension Hyperlipidaemia Previous MI	3	9	25	25	Type 1 MI	PCI to RCA CABG	No	No
52	M	Chest pain	-	No		4	41	8	32	Type 1 MI	PCI to RCA	No	-
47	M	Chest pain	-	Yes	Smoker	3	3,932	10	949	Type 1 MI	PCI to LAD	No	No
75	M	Chest pain	-	Yes	Smoker Hypertension Hyperlipidaemia Previous MI	2	93	9	50	Type 1 MI	PCI to LAD	No	No
MANCHESTER [19]								**Reference assay (URL): Roche hs-TnT >14 ng/L**					
80	M	Chest pain	517	Yes	Hypertension	2	-	-	136	NSTEMI	Angiogram Medical	-	-

Abbreviations: (-) data not available. MI – myocardial infarction, PCI – percutaneous coronary intervention, CABG – coronary artery bypass graft, LAD – left anterior descending artery, RCA – right coronary artery

APPENDIX 2

COMPARISON OF THE EFFICACY AND SAFETY OF EARLY RULE OUT PATHWAYS FOR ACUTE MYOCARDIAL INFARCTION

Appendix 2.1 Baseline characteristics for the APACE external validation cohort

	APACE Study* (n=2,533)
Age	61 (16.0)
Male (%)	1,722 (68.0)
Primary Symptom	
Chest Pain	2,214 (87.4)
Symptom onset	
Minutes since onset	300 (120-720)
Less than three hours (%)	717 (28.5)
Less than six hours (%)	1,338 (53.2)
Over six hours (%)	1,054 (41.9)
Cardiovascular Risk Factors	
Smoker (%)	635 (25.1)
Diabetes mellitus (%)	451 (17.8)
Hypertension (%)	1,591 (62.8)
Hyperlipidaemia (%)	1,293 (51.0)
Family history (%)	987 (40.9)
Known angina (%)	883 (34.9)
Previous MI (%)	621 (24.5)
Previous PCI (%)	646 (25.5)
Previous CABG (%)	228 (9.0)
Stroke (%)	149 (5.9)
Peripheral vascular disease (%)	146 (5.8)
Troponin concentration at presentation	
<5 ng/L (%)	1,348 (53.2)
≥5 ng/L and ≤ 99th centile (%)	735 (29.0)
>99th centile (%)	450 (17.8)
Adjudicated Diagnosis	

Type 1 myocardial infarction (%)	289 (11.4)
All myocardial infarction (%)	378 (14.9)

**The APACE study is a prospective cohort study of patients with suspected acute coronary syndrome presenting to the Emergency Department of Basel and six other centers in Europe between April 2006 and August 2015. Blood samples were obtained on presentation and at 3, and 6h for high-sensitivity cardiac troponin I testing. All diagnoses were adjudicated by two independent cardiologists; the diagnosis of type 1 myocardial infarction required at least one high-sensitivity cardiac troponin I concentration above the sex-specific 99th centile upper reference limit (16 ng/L women, 34 ng/L men). This study was approved by the local ethics committee.*

Appendix 2.2. Patients ruled out by the ESC pathway at 0 and 3 hours meeting the primary outcome

Age	Gender	Time since symptom onset (Minutes)	Troponin concentration, ng/L (hours)	Relative Change (%)	Absolute Change	Presenting Symptom	Index Diagnosis	Risk Factors	Initial ECG	Management
81	Male	444	31 (0) 33 (3) 39 (12)	6.5	2	Chest Pain	Type 1 MI	Hypertension Hyperlipidaemia Ischaemic Heart Disease Previous CABG	Sinus Rhythm ST Depression T wave Inversion	PCI to LCx
57	Male	440	33 (0) 80 (3) 144 (5)	142.4	47	Chest Pain	Type 1 MI	Previous Smoker Family History of CHD	Sinus Rhythm	PCI to OM1
70	Male	375	17 (0) 160 (3) 2583 (6)	841.2	143	Chest Pain	Type 1 MI	Previous Smoker Diabetes Hypertension Hyperlipidaemia Previous MI	Sinus Rhythm RBBB	Medical
84	Male	4900	25 (0) 44 (3)	76.0	19	Chest Pain	Type 1 MI	Hypertension Previous MI Previous PCI Previous CABG Previous Stroke	Atrial Fibrillation	PCI to SVG-D1
82	Female	86	11 (0) 15 (3) 26 (10)	36.4	4	Chest Pain	Type 1 MI	Hypertension	Sinus Rhythm	Medical
62	Male	70	27 (0) 32 (3) 50 (11)	18.5	5	Chest Pain	Type 1 MI	Current Smoker Diabetes Hypertension Hyperlipidaemia Previous MI Previous PCI	Sinus Rhythm	Medical
87	Male	139	5 (0) 16 (3) 691 (10)	220.0	11	Chest Pain	Type 1 MI	Previous Smoker Hypertension Ischaemic Heart Disease Previous CABG	Atrial Fibrillation ST Depression	Medical

73	Male	180	26 (0) 29 (3) 41 (9)	11.5	3	Chest Pain	Type 1 MI	Previous Smoker Hypertension Hyperlipidaemia Family history of CHD Previous MI Previous CABG	Sinus Rhythm LBBB	Medical
58	Male	122	26 (0) 33 (3) 46 (11)	26.9	7	Dyspnoea	Type 1 MI	Previous Smoker Diabetes Family history of CHD Ischaemic Heart Disease Previous MI Previous CABG Previous Stroke	Sinus Rhythm T wave inversion	Medical
63	Female	151	10 (0) 16 (3) 167 (10)	60.0	6	Chest Pain	Type 1 MI	Current Smoker Family history of CHD	Sinus Rhythm ST Depression	PCI to LAD
66	Male	89	12 (0) 31 (3) 202 (10)	158.3	19	Chest Pain	Type 1 MI	Hypertension Previous MI Previous PCI	Sinus Rhythm	Medical
60	Male	81	2 (0) 6 (3) 2932 (11)	200.0	4	Chest Pain	Type 1 MI	Current Smoker Family History of CHD Ischaemic Heart Disease Previous PCI	Sinus Rhythm T wave inversion (old)	PCI to RCA/D1
56	Male	262	8 (0) 14 (3) 307 (10)	75.0	6	Chest Pain	Type 1 MI	Previous Smoker Hypertension Hyperlipidaemia Family history of CHD Ischaemic Heart Disease Previous MI Previous PCI	Sinus Rhythm	Medical
77	Male	272	21 (0) 26 (3) 56 (10)	23.8	5	Chest Pain	Type 1 MI	Previous Smoker Diabetes Hypertension Hyperlipidaemia Family history of CHD Previous MI Previous PCI	Atrial Fibrillation Inferior Q waves	Medical

66	Male	305	22 (0) 36 (3) 50 (8)	63.6	14	Chest Pain	Type 1 MI	Ischaemic Heart Disease Hypertension Hyperlipidaemia Previous MI Previous PCI	Sinus Rhythm Bradycardia	PCI to LCx Instent Restenosis
60	Male	295	14 (0) 14 (3) 170 (8)	0	0	Chest Pain	Type 1 MI	Current Smoker Hypertension Hyperlipidaemia Family history of CHD Ischaemic heart disease Previous MI	Sinus Rhythm Bradycardia	Angiography 70% stenosis OM1 Medical
88	Female	222	15 (0) 19 (3)	26.7	4	Chest Pain	Type 1 MI	Ischaemic heart disease Previous MI Previous PCI Family history of CHD	Sinus Rhythm	Medical
89	Female	165	16 (0) 18 (3) 24 (10)	12.5	2	Chest Pain	Type 1 MI	Hypertension Family history of CHD Ischaemic Heart Disease	Sinus Rhythm	Medical
82	Male	126	19 (0) 20 (3) 22 (11) Re-attendance 52 (0) 44 (3) 24 (10)	5.3	1	Chest Pain	Musculoskeletal Chest Pain	Previous Smoker Diabetes Hyperlipidaemia Family history of CHD Ischaemic Heart Disease Previous MI Previous CABG	Sinus Rhythm First Degree HB Left Axis Deviation RBBB	Re-presented with ongoing chest pain two days post index presentation Missed Type 1 MI
73	Female	425	9 (0) 11 (3)	22.2	2	Chest Pain	Paroxysmal AF	Previous Smoker Hypertension Hyperlipidaemia Ischaemic Heart Disease Previous MI Previous PCI	Atrial Fibrillation RBBB T wave inversion	Re-presented with inferior STEMI 14 days post index presentation PCI to RCA Type 1 MI

Demarcations for missed index events with ≥6 hours symptoms (n=4), <6 hours symptoms (n=14) and 30 day events (n=2)

AF = atrial fibrillation, CABG = coronary artery bypass graft, CHD = coronary heart disease, LBBB = left bundle branch block, RBBB = right bundle branch block, MI = myocardial infarction, PCI = percutaneous coronary intervention, STEMI = ST-segment elevation myocardial infarction.

Appendix 2.3. Patients ruled out by the High-STEACS pathway at 0 and 3 hours meeting the primary outcome

Age	Gender	Time since symptom onset (Minutes)	Troponin concentration, ng/L (hours)	Relative Change (%)	Absolute Change	Presenting Symptom	Index Diagnosis	Risk Factors	Initial ECG	Management
81	Male	444	31 (0) 33 (3) 39 (12)	6.45	2	Chest Pain	Type 1 MI	Hypertension Hyperlipidaemia Ischaemic Heart Disease Previous CABG	Sinus Rhythm ST Depression T wave Inversion	PCI to LCx
60	Male	295	14 (0) 14 (3) 170 (8)	0	0	Chest Pain	Type 1 MI	Current Smoker Hypertension Hyperlipidaemia Family history of CHD Ischaemic heart disease Previous MI	Sinus Rhythm Bradycardia	Angiography 70% stenosis OM1 Medical
82	Male	126	19 (0) 20 (3) 22 (11) Re-attendance 52 (0) 44 (3) 24 (10)	5.3	1	Chest Pain	Musculoskeletal Chest Pain	Previous Smoker Diabetes Hyperlipidaemia Family history of CHD Ischaemic Heart Disease Previous MI Previous CABG	Sinus Rhythm First Degree HB Left Axis Deviation RBBB	Re-presented with ongoing chest pain two days post index presentation Missed Type 1 MI
73	Female	425	9 (0) 11 (3)	22.2	2	Chest Pain	Paroxysmal AF	Previous Smoker Hypertension Hyperlipidaemia Ischaemic Heart Disease Previous MI Previous PCI	Atrial Fibrillation RBBB T wave inversion	Re-presented with inferior STEMI 14 days post index presentation PCI to RCA Type 1 MI

Demarcation for missed index events (n=2) and 30 day events (n=2)

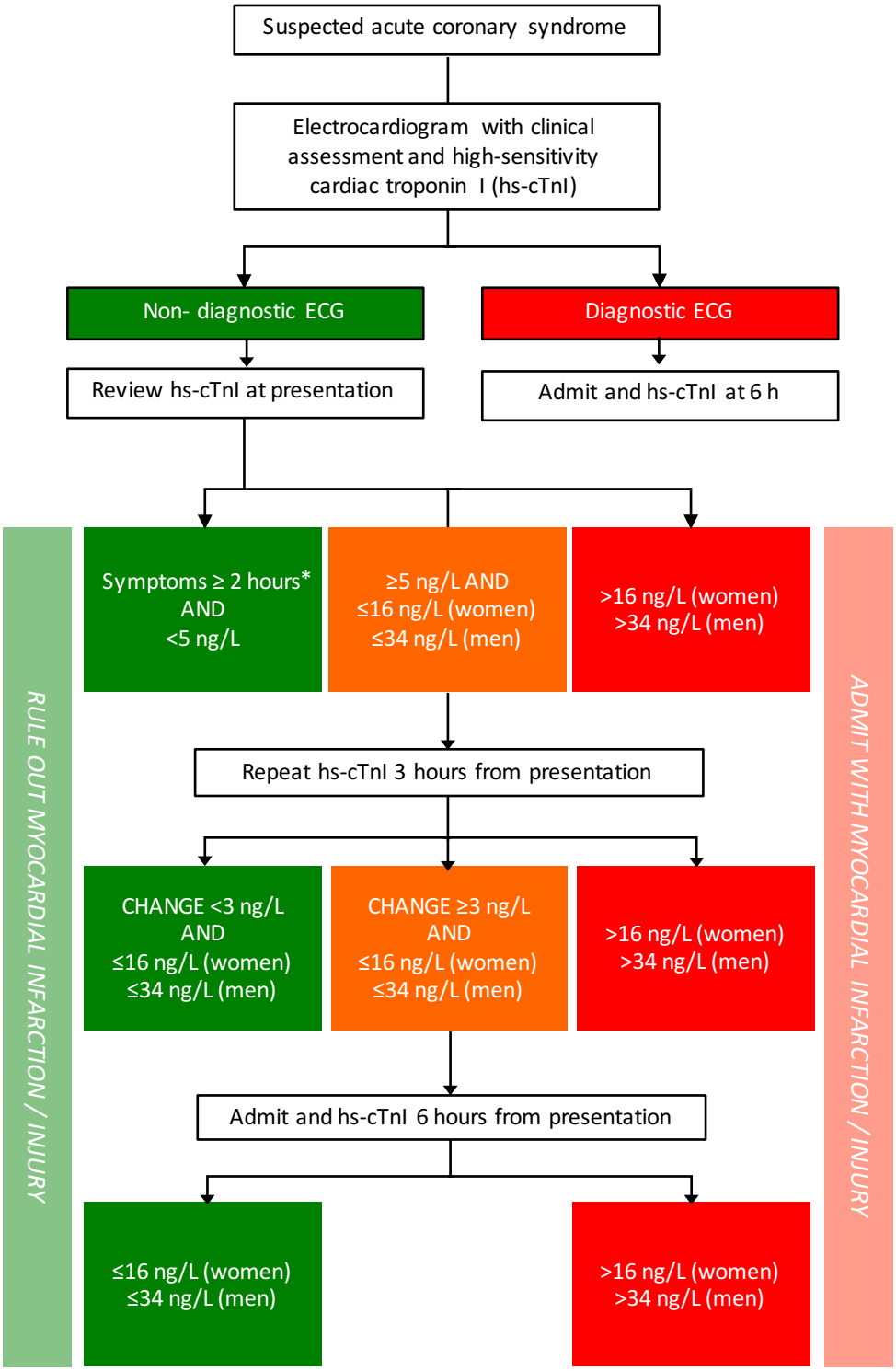
AF = atrial fibrillation, CABG = coronary artery bypass graft, CHD = coronary heart disease, LBBB = left bundle branch block, RBBB = right bundle branch block, MI = myocardial infarction, PCI = percutaneous coronary intervention. STEMI = ST-segment elevation myocardial infarctio

Appendix 2.4 2x2 table with diagnostic performance of the High-STEACS pathway at 6 hours

	Type 1 MI	No Type 1 MI
Pathway rules in	187	88
Pathway rules out	4	939

Patients with cardiac troponin concentrations <5 ng/L who present over two hours from time of symptom onset are ruled out on presentation. Those ≥5 ng/L and <99th centile on presentation, and those who present within two hours of symptom onset are re-tested at three hours. Those with a change in cardiac troponin of <3 ng/L are ruled out if they remain <99th centile, with all other patients admitted for peak testing at six hours.

Appendix 2.5 Diagnostic algorithm of the High-STEACS pathway



*Diagnostic algorithm of the High-STEACS pathway, currently being evaluated as part of a multi-centre stepped-wedge cluster randomised trial in unselected consecutive patients across Scotland. *In the High-STEACS pathway, patients with cardiac troponin concentrations <5 ng/L who present within two hours of symptom onset are retested at three hours.*

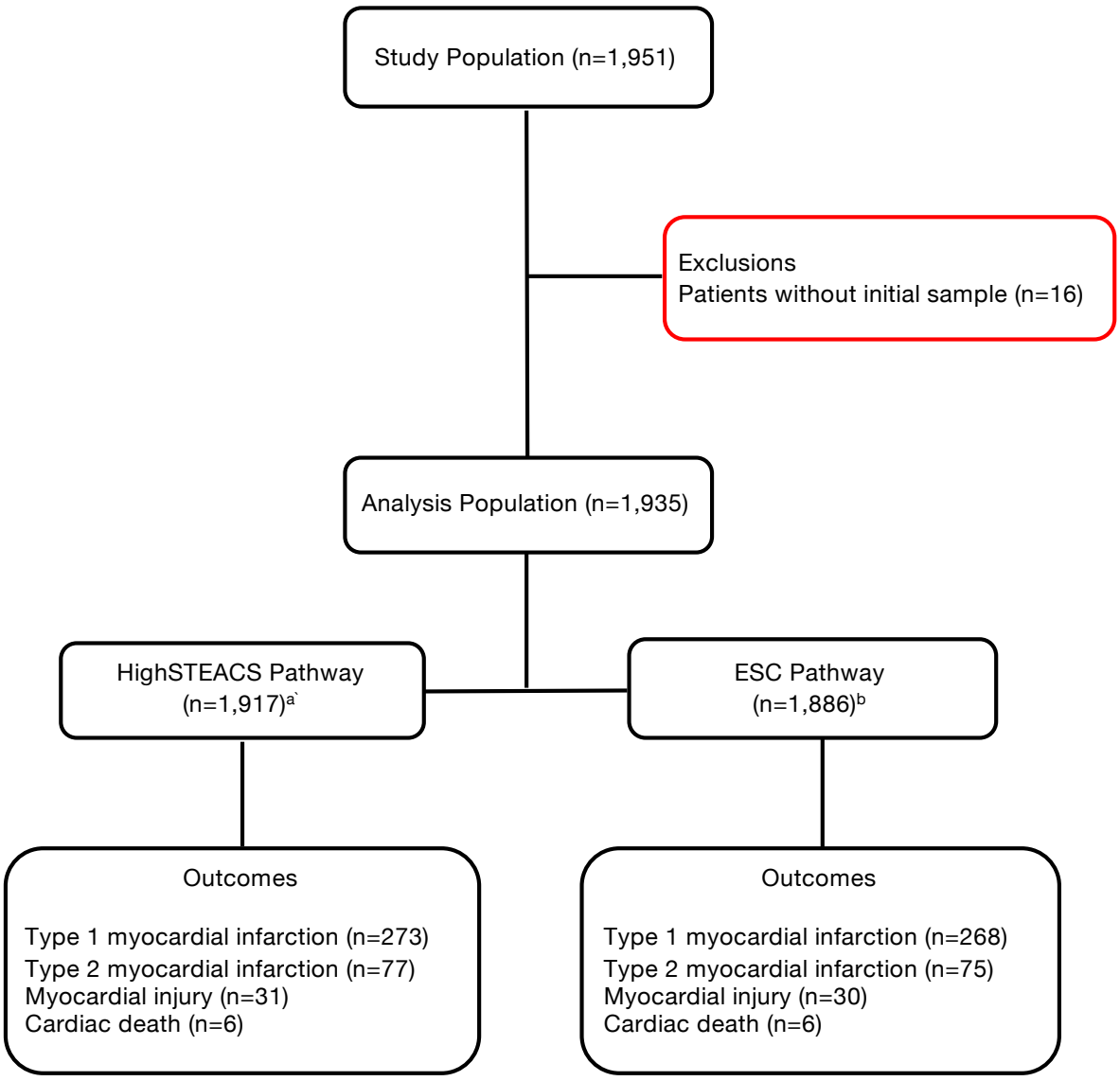
Appendix 2.6. Available blood samples and median time of sampling in the High-STEACS substudy.

	Presentation	**3 hours from presentation**	**6 – 12 hours from presentation**
Time from arrival to sample (minutes) *Median (IQR)*	29 (15 – 49)	180 (160-211)	495 (295-616)
Proportion of patients with samples available *% (n)*	100 (1,218)	95.6 (1,164)	58.2 (709)
Sample rules	All samples included	All samples included	All samples included

APPENDIX 3

HIGH-SENSITIVITY CARDIAC TROPONIN AND CLINICAL RISK SCORES IN PATIENTS WITH SUSPECTED ACUTE CORONARY SYNDROME

Appendix 3.1. Flow diagram illustrating identification of the study population



[a] 18 patients excluded as missing required serial sample for HighSTEACS pathway
[b] 49 patients excluded as missing required serial sample for ESC pathway

Appendix 3.2. Summary of missed index or 30-day events using the ESC 3 hour pathway**.**

Age	Gender	Time since symptom onset (Minutes)	Troponin concentration, ng/L Presentation	Troponin concentration ng/L 3 hours	Troponin concentration ng/L Peak	Presenting Symptom	Diagnosis	TIMI Score	GRACE Score	EDACS Score	HEART Score
82	F	86	11	15	26	Chest pain	Index Type 1 MI	2	169	17	5
62	M	70	27	32	43	Chest pain	Index Type 1 MI	3	107	24	5
73	F	150	35	34	37	Chest pain	Index Type 1 MI	2	129	14	6
64	M	199	42	45	48	Chest pain	Index Type 1 MI	4	129	16	6
89	M	317	68	74	934	Chest pain	Index Type 1 MI	4	214	27	7
85	M	150	16	12	-	Chest pain	Cardiac death (30d)	3	107	23	6
80	F	191	17	17	15	Chest pain	Index Type 1 MI	3	129	19	7
79	M	107	37	35	28	Chest pain	Index Type 1 MI	3	173	22	7
73	M	180	26	29	41	Chest pain	Index Type 1 MI	4	107	24	5
82	M	126	19	20	22	Chest pain	Type 1 MI (30d)	4	169	24	6
66	M	89	12	31	202	Chest pain	Index Type 1 MI	3	107	18	5
56	M	202	8	14	307	Chest pain	Index Type 1 MI	3	65	10	5
65	F	57	47	44	-	Chest pain	Index Type 1 MI	2	79	10	6
88	F	82	18	15	12	Chest pain	Index Type 1 MI	4	141	24	6
66	M	305	22	36	50	Palpitations	Index Type 1 MI	3	79	22	5
60	M	295	14	14	170	Chest pain	Index Type 1 MI	3	65	14	4
88	F	222	15	19	-	Chest pain	Index Type 1 MI	3	129	20	5
89	F	165	16	18	24	Chest pain	Index Type 1 MI	1	129	20	6
80	F	144	21	21	-	Jaw pain	Index Type 1 MI	2	129	16	6

58	M	112	20	33	35	Chest pain	Index Type 1 MI	0	95	14	4
54	M	96	22	32	36	Chest pain	Index Type 1 MI	1	85	16	3
58	F	135	20	21	19	Chest pain	Index Type 1 MI	2	107	16	6
72	M	199	56	58	-	Chest pain	Index Type 1 MI	2	129	25	5
57	M	458	33	80	144	Chest pain	Index Type 1 MI	0	65	14	4
70	M	375	17	160	2583	Chest pain	Index Type 1 MI	4	84	18	6
85	F	1616	7	12	19	Chest pain	Index Type 1 MI	1	129	18	5
61	M	790	15	82	635	Chest pain	Index Type 1 MI	0	65	16	4

Appendix 3.3. Summary of missed index or 30-day events using the High-STEACS pathway**.**

Age	Gender	Time since symptom onset (Minutes)	Troponin concentration, ng/L Presentation	Troponin concentration ng/L 3 hours	Troponin concentration ng/L Peak	Presenting Symptom	Diagnosis	TIMI Score	GRACE Score	EDACS Score	HEART Score
82	M	126	19	20	22	Chest pain	Type 1 MI (30d)	4	169	24	6
60	M	295	14	14	170	Chest pain	Index Type 1 MI	3	65	14	4
64	F	134	1	54	19066	Chest pain	Index Type 1 MI	0	79	15	4

Appendix 3.4. Diagnostic metrics for the European Society of Cardiology 0h / 3h pathway with and without clinical risk scores for a composite outcome of type 1 or type 2 myocardial infarction or cardiac death at 30 days

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95%CI)	Sensitivity (95%CI)	Positive predictive value (95%CI)	Specificity (95% CI)	Proportion low risk (%)
ESC Pathway	327	231	1279	49	96.3 (95.2-97.2)	86.9 (83.4-90.2)	58.6 (54.5-62.6)	84.7 (82.9-86.5)	70.4
ESC Pathway + TIMI (0/1)	368	674	836	8	99.0 (98.2-99.6)	97.7 (96.0-99.0)	35.3 (32.5-38.3)	55.4 (52.8-57.9)	44.8
ESC Pathway + GRACE ≤108	362	600	910	14	98.4 (97.5-99.1)	96.2 (94.0-97.9)	37.6 (34.6-40.7)	60.3 (57.8-62.7)	49.0
ESC Pathway + EDACS <16	363	723	787	13	98.3 (97.3-99.1)	96.4 (94.3-98.0)	33.4 (30.7-36.3)	52.1 (49.6-54.6)	42.4
ESC Pathway + HEART ≤3	374	1044	466	2	99.5 (98.6-99.9)	99.3 (98.3-99.9)	26.4 (24.1-28.7)	30.9 (28.6-33.2)	24.8

ESC – European Society of Cardiology, TIMI – Thrombolysis In Myocardial Infarction, GRACE – Global Registry of Acute Coronary Events, EDACS – Emergency Department Assessment of Chest pain Score, CI – confidence interval

Appendix 3.5. Diagnostic metrics for the HighSTEACS pathway with and without clinical risk scores for a composite outcome of type 1 or type 2 myocardial infarction or myocardial injury or cardiac death at 30 days

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95%CI)	Sensitivity (95%CI)	Positive predictive value (95%CI)	Specificity (95% CI)	Proportion low risk (%)
High-STEACS Pathway	378	295	1238	6	99.5 (99.0-99.8)	98.3 (97.0-99.5)	56.2 (52.4-59.9)	80.7 (78.8-82.7)	64.9
High-STEACS + TIMI (0/1)	382	701	832	2	99.7 (99.2-100)	99.4 (98.3-99.9)	35.3 (32.5-38.2)	54.3 (51.8-56.8)	43.5
High-STEACS + GRACE ≤108	381	627	906	3	99.6 (99.1-99.9)	99.1 (97.9-99.8)	37.8 (34.8-40.8)	59.1 (56.6-61.5)	47.4
High-STEACS + EDACS <16	382	743	790	2	99.7 (99.2-99.9)	99.4 (98.3-99.9)	34.0 (31.3-36.8)	51.5 (49.0-54.0)	41.3
High-STEACS + HEART ≤3	384	1068	465	0	99.9 (99.6-100)	99.9 (99.5-100)	26.5 (24.2-28.8)	30.3 (28.1-32.7)	24.3

High-STEACS = High-Sensitivity Troponin in the Evaluation of patients with Acute Coronary Syndrome, TIMI – Thrombolysis In Myocardial Infarction, GRACE – Global Registry of Acute Coronary Events, EDACS – Emergency Department Assessment of Chest pain Score, CI – confidence interval

Appendix 3.6. Diagnostic metrics for High-STEACS pathway excluding the first 1,218 participants in whom the pathway was derived.

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95%CI)	Sensitivity (95%CI)	Positive predictive value (95%CI)	Specificity (95% CI)	Proportion low risk (%)
High-STEACS Pathway	80	97	463	1	99.7 (99.3-100)	98.2 (95.3-100)	45.2 (38.0-52.6)	82.6 (79.5-85.7)	72.4

Appendix 3.7. Diagnostic metrics for High-STEACS and the ESC pathway excluding all patients who underwent cardiac testing.

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95%CI)	Sensitivity (95%CI)	Positive predictive value (95%CI)	Specificity (95% CI)	Proportion low risk (%)
High-STEACS Pathway	74	326	1176	1	99.9 (99.6-100)	98.0 (94.9-100)	18.6 (14.9-22.5)	78.3 (76.2-80.3)	74.6
ESC Pathway	64	252	1225	11	99.1 (98.5-99.5)	84.9 (76.8-92.5)	20.3 (16.1-24.9)	82.9 (81.0-84.8)	79.6

Of 1,594 patients who did not undergo cardiac testing, serial samples required for the High-STEACS and the ESC Pathways were missing in 17 and 42 patients respectively.

Appendix 3.8. Diagnostic metrics for TIMI, GRACE, EDACS and HEART scores alone

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95%CI)	Sensitivity (95%CI)	Positive predictive value (95%CI)	Specificity (95% CI)	Proportion low risk (%)
TIMI 0/1	213	674	967	63	93.8 (92.3-95.2)	77.1 (72.0-81.8)	24.0 (21.3-26.9)	58.9 (56.5-61.3)	53.7
GRACE ≤108	201	566	1075	75	93.4 (91.9-94.8)	72.7 (67.4-77.8)	26.2 (23.2-29.4)	65.5 (63.2-67.8)	60.0
EDACS <16 *	273	834	767	3	99.5 (99.0-99.9)	98.7 (97.1-99.7)	24.7 (22.2-27.3)	47.9 (45.5-50.4)	41.0
HEART ≤3	268	1136	505	8	98.3 (97.1-99.3)	96.9 (94.6-98.6)	19.1 (17.1-21.2)	30.8 (28.6-33.0)	26.8

** When EDACS is applied in isolation, the following low risk criteria are recommended: 1) EDACS Score <16, 2) No myocardial ischaemia on the ECG and 3) troponin concentrations are ≤99th centile at 0 and 2 hours. Than M et al. Emerg Med Australas. 2014;26:34-44.*

TIMI – Thrombolysis In Myocardial Infarction, GRACE – Global Registry of Acute Coronary Events, CI – confidence interval

Appendix 3.9. Diagnostic metrics for the ESC 0/1 hour pathway with and without clinical risk scores.

	True Positive	False Positive	True Negative	False Negative	Negative predictive value (95% CI)	Sensitivity (95% CI)	Positive predictive value (95% CI)	Specificity (95% CI)	Proportion low risk (%)
ESC 1-hour pathway	33	83	290	0	99.8 (99.3-100)	98.5 (94.4-100)	28.6 (20.8-37.1)	77.7 (73.4-81.8)	71.4
ESC 1-hour + TIMI (0/1)	33	149	224	0	99.8 (99.1-100)	98.5 (94.4-100)	18.3 (13.1-24.2)	60.0 (55.0-64.9)	55.2
ESC 1-hour + GRACE ≤108	33	134	239	0	99.8 (99.2-100)	98.5 (94.4-100)	19.9 (14.3-26.3)	64.0 (59.1-68.8)	58.9
ESC 1-hour + EDACS <16	33	151	222	0	99.8 (97.2-100)	98.5 (94.4-100)	18.1 (12.9-24.0)	59.5 (54.5-64.4)	54.7
ESC 1-hour + HEART ≤3	33	239	134	0	99.6 (98.6-100)	98.5 (94.4-100)	12.3 (8.7-16.4)	36.0 (31.2-40.9)	33.0

TIMI – Thrombolysis In Myocardial Infarction, GRACE – Global Registry of Acute Coronary Events, EDACS – Emergency Department Assessment of Chest pain Score, CI – confidence interval

Appendix 3.10. Available blood samples and median time of sampling in the High-STEACS substudy.

	Presentation	**3 hours from presentation**	**6 – 12 hours from presentation**	**1 hour from first sample**
Time from arrival to sample (minutes) *Median (IQR)*	28 (15 – 46)	176 (146-206)	416 (216-605)	65 (60 – 73)
Proportion of patients with samples available *% (n)*	100 (1,935)	94.9 (1,837)	51.2 (990)	21 (406)
Sample rules	All samples included	All samples included	All samples included	Included if ≥ 30 and ≤90 minutes from time of first sample

Appendix 3.11. Additional information on diagnostic adjudication

Criteria for adjudication of patients with myocardial necrosis

Type 1 myocardial infarction	Myocardial necrosis (any cardiac troponin I [cTnI] concentration above the upper reference limit) with rise and or fall in cTnI concentration where serial testing was available AND symptoms OR signs of myocardial ischaemia
Type 2 myocardial infarction	Myocardial necrosis (any cTnI concentration above the upper reference limit) with rise and or fall in cTnI concentration where serial testing was available AND symptoms OR signs of myocardial ischaemia AND evidence of increased oxygen demand (e.g. tachyarrhythmia, hypertrophy) or reduced supply (e.g. hypotension, hypoxia or anaemia) in context of alternative clinical diagnosis
Myocardial injury	Myocardial necrosis (any cTnI concentration above the upper reference limit) without symptoms OR signs of myocardial ischaemia in context of alternative clinical diagnosis

The process of adjudication was conducted by two cardiologists independently. Both had access to the electronic patient record. The adjudicated diagnosis was reached by evaluating the attending clinicians documentation of the presenting complaint, past medical history, cardiovascular risk factors and clinical examination findings including routine observations (pulse, blood pressure, pulse oximetry, temperature and conscious level). All investigation results undertaken by the attending clinician were available for review, including biochemistry and haematology results, the 12 lead electrocardiogram, echocardiogram, chest X-ray and invasive coronary angiography findings when performed. Both adjudicating cardiologists had access to the final discharge letter documenting the attending clinicians' final diagnosis.

APPENDIX 4

LONG TERM OUTCOMES IN PATIENTS WITH TYPE 2 MYOCARDIAL INFARCTION AND MYOCARDIAL INJURY

Appendix 4.1 Additional information on diagnostic adjudication

Criteria for adjudication of patients with myocardial necrosis

Type 1 myocardial infarction	Myocardial necrosis (any cardiac troponin I [cTnI] concentration above the upper reference limit) with rise and or fall in cTnI concentration where serial testing was available AND symptoms OR signs of myocardial ischaemia
Type 2 myocardial infarction	Myocardial necrosis (any cTnI concentration above the upper reference limit) with rise and or fall in cTnI concentration where serial testing was available AND symptoms OR signs of myocardial ischaemia AND evidence of increased oxygen demand (e.g. tachyarrhythmia, hypertrophy) or reduced supply (e.g. hypotension, hypoxia or anaemia) in context of alternative clinical diagnosis
Myocardial injury	Myocardial necrosis (any cTnI concentration above the upper reference limit) without symptoms OR signs of myocardial ischaemia in context of alternative clinical diagnosis

The process of adjudication was conducted by two cardiologists independently. Both had access to the electronic patient record. The adjudicated diagnosis was reached by evaluating the attending clinicians documentation of the presenting complaint, past medical history, cardiovascular risk factors and clinical examination findings including routine observations (pulse, blood pressure, pulse oximetry, temperature and conscious level). All investigation results undertaken by the attending clinician were available for review, including biochemistry and haematology results, the 12 lead electrocardiogram, echocardiogram, chest X-ray and invasive coronary angiography findings when performed. Both adjudicating cardiologists had access to the final discharge letter documenting the attending clinicians' final diagnosis. We did not apply specific criteria to define supply or demand imbalance,(Saaby et al., 2014) but adjudicated myocardial supply or demand imbalance on an individual patient basis, in line with most studies in this area(Sandoval and Thygesen, 2017)

Appendix 4.2 Additional information on classification of cardiovascular death

ICD Code	Definition
Ischaemic heart diseases	
I20	Angina pectoris
I21	Acute myocardial infarction
I22	Subsequent myocardial infarction
I23	Certain current complications from acute myocardial infarction
I24	Other acute ischaemic heart diseases
I25	Chronic ischaemic heart disease
Other forms of heart disease	
I34	Non-rheumatic mitral valve disorders
I35	Non-rheumatic aortic valve disorders
I36	Non-rheumatic tricuspid valve disorders
I37	Pulmonary valve disorders
I42	Cardiomyopathy
I43	Cardiomyopathy in diseases classified elsewhere
I46	Cardiac arrest
I48	Atrial fibrillation and flutter
I49	Other cardiac arrhythmias
I50	Heart failure
I51	Complications and ill-defined descriptions of heart disease
Cerebrovascular diseases	
I60	Subarachnoid haemorrhage
I61	Intracerebral haemorrhage
I62	Other nontraumatic intracerebral haemorrhage
I63	Cerebral infarction

I64	Stroke, not specified as haemorrhage or infarction
I65	Occlusion and stenosis of precerebral arteries, not resulting in infarction
I66	Occlusion and stenosis of cerebral arteries, not resulting in infarction
I67	Other cerebrovascular diseases
I68	Cerebrovascular disorders in diseases classified elsewhere
I69	Sequelae of cerebrovascular disease

Appendix 4.3 Analysis code

All analysis was performed using R (version 3.2.2) using the *survival* and *cmprsk* packages. For transparency, the analysis code is available open source via GitHub.

Available at https://github.com/a-r-chapman/type_2_outcomes

Appendix 4.4. Most common primary discharge diagnoses in patients with an adjudicated diagnosis of type 2 myocardial infarction or myocardial injury.

Type 2 Myocardial Infarction	*Myocardial Injury*
Arrhythmia (19.1%, 82/429)	Heart Failure (12.8%, 67/522)
Pneumonia (13.5%, 58/429)	Arrhythmia (10.9%, 57/522)
Heart Failure (12.4%, 53/429)	Pneumonia (9.6%, 50/522)
Fracture (4.2%, 18/429)	Fracture (8.0%, 42/522)

Appendix 4.5. Cause-specific hazard ratios for major adverse cardiovascular events in all patients.

	Major Adverse Cardiovascular Events (MACE)	
	Unadjusted HR (95% CI)	***Adjusted HR (95% CI)***
Age (per 10-year increase)	1.60 (1.50-1.70)	-
Sex (male)	0.85 (0.73-0.98)	1.09 (0.93-1.28)
Haemoglobin (per 10 g/L reduction)	1.18 (1.14-1.21)	1.07 (1.03-1.11)
eGFR (per 10 ml/min reduction)	1.20 (1.17-1.24)	-
Smoking	0.66 (0.55-0.79)	1.26 (1.02-1.56)
Diabetes Mellitus	1.77 (1.49-2.10)	1.36 (1.14-1.64)
Hypertension	1.66 (1.42-1.93)	1.05 (0.89-1.24)
Coronary Artery Disease	2.52 (2.16-2.94)	1.80 (1.52-2.14)
Stroke	1.88 (1.53-2.31)	1.10 (0.89-1.38)
Peripheral Vascular Disease	2.07 (1.65-2.59)	1.45 (1.14-1.86)
Validation phase	1.21 (1.04-1.40)	1.16 (0.99-1.35)
Type 1 Myocardial Infarction	1.00	1.00
Type 2 Myocardial Infarction / Myocardial Injury	1.16 (1.00-1.34)	0.82 (0.69-0.96)

Penalised smoothing splines used for age and eGFR (estimated glomerular filtration rate) in multivariate model. Type 1 Myocardial Infarction as referent group.

Appendix 4.6. Adjusted relative risks of primary and secondary outcomes for patients with myocardial injury versus type 2 myocardial infarction

	Myocardial Injury versus Type 2 MI **Adjusted RR (95% CI)**
Death from any cause	1.27 (1.08-1.48)
MACE	0.99 (0.87-1.13)
Non-fatal MI	0.80 (0.61-1.03)
Cardiovascular death	1.07 (0.94-1.22)
Fatal MI	1.18 (0.87-1.58)
Heart failure hospitalization	1.23 (1.03-1.46)
Non-cardiovascular death	1.12 (0.99-1.26)

Models adjusted for age, gender, renal function, haemoglobin and history of hypertension, stroke, peripheral vascular disease, diabetes mellitus, smoking, coronary artery disease and study phase.

APPENDIX 5

EVEREST-MI STUDY PROTOCOL

*Ev*aluating the role of coronary artery disease to *res*olve the diagnosis of *T*ype 2 *M*yocardial *I*nfarction

The EVEREST–MI Study

Co-sponsor	**The University of Edinburgh and Lothian Health Board** **ACCORD** **The Queen's Medical Research Institute** **47 Little France Crescent** **Edinburgh** **EH16 4TJ**
Protocol authors	**Dr Andrew Chapman, Dr Scott Semple, Prof Edwin van Beek, Dr Marc Dweck, Prof Nicholas Mills, Prof David Newby,**
Funder	**British Heart Foundation**
Funding Reference Number	**FS/16/75/32533**
Chief Investigator	**Dr Andrew Chapman, Clinical Research Fellow**
Sponsor number	**AC17042**
REC Number	**17/SS/0078**
Project registration	**NCT03338504**
Version Number and Date	**Version 2.2 11th December 2017**

Trial Start Date: 1st August 2017
Trial Finish Date: 1st August 2020
Trial Report Date: 1st November 2020

Amendment classification and number:	**Summary of change(s)**
Version 2	Updated trial start and finish dates Detailed demographic data to be recorded Updated consent information Included patient preference for coronary angiography modality Detailed the genetic analysis to be undertaken
Version 2.1	Inclusion of stress-perfusion MRI
Version 2.2	Updated exclusion criteria
Version 2.3	Addition of serum sample tube to biomarker sampling

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Contents:

1. Protocol Approval

Evaluating the role of coronary artery disease to resolve the diagnosis of Type 2 Myocardial Infarction

Signatures

Dr Andrew Chapman Chief Investigator	Signature	Date
Prof Nicholas Mills Co-Investigator	Signature	Date

2. Investigator Statement

Evaluating the role of coronary artery disease to resolve the diagnosis of Type 2 Myocardial Infarction

I agree to conduct the study according to this protocol, the principles of International Conference on Harmonisation Tripartite Guidelines for Good Clinical Practice (ICH GCP) and the applicable regulatory requirements. Any changes in procedure will only be made if necessary to protect the safety, rights or welfare of the patients.

I agree to take responsibility for the conduct of the study and ensure that all other staff involved are adequately informed about the protocol and amendments and their study related duties and functions.

Signatures

Signature of Investigator	Date

Name of Investigator (please print)

3. Summary

Myocardial injury is common in patients without acute coronary syndrome, and therefore international guidelines propose a classification of patients with myocardial infarction by aetiology. This differentiates between myocardial infarction due to plaque rupture (type 1) and myocardial oxygen supply-demand imbalance (type 2) in other acute illnesses. However, these guidelines have not been widely adopted as the diagnostic criteria for type 2 myocardial infarction are not clearly defined. Patients with type 2 myocardial infarction have poor long term outcomes, with at least twice the mortality at five years compared to those with an index type 1 myocardial infarction. Despite the majority of deaths being attributable to non-cardiovascular events, the rate of future type 1 myocardial infarction or cardiovascular death is similar regardless of index classification. If this future risk is related to the presence of underlying coronary artery disease, then there may be the potential to improve outcomes through targeted investigation and secondary prevention. We will undertake a systematic evaluation of the mechanism of myocardial injury and the role of coronary artery disease in 100 patients with elevated cardiac troponin concentrations where the diagnosis is likely to be type 2 myocardial infarction. These studies will help improve the assessment of patients with myocardial injury, refine the diagnostic criteria for type 2 myocardial infarction, and aid the design of future therapeutic trials.

4. Background

The definition of acute myocardial infarction has evolved to accommodate increasingly sensitive markers of myocardial necrosis and imaging methods that allow greater understanding of the pathogenic mechanisms of acute coronary syndrome. As such, the universal definition of myocardial infarction now proposes that we classify patients with myocardial infarction based on aetiology.[1] Whilst this classification has been used in clinical trials to refine clinical outcomes[2-4], it has not been widely adopted in clinical practice, and the frequency and implications of subtypes of acute myocardial infarction are uncertain. We believe the diagnostic criteria for type 2 myocardial infarction require clarification and that this is necessary to encourage clinicians to adopt the proposed classification. This can only be achieved through prospective and systematic evaluation of the clinical presentation, pathophysiological mechanisms and outcomes of unselected patients with acute myocardial injury in clinical practice.

Classification of myocardial infarction

The Universal Definition of Myocardial Infarction differentiates between type 1 myocardial infarction due to thrombosis of an atherosclerotic plaque, and type 2 myocardial infarction due to an imbalance in myocardial oxygen supply and demand in another acute illness.[1] The classification describes evidence of myocardial necrosis in the absence of myocardial ischemia as myocardial injury. Myocardial infarction presenting as sudden death (type 3), or after percutaneous coronary intervention (type 4) and coronary artery bypass grafting (type 5) are also defined. This classification is contentious and was based on expert consensus rather than evidence from prospective clinical trials. The most controversial diagnosis is that of type 2 myocardial infarction as these patients are heterogeneous, and have myocardial ischemia secondary to a wide range of primary acute medical or surgical conditions. It is here where the diagnosis of type 2 myocardial infarction has the greatest potential for benefit and for harm. For instance, a patient with type 2 myocardial infarction secondary to a tachyarrhythmia may be identified as having three vessel coronary artery disease and undergo surgical revascularisation. In contrast, the inappropriate use of anti-platelet agents and anti-coagulants in a patient with myocardial ischemia due to hypotension and occult gastrointestinal bleeding is likely to accelerate bleeding and may be fatal.

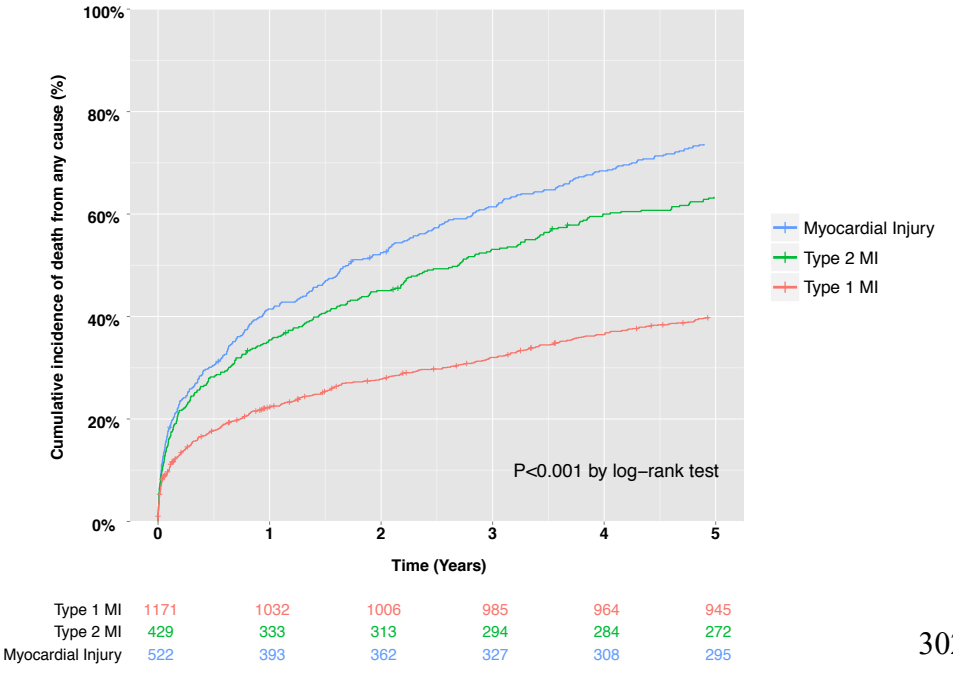
The global task force are reviewing the Universal Definition of Myocardial Infarction and recognise the need to provide clearer diagnostic criteria and guidance.[5] Based on the current guideline the differentiation between patients with type 2 myocardial infarction and myocardial injury is subjective and therefore inconsistent in clinical practice.[6,7] Likewise, in the absence of an accepted definition it is difficult to conduct randomised trials to determine the effectiveness of secondary prevention, such as aspirin or statins.

Incidence of type 2 myocardial infarction and myocardial injury in clinical practice

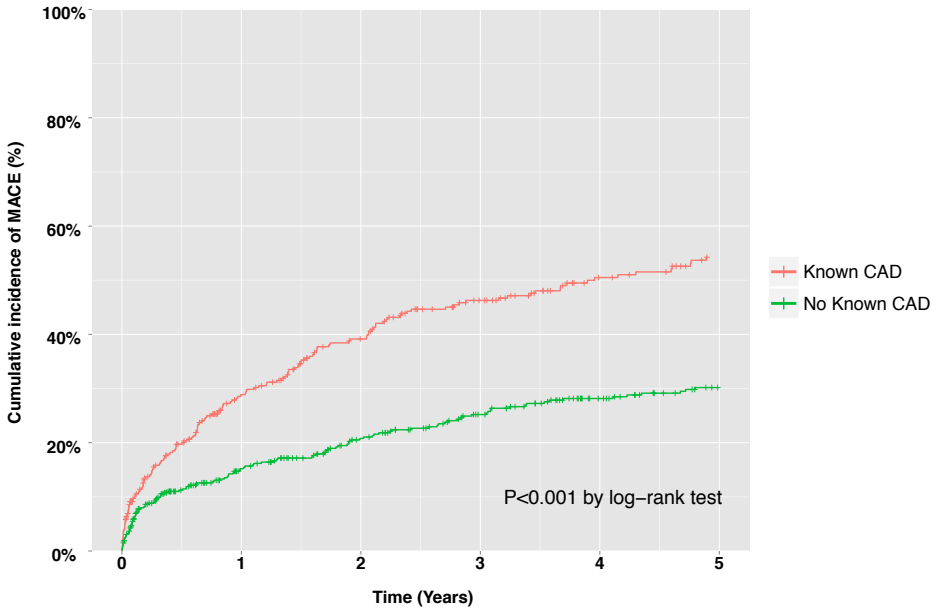
Following improvements in assay performance, we introduced a more sensitive troponin assay at our institution.[8,9] The validation and subsequent implementation of this assay provided an opportunity to assess the impact of lowering the diagnostic threshold on the incidence, management and clinical outcome of patients with type 2 myocardial infarction and myocardial injury.[10] We systematically evaluated all patients with elevated plasma troponin concentrations ***irrespective of their presenting complaint*** who were admitted to the Royal Infirmary of Edinburgh during the validation and implementation of a sensitive cardiac troponin I assay (n=2,122) and made a number of novel observations. First, type 2 myocardial infarction and myocardial injury are as common as type 1 myocardial infarction in clinical practice. The incidence of type 2 myocardial infarction or myocardial injury increases with age and is more common than type 1 myocardial infarction in patients ≥75 years of age. Lowering the diagnostic threshold preferentially increased the number of patients identified with type 2 myocardial infarction or myocardial injury. Indeed, for every additional patient reclassified as type 1 myocardial infarction, we identified three patients with type 2 myocardial infarction or myocardial injury (257 versus 672 patients, P<0.001).[10] This is important as the advent of newer highly sensitive cardiac troponin assays with lower thresholds may identify an even higher proportion of patients with previously undetectable myocardial injury.

Long term outcomes in patients with type 2 myocardial infarction and myocardial injury

Patients with type 2 myocardial infarction have poor outcomes,[11-15] worse than those with type 1 myocardial infarction, with 62.5% (268/429) versus 36.7% (430/1,171) dead at five years (adjusted hazard ratio [HR] 1.36, 95%CI 1.16-1.59). Survival in patients with myocardial injury is worse, even compared to those with type 2 myocardial infarction with 72.4% (378/522) dead at five years (HR 1.25, 95%CI 1.07-1.46 [***Figure 1 - below***].



Despite the excess in five-year mortality being largely attributable to non-cardiovascular death, the risk of future major adverse cardiovascular events (MACE; future type 1 myocardial infarction or cardiovascular death) is similar, regardless of index diagnosis. We found patients with type 2 myocardial infarction or myocardial injury and known coronary artery disease were at significantly increased risk of MACE, yet the majority of patients did not receive invasive coronary investigation nor optimal secondary prevention **[*Figure 2 - below*]**.



Indeed, given patients with type 2 myocardial infarction are older and have a higher prevalence of co-morbidities, it is likely that there is an unrecognised burden of previously clinically quiescent coronary artery disease. It is here where there may be the opportunity to improve patient outcomes through identification and targeted secondary prevention.

5. Study Objectives

We propose to systematically evaluate the mechanisms of acute myocardial injury in unselected patients who present to hospital with an alternative primary illness likely to cause myocardial oxygen supply or demand imbalance. All patients will be assessed by a member of the study team during their index admission and will undergo a detailed assessment of their coronary anatomy with either computed tomography coronary angiography (CTCA), CT calcium scoring and non-invasive fractional flow reserve assessment (CT-FFR) *or* invasive coronary angiography with optical coherence tomography (OCT) and invasive fractional flow reserve (FFR). The pattern of myocardial injury and its functional consequence will be evaluated by cardiac magnetic resonance (CMR) imaging. We will determine the kinetics of cardiac troponin release using serial testing at multiple time points throughout admission, and quantify other proteins and the expression of long non-coding RNA and associated mRNA to identify differences related to the presence of coronary artery disease, which may help to identify new biomarkers.

Understanding the mechanisms of acute myocardial injury in hospitalised patients and the contribution of coronary artery disease will lead to the development of a diagnostic algorithm and framework for clinicians to base their assessment, and will help to guide future therapeutic trials.

5.1 Original Hypothesis

The majority of patients with myocardial injury secondary to oxygen supply or demand imbalance will have evidence of underlying coronary artery disease.

6. Trial Design

Design: Prospective cohort study

Setting: Royal Infirmary of Edinburgh, a tertiary cardiac centre

Study population:
We will identify consecutive patients with acute myocardial injury (defined as a rise and or fall in cardiac troponin concentration on serial testing, with at least one value >99th centile) where the likely mechanism of injury is thought to be myocardial oxygen supply and demand imbalance (e.g secondary to hypoxia, hypotension, tachycardia or anaemia). Patients will be identified through screening of cardiac troponin measurements using the electronic patient record and laboratory databases at the recruiting site. The chief investigator is a clinical research fellow and honorary cardiology registrar in NHS Lothian, and has access to electronic patient records as part of routine clinical care. All patients screened will be recorded in a screening log. In patients that meet our inclusion criteria, but have one or more exclusion criteria and therefore are not eligible for enrolment, we will record demographic and clinical information from the electronic patient record with approval from the local Caldicott Guardian (including age, gender, previous medical history such as hypertension, diabetes, stroke, angina, myocardial infarction, previous angioplasty or bypass surgery, medication history, presenting complaint and ECG findings). Patients who meet both the inclusion and exclusion criteria, will be approached and those who provide consent will comprise the study population and be allocated a unique study number.

Number of participants:
We will recruit one hundred patients from the Royal Infirmary of Edinburgh, Scotland.

Inclusion criteria:

- Unscheduled hospital admission with acute myocardial injury (defined as a rise and or fall in high-sensitivity cardiac troponin I concentrations on blood testing)
- A suspected aetiology of myocardial oxygen supply and demand imbalance

Exclusion criteria:

- Unable or unwilling to give informed consent
- Women who are pregnant, breastfeeding or of child-bearing potential (women who have experienced menarche, are pre-menopausal and have not been sterilised) will not be enrolled into the trial.
- Probable type 1 myocardial infarction
- Renal impairment (estimated glomerular filtration rate ≤30ml/min/1.73m^2)
- Severe hepatic impairment
- Frailty with inability to self-transfer (determined using Katz Index)

Primary objective:

- To determine the prevalence and severity of coronary artery disease (defined as stenosis >50% in a major epicardial vessel) in patients with myocardial injury secondary to oxygen supply demand imbalance using invasive or CT coronary angiography

Secondary objectives:

- To determine the functional significance of coronary artery stenosis using the invasive or CT fractional flow reserve technique.
- To determine the prevalence of intraluminal plaque rupture using optical coherence tomography
- To evaluate the pattern of myocardial injury or infarction using the late gadolinium enhancement technique
- To determine the presence of myocardial ischaemia quantified using stress-perfusion magnetic resonance imaging.
- To validate a prediction model derived in patients with an adjudicated diagnosis of type 2 myocardial infarction identified in the High-STEACS clinical trial.
- To identify novel biomarkers that differentiate between type 1 and type 2 myocardial infarction
- To evaluate the relationship between coronary artery disease and cardiovascular outcomes

Consent

The chief investigator will screen all measurements of cardiac troponin I to identify patients with evidence of myocardial injury within the previous 24 hours, and after review of the electronic patient record will liaise other members of the clinical team to identify patients who may be suitable for recruitment. The clinical team will approach the patient to obtain verbal consent for the researcher to discuss the study. All patients will receive a patient information sheet at the time of screening and will be provided with an opportunity to ask questions. Eligible patients will be given a minimum of one hour to consider the written material and the investigator or another suitably qualified member of the research team will then re-attend to provide an opportunity for further questions. As patients may be recruited from the emergency department or the acute medical unit where there is potential for early discharge, a short time interval is necessary to permit recruitment prior to hospital discharge. Audit data shows it takes 124 minutes from arrival to troponin result at the Royal Infirmary of Edinburgh. Assuming a patient is identified as suitable within 30 minutes of sample result, this leaves only 90 minutes for screening and recruitment prior to possible discharge at four hours, in line with government standards. An independent study observer will be available to discuss any aspect of the study. Written informed consent will be obtained from all participants.

Withdrawal from study

Participation in this study is voluntary and subjects will be free to withdraw from study at any point should they wish to do so. Patients retain the right to ask for blood samples to be destroyed at any time.

7. Study Procedures

All participants will undergo cardiac magnetic resonance imaging of the myocardium where there are no contraindications, in addition to evaluation of coronary anatomy by either invasive or non-invasive coronary angiography dependent on their co-morbidities or patient preference (***Appendix 1*** – Flow Diagram). For example, frail patients or those with severe peripheral vascular disease in whom intra-vascular access may be challenging would be more likely to undergo non-invasive imaging. This clinical decision will be made in discussion with the consultant responsible for the patient's ongoing care by the investigators who are all cardiologists with experience of both invasive and non-invasive diagnostic imaging. Such investigations are often considered by the attending clinician as part of routine clinical care, but decisions are challenging given the lack of evidence to date. All patients will provide informed consent in line with routine clinical practice. We will report the results of all investigations to the patients attending clinician so therapy may be modified where this is felt to be of benefit. The target for completion of all diagnostic studies is 28 days from index presentation, with the aim to perform imaging as early as is feasible.

Cardiac Magnetic Resonance Imaging

Cardiovascular magnetic resonance (CMR) will be performed using a 3T scanner (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany) at the Clinical Research Imaging Centre (CRIC), Edinburgh, where there is established experience in research MRI. The MRI scan will consist of localisers, axial and coronal HASTE images, standard breath-held and ECG-gated cine sequences in 2 chamber, 4 chamber and short axis views. Short-axis cine images will be obtained using a balanced steady-state free precession sequence from the mitral valve annulus to the apex (8 mm parallel slices with 2 mm spacing) for the assessment of left ventricle function and volumes. Left ventricle volumes, mass and ejection fraction will be assessed using dedicated software (Argus Ventricular Function, Siemens AG Healthcare Sector, Erlangen, Germany) and values indexed to body surface area. Breath-held, ECG-gated T2 mapping sequences of the myocardium will be performed in the long-axis as a marker of myocardial inflammation. T1-weighted imaging of the coronary arteries will be performed to look for evidence of recent intraplaque thrombus or haemorrhage using the CATCH sequence. All patients will then receive 0.4mg (5ml) of peripheral Regadenoson (Rapiscan™) as a stress perfusion agent, and a bolus of 0.2 mmol/kg Gadolinium (Gadovist™), to quantify areas of myocardial ischaemia. Stress-perfusion imaging will not be undertaken in patients with severe asthma or chronic obstructive pulmonary disease, those on dipyridamole, theophylline or aminophylline, or those with atrioventricular block. Patients will be asked to withhold caffeine intake for 12 hours prior to imaging. This will be followed by standard late-gadolinium enhancement sequences. The late gadolinium enhancement and T2 mapping techniques will identify regions of new or old myocardial infarction

as well as other patterns of injury such as the mid-wall pattern associated with myocarditis.

Invasive coronary angiography and optical coherence tomography

Coronary angiography will be performed via the femoral or radial artery with 6F arterial catheters. In patients with one or more stenoses in a major epicardial vessel, a coronary pressure guidewire (PressureWire™ Aeris™, St. Jude Medical, St. Paul, Minnesota) will be used to determine distal coronary pressure and the fractional flow reserve (FFR) calculated at maximal adenosine-induced (intravenous 140 μg/kg/min) hyperaemia. As previously described, frequency domain optical coherence tomography (FD-OCT) will be performed in all three coronary vessels using a FastView® coronary imaging catheter (Terumo, Tokyo, Japan) with pullback at 20 mm/s to identify features consistent with vulnerable plaque or recent plaque rupture.[16] If there is evidence of inducible myocardial ischaemia due to coronary artery stenosis, revascularisation with percutaneous coronary intervention may be considered if in the patients best interests.

CT coronary angiography

CT coronary angiography will be performed in the Clinical Research Imaging Centre, according to previously published methodology.[17] Imaging will be performed using a 128 multidetector row CT (Siemens Biograph, Siemens Healthcare, Erlangen, Germany). Patients with a heart rate exceeding 65 beats/min will receive oral beta-blockade (50 or 100 mg metoprolol) 1 hour before computed tomography. Additional intravenous beta blockers will be given depending on heart rate at the time of imaging. All patients will receive sublingual glyceryl trinitrate (300 μg) immediately prior to dual cardiac and respiratory-gated computed tomography imaging of the coronary arteries. We will quantify total plaque burden using CT calcium scoring. A bolus of 80-100 mL of contrast (400 mg/mL; Iomeron, Bracco, Milan, Italy) will be injected intravenously at 5 mL/s. CT angiography will be evaluated jointly by a Radiologist and a Cardiologist with suitable training to determine the extent of coronary atherosclerosis. An assessment of the functional consequences of coronary artery stenosis will be made using the computed tomography fractional flow reserve (CT-FFR) technique, using the HeartFlow™ platform.[18]

High-sensitivity cardiac troponin I assay

Serial blood samples will be obtained on enrolment to the study, at 24 hours, and at last point of contact to the research team (on discharge from hospital or at outpatient visit for study imaging) in two 9 mL lithium-heparin tubes, two 9mL EDTA plasma tubes and two 9mL serum tubes. Samples obtained will facilitate development of novel biomarkers using proteomic and genomic approaches. The maximum sample volume obtained will be 180 ml. We will obtain wastage serum (surplus) from routinely obtained clinical samples. All blood samples will be stored at -80 degrees Celsius for future development, evaluation and audit of novel and existing cardiovascular biomarkers. High-sensitivity cardiac troponin I concentrations will be measured in batch processing using the ARCHITECT$_{STAT}$ high-sensitive troponin I assay (Abbott Laboratories, Abbott Park, IL). This assay has a limit of detection of 1.2 ng/L and the inter-assay CV<10% at 4.7 ng/L. The upper reference limit (99th centile) is 26 ng/L, and is two-fold higher in men (34 ng/L) than in women (16 ng/L).[19,20] All samples will be anonymised and linked by a unique non-identifiable ID.

Genetic analysis
We will extract total RNA from blood samples, and perform qPCR to quantify the expression of long non-coding RNA and associated mRNA. We aim to determine relative expression of candidate transcripts across the multiple patient phenotypes of coronary artery disease.

Data collection and record linkage:
CHI is a population register containing details of all Scottish residents registered with a General Practitioner and will be used to link all data sources. The ***Scottish Morbidity Record (SMR)*** will be used to identify the rate of myocardial infarction or cardiovascular death at 1 year. As in our previous studies, additional clinical information will be obtained through the TrakCare software application (InterSystems Corporation, Cambridge, MA, USA); with further information collected through a standardised *pro forma*.[8,9] This will include details of their presenting complaint, risk factors and past medical history including the following: time of onset of symptoms, time of hospitalisation, patient demographics (e.g. age and sex, cardiovascular risk factors, medical therapy on admission), GRACE score, heart rate, blood pressure, management in the Emergency Department, referral to cardiology, and discharge location. Any change to medical therapy will be extracted from the patients' standardised electronic discharge summary. Reports from diagnostic coronary angiography, percutaneous and surgical coronary revascularisation will be extracted from the ***TOMCAT database*** (Cardiovascular Information Management System, Philips Healthcare). This information is stored locally on NHS Lothian servers. The trial results will be reported in accordance with the CONSORT guidelines and, where possible, the clinical profile of non-recruited and ineligible patients will be recorded with Caldicott approval.

8. Statistical Analysis

Power calculations:
This exploratory analysis will determine the prevalence of coronary artery disease in patients with myocardial injury secondary to oxygen supply and demand imbalance. During our pilot study,[19] 1,126 consecutive patients were recruited over eight weeks. An adjudicated diagnosis of type 2 myocardial infarction or myocardial injury was made in 74 patients, and we therefore anticipate that 9 patients per week will meet the inclusion and exclusion criteria. We aim to recruit 100 patients as this is a feasible sample size, and will complete recruitment over 12-18 months. As this is exploratory analysis, no formal power calculations have been performed.

Statistical analysis:
We will report the prevalence of coronary artery stenosis in those with type 2 myocardial infarction. We will derive linear mixed-effects models to compare the release kinetics of cardiac troponin in patients with type 2 myocardial infarction, with a control cohort of individuals with type 1 myocardial infarction. We will determine whether differences in release kinetics may aid identification of such patients in clinical practice. We will validate a prediction model of coronary disease in patients with type 2 myocardial infarction, derived in the HighSTEACS clinical trial, with observed prevalence of coronary artery disease in the EVEREST-MI study.

9. Expected Results

To date, no study has prospectively evaluated patients with myocardial injury secondary to myocardial oxygen supply or demand imbalance for the presence of obstructive coronary artery disease. From our previous studies, we know patients with these diagnoses are older, and have a higher prevalence of co-morbid conditions such as hypertension and hyperlipidaemia. We anticipate that the majority of patients will have evidence of obstructive coronary artery disease on diagnostic testing. At present, this patient population are understudied and have extremely poor outcomes. By determining the role of coronary artery disease in the pathogenesis of type 2 myocardial infarction and myocardial injury, we can better inform clinicians on the importance of risk stratification for coronary disease in this population. Ultimately, this study will guide the rationale and design of future therapeutic trials of secondary prevention in patients with type 2 myocardial infarction and myocardial injury.

10. Safety Reporting and Study Monitoring

10.1 Trial Management Group

The trial management group will meet regularly and consists of the grant applicants, the trial manager, and research team.

10.2 Safety Reporting

An **adverse event** (AE) is any untoward medical occurrence in a study participant, which does not necessarily have a causal relationship with the study intervention.

An **adverse reaction** (AR) is any untoward and unintended response that has occurred due to the intervention.

A **serious adverse event** (SAE) or **serious adverse reaction** (SAR). Any AE or AR that:

- results in death of the study participant;
- is life threatening*;
- requires in-patient hospitalisation^ or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect;
- results in any other significant medical event not meeting the criteria above.

*Life-threatening in the definition of an SAE or SAR refers to an event where the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

^Any hospitalisation that was planned prior to enrolment will not meet SAE criteria. Any hospitalisation that is planned post enrolment will meet the SAE criteria.

10.3 identifying AEs and SAEs

The risk of AEs/SAEs relates to the investigations to be undertaken as part of the study, namely invasive coronary angiography, CT coronary angiography or cardiac magnetic resonance imaging. Any likely AE or SAE will be identified at or immediately after the time of the investigation taking place. Patients will be given the opportunity to contact the research team should they develop new symptoms. If there is any doubt as to whether a clinical observation is an AE, the event will be recorded.

10.4 Recording AEs and SAEs

When an AE/SAE occurs, the Investigator, or another suitably qualified physician in the research team will review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the Case Record Form and or AE log and on the SAE form (if the AE meets the criteria of serious). The information to be recorded will include the type of event, onset date, assessment of severity and causality, date of resolution and treatment required, investigations needed and outcomes. Pre-existing medical conditions (i.e.

existed prior to informed consent) will be recorded as medical history and only recorded as adverse events if medically judged to have worsened during the study. All AE or SAE will be recorded from the time of first intervention. AEs and SAEs will be followed up until outcome of recovered, recovered with sequelae or death of study participant.

We will not record, notify or report the development of a simple radial or femoral haematoma related to invasive coronary angiography where this is managed conservatively with pressure and where hospital admission was not required.

Worsening of the Underlying Condition during the Trial

Medical occurrences or symptoms of deterioration that are expected due to the participant's underlying condition will be recorded in the patient's medical notes and will only be recorded as AEs on the AE log if medically judged to have unexpectedly worsened during the study. Events that are consistent with the expected progression of the underlying disease will not be recorded as AEs.

10.5 Assessment of AEs and SAEs

Each AE will be assessed for seriousness, causality, severity and ARs will be assessed for expectedness by the Principal Investigator or another suitably qualified physician in the research team who has been delegated this role.

Assessment of Seriousness

The Investigator will make an assessment of seriousness as defined above.

Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to the study intervention according to the definitions below.

- Unrelated: where an event is not considered to have occurred as a result of the study intervention.

- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the study intervention.

If two assessments of causality are made (for example between the Primary and the Chief Investigator), the 'worst case' assessment will be used for reporting purposes.

Assessment of Expectedness

If the AE is judged to be related to the study intervention, the Investigator will make an assessment of expectedness:

Expected: the type of event is expected in line with the study intervention

Unexpected: the type of event was not listed in the protocol or related documents/literature as an expected occurrence.

Assessment of Severity

The Investigator will make an assessment of severity for each AE and record this on the CRF/AE log or SAE form according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

10.6 Reporting of SAEs

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance ***within 24 hours****. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying ACCORD. The SAE report form can be updated when the additional information is received.*

The SAE report will provide an assessment of causality and expectedness at the time of the initial report to ACCORD according to Sections 10.5 Assessment of Causality and 10.5, Assessment of Expectedness.

The SAE form will be transmitted via email to safety@accord.scot

10.7 Follow up procedures

After initially recording an AE or recording and reporting an SAE, the Investigator will make every effort to follow each event until a final outcome can be recorded or reported as necessary. Follow up information on an SAE will be reported to the ACCORD office. If, after follow up, resolution of an event cannot be established, an explanation will be recorded on the CRF or AE log or additional information section of SAE form.

11. Oversight Arrangements

Inspection of Records

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

Risk Assessment

A study specific risk assessment will be performed by representatives of the co-sponsors, ACCORD monitors and the QA group, in accordance with ACCORD governance and sponsorship SOPs. Input will be sought from the Chief Investigator or designee. The outcomes of the risk assessment will form the basis of the monitoring plans and audit plans. The risk assessment outcomes will also indicate which risk adaptions (delete if no adaptations were possible) could be incorporated into to trial design.

Study Monitoring And Audit

The ACCORD Sponsor Representative will assess the study to determine if an independent risk assessment is required. If required, the independent risk assessment will be carried out by the ACCORD Quality Assurance Group to determine if an audit should be performed before/during/after the study and, if so, at what frequency.

Risk assessment, if required, will determine if audit by the ACCORD QA group is required. Should audit be required, details will be captured in an audit plan. Audit of Investigator sites, study management activities and study collaborative units, facilities and 3rd parties may be performed.

12. Good Clinical Practice

Ethical Conduct

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).
Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met.

Investigator Responsibilities

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s).

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's medical notes (if applicable).

Study Site Staff

The Investigator must be familiar with the protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the protocol and their trial related duties.

Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

Investigator Documentation

The Principal Investigator will ensure that the required documentation is available in local Investigator Site files ISFs.

GCP Training

For non-CTIMP (i.e. non-drug) studies all researchers are encouraged to undertake GCP training in order to understand the principles of GCP. However, this is not a mandatory requirement unless deemed so by the sponsor. GCP training status for all investigators should be indicated in their respective CVs.

Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to individuals from the research team treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.

Computers used to collate the data will have limited access measures via user names and passwords. Published results will not contain any personal data that could allow identification of individual participants.

13. Study Conduct Responsibilities

Protocol Amendments

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Amendments will be submitted to a sponsor representative for review and authorisation before being submitted in writing to the appropriate REC, and local R&D for approval prior To Participants Being Enrolled Into An Amended Protocol.

Management of Protocol Non Compliance

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, and local R&D for review and approval if appropriate.

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. All protocol deviation logs and violation forms should be emailed to QA@accord.scot

Deviations and violations are non-compliance events discovered after the event has occurred. Deviation logs will be maintained for each site in multi-centre studies. An alternative frequency of deviation log submission to the sponsors may be agreed in writing with the sponsors.

Serious Breach Requirements

A serious breach is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (seriousbreach@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to research ethics committees as necessary.

Study Record Retention

All study documentation will be kept for a minimum of 3 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

End of Study

The end of study is defined as the last participant's last visit.

The Investigators or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, and R+D Office(s) and co-sponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the co-sponsors via email to resgov@accord.scot.

A summary report of the study will be provided to the REC within 1 year of the end of the study.

Continuation of treatment following the end of study

Detail if intervention will be continued to be provided following the end of the study. If not provide justification

Insurance and Indemnity

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's National Health Service will have the benefit of NHS Indemnity.
- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.

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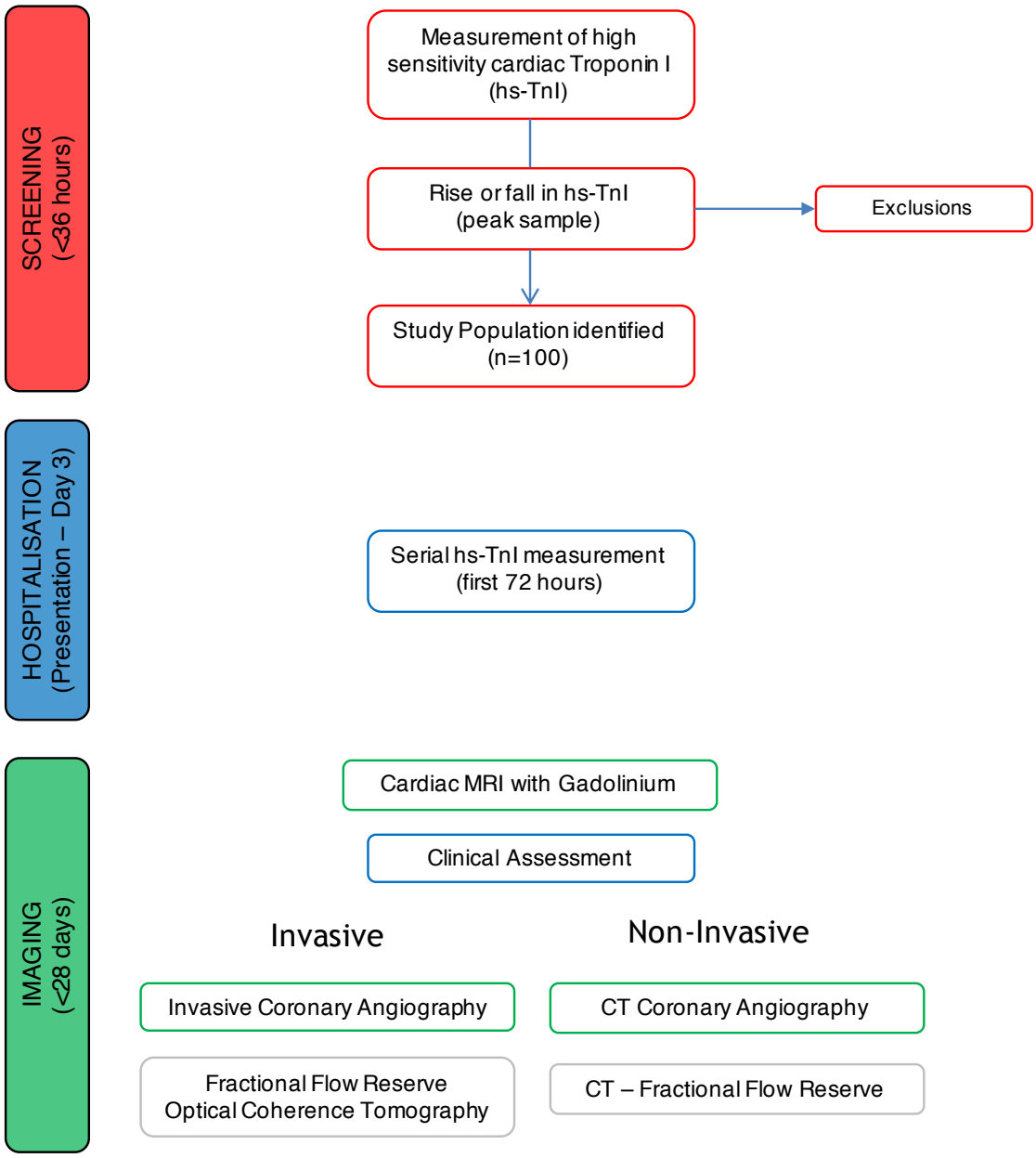
APPENDIX 1



Adjudication and Classification

Step 1: Two cardiologists independently review all clinical information including
- Baseline characteristics
- Cardiac investigations including imaging results
- Outcomes up to 30 days

Step 2: Classification according to the Universal Definition of Myocardial Infarction
Step 3: Discrepant cases resolved by review of source documents and consensus

APPENDIX 6

IDENTIFICATION OF NOVEL BIOMARKERS TO DISTINGUISH TYPE 1 AND TYPE 2 MYOCARDIAL INFARCTION

Josephine Lansdell Grant Application

Identification of novel cardiovascular biomarkers to distinguish type 1 and type 2 myocardial infarction

Dr Andrew R Chapman
Supervisors: Professor Nicholas Mills, Professor David Newby
BHF Centre for Cardiovascular Science, University of Edinburgh

Summary of research project

Type 2 myocardial infarction is common, and occurs due to myocardial oxygen supply or demand imbalance, during an episode of tachyarrhythmia, hypotension or hypoxia secondary to another medical condition. Unlike type 1 myocardial infarction, due to atherosclerotic plaque rupture and intraluminal thrombosis, type 2 myocardial infarction is poorly understood, yet it is responsible for almost half of all detectable myocardial injury in hospitalised patients. At present, distinguishing patients with type 1 and type 2 myocardial infarction is challenging in clinical practice as there are no objective criteria for diagnosis, investigation or management, and clinical outcomes are extremely poor, with as few as 1 in 3 patients alive at five years.

In a prospective cohort study of patients with type 2 myocardial infarction, we aim to screen candidate cardiovascular biomarkers and identify those which could distinguish patients with type 1 and type 2 myocardial infarction. Identification of objective biomarkers has the potential to allow more accurate diagnosis in clinical practice, and would facilitate the identification of patients for therapeutic trials of secondary prevention with the aim of improving cardiovascular outcomes.

Introduction

The definition of acute myocardial infarction has evolved to accommodate increasingly sensitive markers of myocardial necrosis and imaging methods that allow greater understanding of the pathogenic mechanisms of acute coronary syndrome. [1] The third universal definition of myocardial infarction recommends a classification based on aetiology (***Figure 1***), where type 1 myocardial infarction is due to plaque rupture or erosion with intracoronary luminal thrombosis, and type 2 myocardial infarction due to myocardial oxygen supply-demand imbalance in the context of an alternate medical condition such as tachyarrhythmia, hypotension or hypoxia. Patients with elevated cardiac troponin concentrations who do not have overt myocardial ischemia are classified as having myocardial injury.[2]

The universal definition makes a distinction between type 1 and type 2 myocardial infarction based on likely aetiology, but in clinical practice there remains considerable overlap and to date there have been no prospective mechanistic studies to evaluate the range of underlying pathophysiology in these patients. Acute myocardial injury may occur in a variety of cardiac and non-cardiac illnesses (***Table 1***) as a consequence of myocardial oxygen supply-demand mismatch (hypotension, tachycardia or hypoxemia), due to direct injury in sepsis or viral myocarditis, or as part of the pathophysiological process in acute left ventricular failure. However, in some cases the presenting illness may be associated with a pro-inflammatory and pro-thrombotic state with myocardial injury due to embolisation of platelet aggregates and thrombus from an otherwise silent vulnerable plaque. Furthermore, myocardial injury can occur due to myocardial oxygen supply-demand mismatch in the presence of prognostically

important, but unrecognised stable coronary artery disease. Chronic myocardial injury may occur in structural heart disease (hypertensive heart disease, ischaemic or dilated cardiomyopathy) or secondary to other non-cardiac illness such as chronic renal failure. As an example, the detection of chronic myocardial injury may be clinically useful in valvular heart disease, with serum cardiac troponin I concentrations associated with cardiac mass, replacement fibrosis and prognosis in patients with aortic stenosis.[3]

The Global Task Force is reviewing the classification of myocardial infarction, and recognize the need to provide greater clarity for clinicians in practice.[4] Patients with type 2 myocardial infarction and myocardial injury have higher crude rates of all-cause death compared with those with type 1 myocardial infarction, [5-10] with two-thirds of patients dead at five years and an equivalent crude rate of major adverse cardiovascular events (myocardial infarction or cardiac death).[11]At present, we have no objective criteria to guide the diagnosis, investigation and management of patients with type 2 myocardial infarction, and differentiating patients is subjective.[12] Prior studies have suggested significant heterogeneity in the prevalence of type 2 myocardial infarction between centres, reflecting the difficulties in consistent application of the guideline recommendations. If objective criteria could be developed to identify patients with type 2 myocardial infarction, this would allow clinicians to improve the accuracy of diagnosis, and facilitate the development of therapeutic trials of proven secondary prevention therapies in this population with the aim of improving cardiovascular outcomes.

Detection of myocardial injury

Cardiac troponin is an integral component of the contractile apparatus of the cardiomyocyte, expressed exclusively within the myocardium. It is a complex of three subunits, C, I and T, which regulates calcium mediated excitation-contraction coupling. The majority of cardiac troponin is intracellular, with >90% of troponin isoforms located within the sarcomere, and the remainder unbound within the cytoplasmic pool.[13] The mechanisms of cardiac troponin release into the circulation are thought to include myocyte necrosis, apoptosis, formation and release of membranous blebs, increased membrane permeability and release of proteolytic troponin degradation products.[13] However, the underlying cause of troponin release will often differ. Cardiac troponin may be released when cardiomyocytes undergo mechanical stretch in response to pressure or volume overload, through activation of intra-cellular proteases associated with intra-cellular degradation of troponin.[14] Tachycardia may stimulate stress-responsive integrins within the cardiomyocyte, triggering release of intact cardiac troponin I from viable cardiomyocytes in the absence of necrosis.[15] Furthermore, troponin release has also been demonstrated *in vivo* in patients who develop reversible ischaemia during nuclear perfusion imaging with stress testing.[16]

Cardiac troponin is the only recommended biomarker for the detection of myocardial necrosis, and it is integral to the diagnostic criteria for myocardial infarction.[1] However, as the cellular mechanisms of myocardial injury are thought to differ between subtypes of myocardial infarction, there may be opportunities to identify other candidate cardiovascular biomarkers which may distinguish subtypes of myocardial infarction, improving diagnostic accuracy. We hypothesise that in the absence of plaque rupture, platelet aggregation, embolisation and microvascular

obstruction in type 2 myocardial infarction, the profile of sarcomeric and inflammatory proteins released from the myocardium will differ compared to that observed in patients with type 1 myocardial infarction.

Aims and objectives

- To identify candidate biomarkers that differentiate between type 1 myocardial infarction due to plaque rupture or erosion with thrombosis, and type 2 myocardial infarction due to myocardial oxygen supply-demand imbalance

Project design

Design: Prospective observational case-control study

Setting: Royal Infirmary of Edinburgh, a tertiary cardiac centre

Study population: Patients presenting to the Emergency Department of the Royal Infirmary of Edinburgh, a tertiary cardiac centre, with symptoms or signs of myocardial ischaemia and evidence of myocardial necrosis (defined as a rise and or fall in cardiac troponin concentration on serial testing, with at least one value >99th centile).

Type 1 myocardial infarction

We will identify patients presenting to hospital with an adjudicated diagnosis of type 1 myocardial infarction. Patients will be classified as having a type 1 myocardial infarction when myocardial necrosis occurs in the context of an isolated presentation with symptoms suggestive of myocardial ischemia, or evidence of myocardial ischemia on the electrocardiogram. Each case will be reviewed and classified

independently by two cardiologists, and any discrepancies resolved by consensus through in-depth review of all clinical investigations. Ethical approval has been obtained as part of South East Scotland BioResource scheme (SR546, 15/ES/0094).

Type 2 myocardial infarction

We will identify patients with an adjudicated diagnosis of type 2 myocardial infarction enrolled into a trial entitled '*Evaluating the role of coronary artery disease to Resolve the diagnosis of Type 2 myocardial infarction'* (EVEREST-MI). Patients with symptoms or signs of myocardial ischemia that were thought to be due to increased oxygen demand (e.g. tachyarrhythmia or hypertrophy) or decreased supply (e.g. hypotension, hypoxia or anaemia) and myocardial necrosis in the context of an alternative clinical diagnosis are classified as having a type 2 myocardial infarction. Each case is reviewed and classified independently by two cardiologists, and any discrepancies resolved by consensus through in-depth review of all clinical investigations. This clinical trial is using imaging (CT or invasive coronary angiography and cardiac MRI) to better understand the mechanism of myocardial injury. This clinical trial is recruiting, registered at clinicaltrials.gov (NCT:03338504), approved by the national research ethics committee and is being conducted in accordance with the Declaration of Helsinki.

Subjects

We will evaluate 45 patients with type 1 myocardial infarction and 45 patients with type 2 myocardial infarction. As this is a pilot study to identify and validate candidate biomarkers, no formal power calculations have been performed.

Research methods

Blood sampling:

In all patients venous blood samples will be obtained at the time of recruitment and at 24 hours and serum, lithium-heparin and EDTA plasma prepared for storage. All samples will be anonymised and linked by a unique non-identifiable ID. High-sensitivity cardiac troponin I concentrations will be measured in batch processing using the ARCHITECT$_{STAT}$ high-sensitive troponin I assay (Abbott Laboratories, Abbott Park, IL). This assay has a limit of detection of 1.2 ng/L and the inter-assay CV<10% at 4.7 ng/L.[3] The upper reference limit (99th centile) is 26 ng/L, and is two-fold higher in men (34 ng/L) than in women (16 ng/L).[17,18]

Proteomic analysis:

EDTA plasma from 90 subjects (45 type 1 myocardial infarction, 45 type 2 myocardial infarction) will be transferred to Olink Proteomics (Upsalla Science Park, Upsalla, Sweden) for analysis. The Olink Proteomics Inflammation $^{(96x96)}$, Olink Proteomics Cardiovascular II $^{(96x96)}$ and Olink Proteomics Cardiovascular III $^{(96x96)}$ biomarker panels are high-throughput, multiplex immunoassay systems capable of analysing multiple proteins associated with inflammatory or cardiovascular disease, simultaneously. A total 276 candidate proteins will be evaluated. This technology is facilitated by novel proximity extension assay (PEA) technology. Each panel contains 92 oligonucleotide-labelled antibody pairs which bind to the target when present in the sample. Binding by antibody pairs triggers the formation of double-stranded DNA amplicons, which are quantified by real-time polymerase chain reaction (PCR). The resultant relative values are normalized and log transformed, where a high value

correlates to a high protein content. PEA is a robust and reproducible technique, with intra-assay coefficient of variation (CV) ranging between 5% and 13%, and inter-assay CV between 9% and 39% dependent on assay type. Whilst a novel technique, Olink Proteomics have informed the outcomes of several recent cardiovascular studies.[19,20] Further information about reproducibility and validation may be found at http://www.olink.com.

Data analysis:

Baseline characteristics will be summarised as mean (standard deviation, SD) or median (inter-quartile range, IQR) as appropriate. Patient characteristics in those with type 1 myocardial infarction will be compared to those with type 2 myocardial infarction using a chi-square test or Wilcoxon rank sum test as appropriate. We will explore the relationship between high-sensitivity cardiac troponin concentration and candidate biomarkers using pairwise correlation, illustrated in a correlation matrix and ordered by hierarchical clustering.[20] We anticipate a range of candidate biomarkers will be elevated in patients with type 1 and type 2 myocardial infarction, but some may distinguish between diagnoses. The association between biomarker value and diagnosis (type 1 or type 2 myocardial infarction) will be explored by fitting univariate generalised linear models with a binomial link. Candidate biomarkers which are predictive in univariate analysis will be evaluated in a multi-variate generalised linear model with adjustment for age, gender and renal function. A final model will be derived using backward elimination, including co-variates with a P-value of <0.05. We will assess the discriminatory performance of the model by evaluating sensitivity, specificity, and area under the receiver operator curve (C statistic). For cardiac

troponin, and candidate biomarkers which show significant associations with both diagnoses, we will evaluate the time course of biomarker release using a polynomial linear mixed effects model, with a random term for subject. Comparisons will be adjusted for multiple testing using the Bonferroni method. All statistical analyses will be performed using the statistical package R (Version 3.2.2).

Potential of research for patient care

Patients with type 2 myocardial infarction are understudied, commonly excluded from trials of cardiovascular therapies which focus on patients with type 1 myocardial infarction where the mechanisms of disease are better understood. This is despite type 2 myocardial infarction being a common condition, affecting twice as many patients over the age of 75 years than type 1 myocardial infarction, and outcomes for these patients are extremely poor, with as few as one-third alive at five years after index diagnosis.

The international collaboration responsible for the universal definition of myocardial infarction acknowledge the limitations in the current classification.[4] Lack of accurate diagnostic tools limits our ability to do further research into the underlying pathophysiology of this condition. The identification of candidate biomarkers in the proposed study would provide avenues for further research. We will select candidate biomarkers with high discriminatory ability for type 1 or type 2 myocardial infarction, and perform validation studies using immunoassay and ELISA technology in our stored samples. This will facilitate quantification of total protein concentration and development of a diagnostic approach for use in clinical practice. Ultimately, this pilot

study will form the basis of a project of work to provide a more robust, objective means of diagnosis in patients with type 2 myocardial infarction, to allow planning and delivery of prospective clinical trials of cardiovascular therapy with the aim of improving outcomes.

Figure 1. Third universal definition of myocardial infarction[2,21]

Table 1. Causes of myocardial necrosis stratified by aetiology

Primary myocardial ischaemia	***Supply or demand imbalance causing myocardial ischaemia***	***Injury not related to myocardial ischaemia***	***Multifactorial or indeterminate aetiology***
Atherosclerotic Plaque Rupture *Intraluminal Coronary Thrombus* *Distal Microembolisation* *Coronary Artery Dissection*	*Anaemia* *Aortic dissection* *Aortic valve disease* *Tachy- or Brady- Arrhythmias* *Coronary embolism or vasculitis* *Coronary endothelial dysfunction* *Coronary vasospasm* *Hypertension* *Left Ventricular Hypertrophy* *Hypertrophic cardiomyopathy* *Respiratory failure* *Shock* - *Cardiogenic* - *Hypovolaemic* - *Septic*	*Ablation* *Cardiac contusion* *Cardiac surgery* *Cardiotoxic drugs* *Cardioversion* *Cytokine mediated injury* *Myocarditis* *Pacing* *Rhabdomyolysis*	*Acute / Chronic Heart Failure* *Burns* *Critical Illness* *Infiltrative diseases* - *Amyloidosis* - *Sarcoidosis* *Pulmonary embolism* *Pulmonary hypertension* *Acute Kidney Injury* *Chronic Kidney Disease* *Strenuous exercise* *Takotsubo cardiomyopathy* *Stroke* *Subarachnoid Haemorrhage*

Adapted from the Third Universal Definition of Myocardial Infarction [2]

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APPENDIX 7

PUBLICATIONS, PRESENTATIONS AND AWARDS ARISING FROM THIS THESIS

AWARDS AND DISTINCTIONS

PhD Research Prize. CVS Symposium: University of Edinburgh	2018
Commendation. CMVM Innovation Prize: University of Edinburgh	2018
Samuel A. Levine Young Investigator Award: AHA Scientific Sessions	2017
Highest Ranking Abstract: BCS Annual Conference	2017
Autumn Research Prize: Scottish Cardiac Society	2016
British Cardiovascular Society (BCS) Travel Grant. ESC Congress	2016
Emily Taylor Scottish Cardiac Society (SCS) Travel Grant. ESC ACCA	2016

PUBLICATIONS

Chapman AR, Hesse K, Andrews JPM, Lee KK, Anand A, Shah AS, Sandeman D, Ferry AV, Jameson J, Piya S, Stewart S, Marshall L, Strachan FE, Gray AJ, Newby DE. High-sensitivity cardiac troponin I and clinical risk scores in patients with suspected acute coronary syndrome. *Circulation.* 2018. Epub ahead of print.

Chapman AR, Shah AS, Anand A, McAllister D, Strachan F, Newby DE, Mills NL. Long term outcomes of patients with type 2 myocardial infarction or myocardial injury. *Circulation.* 2017. PMID: 29150426

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PRESENTATIONS

High-sensitivity cardiac troponin I and clinical risk scores in patients with suspected acute coronary syndrome
European Society of Cardiology Congress, Munich, August 2018

High-sensitivity cardiac troponin I and risk stratification in suspected acute coronary syndrome
American Heart Association Congress, Anaheim, California, November 2017

High-sensitivity cardiac troponin and the classification of myocardial infarction.
American College of Cardiology Congress, Washington D.C., 2017

Optimal risk stratification pathways for patients with suspected acute coronary syndrome.
American College of Cardiology Congress, Washington D.C., 2017

Improving the characterisation of patients with myocardial injury and infarction
ESC Acute Cardiovascular Care Congress, Lisbon, October 2016

Long term outcomes of patients with type 2 myocardial infarction and myocardial injury
European Society of Cardiology Congress, Rome, August 2016

Comparison of the efficacy and safety of novel rule out pathways for acute coronary syndrome
European Society of Cardiology Congress, Rome, August 2016